Harnessing Hydroxylating Enzymes for the Synthesis of Natural Products and Their Analogues with Relevance to Dementia



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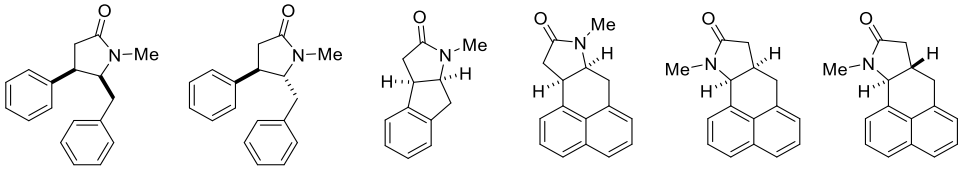
Abstract

Chapter 1

The major synthetic studies towards (–)-clausenamide and its analogues are reviewed. The significance of C–H functionalisation and various aspects of the application of cytochrome $P450_{BM3}$ enzymes are discussed.

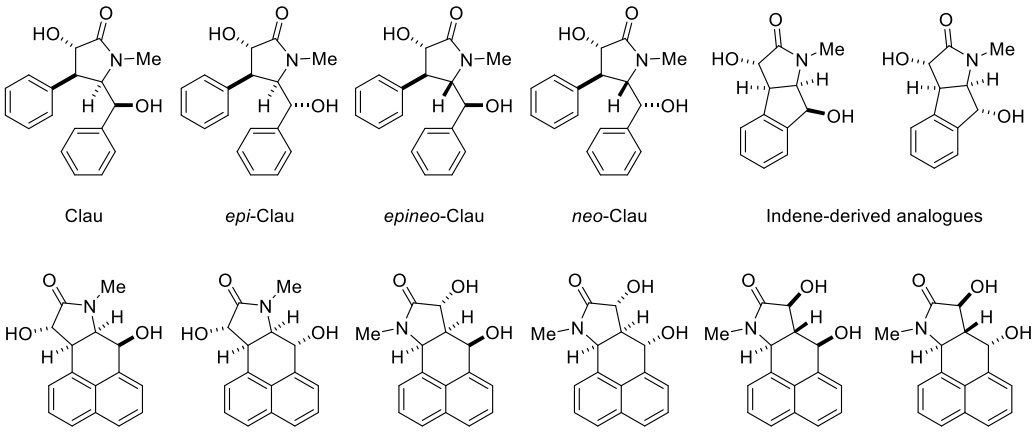
Chapter 2

The chemical syntheses of six target lactams: diphenyl-derived *cis*- and *trans*-lactams, indene-derived lactam, phenalene-derived lactam, and phenalene-derived *cis*- and *trans*-isomer lactams are described.



Chapter 3

The range of metabolites obtained from hydroxylation of the six target lactams by $P450_{BM3}$ variants are discussed, with a focus on the benzylic alcohols. Strategies to access the clausenamide analogues shown below are validated.



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Abbreviations

Ac	acetyl
ACN	acetonitrile
APCI	atmospheric pressure chemical ionisation
aq.	aqueous
Boc	*tert*-butyloxycarbonyl
br	broad
Bu	butyl
Bz	benzoyl
calc.	calculated
cat.	catalytic
cf.	compare
cm^{-1}	wavenumbers
conv.	conversion
COSY	correlation spectroscopy
CYP	cytochrome P450
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
DG	directing group
DMAP	4-dimethylaminopyridine
DME	dimethyoxyethane
DMF	*N*,*N*-dimethylformamide
DMP	Dess–Martin periodinane
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
dr	diastereomeric ratio
e^-	electron
EDCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	ethylenediaminetetraacetic acid
ee	enantiomeric excess
equiv.	equivalents
ESI	electrospray ionisation
Et	ethyl
EWG	electron-withdrawing group
FAD	flavin adenine dinucleotide
FID	Flame ionisation detector
FMN	flavin mononucleotide

g	grams
GC	gas chromatography
GDH	glucose dehydrogenase
h	hours
H^+	proton
HAT	hydrogen atom transfer
HFIP	hexafluoro-2-propanol
HMBC	heteronuclear multiple bond correlation
HMDS	bis(trimethylsilyl)amine
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
HTIB	hydroxy(tosyloxy)iodobenzene
Hz	Hertz
h*v*	radiation
i-	*iso*-
IR	infrared spectroscopy
J	coupling constant
L_n	ligands (chemical structure)
L	litres (volume)
LDA	lithium diisopropylamide
LiHMDS	lithium bis(trimethylsilyl)amide
lit.	literature
m	multiplet (NMR)
m	medium (IR)
m	milli (metric SI prefix)
m	metre (length)
M	molar (concentration)
M	metal (chemical structure)
m-	*meta*-
*m*CPBA	3-chloroperoxybenzoic acid
Me	methyl
Mes	mesityl
MHz	Mega Hertz
min	minutes
mol	moles
MOM	methoxymethyl ether
$mP450_{BM3}$	engineered $P450_{BM3}$
MS	Molecular sieves (reagent)
Ms	methanesulfonyl
MTT	3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/*z*	mass/charge ratio
n	nano

$NADP^+$	nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
n.r.	no reaction
o-	*ortho*-
OD	optical density
p-	*para*-
PCR	polymerase chain reaction
PEG	polyethylene glycol
Ph	phenyl
*p*Ka	acid dissociation constant
*p*KaH	*p*Ka of the conjugate acid
ppm	parts-per-million
Pr	propyl
py	pyridine
q	quartet
quant.	quantitative yield
R	generic group
R_f	retention factor
R_t	retention time
rpm	revolutions per minute
RT	room temperature
s	singlet (NMR)
s	strong (IR)
s	seconds (time)
SAR	structure-activity-relationship
sat.	saturated
S_N1	unimolecular nucleophilic substitution
S_N2	bimolecular nucleophilic substitution
t	triplet
t-	*tert*-
TBS	*tert*-butyldimethylsilyl
TBSOP	*tert*-butyl 2-((*tert*-butyldimethylsilyl)oxy)-1*H*-pyrrole-1-carboxylate
TEA	triethylamine
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transition metal
TMS	trimethylsilyl

TOCSY	total correlation spectroscopy
Ts	4-toluenesulfonyl
U/mL	Units per millilitres
UV-vis	ultraviolet-visible
WT	wild-type
15-mer	a peptide with 15 amino acid residues
Å	Angstrom
°C	degree Celsius
δ	chemical shift
µ	micro
ν_{max}	infrared absorption maximum
λ_{max}	wavelength absorption maximum
1°	primary
2°	secondary
3°	tertiary

Amino acid codes

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

Abbreviations for $P450_{BM3}$ variants

There are 477 amino acid residues in the haem domain of wild-type $P450_{BM3}$, of which 47 residues are replaced in this thesis. The replacement is denoted as AnB, in which the nth residue (A) has changed to a different amino acid residue (B). For example, the denotation of A264G indicates that the alanine (A) residue at the 264th position has been replaced with glycine (G). On the other hand, denotation of A264AV indicates an insertion of a valine (V) residue in between A264 and G265 in the wild-type sequence; however, the numbering of subsequent amino acid residues is not affected; as such, His266 remains as His266 in the inserted variant. The table below shows the residues present in the commonly used abbreviations for $P450_{BM3}$ variants:

Abbreviation	Relationship between abbreviations	Variant
GI	-	A74G/F87I
GIQ	**GI** + L188Q	A74G/F87I/L188Q
GL	-	A74G/F87L
GLQ	**GL** + L188Q	A74G/F87L/L188Q
GV	-	A74G/F87V
GVQ	**GV** + L188Q	A74G/F87V/L188Q
K19	-	H171L/Q307H/N319Y
RLYF	-	R47L/Y51F
KSK19	**K19** + F87A	H171L/Q307H/N319Y/F87A
R19	**K19** + **RLYF**	H171L/Q307H/N319Y/R47L/Y51F
RK	**R19** + F87A **KSK19** + **RLYF**	H171L/Q307H/N319Y/R47L/Y51F/ F87A
RP	**RLYF** + I401P	R47L/Y51F/I401P
KU3	-	N239H/I259V/A276T
RT2	**RLYF** + **KU3** + A191T/L353I	R47L/Y51F/N239H/I259V/A276T /A191T/L353I

Table of Contents

Abstract.....i

Acknowledgements.....ii

Abbreviations.....iv

Amino acid codes.....viii

Abbreviations for P450$_{BM3}$ variants.....ix

Chapter 1: Introduction.....1

1.1 Total synthesis of natural products.....1

1.2 Clausenamide (clau).....2

1.2.1 Isolation, biological activity.....2

1.2.2 Previous synthetic strategies towards clau **1.1** and the *trans*-variant clau (*epineo*-clau) **1.2**.....5

1.3 Chemical C(sp^3)–H activation.....13

1.3.1 Transition-metal-catalysed C–H activation.....14

1.3.2 Metal carbenoid and nitrenoid insertion reactions.....15

1.3.3 Intramolecular homolytic hydrogen atom transfer (HAT).....17

1.4 Cytochrome P450$_{BM3}$ (CYP102A1).....20

1.4.1 Biological function.....20

1.4.2 Active site structure and catalytic cycle.....21

1.4.3 Applications in chemical synthesis.....25

1.4.4 Collaborative research in the Wong and Robertson groups.....27

1.5 Aims and proposed work.....28

Chapter 2: Substrate synthesis.....30

2.1 Initial route to the lactams.....30

2.1.1 Synthesis of the [2+2] cycloaddition precursors.....30

2.1.2 [2+2] Cycloaddition.....33

2.1.3 Lactam formation.....36

2.2 Synthesis of cyclobutanones.....37

2.3 Synthesis of lactams.....41

2.4 Conclusions.....52

Chapter 3: Regio- and diastereoselective oxidation of lactams by P450$_{BM3}$ variants.....54

3.1 Enzymatic screening.....54

3.1.1 Diphenyl-derived *cis*-lactam **1.126**.....56

3.1.2 Diphenyl-derived *trans*-lactam **1.127**.....61

3.1.3 Indene-derived lactam **1.128**.....67

3.1.4 Phenalene-derived lactam **1.129** 71
3.1.5 Phenalene-derived *cis*-isomer lactam **2.85** 73
3.1.6 Phenalene-derived *trans*-isomer lactam **2.86** 77
3.2 Chemical elaboration post-hydroxylation 79
3.3 Conclusions and future work 84
Chapter 4: Experimental 88
4.1 General details 88
4.2 Protein Synthesis 89
4.2.1 Preparation of growth media and buffers 89
4.2.2 *E. coli* transformation 90
4.2.3 Protein expression, harvest, purification and quantification 91
4.2.4 Substrate screening 92
4.2.5 Preparative scale reactions 94
4.3 Synthesis of compounds 95
Appendices 194
References 233

Chapter 1: Introduction

1.1 Total synthesis of natural products

Many natural products possess therapeutic benefit and are often used as lead compounds in drug discovery, with synthetic modifications being introduced to reduce side effects and increase bioavailability.[1, 2] The activity of the vast majority of biologically-active organic molecules, for example in pharmacology, results from the interactions of functional groups dispersed around the carbon-rich core. The functional groups are usually oxygen-containing, or are derived from or attached to oxygen as hydroxyl or carbonyl. Alcohols and ketones act as the fundamental units for further transformations. Each molecule's surface bears a unique profile of polar and non-polar regions that interact in a specific way with biological targets and receptors, leading to activity.

The following chapters describe research directed towards the total synthesis of the natural product (–)-clausenamide **1.1**, a potential anti-dementia drug,[3-5] and its analogues **1.2**–**1.4** (*Figure 1.1*). Work is still establishing the structure-activity-relationship (SAR) profile of clausenamide isomers;[6-8] hence, the current study was designed to add to this SAR profile by preparing rigidified clausenamide analogues in which the two pendant phenyl groups are combined into one or fused in order to provide information about the contribution of the phenyl groups and their relative spatial distribution to the observed activity. Besides that, this project was also designed to expand our knowledge of the reactivity profile of $P450_{BM3}$ variants, in which regio- and diastereoselective oxidation of lactams by $P450_{BM3}$ variants was featured (see *Chapter 3*).

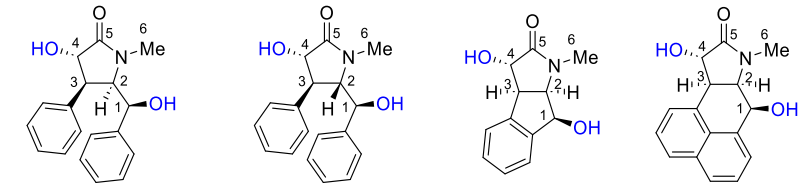


Figure 1.1 The chemical structures of (–)-clausenamide (**1.1**) and its analogues (**1.2**–**1.4**).

1.2 Clausenamide (clau)

1.2.1 Isolation, biological activity

Extracts of the leaves of *Clausenia lansium* ('wampee') have long been used in Chinese herbal medicine.[4, 9, 10] Clausenamide (clau) is one of seven amides isolated from the aqueous extract of *Clausena lansium* leaves.[11] It was found to be a major contributor to the reputed hepatoprotective effect against chemical toxins, including carbon tetrachloride and thioacetamide.[12, 13] Further research found a range of other interesting biological effects of this molecule including enhancing human cognition and treating memory impairment in neurodegenerative diseases, through a multi-target action, which advocates it as a candidate for the treatment of dementia.[14-17]

The chemical structure of clau was elucidated with a combination of spectroscopy and chemical correlations by Huang's group.[11] Clau is a γ-lactam, naturally occurring in racemic form with relative configuration (1*S**,2*R**,3*R**,4*S**). With four stereogenic centres, clau has 16 stereoisomers,[18] as shown in *Figure 1.2*.

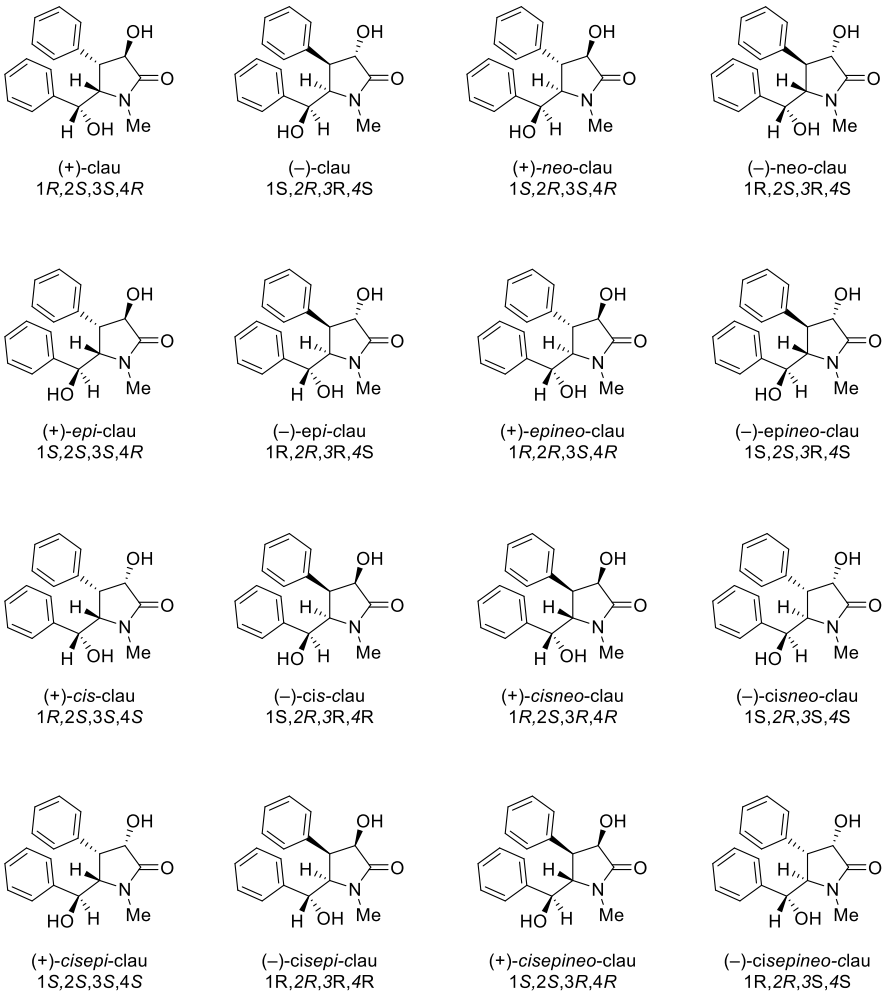


Figure 1.2 The structure and configuration of clau stereoisomers.

The (–)-(1*S*,2*R*,3*R*,4*S*)-clau enantiomer was reported to be the primary nootropic active ingredient as a promising chiral drug candidate for treatment of Alzheimer's disease and other neurodegenerative disorders; the (+)-clau enantiomer shows no detectable effects in comparable assays and exhibits greater toxicity than (–)-clau. This chirality-dependent neuroprotective effect was demonstrated in studies with animal models in which cognitive functions of the animals were impaired by β-amyloid peptide (25-35) ($A\beta_{25\text{-}35}$),[19] transient focal cerebral ischemia,[20] anisodine[21] and okadaic acid.[19] The *in vitro* MTT assay is a colorimetric assay for assessing cell viability based on the ability of NADPH-dependent cellular oxidoreductase enzymes to reduce the yellowish tetrazolium dye to its insoluble

purple formazan form. This assay indicated that (–)-clau exhibits a neuroprotective effect when tested with neuron-like PC12 cells induced by aggregated A$\beta_{25\text{-}35}$.[5]

Mechanistically, the nootropic effect of (–)-clau was shown to be mediated by its multi-target actions, which include mild elevation of intracellular Ca^{2+} concentrations,[22] modulation of the cholinergic system, regulation of synaptic plasticity, and activation of the signalling transduction pathways involved in cognition. Apart from that, (–)-clau suppresses the pathogenesis of Alzheimer's disease by inhibiting multiple etiological processes: (1) Aβ-induced intracellular Ca^{2+} overload and apoptosis (programmed cell death), and (2) tau hyperphosphorylation and neurodegeneration.[4]

Although about 4 g of clau can be isolated from 10 kg of dried leaves, total synthesis of clau has been explored to produce larger quantities for more detailed pharmacological studies.[12] Because clau occurs as the racemate in nature,[11] access to (–)-clau is dependent on chemical synthesis or resolution. Over 20 papers have so far been published from 1987 on the total synthesis of clau and its stereoisomers.[6, 12, 23-32] A number of clau analogues **1.5**–**1.10** (*Figure 1.3*) have also been synthesised to analyse the relationship between the precise three-dimensional structure and activity.[6-8] So far, it has been found that the configuration around the pyrrolidin-2-one and the structure of the lactam *N*-substituent have great impact on the nootropic activities of clau analogues.

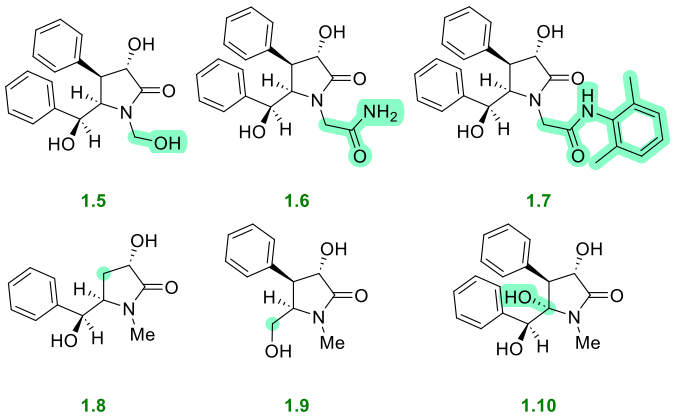


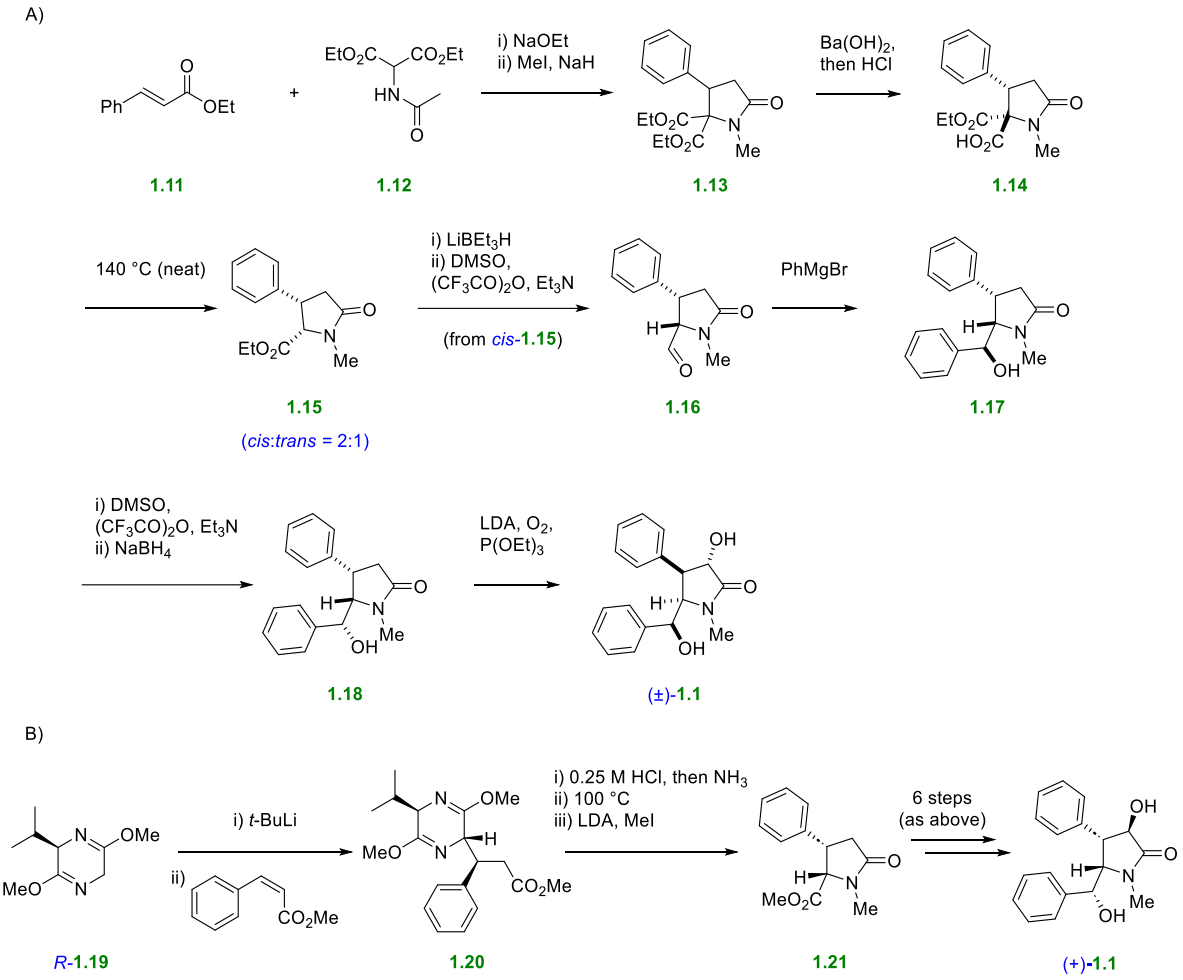
Figure 1.3 Reported clau analogues.

1.2.2 Previous synthetic strategies towards clau 1.1 and the *trans*-variant clau (*epineo*-clau) 1.2

In 1987, total syntheses of (±)-clau (*Scheme 1.1A*) and its separate enantiomers (*Scheme 1.1B*) were first reported by Hartwig and Born.[12] Michael addition between starting materials **1.11** and **1.12**, followed by methylation gave adduct **1.13**. Selective hydrolysis of the ester group *trans* to the phenyl group (→ **1.14**) and decarboxylation yielded the desired *cis*-isomer **1.15** in a ratio of 2:1 to the *trans*-isomer (not shown). Subsequent reduction of *cis*-isomer **1.15** with sterically hindered Super-Hydride® ($LiBEt_3H$), followed by Swern oxidation gave aldehyde **1.16**. Addition of the phenyl residue to the aldehyde gave only one diastereomer **1.17**, a stereochemical outcome aligning with the Felkin–Anh model. The configuration at the new alcohol site was then inverted by oxidation and reduction to give the epimer **1.18** which corresponds to that of clausenamide. Finally, stereoselective carbonyl α-hydroxylation completed the total synthesis of (±)-clau **1.1** in a ten-step route with an overall yield of 6%.

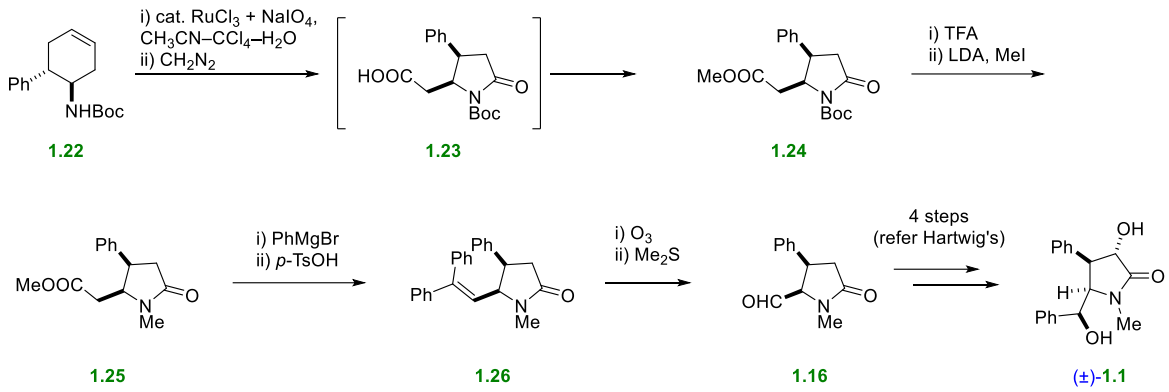
The synthesis of enantiopure clau was developed from the Schöllkopf chiral auxiliary (2*R*)-(–)-dihydropyrazine **1.19**. Metalation with *tert*-butyllithium and subsequent reaction with methyl *cis*-cinnamate afforded adduct **1.20**. Sequential hydrolysis, neutralisation, heating at

100 °C, and methylation gave enantiopure key intermediate **1.21**, which was transformed to (+)-clau **1.1**, following the steps described above for (±)-clau.



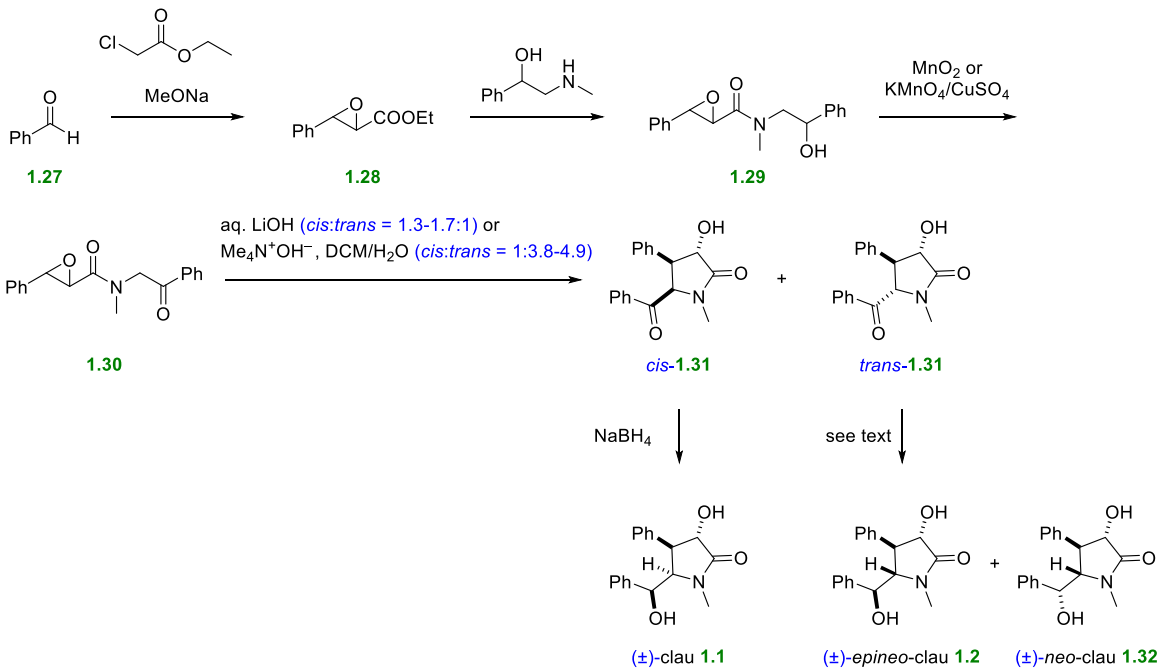
Scheme 1.1 Hartwig and Born's route to (±)-clau **1.1** (A) and (+)-clau **1.1** (B).

In 1991, Ikeda and co-workers[26] reported an alternative route to (±)-clau **1.1** (*Scheme 1.2*). Oxidation of *trans*-disubstituted cyclohexene derivative **1.22** by RuO_4, generated *in situ*, gave lactam-carboxylic acid **1.23**, isolated as its methyl ester **1.24**. Boc deprotection, followed by *N*-methylation and Barbier–Wieland degradation gave key intermediate **1.16**, which was taken on to give (±)-clau **1.1** following the steps described in the Hartwig and Born synthesis.



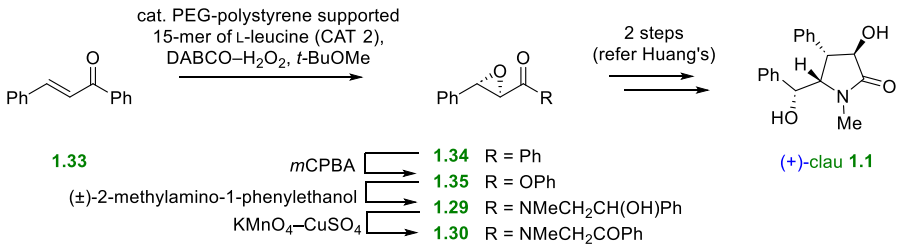
Scheme 1.2 Ikeda's route to (±)-clau **1.1.**

Following that, in 1994, Huang and coworkers[33] reported a five-step biomimetic route to (±)-clau **1.1** (*Scheme 1.3*). α,β-Epoxy ester **1.28** was prepared by Darzens condensation, then amide formation, oxidation, cyclisation, and highly stereoselective reduction gave (±)-clau **1.1** with an overall yield of ~16%. The authors also reported a route to (±)-*epineo*-clau and (±)-*neo*-clau with slight modifications in reagents.[34] Cyclisation with aq. LiOH slightly favoured the *cis*-isomer **1.31** whereas cyclisation in a biphasic (DCM/H_2O) phase transfer system, with base $Me_4N^+OH^-$, favoured the *trans*-isomer **1.31**; no explanations were provided by the authors for the different outcomes, however, in separate work[24] the authors showed that the *cis*-isomer was the major product at equilibrium (aq. LiOH, Et_2O–THF, RT). From *trans*-**1.31**, different reducing agents gave rise to different ratios of (±)-*epineo*-clau **1.2** and (±)-*neo*-clau **1.32**; $NaBH_4$ and L-Selectride® favoured the formation of (±)-*neo*-clau **1.32**, whereas Meerwein–Ponndorf–Verley reduction with Al(*i*-OPr)$_3$/*i*-PrOH produced more (±)-*epineo*-clau **1.2**.



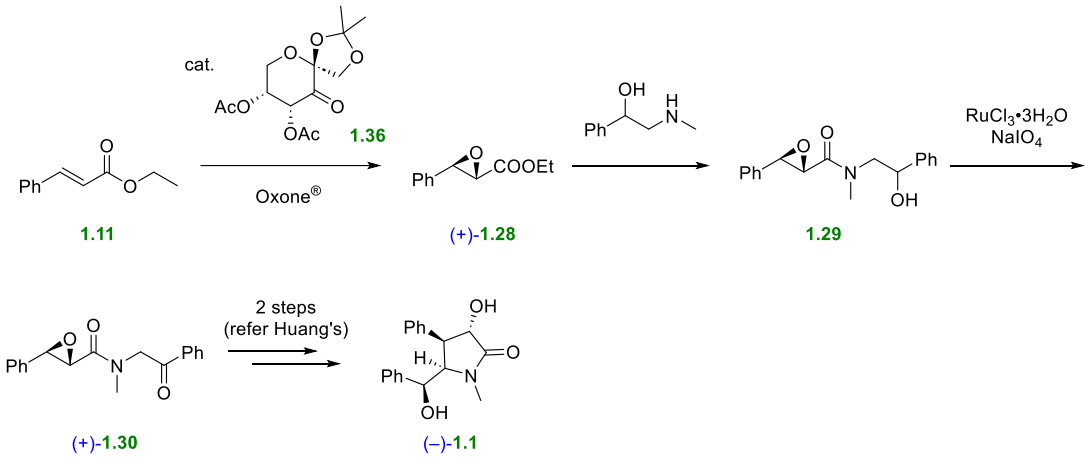
Scheme 1.3 Huang's route to (±)-clau **1.1**, (±)-*epineo*-clau **1.2**, and (±)-*neo*-clau **1.32**.

Roberts and co-workers'[24] route to (+)-clau **1.1**, reported in 1998, followed a similar approach to Huang's (*Scheme 1.4*). Juliá–Colonna asymmetric epoxidation of chalcone **1.33** with poly-L-leucine and hydrogen peroxide afforded epoxide **1.34** in >98% *ee*, which was elaborated to (+)-clau **1.1** as shown.



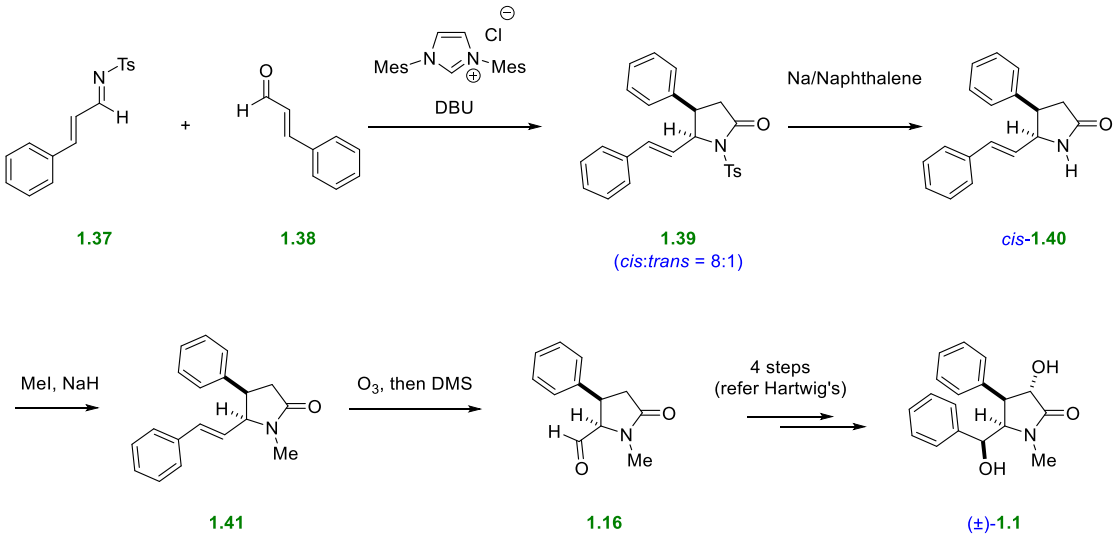
Scheme 1.4 Roberts' route to (+)-clau **1.1**.

In 2011, in another similar approach to Huang's, Shi and co-workers[35] devised a five-step route to (–)-clau (*Scheme 1.5*) based on asymmetric epoxidation mediated by catalyst **1.36**, with overall yield of 19%.



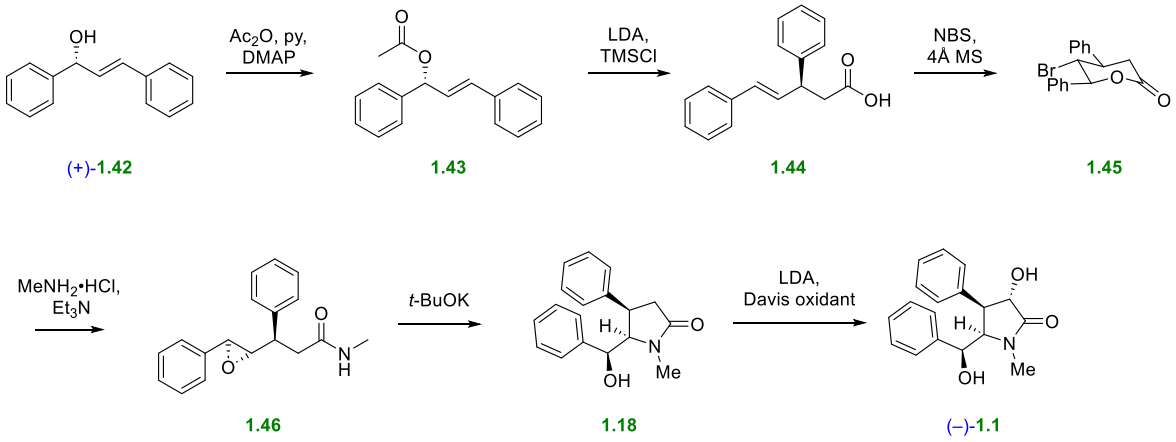
Scheme 1.5 Shi's route to (–)-clau **1.1**.

An alternative strategy to (±)-clau **1.1** was described by Bode and co-workers[36] in their 2012 report (*Scheme 1.6*). Diastereoselective *N*-heterocyclic carbene (NHC)-catalysed annulation of unsaturated imine **1.37** and cinnamaldehyde **1.38** gave lactam **1.39** as a mixture of diastereomers. Removal of the sulfonamide group enabled the *cis*-diastereomer **1.40** to be separated by column for methylation and ozonolysis to give lactam **1.16**. This lactam is an intermediate in the Hartwig and Born route, thus completing a formal synthesis.



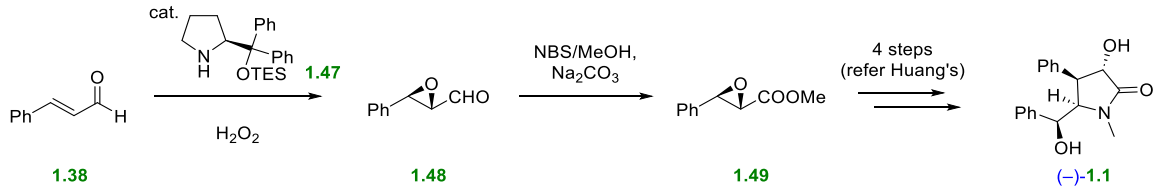
Scheme 1.6 Bode's route to (±)-clau **1.1**.

In 2013, Yu's group[37] reported a seven-step synthesis of (–)-clau (*Scheme 1.7*) from (+)-alcohol **1.42**, which was prepared by kinetic resolution of the racemic alcohol[38] under Sharpless asymmetric epoxidation conditions. Ireland–Claisen rearrangement gave γ,δ-unsaturated acid **1.44** with high stereoselectivity arising from the concerted (chairlike, suprafacial) [3,3]-sigmatropic process. Diastereoselective bromolactonisation resulted in the all-equatorial substituted δ-lactone **1.45**, aminolysis of which formed γ,δ-epoxy-amide **1.46**. Base-promoted cyclisation (→ **1.18**) and Davis oxidation proceeded as in previous syntheses, giving (–)-clau **1.1** with 34% overall yield.



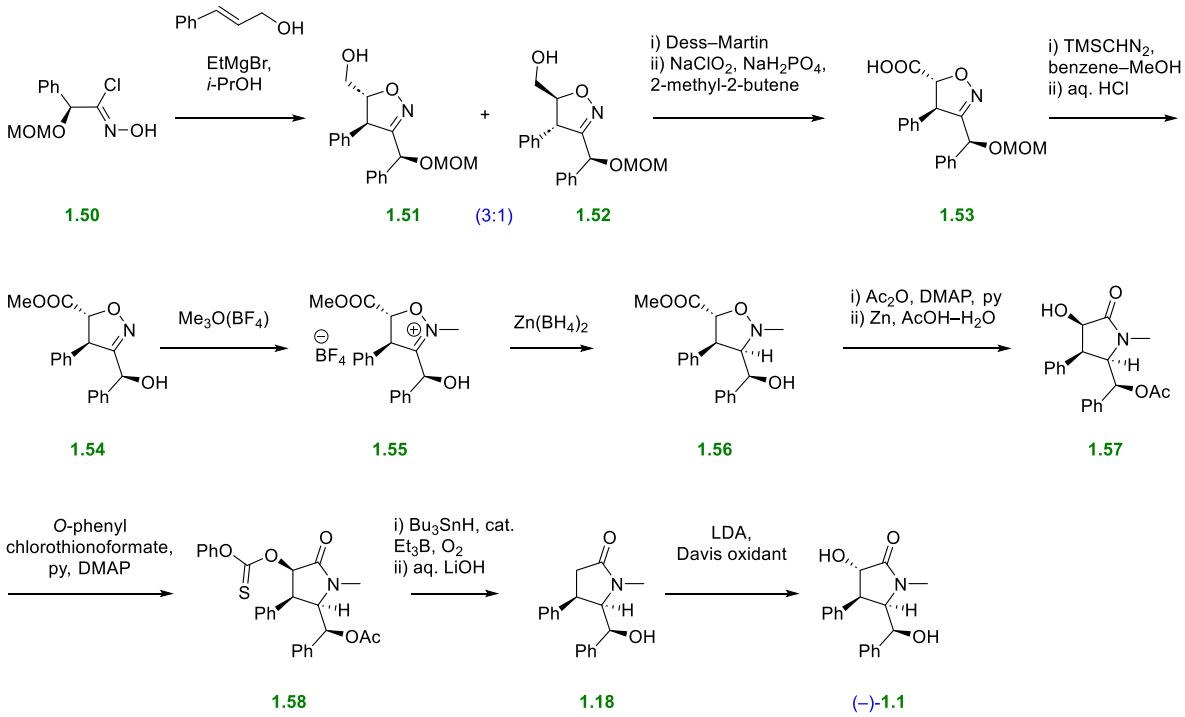
Scheme 1.7 Yu's synthesis of (–)-clau **1.1**.

In the same year, Xuan and Yan[39] reported a six-step synthesis of (–)-clau **1.1** (*Scheme 1.8*) with an overall yield of 20%. One-pot organocatalytic epoxidation and oxidative esterification afforded ester **1.49** from which point the route followed Huang's method with minor variations in the reagents used.



Scheme 1.8 Xuan and Yan's route to (–)-clau **1.1**.

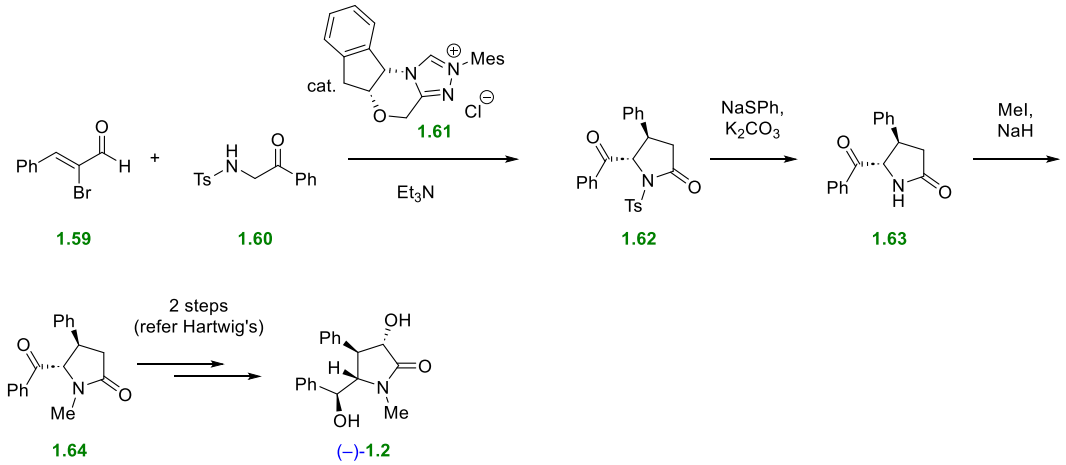
One year later, Tanda and Yamasaki[40] reported a 13-step route to (–)-clau **1.1**, with an overall yield of 7% (*Scheme 1.9*). 1,3-Dipolar cycloaddition of (*E*)-cinnamyl alcohol with the nitrile oxide generated *in situ* from hydroximoyl chloride **1.50**, readily prepared from known ester methyl L-mandelate, gave a 3:1 mixture of separable cycloadducts **1.51** and **1.52**, respectively. Oxidation of the desired diastereomer (**1.51**) gave the carboxylic acid **1.53** which was esterified and deprotected to alcohol **1.54**. *N*-methylation (→ iminium **1.55**) and reduction *anti*- to the phenyl substituent afforded intermediate **1.56** as a single diastereomer. Following acetylation, reductive cleavage of the N–O bond set up cyclisation of the resulting amino ester to give pyrrolidinone **1.57**. Overall inversion of the carbonyl α-alcohol was achieved by Barton–McCombie deoxygenation[41, 42] then Davis oxidation as before to give (–)-clau **1.1**.



Scheme 1.9 Tanda and Yamasaki's route to (–)-clau **1.1.**

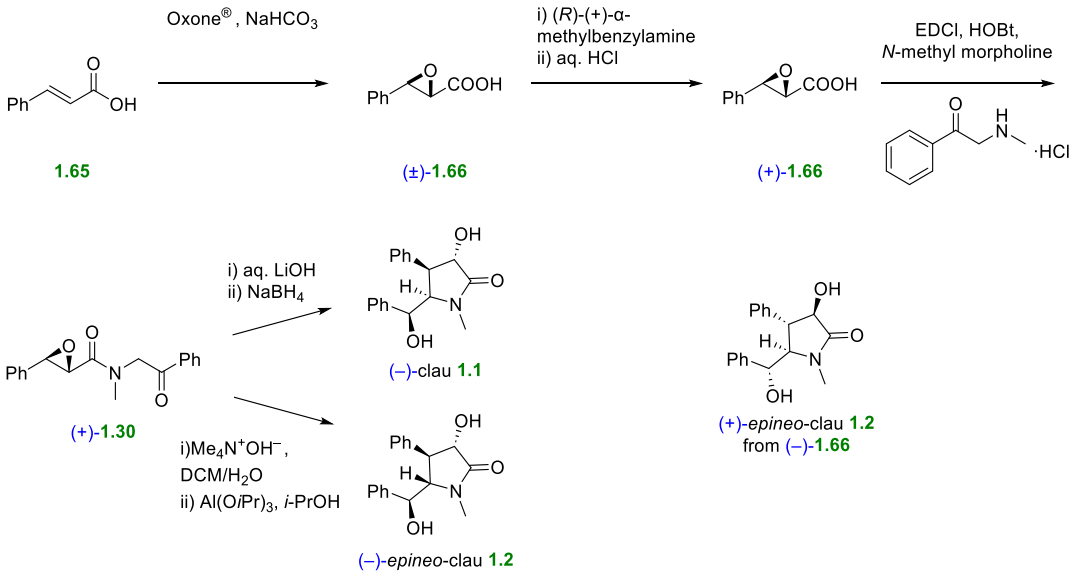
25 years after L. Huang's 1994 route to *trans*-variant clau **1.2** [(1*S**,2*S**,3*R**,4*S**)-*epineo*-clau] (*Scheme 1.3*), an alternative synthesis was reported by Fu and W. Huang[31] (*Scheme*

1.10). Pyrrolidinone **1.62** (with 99% *ee*) was synthesised *via* NHC-catalysed (3+2) annulation between bromoenal **1.59** and α-amino ketone **1.60**, then *N*-deprotection and methylation gave lactam **1.64**. Following the well-established precedent, stereoselective reduction of the ketone and α-hydroxylation gave the clau epimer **1.2**.



Scheme 1.10 Fu and Huang's route to (–)-*epineo*-clau **1.2**.

More recently, in 2021 Lin and co-workers[43] reported routes to (–)-clau **1.1**, (+)-*epineo*-clau **1.2** and (–)-*epineo*-clau **1.2**. In essence, these followed Huang's strategy with slight modification of the reagents used (*Scheme 1.11*), starting from the resolution of epoxy acid **1.66** with either (*R*)- or (*S*)-α-methylbenzylamine.



Scheme 1.11 Lin's route to (–)-clau **1.1** and *epineo*-clau enantiomers (+)-**1.2** and (–)-**1.2**.

1.3 Chemical C(sp^3)–H activation

Selective and direct activation of C–H bonds in organic synthesis is highly desirable, allowing atom- and step-economic approaches for late-stage functionalisation and diversification of complex molecules.[44-47] This strategy allows, for example, methane, the main constituent of natural gas, to be converted directly into derivatives of methanol, the starting point for many industrial processes. Direct C–H bond functionalisation reactions remain, however, a major challenge for chemists due to the inert nature of most C–H bonds and the difficulties in activating a specific C–H site selectively in molecules which contain many other similar C–H bonds.[48, 49] Three main classes of C–H functionalisation reactions have been developed, especially recently: transition metal-catalysed C–H activation (*Figure 1.4A*), metal carbenoid and nitrenoid insertion reactions (*Figure 1.4B*), and hydrogen atom transfer (HAT) by radical species (*Figure 1.4C*).[45]

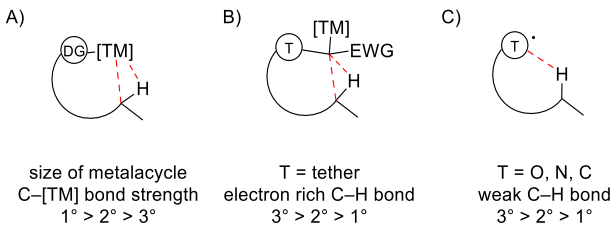


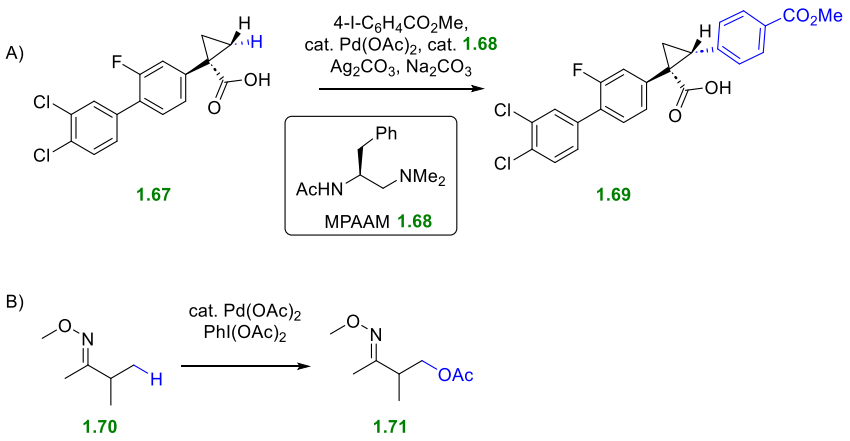
Figure 1.4 Three different modes of remote C–H functionalisation: A) transition metal-catalysed; B) metal carbenoid and nitrenoid insertion reactions; C) HAT by radical species.

1.3.1 Transition-metal-catalysed C–H activation

In order to deliver selective functionalisation of a single C–H bond within a complex molecule, transition metal-catalysed C–H activation usually relies upon substrates containing a coordinating ligand or 'directing group'. Directing groups (DG) bind transiently to the metal centre to position the transition metal centre to an appropriately disposed C–H bond which lowers the energy barrier for the cleavage of this C–H bond.[45]

These stoichiometric ligand-directed C–H activation reactions have been reported with many different transition metals, including Ni,[50] Rh,[51] and Co.[52] Various directed transition-metal catalysed C–H activation methodologies have been comprehensively and extensively reviewed.[45, 47, 53]

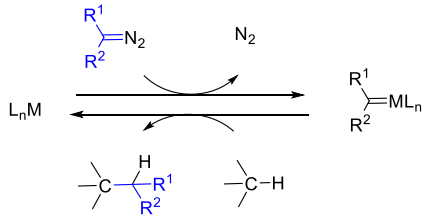
For example, Yu and co-workers[54] reported Pd-catalysed enantioselective C–H functionalisation with a carboxylic acid directing group (*Scheme 1.12A*). The chiral ligand **1.68** was crucial for achieving reactivity and enantioselectivity in this reaction. Besides C–C bond formations, metal-catalysed C–O bond formations have been accomplished. Sanford and co-workers[55] demonstrated the use of an oxime ether to direct Pd-catalysed C–H to C–O bond formation, using $PhI(OAc)_2$ as the terminal oxidant (*Scheme 1.12B*).



Scheme 1.12 Pd-catalysed C–H activation: A) Yu's C–H arylation; B) Sanford's C–H acetoxylation.

1.3.2 Metal carbenoid and nitrenoid insertion reactions

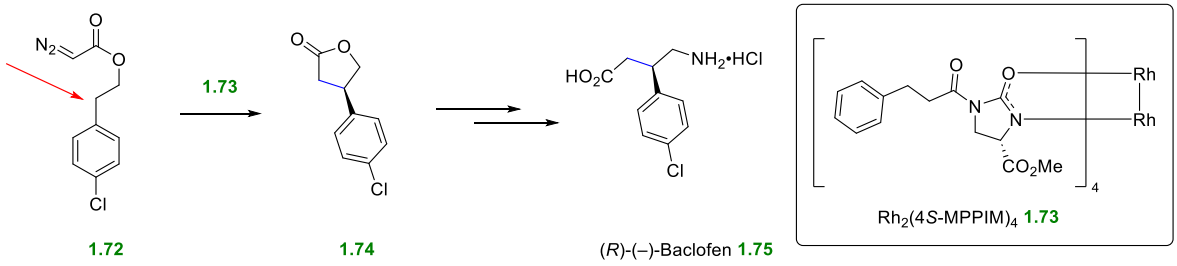
First used synthetically by Doering in 1954,[56] carbenes have also been explored as reactive intermediates for C–H functionalisation. Along with nitrenes, the lack of an octet configuration renders them highly electrophilic, highly unstable and sufficiently reactive to break certain inactivated C–H bonds.[45] The stabilisation of carbenes/nitrenes with transition metals as metal carbenoids/nitrenoids leads to more controlled reactions, especially with respect to site selectivity and stereochemistry. Metal carbenoids are, for example, generated by metal-induced extrusion of nitrogen from diazo precursors (*Scheme 1.13*).[45]



Scheme 1.13 The overall catalytic cycle of metal carbenoid insertion reaction.

Doyle and co-workers[57] demonstrated the synthesis of $GABA_B$ receptor agonist (*R*)-(–)-baclofen **1.75**, which made use of metal carbenoid enantioselective intramolecular C–H insertion as indicated (*Scheme 1.14*). The rhodium carbenoid and the reacting C–H are

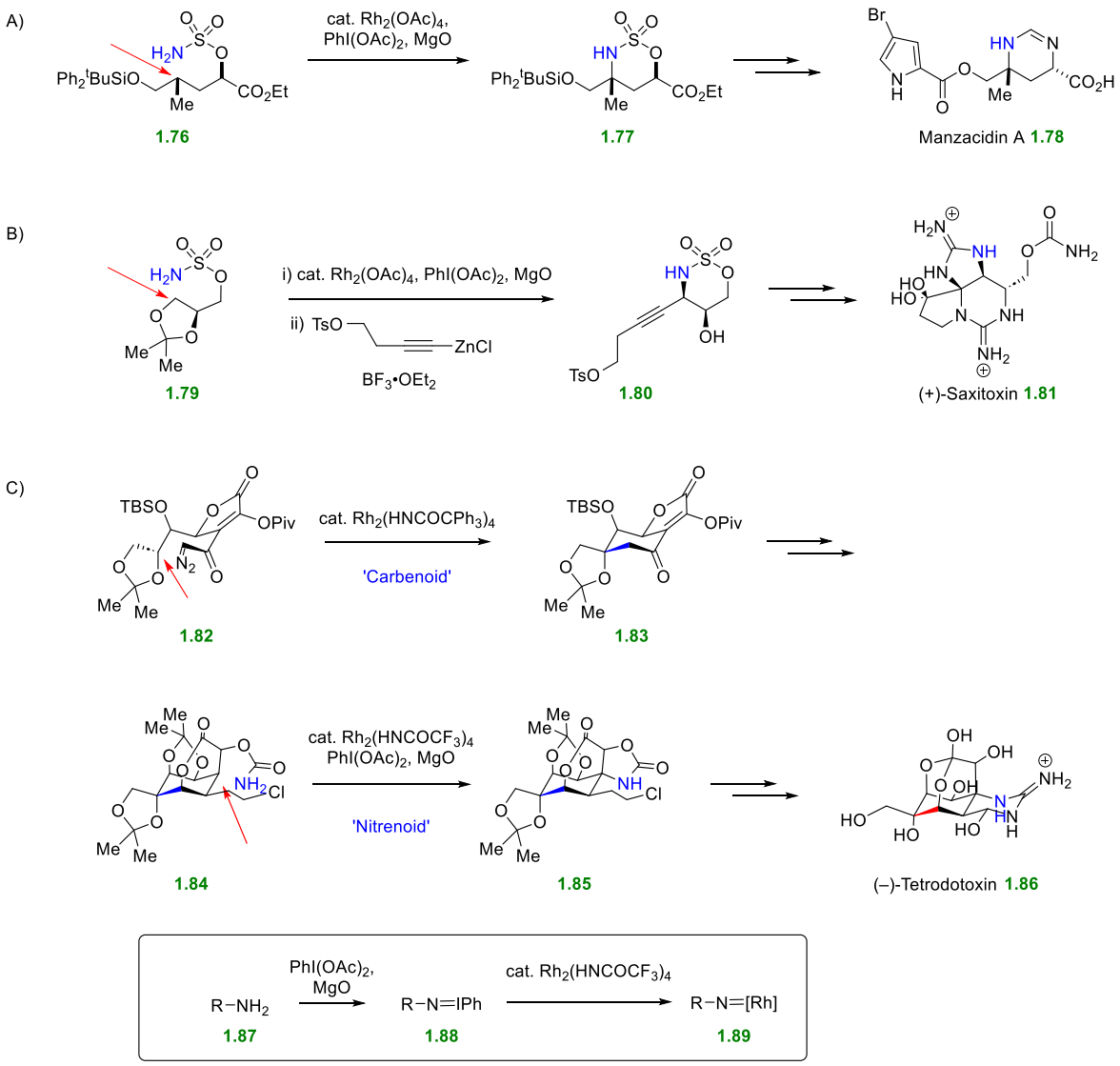
brought into close proximity by the tethering group. The chiral catalyst **1.73** achieved high enantioselectivity (up to 95% *ee*) in this transformation.



Scheme 1.14 Metal carbenoid insertion as a key step in the synthesis of (*R*)-(–)-baclofen **1.75**.

C–H functionalisation by metal nitrenoids using sulfamates and carbamates as precursors is a useful approach to perform amination.[58] Sulfamate and carbamate precursors display different regioselectivity; sulfamates favour the formation of six-membered rings due to the strain associated with a compressed N–S–O bond angle in a five-membered ring; carbamates favour the formation of five-membered rings.[58]

For example, Du Bois and co-workers observed this selectivity with Rh(II)-catalysed stereospecific or stereoselective nitrene insertions within total syntheses of manzacidin A (*Scheme 1.15A*),[59] (+)-saxitoxin (*Scheme 1.15B*),[60, 61] and (–)-tetrodotoxin, which also included an example of stereospecific carbenoid C–H insertion (*Scheme 1.15C*).[62] Mechanistically, sulfamate and carbamate precursors **1.87** react with the $PhI(OAc)_2$ in the presence of base, MgO, to form iminobenzene **1.88**, which then reacts with the Rh(II) catalyst to form rhodium nitrenoid **1.89** (*Scheme 1.15*). The insertion of the rhodium nitrenoid into the C–H is believed to be concerted but asynchronous.[58]



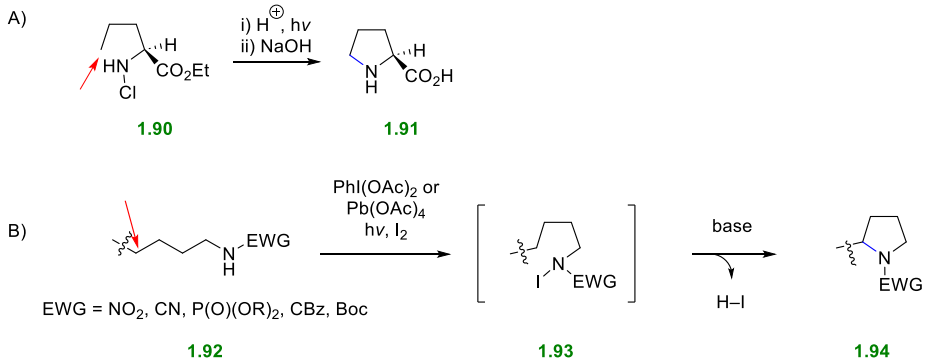
Scheme 1.15 Rh(II)-catalysed intramolecular C–H aminations in total syntheses reported by Du Bois.

1.3.3 Intramolecular homolytic hydrogen atom transfer (HAT)

HAT refers to the abstraction of a hydrogen atom (H$^{\bullet}$) from a C–H bond by a reactive radical species usually centred on carbon, nitrogen or oxygen and driven by the formation of a stronger C/O/N–H bond than the broken C(sp^3)–H bond.[45] HATs from 3° C–H sites, with a weaker bond, are faster than those from 2° and 1° C–H sites. This selectivity trend is somewhat complementary to that found in the transition metal-catalysed approach, which tends to favour 1° C–H sites.[46]

Apart from the thermodynamic driving force, kinetically, intramolecular HATs are usually faster than analogous intermolecular HATs, due to the lower entropic cost in achieving the transition state. In most cases, 1,5-hydrogen atom transfer (1,5-HAT) is the predominant pathway since a favourable near-linear arrangement of [C---H---X]$^{\bullet}$ can just be achieved within a six-membered cyclic transition state, and the entropic cost is less than for more remote 1,n-abstractions.[45]

The radical approach to C–H functionalisation was developed in the mid-20th century but continues to be used. Lavergne and co-workers[63] reported the application of a Hofmann–Löffler–Freytag reaction (discovered in 1883) to prepare L-proline **1.91** (*Scheme 1.16A*). Traditionally, this reaction requires strongly acidic conditions, which limits the reaction's substrate scope. Over the years, several modifications have been introduced, the most significant modification being reported by Suarez and co-workers,[64] in which mild neutral conditions were introduced for the reaction of *N*-nitroamides. The Suarez modification has expanded the substrate scope to include, for example, *N*-cyanamides and *N*-phosphoramidates.[65-68]

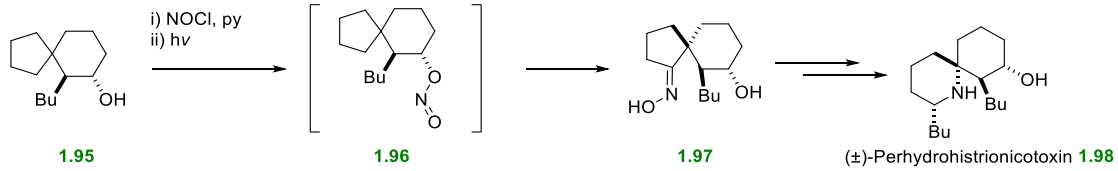


Scheme 1.16 Hofmann–Löffler–Freytag reaction: A) classical conditions; B) Suarez's modified conditions.

With the Suarez modification, the unstable nitrogen-halogen bond is generated *in situ* from an N–H bond, in the presence of elemental halogens, along with $Pb(OAc)_4$ or $PhI(OAc)_2$ (*Scheme 1.16B*). The presence of an attached electron-withdrawing group (EWG) increases

the reactivity of the nitrogen radical towards HAT. Mechanistically, the substrate reacts with, for example, the hypervalent iodine reagent and iodine (I_2) to form iodoamide intermediate **1.93**. Homolytic fragmentation of the iodoamide intermediate **1.93** generates the nitrogen-centred radical *in situ* to initiate intramolecular 1,5-hydrogen abstraction, forming alkyl radical. Alkyl radical abstracts a halogen atom from iodoamide intermediate **1.93** to afford protonated δ-halogenated amine. Intramolecular S_N2 reaction followed by deprotonation gives pyrrolidine **1.94**.

A classic HAT process was reported by Barton in his eponymous nitrite ester reaction (discovered in 1960), which involves 1,5-HAT by an alkoxy radical to functionalise inactivated C–H positions. Corey and co-workers[69] employed this reaction to introduce an oxime selectively as a key step in the synthesis of (±)-perhydrohistrionicotoxin **1.98** (*Scheme 1.17*).



Scheme 1.17 The Barton nitrite ester photolysis applied to the synthesis of (±)-perhydrohistrionicotoxin **1.98**.

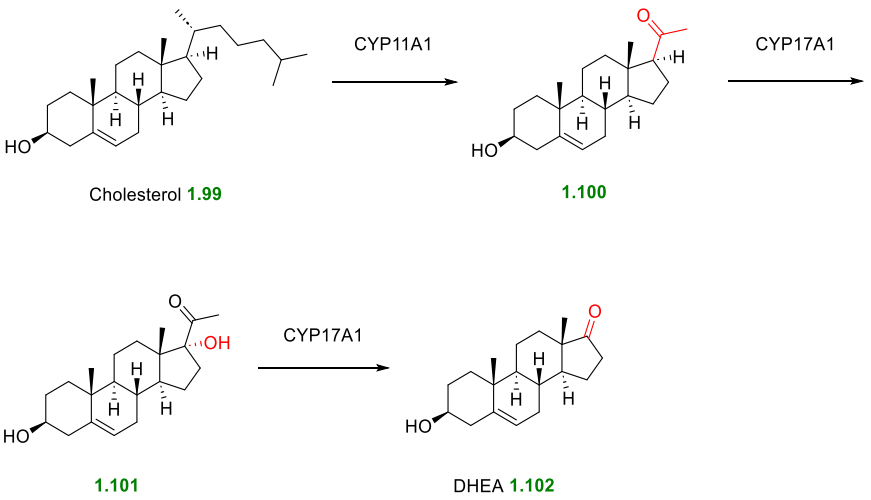
Complementary to the use of chemical reagents, biocatalysts (enzymes) have been explored to facilitate chemical transformations which may be otherwise inaccessible. Because the orientation of the substrate is constrained within an enzyme's active site, biocatalytic transformations usually proceed with high regio- and stereoselectivity. In order to achieve a particular reactivity, enzymes can be engineered by replacing the amino acid residues in and around the active site.[70, 71]

1.4 Cytochrome $P450_{BM3}$ (CYP102A1)

$P450_{BM3}$ (CYP102A1) belongs to the P450 cytochrome (CYP) superfamily of haem *b*-dependent monooxygenases. As the third P450 to be isolated from *Bacillus megaterium*, it was named as $P450_{BM3}$. This enzyme, as other P450s, has a characteristic absorption band at 450 nm when the reduced haem centre is complexed to carbon monoxide. Amongst P450s, $P450_{BM3}$ has drawn the most attention as a promising biocatalyst, owing to its exceptionally high monooxygenase activity and ability to perform regio-, chemo- and enantioselective oxidation of inactivated $C(sp^3)$–H through enzyme engineering.[70, 72-74]

1.4.1 Biological function

Discovered in the late 1950s, P450s have been identified in all kingdoms of life[75] and shown to play important roles in biological functionalisation in living organisms. For example, in humans, cytochrome P450s are involved in making cholesterol[76] and other steroids,[77] and lipids.[78] Besides that, they metabolise drugs, converting them to oxidised derivatives which can more easily be excreted by the body.[78, 79] For instance, P450s catalyse the conversion of cholesterol **1.99** to dehydroepiandrosterone (DHEA) **1.102**, which acts as a precursor to other steroid hormones such as the androgens and oestrogens (*Scheme 1.18*).[80]



Scheme 1.18 P450s involved in the biosynthesis of dehydroepiandrosterone (DHEA).

1.4.2 Active site structure and catalytic cycle

The active site of P450s contains a haem group which is critical for C–H bond activation (*Figure 1.5*), with an axially ligated cysteine thiolate and a water molecule at the hexacoordinate Fe(III) centre.[81]

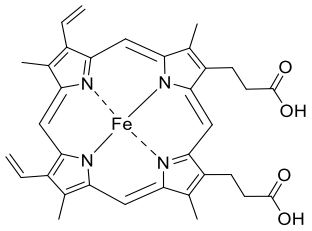
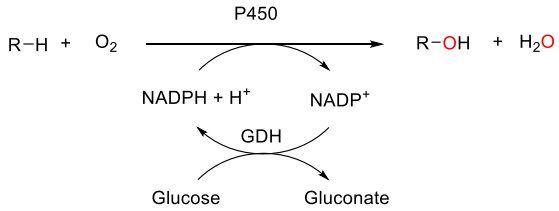


Figure 1.5 Structure of iron-protoporphyrin located in P450 active site.

The overall reaction performed by this enzyme involves the insertion of a single oxygen atom from molecular dioxygen into a C–H bond to give the corresponding alcohol, and the second oxygen atom being liberated as water (*Scheme 1.19*).[72] The $2e^-$ and $2H^+$ (overall H_2) derive from glucose, which is included in the reaction mixture along with glucose dehydrogenase (GDH) to recycle the cofactor $NADP^+$ back to NADPH.[72]



Scheme 1.19 The overall reaction achieved by $P450_{BM3}$-facilitated hydroxylation.

The P450 catalytic cycle is broadly agreed to be as shown in *Figure 1.6*:[82-85]

- From the resting state (**A**), substrate binding drives the displacement of the axial water molecule, resulting in the switch of the hexacoordinated state to a pentacoordinated state (**B**), inducing a change of the ferric spin state, from a low-spin (S = 1/2) to high spin (S = 5/2) state, which enhances the haem reduction potential.
- An electron from a molecule of NADPH being passed to FAD and then to FMN before being shuttled to the haem-containing oxidase domain (see below), is donated to the Fe(III), which reduces the iron centre from Fe(III) to Fe(II) species (**C**). Fe(II) binds O_2 more effectively than Fe(III). This electron transfer step is a rate-determining step in the catalytic cycle.
- Molecular oxygen binds to the species to form an oxy-complex (**D**), which then receives another electron to form Fe(III)-peroxy complex (**E**). Protonation of the terminal oxygen atom gives Compound 0, an Fe(III)-hydroperoxy adduct (**F**).
- Further protonation triggers heterolytic cleavage of the O–O bond, resulting in the loss of a water molecule to give Compound I, an Fe(IV)=O (‘ferryl’) intermediate (**G**), which is the active species in P450-catalysed oxidations.[86] The radical cation is delocalised over the porphyrin and thiolate ligands.

- Abstraction of a hydrogen atom from the substrate forms an alkyl radical and Fe(IV) hydroxide complex (**H**). Rebounding of the newly-formed alkyl radical onto the Fe(IV) –OH gives the hydroxylated product and regenerates the Fe(III) centre (**I**).
- Lastly, water or substrate binding returns the enzyme to states **A** or **B**, respectively.

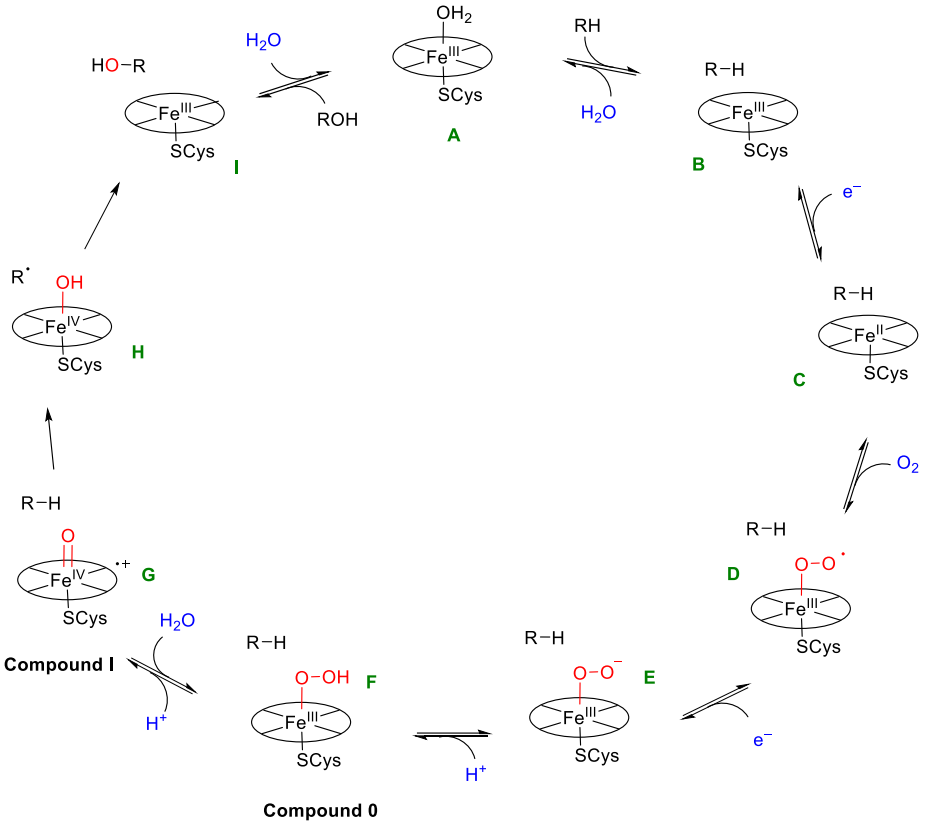


Figure 1.6 Catalytic cycle of P450.

As illustrated in *Figure 1.7*, P450$_{BM3}$ was the first identified self-sufficient P450, having its own reductase domain fused to the haem-containing oxidase domain which means that it only requires an NADPH cofactor to operate.[87] The reductase domain contains two flavin groups: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) which are connected by a linking domain and a flexible hinge, used to shuttle the electrons needed for catalysis.[88] The electron shuttle system of P450$_{BM3}$ is demonstrated in *Scheme 20* where NADPH donates hydride to FAD. FAD then passes two electrons to FMN in separate steps,

following each of which the reduced FMN transfers the electrons to the haem domain of P450 to complete the catalytic cycle.[89-93]

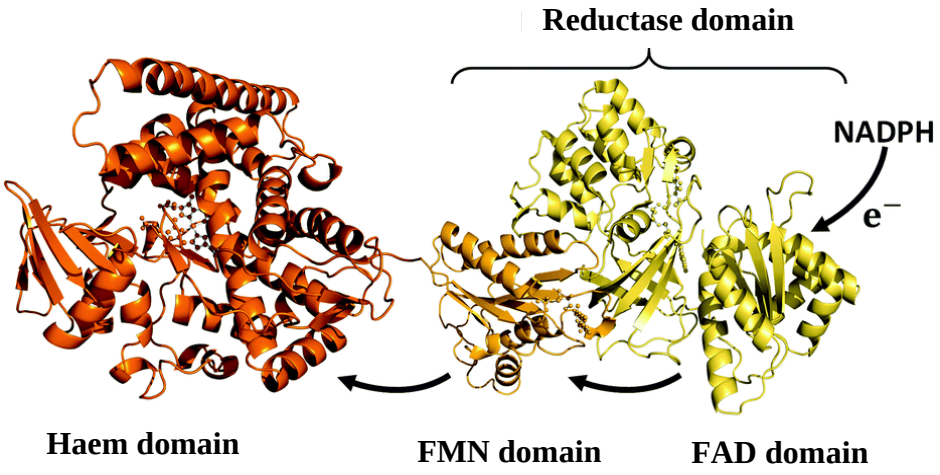
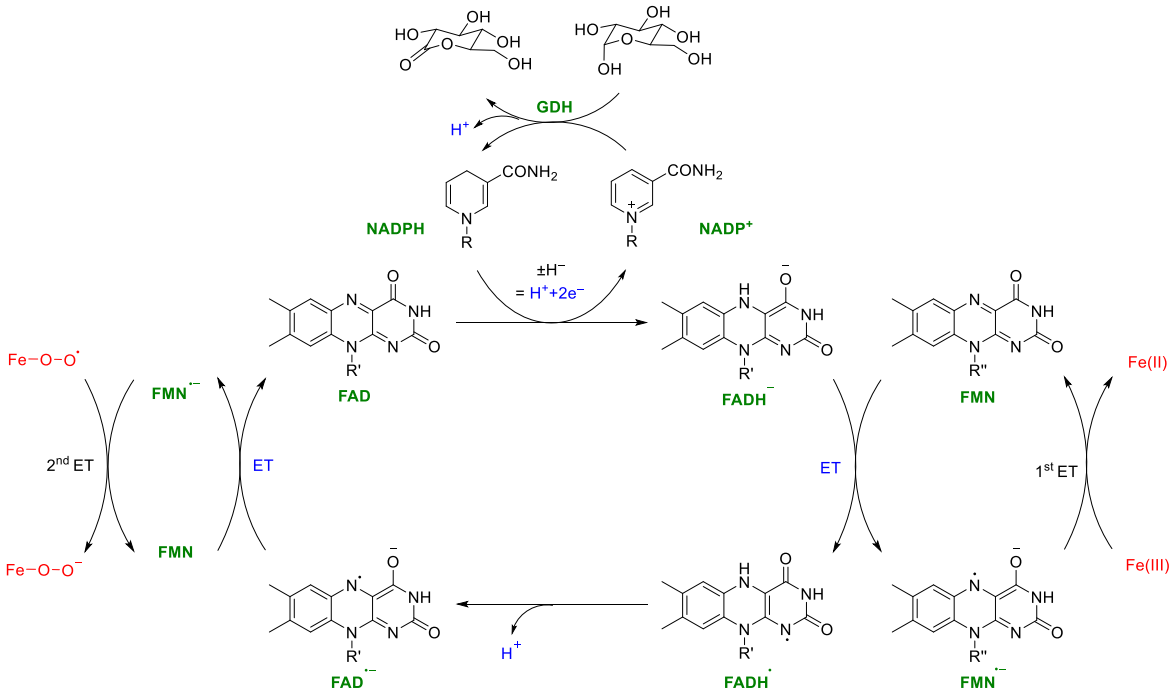


Figure 1.7 Cartoon of the structure of self-sufficient $P450_{BM3}$. (Source: DOI: 10.1039/C8CC02760A)



Scheme 1.20 Electrons pass from NADPH to FAD and then to FMN before being shuttled to the haem-containing oxidase domain.

1.4.3 Applications in chemical synthesis

The use of enzymes in chemical synthesis offers a number of advantages over 'traditional' chemical synthesis because enzymes are able to perform a variety of reactions under mild reaction conditions with high efficiency, high selectivity (chemo-, regio-, and stereoselectivity), and high catalyst total turnover number. By constraining the substrate molecule in the active site, cytochrome P450s achieve the hydroxylation of sterically hindered and electronically inactivated C–H bonds which, in general, is not possible by chemical reagents.[72]

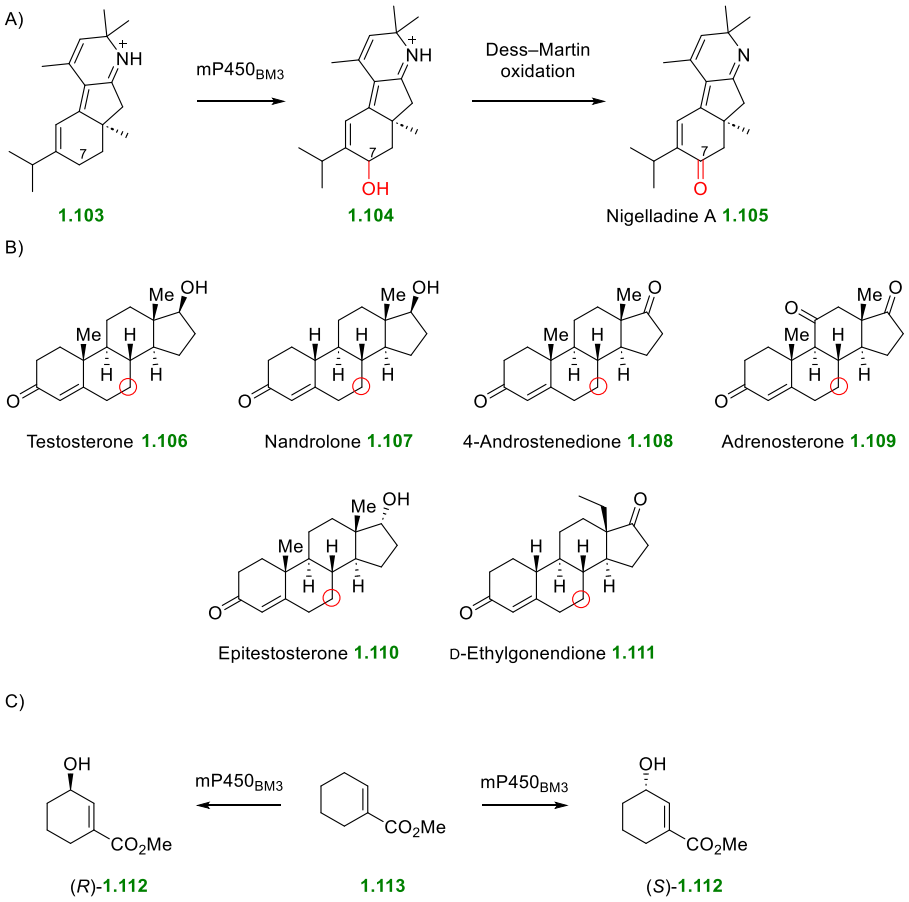
$P450_{BM3}$ stands out amongst other P450s as it is expressed in a soluble form, which enables it to be readily expressed in large quantities in *Escherichia coli* and is stable.[94] Besides that, as mentioned above, it is catalytically self-sufficient whereas other P450s require a redox partner, such as an iron-sulfur containing ferredoxin in bacterial P450s, or a separate FAD/FMN-dependent NADPH-cytochrome P450 oxidoreductase (CPR) in mammalian microsomal enzymes.[95]

Both wild-type (WT) and engineered $P450_{BM3}$ ($mP450_{BM3}$) have been studied extensively for the catalysis of a variety of synthetically useful transformations, and are already utilised in the production of high-value chemicals.[96-102] The mutations are introduced to create space within the active site of the wild-type $P450_{BM3}$ to accept a wider substrate scope and to improve reactivity. For example, the engineered L75A/L181A $P450_{BM3}$ variant was employed to catalyse a regioselective allylic oxidation at the C7 position in the total synthesis of nigelladine A **1.105** (*Scheme 1.21A*),[103] a potential inhibitor against the Omicron variant of SARS-CoV-2.[104]

$P450_{BM3}$ has also been engineered to catalyse the hydroxylation of steroids **1.106**–**1.111** (*Scheme 1.21B*), among others, with high C7-regio- and β-stereoselectivity. These

transformations are not readily accessible by chemical hydroxylation, as C7 is spatially remote from any functional group that could direct C–H activation. Steroidal C7β alcohols and their respective esters are promising neuroprotective and anti-inflammatory agents for the treatment of chronic neuronal damage, such as stroke, brain trauma and cerebral ischemia.[105]

Another important feature is that the incorporation of enzymes in synthesis enables access to both product enantiomers by enantioselective hydroxylation. This is especially useful in searching for SAR during drug development. For example, Reetz and workers[106] reported enantioselective $P450_{BM3}$-catalysed allylic C–H hydroxylation of cycloalkenes (*Scheme 1.21C*). The F87V/A328N $P450_{BM3}$ variant gave (*R*)-selective hydroxylation (up to 96% *ee*) whereas the I263G/A328S variant gave (*S*)-selective hydroxylation (up to 96% *ee*).



Scheme 1.21 Chemoenzymatic synthesis using engineered $P450_{BM3}$ ($mP450_{BM3}$): A) regioselective hydroxylation in the total synthesis of nigelladine A **1.105**; B) regio- and stereoselective C7β hydroxylation of six different steroids **1.106**–**1.111**; C) regio- and enantioselective hydroxylation of a simple cycloalkene.

1.4.4 Collaborative research in the Wong and Robertson groups

During the past 20 years, the Wong group has studied extensively on protein engineering to achieve selective $P450_{BM3}$-catalysed oxidation of small molecules.[72] Within the past ten years, the Wong and Robertson groups have collaborated[107, 108] to explore the hydroxylation of a variety of lactams and *N*-protected cyclic amines, including monocyclic, and bridged-, fused- and spiro-bicyclic substrates, as well as small-ring carbocycles. Some examples of the spirocyclic lactams studied in the group, with the hydroxylated position highlighted in red, are shown in *Figure 1.8A*. In parallel, this research has investigated applications of $P450_{BM3}$ variants in natural product syntheses, with examples shown in *Figure 1.8B*.[109, 110]

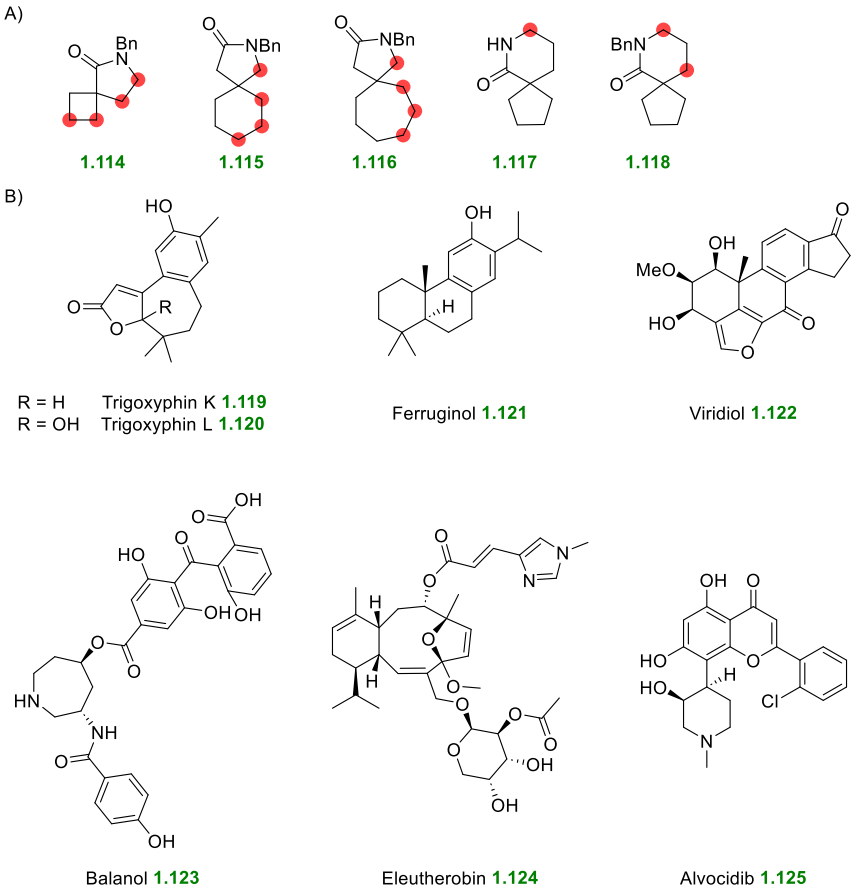


Figure 1.8 Examples of: (A) spirocyclic lactams and (B) natural products synthesised *via* chemoenzymatic reactions in Robertson and Wong collaborative research.

1.5 Aims and proposed work

This project aimed to further explore how chemical synthesis can be made more efficient by harnessing the possibilities of enzymes to achieve C–H bond hydroxylation, with the focus on syntheses of clausenamide **1.1** and its analogues **1.2**–**1.4**.

The syntheses of clausenamide **1.1** and its analogues **1.2**–**1.4** would follow a general approach whereby the lactam cores (*Figure 1.9*) are chemically synthesised, then enzymatic hydroxylation effected to access various regio- and stereoisomers of the monohydroxylated products, with the target precursors being the C1-hydroxylated products (red highlighted). The second (C4) hydroxyl group (blue highlighted) would then be installed in a separate chemical oxidation step.

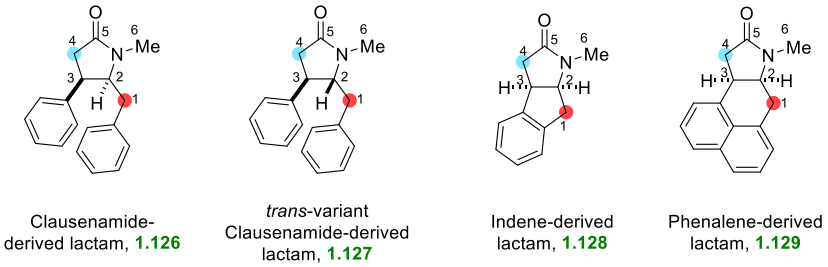


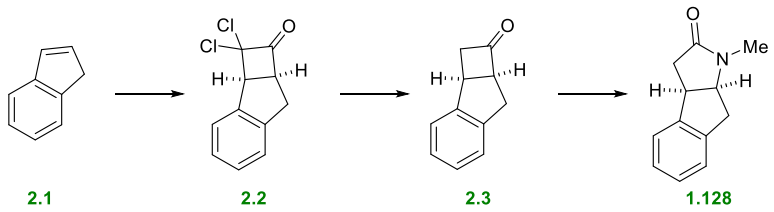
Figure 1.9 The chemically synthesised lactam cores of clausenamide and its analogues.

Through conducting this project, the results from the hydroxylation of a wider range of substrates was expected to add to the groups' understanding of the reactivity and selectivity of our $P450_{BM3}$ variants, giving more case studies and data points for eventual predictive application as general oxidation catalysts.

Chapter 2: Substrate synthesis

2.1 Initial route to the lactams

An initial three-step route was identified (*Scheme 2.1*) for the indene-derived lactam **1.128**, in which key steps involve [2+2] cycloaddition,[111-115] reductive dechlorination,[111] and ring expansion to the lactam.[116-120]



Scheme 2.1 Initial route for the indene-derived lactam **1.128**.

The synthesis of clausenamide and its analogues would then follow the same strategy, proceeding from the starting materials shown in *Figure 2.1*.

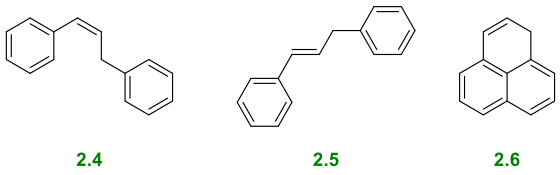
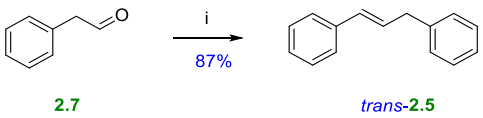


Figure 2.1 Starting materials for clausenamide (**2.4**), the *trans*-variant clausenamide (**2.5**), and the phenalene-derived analogue (**2.6**), respectively.

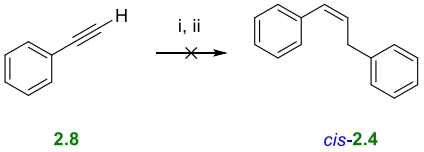
2.1.1 Synthesis of the [2+2] cycloaddition precursors

trans-Diphenylpropene **2.5** was obtained from aldehyde **2.7** *via* aldol dimerisation and dehydration/deformylation (*Scheme 2.2*) using a known procedure,[121] as a single stereoisomer with data which matched those published.[122]



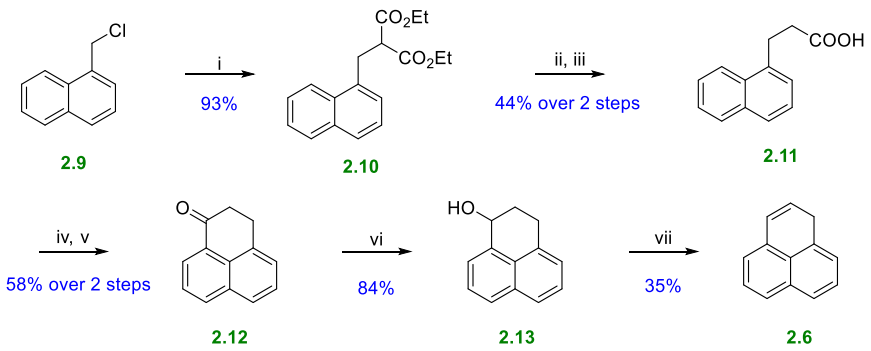
Scheme 2.2 Aldol dimerisation and dehydration/deformylation to *trans*-diphenylpropene **2.5**. Reagents and conditions: i) KOH, EtOH, reflux, 23 h.

Benzylation of alkyne **2.8** (*Scheme 2.3*) to give the *cis*-alkene **2.4** was attempted, as reported,[123] with $(PhCH_2)_3In_2I_3$ generated *in situ*; in our hands, the products were indefinable and an alternative approach was eventually found (see *section 2.3*).



Scheme 2.3 Attempted synthesis of clausenamide precursor, *cis*-diphenylpropene **2.4**. Reagents and conditions: i) In powder, benzyl iodide, THF, RT, 1 h; ii) **2.8**, reflux, 2 d.

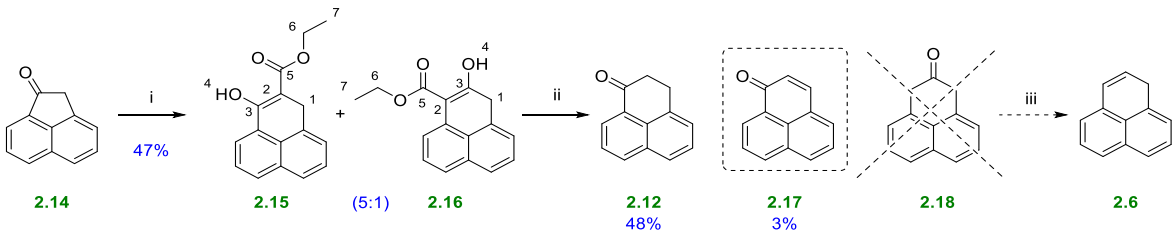
The synthesis of phenalene **2.6**, as reported,[124] began with 1-(chloromethyl)naphthalene **2.9** (*Scheme 2.4*) as the electrophile in a malonate synthesis (→ **2.10**); hydrolysis and decarboxylation then gave acid **2.11**. Friedel–Crafts acylation of the derived acid chloride gave ketone **2.12**, which was reduced and dehydrated to phenalene **2.6** as shown.



Scheme 2.4 Synthesis of phenalene **2.6**. Reagents and conditions: i) diethyl malonate, NaOEt, EtOH, reflux, 1 h; ii) NaOH, EtOH, reflux, 4 h, then aq. HCl; iii) 175 °C (neat), 30 min; iv) $SOCl_2$, reflux, 3h; v) $AlCl_3$, DCM, 0 °C, 30 min; vi) $NaBH_4$, THF, MeOH, 0 °C to RT, 1.5 h; vii) 4 M HCl, THF, reflux, 6 h.

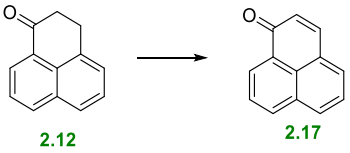
The route was low-yielding and lengthy, so an alternative route (*Scheme 2.5*) was developed. Ring expansion of commercially available 1-acenaphthenone **2.14** afforded the keto-ester regioisomers **2.15** and **2.16** in 5:1 ratio. The enol forms of these regioisomers were confirmed by a series of characteristic features in the NMR spectroscopic data: (a) singlet peaks at 12.57/13.91 ppm for H-4; (b) resonances at 96.2/99.9 ppm and 163.0/172.3 ppm for C-2 and C-3, respectively; (c) the relative integrations for the singlet at 4.19/4.05 ppm (H-1) were 2.00:0.38 (~84:16). Assignment of regioisomer **2.15** as the major product is based on ketone **2.12** being obtained in a reasonable yield following acidic hydrolysis and decarboxylation steps without the presence of ketone **2.18**. Ketone **2.18** is reported to be labile, air-sensitive, and chromatographically unstable to silica gel, alumina, and charcoal.[125, 126] Enone **2.17**, formed as a minor side-product during the reaction to form **2.12**, was found to be formed from ketone **2.12** in the presence of 1 M HCl (*Table 2.1, entries 1–4*). The overall route to ketone **2.12** has been shortened from five steps to two steps and was more efficient compared to the route in *Scheme 2.4*.

Thiemann[127] reported the direct conversion of indanones and tetralones to indenes and dihydronaphthalenes, respectively, by heating with neopentyl glycol under acid catalysis; however, in our hand, this direct conversion of ketone **2.12** into phenalene **2.6** was not a clean process, and the product could only be obtained in a very impure form.



Scheme 2.5 Alternative route to phenalene **2.6**. Reagents and conditions: i) ethyl diazoacetate, $BF_3{\cdot}OEt_2$, Et_2O, RT, 4 h; ii) 1 M HCl, *i*-PrOH, 85 °C, 26 h; iii) neopentyl glycol, *p*-$TsOH{\cdot}H_2O$, toluene, reflux, 29 h.

Table 2.1 Investigation of the formation of side product **2.17.**

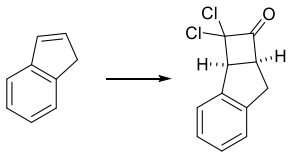


Entry	Reaction conditions	Result
1	RT (neat), 2 days	**2.12**
2	isopropanol, reflux, 19 h	**2.12**
3	1 M HCl, RT, 2 days	**2.12** + **2.17**
4	1 M HCl, isopropanol, reflux, 19 h	**2.17**

2.1.2 [2+2] Cycloaddition

The [2+2] cycloaddition from indene **2.1** (*Table 2.2*) was carried out with dichloroketene generated *in situ via* the dehalogenation of trichloroacetyl chloride with Zn. Krepski's group[112] reported that the reaction of indene **2.1** under the conditions summarised in *entry 1* gave the cycloadduct **2.2** in 81% yield; however, in our hands this procedure, or a variant using Zn powder (*entry 2*) led to complex product mixtures. No conversion of indene occurred when the reaction was attempted in the absence of $POCl_3$ with sonication (*entry 3*) but when Zn dust was used instead, a 57% yield of cycloadduct **2.2** was obtained (*entry 4*) with spectroscopic data matching those reported.[114]

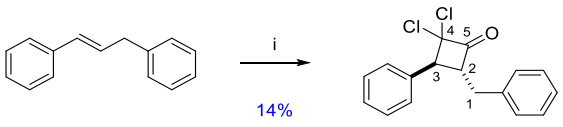
Table 2.2 [2+2] Cycloaddition of indene **2.1** and dichloroketene, generated *in situ via* the dehalogenation of trichloroacetyl chloride.



2.1 **2.2**

Entry	Zn (equiv.)	$POCl_3$ (equiv.)	Cl_3CCOCl (equiv.)	Solvent	Reaction conditions	Result
1	activated Zn dust (1.1)	1.05	1.05	Et_2O	reflux, 1 h	complex
2	activated Zn powder (1.1)	1.05	1.05	Et_2O	reflux, 1 h	complex
3	Zn powder (2)	–	1.5	Et_2O	sonication, 23 h	n.r.
4	Zn dust (2)	–	1.5	Et_2O	sonication, 4 h	*cis*-**2.2** (57%)

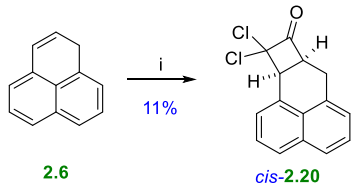
Application of the same reaction conditions to *trans*-diphenylpropene **2.5** led to no reaction. Cycloadduct **2.19** was obtained as a single diastereomer in 14% yield, along with 42% of recovered starting material, when freshly-activating Zn dust was used and the reaction time was increased to 17 h (*Scheme 2.6*). The *trans*-configuration of cycloadduct **2.19** was assigned by comparison of the spectroscopic data with those for a reported close analogue.[128]



2.5 *trans*-**2.19**

Scheme 2.6 The [2+2] cycloaddition of *trans*-diphenylpropene **2.5** with dichloroketene. Reagents and conditions: i) Zn-Cu, Cl_3CCOCl, Et_2O, sonication, 17 h (24% based on recovered alkene **2.5**).

When the conditions used for the indene reaction (*Table 2.2*, *entry 4*) were applied to phenalene **2.6** (*Scheme 2.7*), with a less-strained alkene, only an 11% yield of dichlorocyclobutanone **2.20** was obtained, as a single diastereomer, with 50% of starting material recovered.



Scheme 2.7 The [2+2] cycloaddition of phenalene **2.6** with dichloroketene. Reagents and conditions: i) Zn dust, Cl_3CCOCl, Et_2O, sonication, 4 h (22% based on recovered starting material).

In order to avoid the use of the (noisy) sonicator for extended periods, which requires monitoring and periodic cooling, reactions in which the dichloroketene is generated *in situ via* dehydrochlorination of dichloroacetyl chloride were tested and these gave generally similar results (*Table 2.3*). In this way, a 46% yield of cycloadduct **2.2** was obtained from indene **2.1** (*entry 1*) but no reaction resulted with *trans*-diphenylpropene **2.5** as substrate (*entry 2*); with a large excess of the reagents (*entry 3*), a complex product mixture resulted. A similarly complex product mixture was obtained from phenalene **2.6** (*entry 4*).

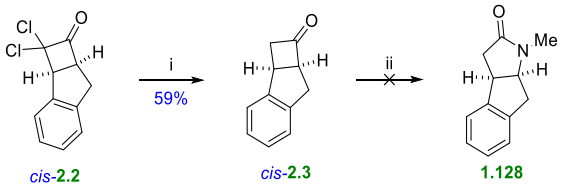
Table 2.3 Attempted [2+2] cycloaddition reactions of dichloroketene, generated *in situ via* dehydrochlorination of dichloroacetyl chloride, with indene **2.1**, *trans*-diphenylpropene **2.5** and phenalene **2.6**.

Entry	Starting material	TEA (equiv.)	$Cl_2CHCOCl$ (equiv.)	Solvent	Reaction conditions	Result
1	**2.1**	1.2	1.2	hexane	reflux, 14 h	*cis*-**2.2** (46%)
2	**2.5**	1.5	1.5	hexane	reflux, 17 h	n.r.
3	**2.5**	10	10	hexane	reflux, 20 h	complex
4	**2.6**	1.5	1.5	hexane	reflux, 15 h	complex

No further attempts were made to optimise the [2+2] cycloadditions and the downstream chemistry was evaluated based on the most available cycloadduct, that from indene **2.1**.

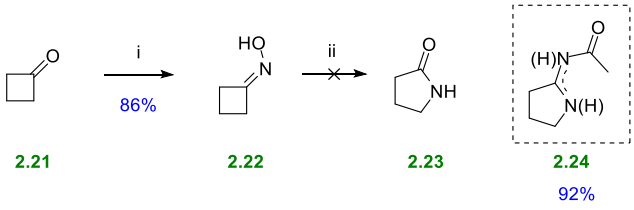
2.1.3 Lactam formation

The conversion of cyclobutanone **2.3**, obtained from precursor **2.2**, into the desired lactam **1.128** with tosyl chloride and *N*-methylhydroxylamine in wet pyridine has been reported (*Scheme 2.8*);[116, 117] however, in our hands the lactam was not obtained and ^{1}H NMR spectroscopic analysis of the crude product showed mainly starting material **2.3**.

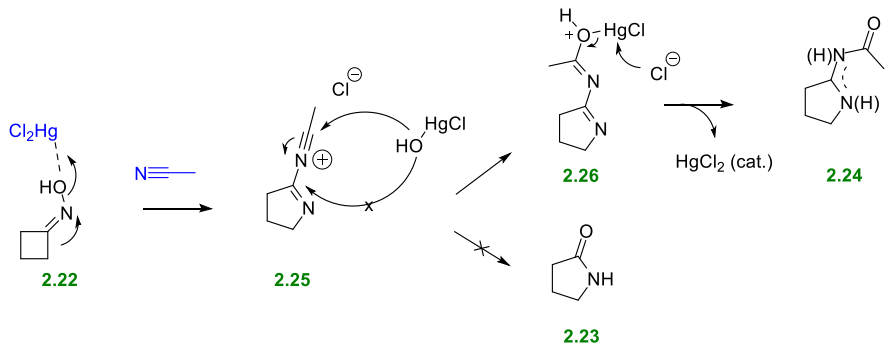


Scheme 2.8 Attempted Beckmann-type ring-expansion of cyclobutanone **2.3**. Reagents and conditions: i) Zn dust, NH_4Cl, MeOH, reflux, 2 h; ii) MeNHOH·HCl, TsCl, wet py, 44 h.

Ramalingan[118] reported that $HgCl_2$ could catalyse the conversion of a wide range of oximes, mainly those derived from 5- and 6-membered cyclic ketones, into their corresponding lactams. A model study was performed using the cyclobutanone oxime **2.22** (*Scheme 2.9*), prepared as shown. During the rearrangement step, *N*-acetylamidine **2.24** was obtained instead of lactam **2.23**, formed *via* interception of the forming cyclopentenyl cation by the solvent prior to hydrolysis, as shown in *Scheme 2.10*. The structure of *N*-acetylamidine **2.24** was confirmed by HRMS and a singlet resonance in the ^{1}H NMR spectrum at 1.94 ppm corresponding to the CH_3CO- group; the specific tautomeric form of amidine **2.24** was not assigned.

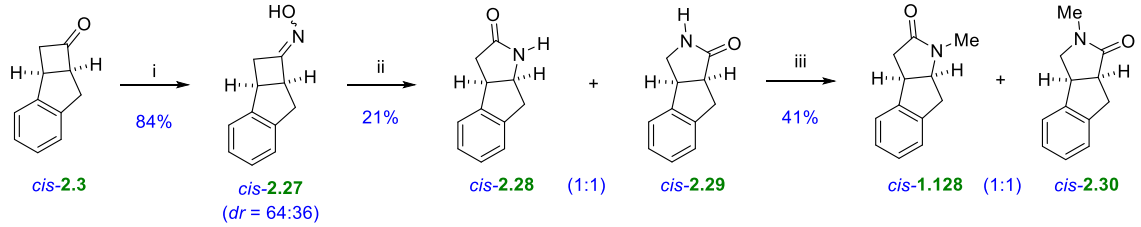


Scheme 2.9 Model study of the Beckmann rearrangement. Reagents and conditions: i) NH_2OH·HCl, NaOAc, MeOH, reflux, 18 h; ii) $HgCl_2$ (30 mol%), CH_3CN, reflux, 20 h.



Scheme 2.10 Proposed mechanism for the formation of amidine **2.24**.

Finally, classical conditions were employed for the Beckmann rearrangement of the ketoxime **2.27** (*dr* = 64:36, unassigned) (*Scheme 2.11*) formed from cyclobutanone **2.3**. This reaction afforded regioisomers **2.28** and **2.29** in a 1:1 ratio, in which the regioisomers were distinguished by comparison with reported NMR data.[119, 129] Attempted *N*-methylation under mild conditions (triethylamine, methyl iodide, DMAP, DCM) was unsuccessful but with NaH as base, lactams **1.128** and **2.30** were obtained, again in a 1:1 ratio. At this stage in the project, the separation of lactams **1.128** and **2.30** was not attempted and they were assigned based on the reported data.[119, 130]

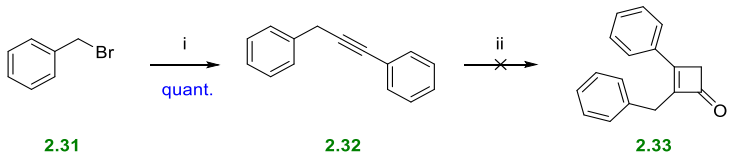


Scheme 2.11 Classical Beckmann rearrangement conditions to form lactams **1.128** and **2.30**. Reagents and conditions: i) $NH_2OH{\cdot}HCl$, py, RT, 4 h; ii) $SOCl_2$, dioxane, RT, 22 h; iii) MeI, NaH, THF, RT, 2 h.

2.2 Synthesis of cyclobutanones

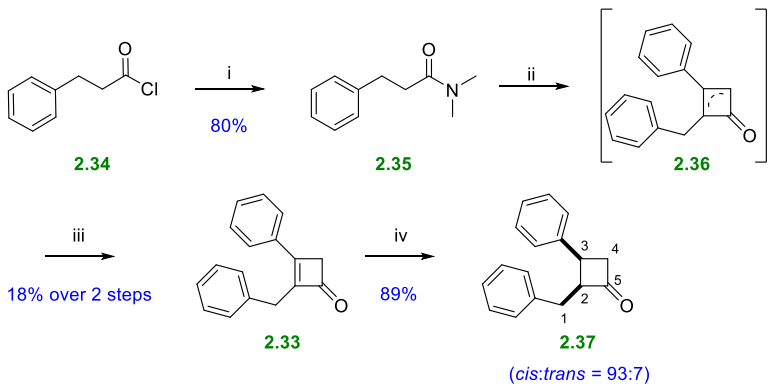
The dichloroketene cycloaddition had been found to be generally inefficient, especially with the less strained acyclic alkenes; therefore, cycloadditions of alternative ketene equivalents were briefly explored (*Schemes 2.12–2.15*) before the whole strategy was eventually

reviewed (see *section 2.3*). From benzyl bromide **2.31** (*Scheme 2.12*), copper-catalysed cross coupling of phenylacetylene with benzyl bromide, using a known procedure,[131] gave diphenylpropyne **2.32**. Attempts to effect a reported[132] (for an analogous compound) metal free, triflic anhydride mediated [2+2] cycloaddition, with acetonitrile serving as the two-carbon building block, did not afford cyclobutenone **2.33**, a complex product mixture resulting instead.



Scheme 2.12 An unsuccessful attempt to prepare cyclobutenone **2.33**. Reagents and conditions: i) phenylacetylene, LiHMDS, CuI or CuCl (5 mol%), toluene, reflux, 3 h; ii) Tf_2O, 1,8-bis(dimethylamino)naphthalene, CH_3CN/toluene (1:1), 70 °C, 23 h, then H_2O, 70 °C, 4 h.

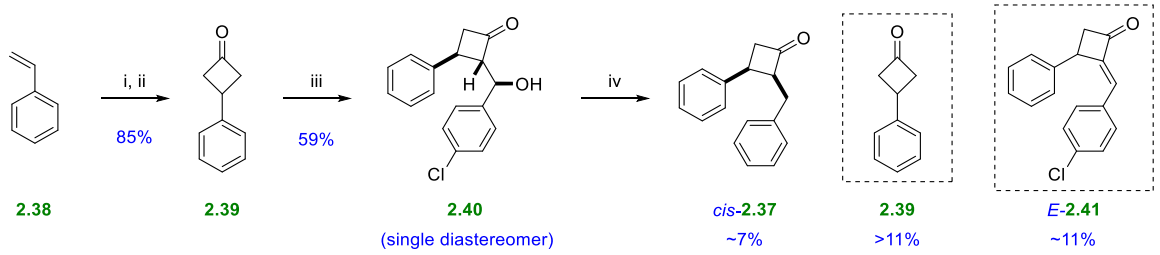
Next, from amide **2.35**, prepared trivially from acid chloride **2.34** (*Scheme 2.13*), [2+2] cycloaddition between the derived keteniminium ion and phenylacetylene, followed by alkene isomerisation gave cyclobutenone **2.33** in low overall yield. Catalytic hydrogenation formed a pair of cyclobutanone diastereomers **2.37** (*dr* = 93:7), with the major product – but not the minor product – showing a NOESY correlation between H-2 and H-3.



Scheme 2.13 Synthesis of cyclobutanone **2.37** from acid chloride **2.34**. Reagents and conditions: i) Me_2NH, THF, RT, 3 h; ii) phenylacetylene, Tf_2O, 2-fluoropyridine, DCM, reflux, 18 h, then 1 M aq. NaOH, 0 °C, 5 h; iii) KO*t*-Bu, EtOH, RT, 16 h; iv) H_2 (balloon), Pd/C (10 mol%), EtOAc, RT, 19 h.

Cyclobutanone **2.37** was also obtained from styrene **2.38** (*Scheme 2.14*) *via* keteniminium [2+2] cycloaddition[133] (→ **2.39**) then aldol reaction[134] (→ **2.40**). An attempt to remove the hydroxyl and chlorine substituents by heterogeneous Pd(0)-catalysed transfer and hydrogenolysis with formic acid as hydrogen source (Pd/C, $HCOONH_4$, aq. EtOH then HCOOH, RT/80 °C)[135] was unsuccessful, with no reaction resulting. Partial success was eventually achieved with more conventional conditions, although the added base gave ketone **2.39** resulting from retro-aldol reaction and subsequent dechlorination. Enone **2.41** and cyclobutanone **2.37** were both produced as minor products. Cyclobutanone **2.37** was formed as a single *cis*-isomer with NMR data matching those of the major product from *Scheme 2.13*. The (*E*)-configuration of enone **2.41** was assigned by comparison of spectroscopic data with those of a reported close analogue.[136]

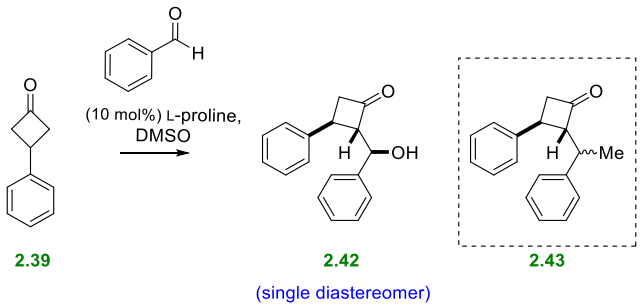
In the aldol step, 20 equivalents of chlorobenzaldehyde were used to speed up the reaction because, with just a single equivalent of the aldehyde, only a 6% yield of aldol adduct **2.40** was observed, even after 6 days of reaction. This highly stereoselective organocatalysed aldol step afforded adduct **2.40** as a single diastereomer with characterisation data matching those reported.[134] Although 96% *ee* was reported, this *ee* was not confirmed for aldol adduct **2.40**.



Scheme 2.14 Alternative route to *cis*-cyclobutanone **2.37**. Reagents and conditions: i) DMA, Tf_2O, 2,4,6-collidine, DCE, 5 °C to reflux, 17 h; ii) H_2O, DCM, reflux, 27 h; iii) chlorobenzaldehyde, L-proline (10 mol%), DMSO, RT, 45 h; iv) H_2 (balloon), $Pd(OAc)_2$ (10 mol%), KO*t*-Bu, chlorobenzene, RT, 5 h.

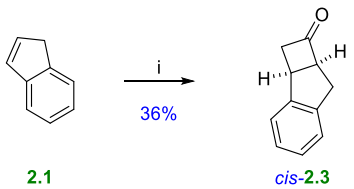
Chlorobenzaldehyde was used in the aldol reaction because the less electrophilic benzaldehyde was found to be less reactive as shown by the results in *Table 2.4*. The methyl addition product **2.43** (*dr* = 58:42, unassigned) was presumably formed by incorporation of a methyl source from DMSO during the prolonged reaction (*entry 1*) by either an S_N1-like substitution (of **2.42**) or, more likely, conjugate addition (to the enone dehydration product of **2.42**). Even with a shorter reaction time the major product was still methylated **2.43** (*dr* = 45:55, unassigned), although this time some of the targeted aldol adduct **2.42** was obtained (*entry 2*), again as a single diastereomer in which the stereochemical configuration was assumed to be the same as that in adduct **2.40**.

Table 2.4 Aldol reaction of **2.39** with benzaldehyde.



Entry	Reaction conditions	Result
1	RT, 45 h	**2.43** (35%, *dr* = 58:42, unassigned)
2	RT, 29 h	**2.43** (68%, *dr* = 45:55, unassigned), **2.42** (14%)

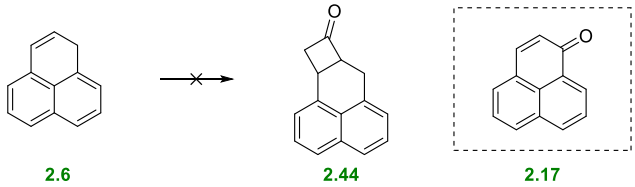
Because the keteniminium cycloaddition with styrene worked well, the same conditions were tested with indene **2.1**, resulting in a 36% isolated yield of the bicyclic cyclobutanone **2.3** (*Scheme 2.15*).



Scheme 2.15 Keteniminium cycloaddition to form cyclobutanone **2.3**. Reagents and conditions: i) DMA, Tf_2O, 2,4,6-collidine, 1,2-DCE, 5 °C to reflux, 2 h, then H_2O, DCM, reflux, 24 h.

On the other hand, the same conditions applied to phenalene **2.6** (*Table 2.5, entry 1*) gave enone **2.17** only, which is a known compound.[137] Use of the much weaker base, 2-fluoropyridine[138] [*p*KaH (H_2O) = –0.44,[139] *cf.* collidine *p*KaH (H_2O) = 7.5[140]] (*entry 2*) gave no reaction. A TLC sample of phenalene **2.6** which had been exposed to the air for a few hours was found to have converted into enone **2.17**.

Table 2.5 Attempted keteniminium [2+2] cycloaddition with phenalene **2.6**.

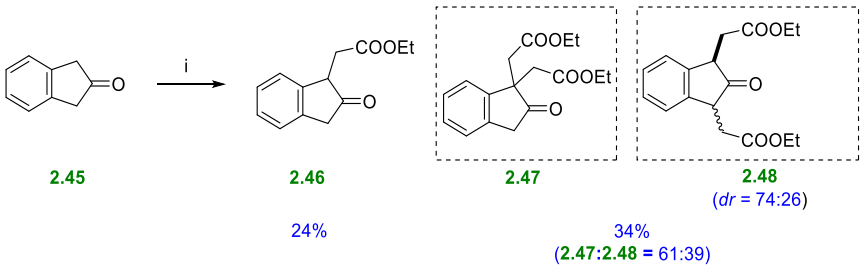


2.6 **2.44** **2.17**

Entry		Reagents and conditions (equiv.)	Result
1	i)	DMA (1.2), Tf_2O (1.4), 2,4,6-collidine (1.4), DCE, 5 °C to reflux, 23 h	**2.17**
	ii)	H_2O, DCM, reflux, 43 h	
2	i)	DMA (1.2), Tf_2O (1.1), 2-fluoropyridine (1.2), DCE, –40 °C to –10 °C, 30 h	n.r.
	ii)	sat. aq. $NaHCO_3$, RT, 18 h	

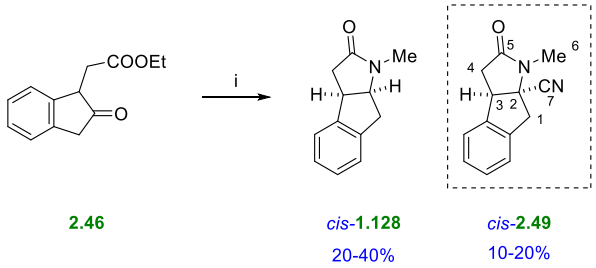
2.3 Synthesis of lactams

A variety of partially successful routes to the lactams and precursor cyclobutanones had been established but a more practical approach was sought; therefore, the strategy was reviewed, and a short route was investigate based on alkylation[141] and reductive amination.[142] From indan-2-one **2.45**, enolate alkylation gave the mono-alkylated ketone **2.46** in 24% yield, along with a mixture of dialkylated side products **2.47** and **2.48** in 34% (*Scheme 2.16*).



Scheme 2.16 Enolate alkylation of indan-2-one **2.45**. Reagents and conditions: i) LiHMDS, ethyl bromoacetate, THF, –78 °C to –20 °C to RT, 17 h.

From keto-ester **2.46**, reductive amination and cyclisation *in situ* gave the lactams **1.128** and **2.49** (*Scheme 2.17*). Both lactams were formed as single diastereomers, in which the spectroscopic data for lactam **1.128** matched those reported;[130] the structure of lactam **2.49** was confirmed by HRMS, IR, and the presence of resonances at 65.2 ppm and 119.0 ppm for quaternary carbon corresponding to the substituted C-2 and nitrile, respectively. Although the overall yield of this two-step approach was low, it was relatively short and allowed access to lactam **1.128** without any undesired regioisomer (*cf.* Beckmann rearrangement approach in *Scheme 2.11*).

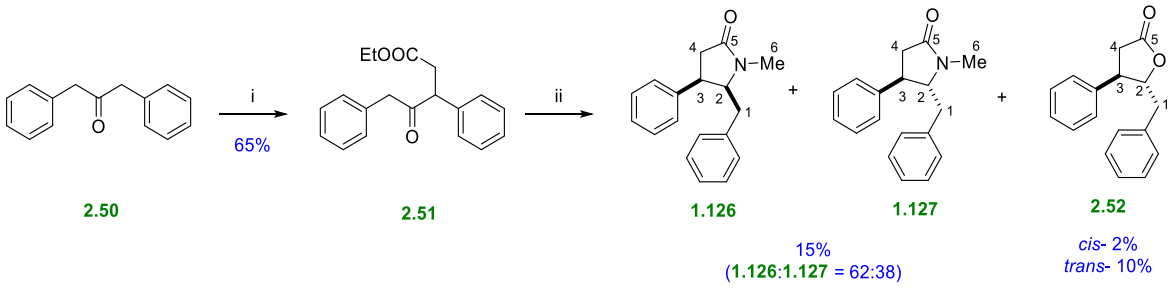


Scheme 2.17 Reductive amination, followed by cyclisation *in situ*, to give target lactam **1.128** and side product **2.49**. Reagents and conditions: i) $MeNH_2$, $NaBH_3CN$, AcOH, MeOH, reflux, 4 h.

Next, from diphenylpropanone **2.50** (*Scheme 2.18*), monoalkylated diphenylpropanone **2.51** was produced free from dialkylated side-products; however, reductive amination and subsequent cyclisation *in situ* gave only a 15% combined yield of lactam diastereomers **1.126** and **1.127**, and a similar quantity of lactone diastereomers **2.52**, along with 21% of recovered starting material. The spectroscopic data for the lactam diastereomers **1.126** and

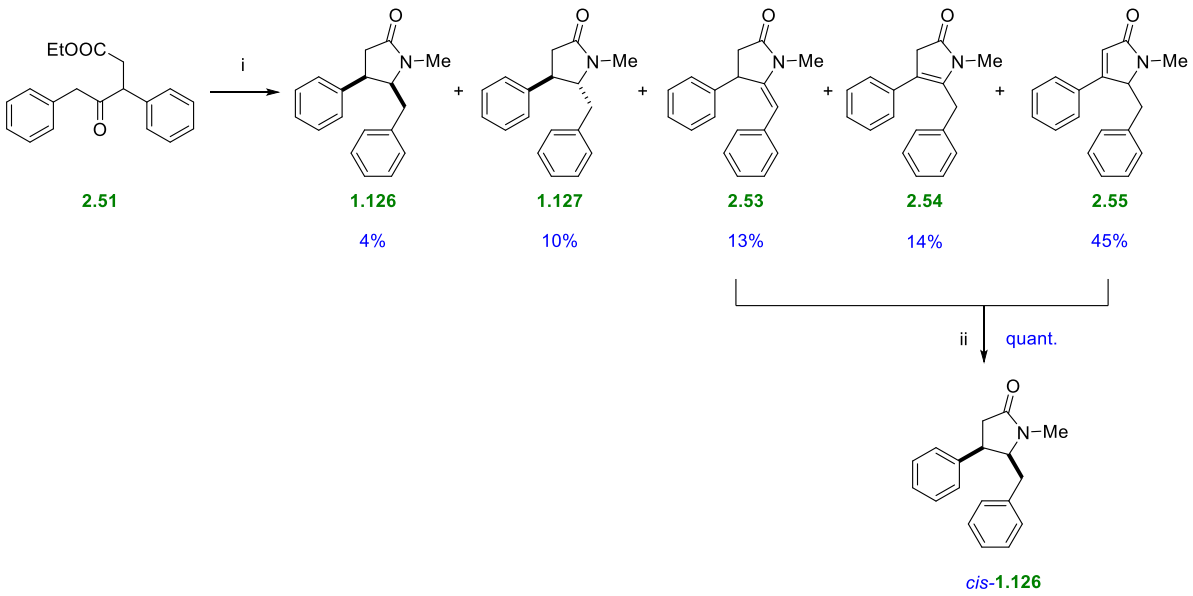
1.127 are reported;[143] however, we suspected that the literature NMR data were wrongly assigned and this was supported by a NOESY correlation between H-2 and H-3 in the major isomer, not found in the minor isomer.

The configurations for the lactone diastereomers were assigned based on data reported for *trans*-lactone **2.52**.[144] The small vicinal coupling constant of J = 6.5 Hz and a NOESY correlation between H-2 and H-3 showed that the novel diastereomer was, indeed, the *cis*-lactone **2.52**. These lactones are apparently formed by premature reduction of the ketone (and cyclisation) in competition with iminium formation and reduction; this may be partly attributable to electronic activation of the ketone towards reduction by the nearby electon-withdrawing ester substituent.



Scheme 2.18 Enolate alkylation of diphenylpropanone **2.50**, followed by reductive amination and cyclisation *in situ*. Reagents and conditions: i) LiHMDS, ethyl bromoacetate, THF, -78 °C to -20 °C to RT, 22 h; ii) $MeNH_2$ (1.5 equiv.), $NaBH_3CN$, AcOH, MeOH, reflux, 21 h.

Eventually, use of an excess of methylamine eliminated the formation of lactones **2.52** but the target lactams **1.126** and **1.127** were produced in low yield (*Scheme 2.19*), with the majority of the product deriving from cyclisation of the imine before hydride reduction. Although the lactams **1.126**, **1.127**, and **2.53**–**2.55** were all separable by chromatography, catalytic hydrogenation converted the combined side products **2.53**–**2.55** into *cis*-lactam **1.126** only and in quantitative yield.

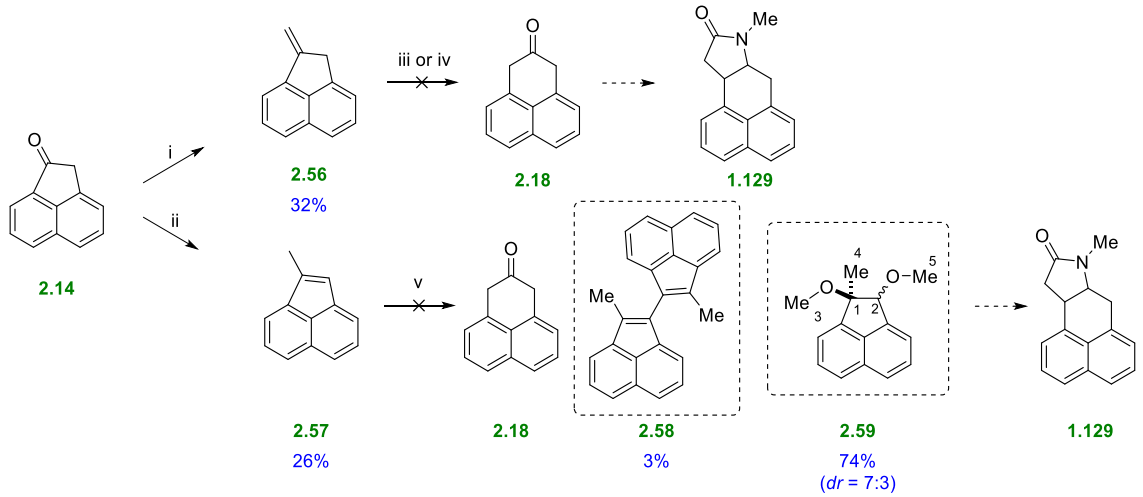


Scheme 2.19 Reductive amination then cyclisation *in situ* to give target lactam diastereomers **1.126** and **1.127**. Reagents and conditions: i) $MeNH_2$ (3 equiv.), $NaBH_3CN$, AcOH, MeOH, reflux, 6 h; ii) H_2 (balloon), Pd/C (10 mol%), EtOAc, RT, 23 h.

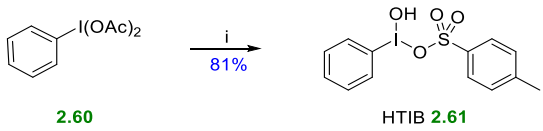
The preparation of the remaining target lactam **1.129** (*Scheme 2.20*) by this route required access to ketone **2.18**. Wittig olefination (→ **2.56**) and oxidative ring expansion with HTIB (→ **2.18**), generated *in situ*, was tested in an extension of Silva's work.[145] The ring expansion reaction resulted in a complex mixture with no ketone **2.18** evident by ^{1}H NMR analysis and a similar result was obtained using separately prepared HTIB[146] (*Scheme 2.21*). As mentioned above, ketone **2.18** is known to be labile, air-sensitive, and chromatographically unstable and it is likely that over-oxidation resulted in the loss of any ketone **2.18** formed.[125, 126]

The low yield of alkene **2.56** was balanced by 57% starting material **2.14** being recovered after 72 h of reaction time. When double the quantities of reagents were used, regioisomer **2.57** was obtained, with data matching those reported.[147] When reacting regioisomer **2.57** with separately prepared HTIB **2.61**, a 3% yield of dimer **2.58** and a 74% yield of diastereomers **2.59** were obtained, along with 34% of recovered starting material. HRMS supported the structure assigned to dimer **2.58**. For the diastereomers of **2.59**, ^{13}C NMR

analysis showed two downfield shifted peaks at 59.6/59.5 ppm and 53.7/51.8 ppm which, combined with the methyl singlets in the ^{1}H NMR spectrum at 3.86–3.08 ppm, indicated the presence of two OMe groups; the ratio of the diastereomers **2.59** was assigned by integration of the H-3, -4, and -5 singlets in the ^{1}H NMR spectrum.



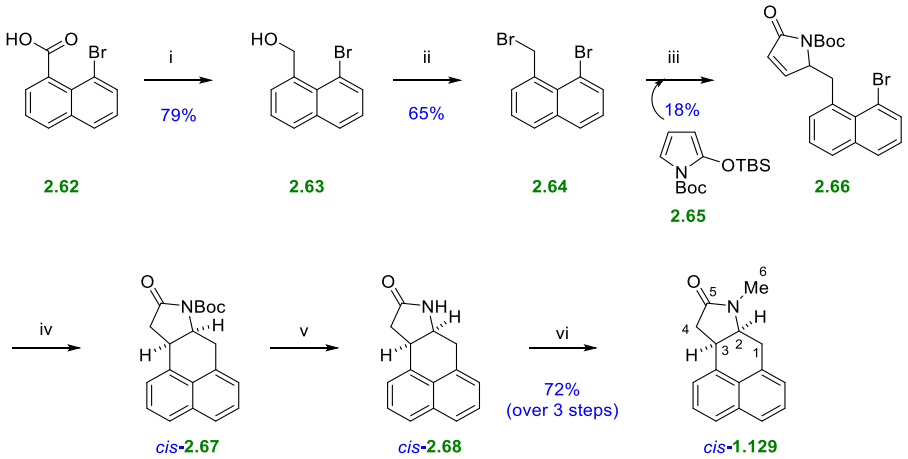
Scheme 2.20 Attempted synthesis of phenalene-derived lactam **1.129** *via* ketone **2.18**. Reagents and conditions: i) Ph_3PCH_3Br (1.5 equiv.), KO*t*-Bu (1.5 equiv.), Et_2O, RT, 72 h (74% based on recovered starting material); ii) Ph_3PCH_3Br (3.0 equiv.), KO*t*-Bu (3.0 equiv.), Et_2O, RT, 20 h; iii) PhI, *m*CPBA, $TsOH{\cdot}H_2O$, HFIP:DCM, RT, 4 h; iv) HTIB **2.61**, MeOH, RT, 20 min; v) HTIB **2.61** (*in situ*), MeOH, RT, 3 h.



Scheme 2.21 Preparation of HTIB **2.61**. Reagents and conditions: i) $TsOH{\cdot}H_2O$, CH_3CN, 1 min.

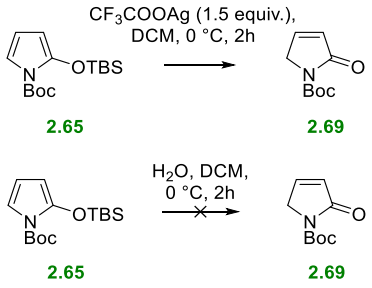
An alternative route was explored for the phenalene-derived lactam **1.129** (*Scheme 2.22*). Reduction of bromonaphthoic acid **2.62** gave alcohol **2.63**, which was converted into bromide **2.64**. By adapting the procedure for the synthesis of an analogous compound,[148] coupling between bromide **2.64** and TBSOP **2.65** in the presence of silver(I) trifluoroacetate gave a crude mixture containing target adduct **2.66** along with hydrolysis products **2.63** and **2.69** in a ratio of 13:63:24, respectively. Target adduct **2.66** was isolated from this mixture with 18% yield. Reductive Heck reaction gave lactam **2.67** as a single diastereomer from

which deprotection (→ **2.68**) and methylation gave the sought-after phenalene-derived *cis*-lactam **1.129**. NOESY correlations between H-2 and H-3, and between *endo*-situated H-1 and *endo*-situated H-4 confirmed the assigned *cis*-configuration.



Scheme 2.22 Route to phenalene-derived lactam **1.129**. Reagents and conditions: i) $B(OMe)_3$, $BH_3{\cdot}SMe_2$, THF, reflux, 4 h; ii) MsBr, TEA, RT, 23 h; iii) CF_3CO_2Ag, **2.65**, DCM, 0 °C, 3 h; iv) $Pd(OAc)_2$ (20 mol%), Et_3N, PPh_3 (20 mol%), HCOOH, THF, reflux, 4 h; v) CF_3COOH, DCM, RT, 2 h; vi) NaH, MeI, DMF, RT, 3 h.

During attempts to improve the coupling step (→ **2.66**), it was found that hydrolysis to form lactam **2.69** occurred quickly in the presence of the Lewis acid but not by water alone (*Scheme 2.23*).

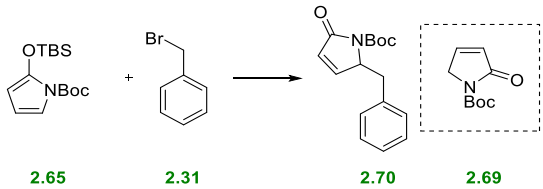


Scheme 2.23 Formation of hydrolysis product **2.69** originates with the Lewis acid, rather than by water alone.

Model studies were carried out with benzyl bromide **2.31** and the attempts made are tabulated in *Table 2.6*. When the Lewis acid was dried beforehand, the result was similar to that with non-dried reagent (*entries 1 and 2*). Different Lewis acids (AgOTf, $ZnCl_2$,[149]

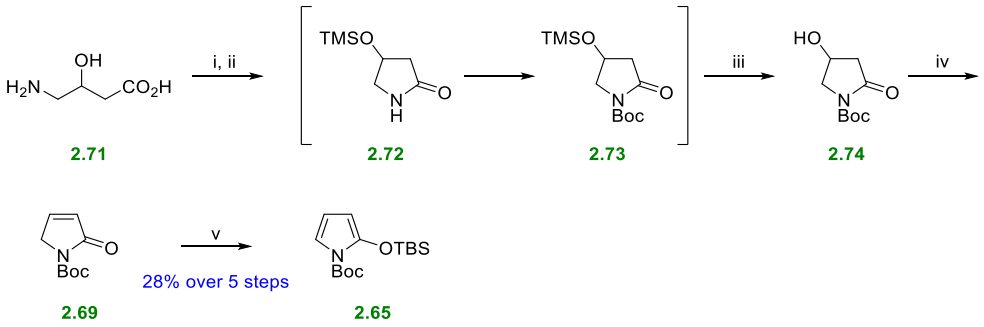
$AgNO_3$ and $AgBF_4$) were tested (*entries 3–6*) but none gave target compound **2.70**. Eventually, because the side products **2.63** and **2.69** could be recycled back into the route, no further reaction optimisation was carried out.

Table 2.6 Model study of Lewis acid-mediated coupling of benzyl bromide **2.31** with TBSOP **2.65**.



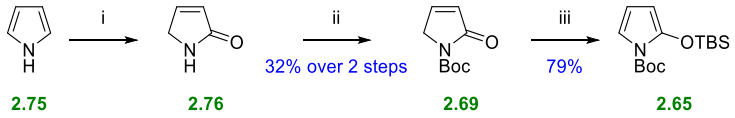
Entry	Lewis acid	Reaction conditions	Result
1	CF_3CO_2Ag	DCM, 0 °C, 2 h	**2.70** (22%), **2.69** (24%)
2	dried CF_3CO_2Ag	DCM, 0 °C, 2 h	**2.70** (28%), **2.69** (27%)
3	CF_3SO_2Ag	DCM, 0 °C, 4 h	complex
4	$ZnCl_2$	DCM, 0 °C, 2 h	n.r.
5	$AgNO_3$	DCM, 0 °C, 2 h	n.r.
6	$AgBF_4$	DCM, 0 °C, 2 h	n.r.

The TBSOP **2.65** was originally prepared (*Scheme 2.24*) by cyclisation of amino-hydroxybutyric acid **2.71**, then Boc protection (→ **2.73**) and desilylation (→ **2.74**), following known procedures.[150] This one-pot three step sequence gave a clean reaction, and the hydroxy-lactam was used without purification. Subsequent dehydration (→ **2.69**) and *O*-silylation afforded TBSOP **2.65** in 28% overall yield.



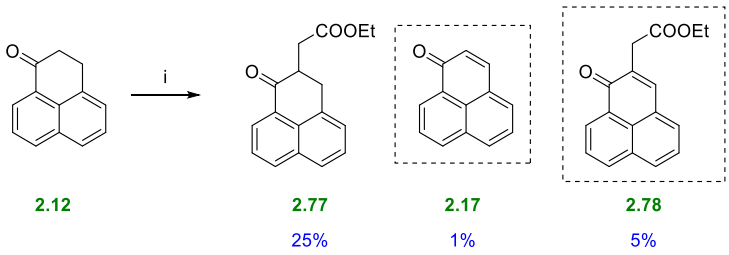
Scheme 2.24 Route to TBSOP **2.65**. Reagents and conditions: i) HMDS, py, *o*-xylenes, reflux, 5 h; ii) Boc_2O, DMAP (5 mol%), CH_3CN, 0 °C to RT, 18 h; iii) $Et_3N{\cdot}3HF$, RT, 1 h, then heptane, 0 °C, 3 h; iv) MsCl, Et_3N, THF, 0 °C, 0.5 h then RT, 2 h; v) TBSOTf, Et_3N, Celite®, heptane, DCM, 0 °C, 1 h.

A shorter synthetic path to TBSOP **2.65** was evaluated as a comparison (*Scheme 2.25*). Oxidation of pyrrole **2.75** formed pyrrolinone **2.76**, then Boc protection and silylation gave TBSOP **2.65** in similar overall yield to the earlier route.

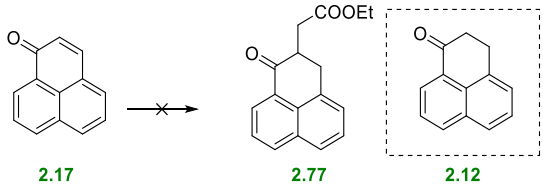


Scheme 2.25 Shorter route to TBSOP **2.65**. Reagents and conditions: i) H_2O_2, $BaCO_3$ (10 mol%), H_2O, reflux, 5 h; ii) Boc_2O, DMAP (5 mol%), CH_3CN, RT, 20 h; iii) TBSOTf, 2,6-lutidine, DCM, RT, 1 h.

It was decided to additionally prepare the isomeric phenalene-derived lactam because the necessary precursors were available from previous reactions and it was thought that this isomer could also contribute to understanding the (eventual) SAR profile of clausenamide analogues. Enolate alkylation of ketone **2.12** (*Scheme 2.26*) as before yielded only 25% of keto-ester **2.77**, with 28% recovered starting material. As mentioned earlier, ketone **2.12** tends to form enone **2.17** easily (*cf. Table 2.1*), and keto-ester **2.77** similarly tends to form enone-ester **2.78** in the presence of acid or base. Treatment of enone **2.17** with L-Selectride® effected selective conjugate reduction back to ketone **2.12**; attempts to alkylate the intermediate enolate from this reduction with iodomethane, ethyl bromoacetate or ethyl iodoacetate were unsuccessful (*Scheme 2.27*).



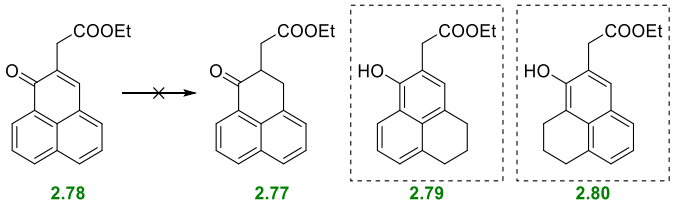
Scheme 2.26 Alkylation of ketone **2.12**. Reagents and conditions: i) LiHMDS, ethyl bromoacetate, THF, –78 °C to –20 °C to RT, 4 d.



Scheme 2.27 Attempted enone **2.17** reduction and alkylation. Reagents and conditions: i) L-Selectride® then iodomethane or ethyl bromoacetate or ethyl iodoacetate, THF, RT, 53 h.

Attempts to reduce the enone-ester **2.78** back to keto-ester **2.77** were unsuccessful (*Table 2.7*). Catalytic hydrogenation of enone-ester **2.78** gave a similar amount of regiosomers **2.79** and **2.80** (*entry 1*), the structures being assigned based on HMBC, a lack of a ketone carbonyl resonance in the ^{13}C NMR spectrum, and -$(CH_2)_3$- evident from the ^{1}H NMR spectra of both isomers.

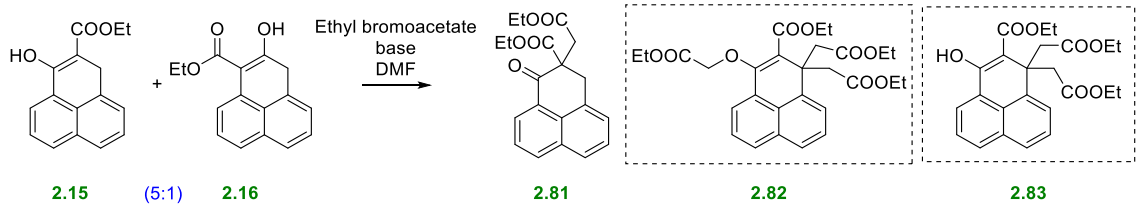
Table 2.7 Attempted enone-ester **2.78** reduction.



Entry	Reagent	Reaction conditions	Result
1	H_2 (balloon), Pd/C (10 mol%)	EtOAc, RT, 4 h	**2.79** (28%), **2.80** (28%)
2	L-Selectride®	THF, –78 °C, 23 h	n.r.

In an alternative route to keto-ester **2.77**, alkylation and Krapcho decarboxylation of the keto-ester regioisomers **2.15** and **2.16** was evaluated. When K_2CO_3 was used as a base, a 69% yield of target compound **2.81** was obtained along with an 11% yield of over-alkylated product **2.82** (*Table 2.8*, *entry 1*). Aiming to reduce the extent of *O*-alkylation, Li_2CO_3 was tested as base but found to be ineffective, with recovered starting material and unidentified side product being produced (*entry 2*). In all cases, there was no evidence for alkylation of regioisomer **2.16**.

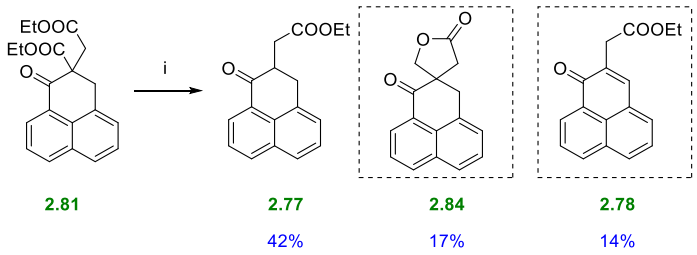
Table 2.8 Enolate alkylation of mixture of keto-ester regioisomers **2.15** and **2.16** with different bases**.**



2.15 (5:1) **2.16** **2.81** **2.82** **2.83**

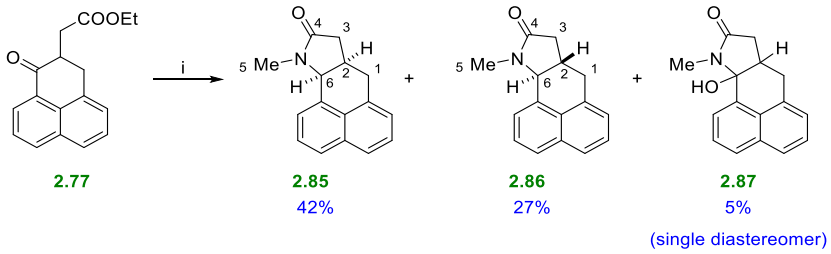
Entry	Base	Reaction conditions	Result
1	K_2CO_3	RT, 2 h	**2.81** (69%), **2.82** (11%)
2	Li_2CO_3	RT, 3 h	**2.81** (3%), **2.83** (8%), 65% recovered starting material

Moving on, Krapcho decarboxylation of keto-ester **2.81** (*Scheme 2.28*) gave keto-ester **2.77** and side products **2.84** and **2.78**. Side product **2.84** was eliminated when freshly distilled DMSO was used (**2.77,** 20% yield; **2.78**, 45% yield). Attempting to optimise the Krapcho decarboxylation step, LiCl was used in place of NaCl; however, unidentified products were obtained. Changing the solvent from DMSO to DMF resulted in no target compound.



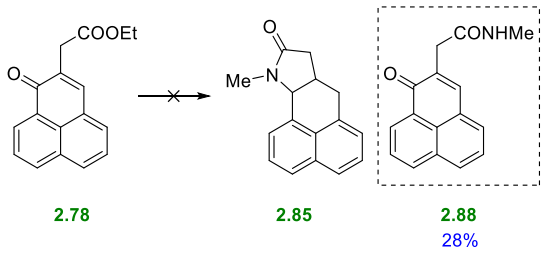
Scheme 2.28 Krapcho decarboxylation of keto-ester **2.81**. Reagents and conditions: i) NaCl, H_2O, DMSO, reflux, 6 h.

Finally, reductive amination of keto-ester **2.77** (*Scheme 2.29*), with cyclisation *in situ*, gave lactam diastereomers **2.85** and **2.86** in satisfactory overall yield, along with a small amount of incomplete reduction product **2.87** (single diastereomer, unassigned). The vicinal coupling constants between H-2 and H-6 for major and minor diastereomers were J = 4.5 Hz and 10.5 Hz respectively; hence, the major diastereomer was assigned as *cis*- and the minor as *trans*-fused.



Scheme 2.29 Reductive amination and cyclisation *in situ* from **2.77**. Reagents and conditions: i) $MeNH_2$, $NaBH_3CN$, AcOH, MeOH, reflux, 53 h.

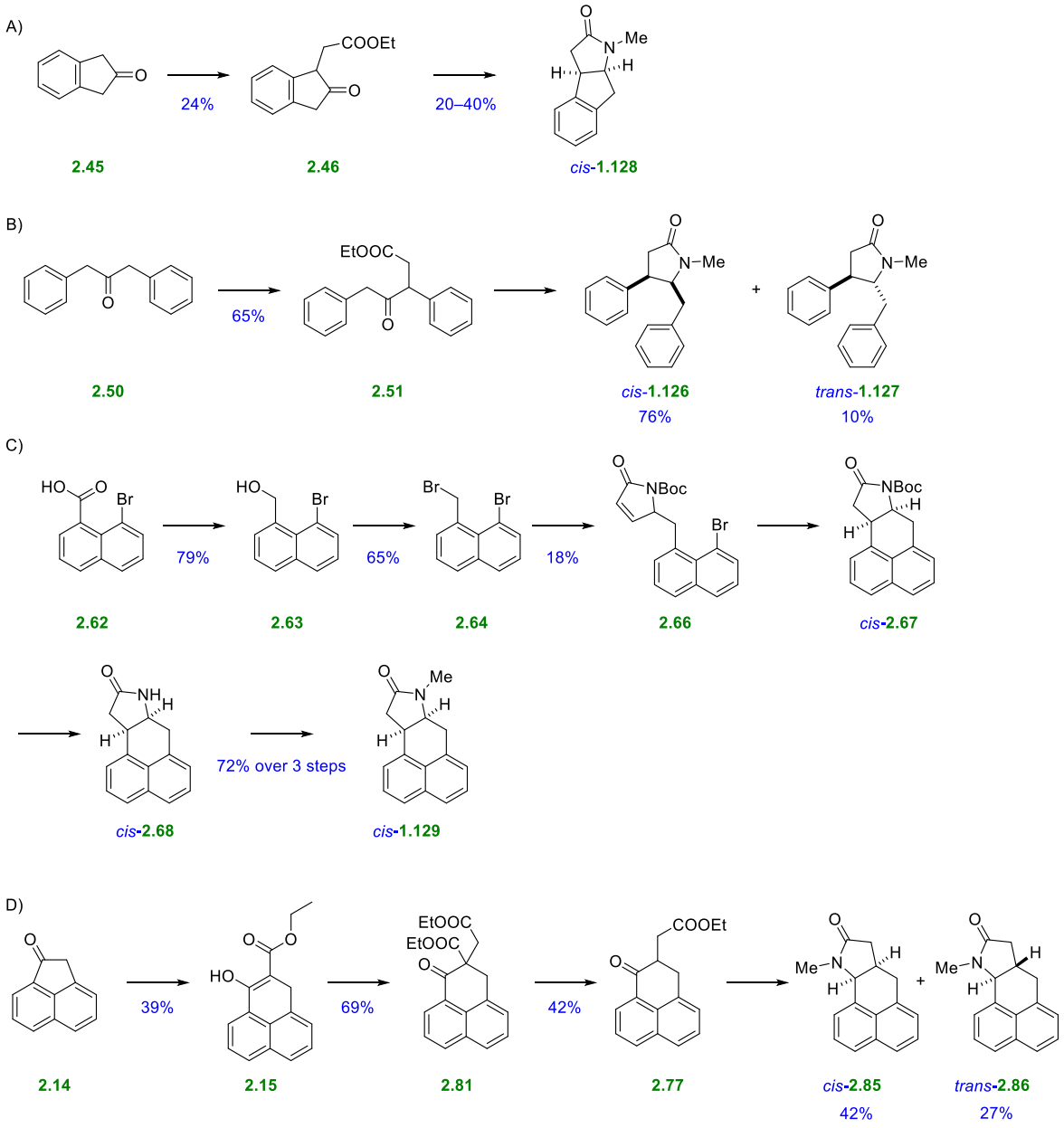
Application of the same reaction conditions to side product **2.78** led only to enone-amide **2.88** in 28% yield, along with 60% recovered starting material (*Scheme 2.30*).



Scheme 2.30 Attempted reductive amination of enone-ester **2.78**. Reagents and conditions: i) $MeNH_2$, $NaBH_3CN$, AcOH, MeOH, reflux, 43 h (70% based on recovered starting material).

2.4 Conclusions

Six lactams were synthesised as summarised in *Scheme 2.31*.



Scheme 2.31 Summary of the routes to target lactams **1.126**–**1.129**, **2.85**, and **2.86**.

Chapter 3: Regio- and diastereoselective oxidation of lactams by $P450_{BM3}$ variants

3.1 Enzymatic screening

All the substrates were screened initially with 36 chosen $P450_{BM3}$ variants derived from eight base variants:

GV	A74G/F87V
GVQ	A74G/F87V/L188Q
K19	H171L/Q307H/N319Y
KSK19	H171L/Q307H/N319Y/F87A
KU3	N239H/I259V/A276T
RP	R47L/Y51F/I401P
R19	R47L/Y51F/H171L/Q307H/N319Y
RK	R47L/Y51F/H171L/Q307H/N319Y/F87A

These variants had given a range of metabolite profiles in the oxidation of structurally diverse organic substrates, including small-ring carbocycles, steroids and amines.[94, 107, 108, 151-154] Other variants were also introduced at the active site residues such as S72, V78, A82, F87, A184, N239, T260, I263, A264, E267, A328, A330 and L437 to generate diversity in the size and shape of substrate binding pockets.

Figure 3.1 shows the active site of wild-type $P450_{BM3}$ with its natural substrate, *N*-palmitoglycine (NPG) binding to it, showcasing the key residues at the substrate binding pocket. R47 and Y51, located at the entrance of the substrate access channel, were proposed to be involved in fatty acid substrate recognition. The R47L/Y51F combination of hydrophobic substitutions was reported to promote the entry, binding and oxidation of hydrophobic compounds, including polycyclic aromatic hydrocarbons (PAHs) by up to 40-fold.[155] F87, situated directly above and in contact with the haem, is one of the most mutated residues of $P450_{BM3}$ as its side chain extends into the substrate access channel which strongly

influences the substrate specificity and product selectivity.[72, 156, 157] Changing F87 to smaller residues, such as Ala (A) and Val (V), expands the active site and allows a wider range of substrate to enter the substrate binding pocket.[72] A264, on the I helix, is hydrogen-bonded to the axial water ligand. Replacing A264 with Gly (G) creates space close to the haem. The combination of F87A/A264G generally improves the oxidation of PAHs, such as pyrene.[155] Residue A328, situated above the porphyrin ring and opposite F87, was reported to dramatically change the regio- and enantioselectivity of hydroxylation for alkanes when it was replaced with Val (V).[158] T268, a highly conserved residue, plays an important role in stabilising the dioxygen binding and the delivery of two protons for O–O bond cleavage during the enzymatic oxidation. The various combinations of replacements create diverse binding pocket topologies that result in a range of oxidised products.

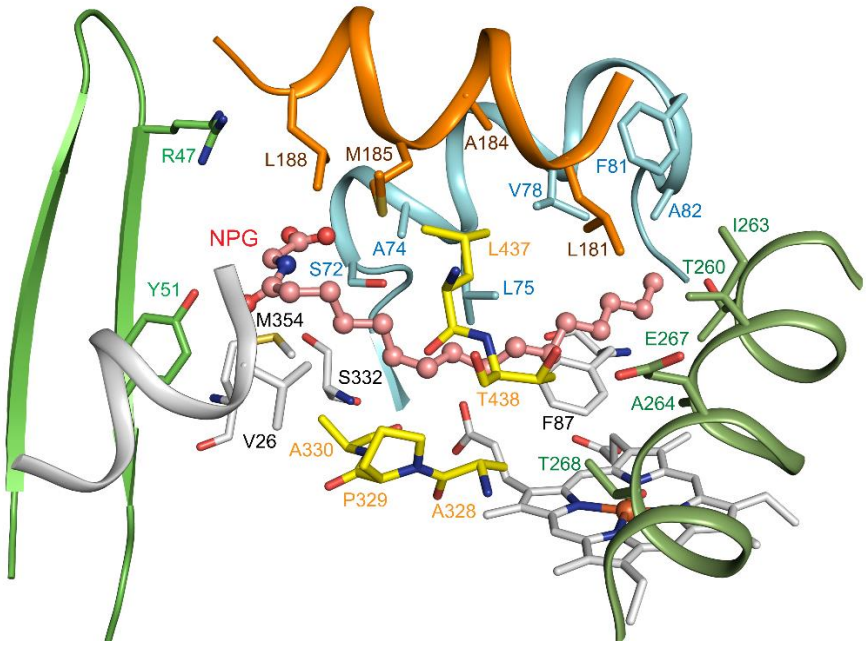


Figure 3.1 The active site of NPG-bound wild-type $P450_{BM3}$, with key amino acid residues labelled. PDB ID: 1JPZ.

Screening reactions (0.5 μmol substrate) were carried out at a 500:1 substrate/enzyme ratio in phosphate buffer (pH 7.9), shaking at 120 rpm for 16 h, at 20 °C. $NADP^+$, GDH and

glucose were added to regenerate the NADPH cofactor. The reactions were analysed by GC to study the conversion and product selectivity profiles of the substrates. Based on these preliminary data, preparative scale reactions (ranging from 0.015–0.38 mmol substrate) were carried out using selected variants to allow the isolation and characterisation of the metabolites by NMR spectroscopy and mass spectrometry.

In the preparative scale reactions, the ratios of reagents used were the same as in the screening procedures, unless otherwise stated, and the product profiles were consistent with those in screening. The relative configuration of the products was determined based on the NMR spectroscopic data; however, the absolute configuration and enantioselectivity of the chiral compounds were not determined during the screening due to difficulties in separating enantiomers using chiral GC and a lack of racemic standards. All products were isolated as single diastereomers unless stated otherwise. Some metabolites were inseparable by GC (coeluting peaks) and, hence, they were analysed as one unit.

3.1.1 Diphenyl-derived *cis*-lactam 1.126

The initial screening studies of diphenyl-derived *cis*-lactam **1.126** with 36 variants showed 13 products, as detected by GC analysis. Enzymes GVQ/A328G and K19/F87V were chosen for preparative scale reactions because of their near perfect conversion and the wide range of products offered; in other words, multiple metabolites were able to be isolated and characterised upon these scale-ups (*Table 3.1*). The variants were prepared according to the general procedure (see *Chapter 4, section 4.2 Protein Synthesis*).

Table 3.1 Enzymes selected for preparative scale reactions with diphenyl-derived cis-lactam **1.126**. GC retention time for substrate (7.0 min) and metabolites (5.9–13.0 min). Percentages refer to the fractional area of the total GC peak integration.

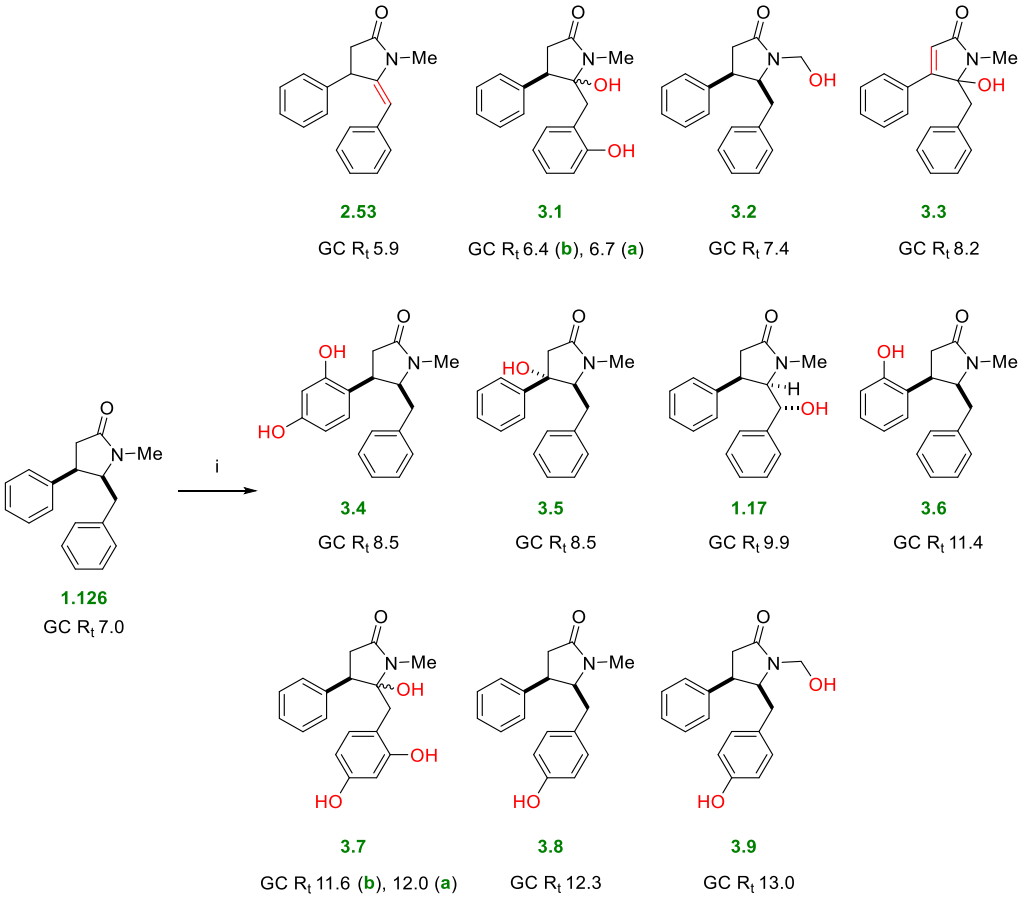
Variant	Retention time (min)													
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**Others**
GVQ/A328G	2	3	18	8	17	2	9	20	13	-	-	1	-	7
K19/F87V	1	5	7	10	34	2	2	-	12	-	-	14	8	5

A, 5.9; B, 6.4; C, 6.7; D, 7.0; E, 7.4; F, 8.2; G, 8.5; H, 9.9; I, 11.4; J, 11.6; K, 12.0; L, 12.3; M, 13.0
H: the target metabolite

The structures of 13 metabolites isolated and characterised from the two scale-up reactions are demonstrated in *Scheme 3.1*. These products were first identified by the appearance of additional resonances in the 60–80 ppm and/or, in more general case, 200–210 ppm regions to indicate the addition of alcohol and/or ketone functionality, respectively.

Upfield shifting of ^{13}C NMR aromatic resonances from 120–135 ppm to 100–110 ppm indicated hydroxylation in the aromatic rings; additional quaternary carbon resonances observed in the 155–160 ppm region correspond to the hydroxylated carbons on the aromatic rings. In ^{1}H NMR spectra, the peaks corresponding to the protons in hydroxylated rings were also found to be upfield shifted from 7.00–8.00 ppm to 6.50–7.00 ppm.

The configuration of metabolite **1.17** was assigned based on the reported data.[159] The structure of metabolite **3.2**, formed as an intermediate of demethylation, was confirmed by HRMS and the missing NCH_3 singlet peak. Two pairs of diastereomers **3.1a/b**, and **3.7a/b** were isolated which corresponded to four separate peaks on GC; however, the configurations of these two pairs of diastereomers were unassigned. The target metabolite, in this case, was the benzylic alcohol **1.17** that could give access to the natural product, clausenamide eventually.



Scheme 3.1 Metabolites isolated from $P450_{BM3}$ preparative scale reactions of diphenyl-derived *cis*-lactam **1.126**, with their retention time (R_t) on GC. Reagents and conditions: i) $P450_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C, 6 h.

Selected initial screening data, based on the selectivity for metabolite **1.17**, are tabulated in *Table 3.2*. Only GVQ/A328G showed some selectivity (21%; isolated yield from scale-up, 5%) to metabolite **1.17** (*entry 1*) with high conversion (87%). The A328G replacement appeared significant to the selectivity for metabolite **1.17**. Without A328G, GVQ and GVQ/A330W favoured the formation of metabolite **3.2**, and gave no selectivity for metabolite **1.17** (*entries 9* and *10*). Other base variants show almost no selectivity for metabolite **1.17** (*entries 2–8*, and *11*). Wild-type $P450_{BM3}$ showed only 3% of conversion (*entry 12*).

Table 3.2 Selected conversion and selectivity data for initial $P450_{BM3}$ screening of diphenyl-derived *cis*-lactam **1.126**, organised first by decreasing selectivity for metabolite **1.17**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **1.17**. Selectivity is calculated by peak area ((single metabolite/all metabolites) x 100).

Entry	Variant	Conv. (%)	Selectivity (%)												
			a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**Others**
1	GVQ/A328G	87	2	10	10	17	-	9	21	13	-	-	1	-	18
2	KU3/A330P/I263G	7	-	-	-	80	-	8	5	-	-	-	-	-	7
3	RP/A82M/I263A/L181F/A328G/A330V	82	4	3	15	61	-	11	2	-	-	-	-	-	4
4	RK/A328L	10	-	-	-	86	-	6	2	-	-	-	-	-	6
5	RK/A328I/S72W	16	1	-	3	94	-	1	1	-	-	-	-	-	1
6	R19/F87I/L437LV	18	-	-	1	81	-	1	1	1	-	-	3	-	13
7	R19/F87I	21	-	-	2	80	-	1	1	-	-	-	5	-	11
8	RK/A184I	74	6	27	24	27	-	2	1	-	-	-	1	-	11
9	GVQ/A330W	78	2	8	10	32	-	2	-	13	-	-	7	4	19
10	GVQ	52	1	2	7	57	-	1	-	20	-	-	3	1	6
11	K19/F87V/E267V	29	2	1	8	60	-	4	-	11	-	-	5	4	5
12	WT	3	-	-	-	49	-	23	-	-	-	-	-	-	28

a, **2.53**; b, **3.1b**; c, **3.1a**; d, **3.2**; e, **3.3**; f, **3.4**, **3.5**; g, **1.17**; h, **3.6**; i, **3.7b**; j, **3.7a**; k, **3.8**; l, **3.9**

Further screening, focusing on the A328G and/or GVQ variants was carried out. Out of 280 variants screened (see *Appendices*, *Table 5.1*), only GVQ/A328G, GVQ/A264G, GV/A184I/A264G, GVQ/A264G/A328G, and GV/A328G gave >20% selectivity for metabolite **1.17** with the highest selectivity achieved by GVQ/A264G/A328G (52%; isolated yield from scale-up, 11%). Other base variants, even with the presence of A328G, showed poor selectivity for metabolite **1.17**.

Looking further into the conversion and selectivity profiles of GV base variant (*Table 3.3*), GV/A184I/A264G gave the highest selectivity for metabolite **1.17** (27%, *entry 2*), followed by GV/A328G (24%, *entry 12*). Without A264G, GV/A184I gave no selectivity (*entry 1*). Further introduction of A328G or I263G to GV/A184I/A264G diminished the selectivity for metabolite **1.17** (*entries 3* and *4*). On the other hand, the addition of A328G to GV/A184I gave only 7% selectivity for metabolite **1.17** but increased the conversion from 20% to 62% (*entry 5*). Additional replacement from GV/A184I/A328G resulted in a drop in conversion and again, no selectivity for metabolite **1.17** was observed (*entries 6–11*). Without A184I, GV/A328G gave a near perfect conversion and a selectivity of 24% for metabolite **1.17**

(*entry 12*). GV/A330F gave only 26% of conversion and no selectivity for metabolite **1.17** (*entry 13*).

Table 3.3 Selected conversion and selectivity data for GV base variant screening of diphenyl-derived *cis*-lactam **1.126**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)												
			a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**Others**
1	GV/A184I	20	2	-	6	59	-	1	-	14	-	-	7	-	10
2	GV/A184I/A264G	26	1	-	6	40	-	6	27	6	-	-	5	1	8
3	GV/A184I/A264G /A328G	63	4	1	34	19	2	5	6	4	-	-	-	-	24
4	GV/A184I/A264G /I263G	39	-	-	1	28	-	2	2	-	-	-	57	4	7
5	GV/A184I/A328G	62	2	4	20	20	2	6	7	12	-	-	1	-	26
6	GV/A184I/A328G /I263G	32	6	1	16	49	1	1	1	-	-	-	14	2	10
7	GV/A184I/A328G /I263G/A330F	43	11	-	27	50	-	-	-	-	-	-	7	-	4
8	GV/A184I/A328G /I263G/L75F	38	6	1	21	49	1	1	1	-	-	-	3	-	18
9	GV/A184I/A328G /I263G/L437S	5	23	-	15	62	-	-	-	-	-	-	-	-	-
10	GV/A184I/A328G /I263G/S72F	7	22	-	24	51	-	-	-	-	-	-	-	-	3
11	GV/A184I/A328G /T260G	11	8	-	-	25	6	12	-	14	-	-	-	-	35
12	GV/A328G	98	-	3	3	10	2	7	24	4	-	-	3	2	42
13	GV/A330F	26	2	1	7	64	-	2	-	7	-	-	4	-	13

a, **2.53**; b, **3.1b**; c, **3.1a**; d, **3.2**; e, **3.3**; f, **3.4**, **3.5**; g, **1.17**; h, **3.6**; i, **3.7b**; j, **3.7a**; k, **3.8**; l, **3.9**

From GVQ as base variant, GVQ/A264/A328G gave the best performance in terms of conversion (94%) and selectivity (52%) (*Table 3.4*, *entry 3*). Without A328G, both conversion and selectivity dropped significantly to 41% and 19%, respectively (*entry 2*); without A264G, the conversion and selectivity were 87% and 21%, respectively (*entry 8*), similar to GV/A328G. The A264G/A328G combination significantly improved enzyme performance and selectivity. Additional replacement from GVQ/A264/A328G abolished the activity (*entry 4*). These observations imply complex interactions between residues in the three-dimensional structure of the enzyme.

With GVQ/A328G, the insertion of T260G resulted in a complete loss of conversion from 87% to 0% (*entry 9*). The T260G replacement tends to kill enzyme activity with lactam

1.126, as shown also in GVQ/T260G (*entry 13*). Additional I263G or A330P or A330W/P329G replacements from GVQ/A264G worsen the enzyme conversion (entries *5*−*7*). Without A264G or A328G, GVQ base variant gave no selectivity for metabolite **1.17** (*entries 1* and *10*−*13*).

Table 3.4 Selected conversion and selectivity data for GVQ base variant screening of diphenyl-derived *cis*-lactam **1.126**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)												
			a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**Others**
1	GVQ	52	1	2	7	57	-	1	-	20	-	-	3	1	6
2	GVQ/A264G	41	2	-	11	43	-	18	19	4	-	-	3	-	-
3	GVQ/A264G/A328G	94	-	4	5	12	4	6	52	3	-	-	1	1	12
4	GVQ/A264G/A328G/ I263G	17	14	-	5	33	1	2	2	3	-	-	3	-	36
5	GVQ/A264G/A330P	39	5	3	20	47	1	5	5	-	-	-	1	-	13
6	GVQ/A264G/A330W /P329G	12	4	-	20	34	-	10	5	4	-	-	1	-	21
7	GVQ/A264G/I263G	19	7	-	23	40	1	1	2	-	-	-	12	-	13
8	GVQ/A328G	87	2	10	10	17	-	9	21	13	-	-	1	-	18
9	GVQ/A328G/T260G	0	-	-	-	-	-	-	-	-	-	-	-	-	-
10	GVQ/A330P	37	5	3	24	53	1	2	-	-	-	-	1	-	11
11	GVQ/A330W	78	2	8	10	32	-	2	-	13	-	-	7	4	19
12	GVQ/S72W	94	-	3	3	31	-	1	-	13	-	-	16	-	32
13	GVQ/T260G	4	16	-	9	39	-	3	-	7	-	-	2	-	24

a, **2.53**; b, **3.1b**; c, **3.1a**; d, **3.2**; e, **3.3**; f, **3.4**, **3.5**; g, **1.17**; h, **3.6**; i, **3.7b**; j, **3.7a**; k, **3.8**; l, **3.9**

3.1.2 Diphenyl-derived *trans*-lactam 1.127

Following the same procedure as for diphenyl-derived *cis*-lactam **1.126**, initial screening studies with the same library of 36 variants were carried out with diphenyl-derived *trans*-lactam **1.127**. GVQ/A328G was again selected for the preparative scale reaction (*Table 3.5*).

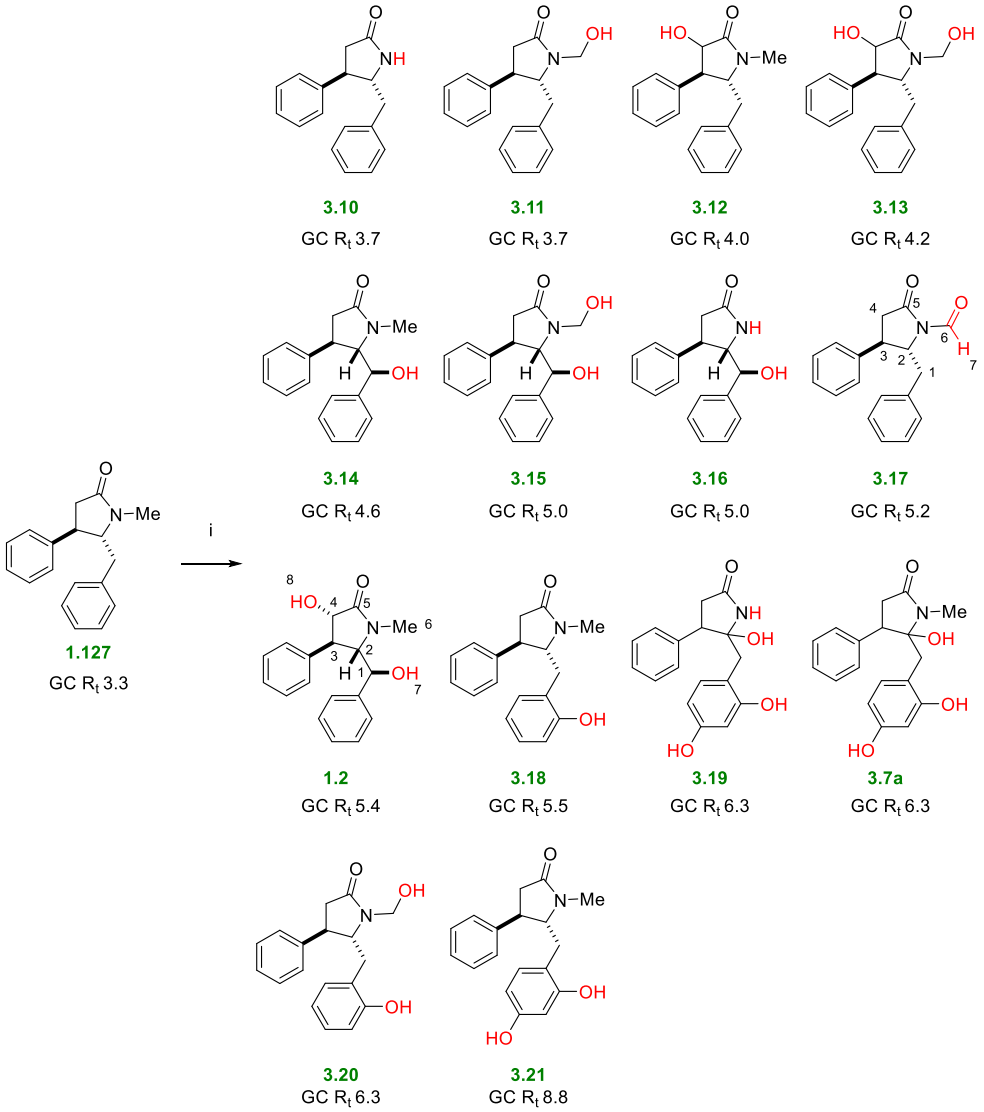
Table 3.5 Enzyme selected for preparative scale reaction with diphenyl-derived *trans*-lactam **1.127**. GC retention time for substrate (3.3 min) and metabolites (3.7–8.8 min). Percentages refer to the fractional area of the total GC peak integration.

Variant	Retention time (min)										
	3.3	**3.7**	**4.0**	**4.2**	**4.6**	**5.0**	**5.2**	**5.4**	**5.5**	**6.3**	**8.8**
GVQ/A328G	2	6	2	14	13	30	3	10	3	5	12

In total, 14 metabolites were isolated and characterised, as shown in *Scheme 3.2*. Similar metabolite profiles were observed as in diphenyl-derived *cis*-lactam **1.126**, except, in this

case, hydroxylation also took place at the carbonyl α-position. Both demethylated **3.10** and the intermediate **3.11** metabolites were isolated, confirmed by HRMS. The relative configuration of metabolite **1.2** was assigned based on the reported data for (1*S**,2*S**,3*R**,4*S**)-*epineo*-clau.[29, 160] Furthermore, the assigned configuration of benzylic alcohol was further proven by the published data for metabolite **3.16**.[161] Although a time course reaction was performed, it was not able to identify whether metabolites **3.15**, **3.16**, and **1.2** were derived from over-oxidation or demethylation of metabolite **3.14**, or the other way round. The primary oxidised metabolite **3.14** was selected as the target metabolite when optimising the enzyme selectivity.

The structure of metabolite **3.17** was supported by HRMS; moreover, an additional resonance was observed at 176.6 ppm in the ^{13}C NMR spectrum, indicating the presence of an aldehyde group; in the ^{1}H NMR spectrum, an additional singlet peak was observed at 9.24 ppm corresponding to H-7. Only one of the diastereomers, metabolite **3.7a** in diphenyl-derived *cis*-lactam **1.126**, was isolated with diphenyl-derived *trans*-lactam **1.127**.



Scheme 3.2 Metabolites isolated from P450$_{BM3}$ preparative scale reaction of diphenyl-derived *trans*-lactam **1.127**, with their retention time (R_t) on GC. Reagents and conditions: i) P450$_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C, 3 h.

Table 3.6 shows selected initial screening data for diphenyl-derived *trans*-lactam **1.127**. Similar to its epimer lactam **1.126**, only GVQ/A328G showed some selectivity (12%; isolated yield from preparative scale reaction, 12%) for metabolite **3.14** (*entry 1*) with high conversion (98%). Again, A328G is the key point replacement which gives selectivity for metabolite **1.17**. Without A328G, demethylation appears to be more favourable (*entries 11* and *12*). Again, other base variants showed negligible selectivity for metabolite **3.14** (*entries*

2−10). Only 3% conversion was observed in wild-type $P450_{BM3}$, as with epimer **1.126** (*entry 13*).

Table 3.6 Selected conversion and selectivity data for initial $P450_{BM3}$ screening of diphenyl-derived *trans*-lactam **1.127**, organised first by decreasing selectivity for metabolite **3.14**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.14**. Selectivity is calculated by peak area ((single metabolite/all metabolite) x 100).

Entry	Variant	Conv. (%)	Selectivity (%)										
			a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**Others**
1	GVQ/A328G	98	6	2	14	12	29	-	10	-	5	11	11
2	R19/A328L/I263A	24	58	5	8	7	8	-	-	-	-	-	13
3	A330P	2	34	-	-	7	-	-	-	-	-	-	60
4	KSK19/I263A	63	67	-	2	5		-	-	-	-	-	26
5	RK/A328G/P329G/A330G	4	52	4	3	4	-	-	-	-	-	-	37
6	RK/F81W/T260G/A328G	35	81	3	1	4	1	-	-	-	-	-	10
7	RK/A328I/S72W	6	79	-	-	2	-	-	-	-	-	-	19
8	KSK19/A82M/I263G	68	75	3	1	2		-	-	-	-	-	19
9	RK/A328G	60	65	10	6	1		-	-	-	-	-	17
10	S72A/A82M/F87A/A184I	98	56	-	2	1	8	-	-	-	1	-	31
11	GVQ	75	86	1	2	1	-	-	1	-	1	-	10
12	GVQ/A330W	48	80	2	2	-	-	-	-	-	1	-	14
13	WT	3	77	-	-	-	-	-	-	-	-	-	23

a, **3.10**, **3.11**; b, **3.12**; c, **3.13**; d, **3.14**; e, **3.15**, **3.16**; f, **3.17**; g, **1.2**; h, **3.18**; i, **3.19**, **3.7a**, **3.20**; j, **3.21**

Further screening was performed, focusing on the A328G and/or GVQ. Variants that gave high selectivity (45%−55%) to benzylic alcohol **3.14** were from RK/A328G/T260G/A82M and KSK19/A82M/T260G/A328G. Preparative scale reactions were not carried out with these variants to assess their enzymatic performance on scale-up due to the limited amount of substrate **1.127** available. Screening of diphenyl-derived *cis*-lactam **1.126** with these two variants gave ~10% selectivity for the benzylic alcohol only. Full screening data of 214 variants with diphenyl-derived *trans*-lactam **1.127** can be found in *Appendices*, *Table 5.2*.

RK/A328G/T260G/A82M gave full conversion of substrate with 55% selectivity for metabolite **3.14** (*Table 3.7*, *entry 5*). In the absence of A82M, conversion halved and the selectivity for metabolite **3.14** diminished (*entry 4*). When replacing A82M with A184I, conversion dropped from 100% to 71%, and no selectivity for metabolite **3.14** was observed (*entry 13*). A82M is a key residue change in boosting selectivity for metabolite **3.14**, with

the combination of A82M/A184I giving 30% selectivity for metabolite **3.14** (*entry 6*). Further addition of A330I/L/V or S72W boosted the selectivity back to ~50% and increased the conversion significantly to near perfect (*entries 8–10*, and *12*). A significant drop in both conversion and selectivity were observed when A330 and S72 were changed to Phe (F) (*entries 7* and *11*).

Furthermore, the A328I replacement reduced the activity of the enzyme; RK/A264G gave 65% conversion, but this dropped to 19% when A328I was added (*entries 1* and *2*); RK/A328G gave 81% conversion which dropped to 35% when Gly (G) was replaced with Ile (I), with longer chain (*entries 3* and *14*).

Table 3.7 Selected conversion and selectivity data for RK base variant screening of diphenyl-derived *trans*-lactam **1.127**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)										
			a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**Others**
1	RK/A264G	65	73	2	2	10	6	-	-	-	-	-	7
2	RK/A264G/A328I	19	44	-	-	41	1	-	-	-	-	-	14
3	RK/A328G	81	58	17	13	2	4	-	-	-	2	-	5
4	RK/A328G/T260G	48	96	2	-	1	-	-	-	-	-	-	1
5	RK/A328G/T260G/A82M	100	11	1	1	55	15	-	-	6	-	-	10
6	RK/A328G/T260G/A82M/ A184I	74	56	1	1	30	6	-	-	2	1	-	4
7	RK/A328G/T260G/A82M/ A184I/A330F	7	82	4	-	8	-	-	-	6	-	-	-
8	RK/A328G/T260G/A82M/ A184I/A330I	98	18	1	3	46	19	-	-	2	3	-	9
9	RK/A328G/T260G/A82M/ A184I/A330L	96	19	-	2	50	15	-	-	3	4	-	6
10	RK/A328G/T260G/A82M/ A184I/A330V	100	4	-	4	51	23	-	-	1	2	-	15
11	RK/A328G/T260G/A82M/ A184I/S72F	49	65	2	1	24	4	-	-	2	-	-	2
12	RK/A328G/T260G/A82M/ A184I/S72W	95	31	1	2	40	17	-	-	1	2	-	5
13	RK/A328G/T260G/A184I	71	74	14	4	-	-	-	-	-	-	-	8
14	RK/A328I	35	98	-	-	-	-	-	-	-	-	-	2

a, **3.10**, **3.11**; b, **3.12**; c, **3.13**; d, **3.14**; e, **3.15**, **3.16**; f, **3.17**; g, **1.2**; h, **3.18**; i, **3.19**, **3.7a**, **3.20**; j, **3.21**

From KSK19/A82M, the inclusion of A264G/A328G switched the major product from metabolite **3.10** and/or **3.11** to the target metabolite **3.14** (44%) (*Table 3.8*, *entries 1* and *2*).

KSK19/A82M/I263G favoured demethylation (*entry 3*). Further addition of A264G/A328G or just A264G boosted the selectivity for metabolite **3.14**, but A328G alone reduced the selectivity from ~42% to 12% (*entries 4*, *5*, and *10*).

A82M seemed to play an important role in giving selectivity for metabolite **3.14**, by comparing KSK19/A82M/I263G/A264G (42%) and KSK19/I263G/A264G (13%) (*entries 4* and *16*). Further addition of A330F/I/V on KSK19/A82M/I263G/A264G/A328G maintained the selectivity for metabolite **3.14**, and boosted the conversion (*entries 6*–*8*). Further addition of P329G, on the other hand, diminished the enzyme activity (*entry 9*).

Further addition of A264G or A264G/I263G to KSK19/A82M/T260G showed no activity (*entries 11* and *12*). When A328G was added instead, the conversion and selectivity for metabolite **3.14** were both raised significantly (*entry 13*). Addition of I263G or T268S to KSK19/A82M/T260G favoured demethylation (*entries 14* and *15*).

Table 3.8 Selected conversion and selectivity data for KSK19 base variant screening of diphenyl-derived *trans*-lactam **1.127**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)										
			a	b	c	d	e	f	g	h	i	j	Others
1	KSK19/A82M	58	70	8	3	-	-	-	-	-	-	-	19
2	KSK19/A82M/A264G/A328G	76	11	3	1	44	14	-	-	-	2	2	23
3	KSK19/A82M/I263G	68	75	3	1	2	-	-	-	-	-	-	19
4	KSK19/A82M/I263G/A264G	68	43	2	1	42	8	-	-	-	-	-	5
5	KSK19/A82M/I263G/A264G/A328G	58	49	3	2	33	6	-	-	-	-	-	8
6	KSK19/A82M/I263G/A264G/A328G/A330F	89	37	8	8	22	18	-	1	1	-	-	5
7	KSK19/A82M/I263G/A264G/A328G/A330I	96	23	1	4	39	25	-	-	-	1	-	8
8	KSK19/A82M/I263G/A264G/A328G/A330V	78	40	2	2	38	10	-	-	1	1	-	7
9	KSK19/A82M/I263G/A264G/A328G/P329G	0	-	-	-	-	-	-	-	-	-	-	-
10	KSK19/A82M/I263G/A328G	78	63	9	3	12	4	-	1	-	1	1	7
11	KSK19/A82M/T260G/A264G	0	-	-	-	-	-	-	-	-	-	-	-
12	KSK19/A82M/T260G/A264G/I263G	7	62	-	-	33	-	-	-	-	-	-	4
13	KSK19/A82M/T260G/A328G	86	22	2	1	53	7	-	-	8	3	-	4
14	KSK19/A82M/T260G/I263G	11	80	-	3	6	-	-	-	-	-	-	11
15	KSK19/A82M/T260G/T268S	7	80	-	4	-	-	-	-	-	-	-	17
16	KSK19/I263G/A264G	62	69	1	1	13	4	-	-	-	-	-	12

a, **3.10**, **3.11**; b, **3.12**; c, **3.13**; d, **3.14**; e, **3.15**, **3.16**; f, **3.17**; g, **1.2**; h, **3.18**; i, **3.19**, **3.7a**, **3.20**; j, **3.21**

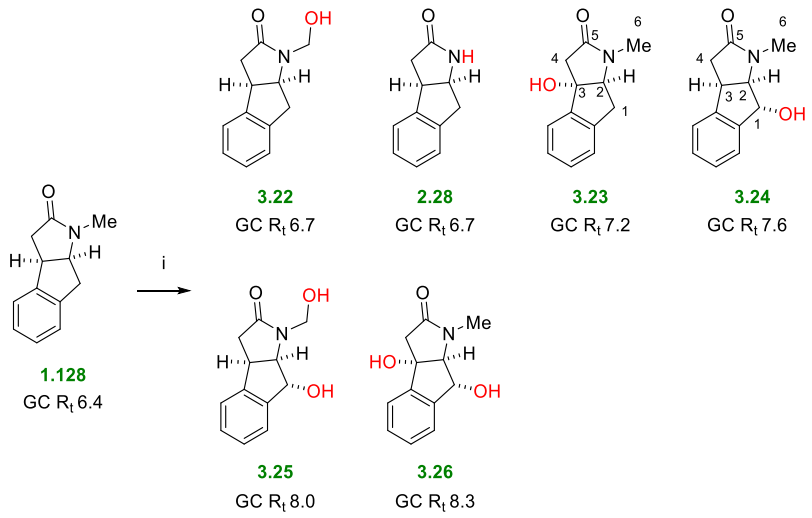
3.1.3 Indene-derived lactam 1.128

Indene-derived lactam **1.128** was screened against the same 36 variants. Scale-up reactions with RP/A82M/I263A and R19/A328L/I263A were carried out to isolate the four major products with retention time 6.7–7.6 min, as tabulated in *Table 3.9*. Products with retention time 8.0 min and 8.3 min were isolated in further scale-up reactions, discussed below.

Table 3.9 Enzymes selected for preparative scale reactions with indene-derived lactam **1.128**. GC retention time for substrate (6.4 min) and metabolites (6.7–8.3 min). Percentages refer to the fractional area of the total GC peak integration.

Variant	Retention time (min)						
	6.4	**6.7**	**7.2**	**7.6**	**8.0**	**8.3**	**Others**
RP/A82M/I263A	27	2	26	29	2	-	14
R19/A328L/I263A	1	67	1	2	-	-	29

The structures of the metabolites isolated are illustrated in *Scheme 3.3*. The structure of metabolite **3.23** was confirmed by the appearance of an additional quaternary carbon peak at 84.2 ppm in the ^{13}C NMR spectrum, corresponding to C-3. No NOESY correlation between H-1 and H-2 was observed in metabolite **3.24** but this was found in the C-1 epimer **3.46** (see *section 3.2 Chemical elaboration post-hydroxylation*), confirming the assigned *trans*- relationship between H-1 and H-2 in metabolite **3.24**.



Scheme 3.3 Metabolites isolated from P450$_{BM3}$ preparative scale reactions of indene-derived lactam **1.128**, with their retention time (R_t) on GC. Reagents and conditions: i) P450$_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C, 20–24 h.

Table 3.10 shows the top 10 variants that gave selectivity for benzylic alcohol **3.24** from initial screening. RP/A82M/I263A gave the highest selectivity for metabolite **3.24** (38%; isolated yield from preparative scale reaction, 23%) (*entry 1*). Unlike previously discussed lactam substrates, screening of indene-derived lactam with wild-type P450$_{BM3}$ gave near

perfect conversion and a selectivity of 13% towards metabolite **3.24** (*entry 5*). Other base variants screened showed a preference for demethylated and intermediate metabolites **3.22** and/or **2.28**, and/or metabolite **3.23** (*entries 2–4* and *6−10*).

Table 3.10 Conversion and selectivity data for the top 10 variants that gave selectivity for metabolite **3.24** in the initial screening with indene-derived lactam **1.128**, in order of decreasing selectivity for metabolite **3.24**. Selectivity is calculated by peak area ((single metabolite/all metabolite) x 100).

Entry	Variant	Conv. (%)	Selectivity (%)					
			3.22, 2.28	3.23	3.24	3.25	3.26	Others
1	RP/A82M/I263A	74	3	34	38	1	-	24
2	KSK19/A82M	68	6	60	17	-	-	17
3	KT2/L188G/I263G	60	37	28	15	-	-	19
4	S72A/A82M/F87A/A184I	66	14	19	14	-	-	53
5	WT	94	18	21	13	3	-	45
6	RK/A184I	33	26	48	9	-	-	16
7	KSK19/A82M/I263G	28	7	57	9	-	-	27
8	KSK19/A82M/I263G/A330W	18	16	29	7	-	-	48
9	RK/F81W/T260G/A328G	13	24	39	6	-	-	31
10	R19/A330W	44	56	11	5	-	-	27

Further screening focusing on RP/A82M/I263A base variant revealed that the highest selectivity for metabolite **3.24** was achieved by RP/A82M/I263A/L181F/A328G/A330L, with 58% selectivity and a conversion of 90% (*Table 3.11*, *entry 6*). The L181F replacement proved to be important in influencing the conversion, and selectivity for metabolite **3.24**. Without L181F, conversion dropped from 90% to 10% and selectivity was compromised as well (*entries 2−4*). Apart from that, when A328 was replaced with Leu (L), the conversion and selectivity dropped drastically (*entry 14*); when A330 was changed to Val (V), selectivity for metabolite **3.25** remained similar, but the conversion dropped to 41% (*entry 13*).

RP/A82M/I263A gave 38% selectivity for metabolite **3.24** and a conversion of 74% (*entry 1*). When I263 was mutated to Gly (G) or Phe (F), similar reactivities were observed (*entries 16* and *17*). Lacking A328G/A330L, variants RP/A82M/I263(A/G)/L181F gave low activities (*entries 5*, *15*, and *18−21*). Additional replacements E267Q or M354F or Q359A

or K349L or K440F or V26H from RP/A82M/I263A/L181F/A328G/A330L also affected the enzyme activity negatively (*entries 7−12*). The full screening data with 250 variants are detailed in *Appendices*, *Table 5.3*.

Preparative scale reactions were carried out with the three variants which gave the highest selectivity for metabolite **3.24** (51%−58%) during screening (*entries 6*, *11*, and *13*). A scale-up reaction of indene-derived lactam **1.128** with RP/A82M/I263A/L181F/A328G/A330L gave target metabolite **3.24** and the over-oxidised metabolite **3.25**, 20% and 3% yield, respectively, after 30 h of reaction time. Additional replacement of Q359A from RP/A82M/I263A/L181F/A328G/A330L, on the other hand, gave 10% isolated yield of metabolite **3.24** and 3% yield of a mixture of over-oxidised metabolites **3.25** and **3.26**, after 28 h of reaction time. The isolated yield of metabolite **3.24** was improved to 20% when the reaction time was shortened to 20 h and 1.5 times more of the enzyme was added at the beginning of the reaction. RP/A82M/I263A/L181F/A328G/A330V gave a complex crude product (as ascertained by ^{1}H NMR spectroscopy) which was not purified by column chromatography.

Table 3.11 Selected conversion and selectivity data for RP/A82M base variant screening of indene-derived lactam **1.128**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)					
			a	b	c	d	e	Others
1	RP/A82M/I263A	74	3	34	38	1	-	24
2	RP/A82M/I263A/A328G/A330L	10	10	5	14	-	-	70
3	RP/A82M/I263A/A328G/A330V	7	21	9	24	-	-	46
4	RP/A82M/I263A/A328G/A330W	4	46	13	5	-	-	36
5	RP/A82M/I263A/L181F	27	10	32	42	-	-	16
6	RP/A82M/I263A/L181F/A328G/A330L	90	6	9	58	13	-	14
7	RP/A82M/I263A/L181F/A328G/A330L/E267Q	13	20	11	31	-	-	38
8	RP/A82M/I263A/L181F/A328G/A330L/K349L	25	11	10	45	2	-	33
9	RP/A82M/I263A/L181F/A328G/A330L/K440F	42	8	11	49	3	-	29
10	RP/A82M/I263A/L181F/A328G/A330L/M354F	18	9	21	30	-	-	41
11	RP/A82M/I263A/L181F/A328G/A330L/Q359A	44	11	6	51	4	-	29
12	RP/A82M/I263A/L181F/A328G/A330L/V26H	19	10	11	39	1	-	38
13	RP/A82M/I263A/L181F/A328G/A330V	41	13	6	56	4	-	22
14	RP/A82M/I263A/L181F/A328L/A330L	14	47	3	2	-	-	47
15	RP/A82M/I263A/L181F/L188F	59	4	37	36	2	-	21
16	RP/A82M/I263F	68	10	29	35	1	-	25
17	RP/A82M/I263G	43	8	30	32	-	-	30
18	RP/A82M/I263G/L181F	53	7	27	44	1	-	22
19	RP/A82M/I263G/L181F/L188F	20	9	34	24	-	-	33
20	RP/A82M/I263G/L181F/V26F	33	7	30	36	1	-	27
21	RP/A82M/I263G/L181F/V26M	29	8	31	41	1	-	19

a, **3.22**, **2.28**; b, **3.23**; c, **3.24**; d, **3.25**; e, **3.26**

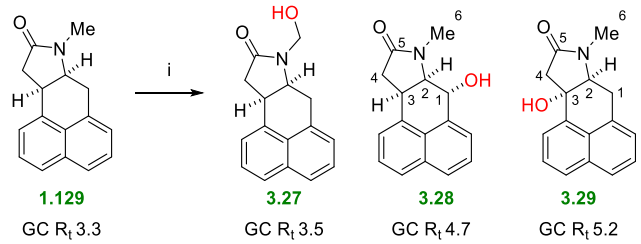
3.1.4 Phenalene-derived lactam 1.129

The phenalene-derived lactam **1.129** was screened against the same 36 variants. Full screening data can be found in *Appendices*, *Table 5.4*. Scale-up reactions with K19/F87V/E267V/V78I and KSK19/A82M were performed to isolate the three major metabolites, as tabulated in *Table 3.12*. Screening with RP/A82M/I263A/L181F/A328G/A330V gave 100% conversion of substrate but no product peak was detected on GC. A scale-up reaction with this enzyme revealed that unstable metabolite(s) was/were formed; the ^{1}H NMR spectrum of the crude product showing no obvious metabolites. In this case, the reported GC-analysed screening data might not fully reflect the performance of the variants with this lactam.

Table 3.12 Enzymes selected for preparative scale reactions with phenalene-derived lactam **1.129**. GC retention time for substrate (3.3 min) and metabolites (3.5–5.2 min). Percentages refer to the fractional area of the total GC peak integration.

Variant	Retention time (min)				
	3.3	**3.5**	**4.7**	**5.2**	**Others**
K19/F87V/E267V/V78I	15	54	-	5	26
KSK19/A82M	-	-	93	-	7
RP/A82M/I263A/L181F/A328G/A330V	-	-	-	-	-

The structures of the metabolites isolated are shown in *Scheme 3.4*. The configuration of target metabolite **3.28** was determined by the large vicinal coupling constant of J = 7.5 Hz between H-1 and H-2, which is consistent with the almost *trans*-diaxial coupling in the *exo*-alcohol, rather than the axial-equatorial coupling expected for the *endo*-alcohol (in epimer **3.49** the corresponding coupling constant J = 3.0 Hz, see *section 3.2 Chemical elaboration post-hydroxylation*). Additionally, NOESY correlations were observed between H-1 and *endo*-situated H-4, and between H-3 and *exo*-situated H-4 in metabolite **3.28**, further confirming the assigned *exo*-configuration.



Scheme 3.4 Metabolites isolated from $P450_{BM3}$ preparative scale reactions of phenalene-derived lactam **1.129**, with their retention time (R_t) on GC. Reagents and conditions: i) $P450_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C, 2–3 h.

Based on the initial screening data (*Table 3.13*), KSK19/A82M/I263G gave perfect selectivity for the target benzylic alcohol **3.28** with 74% conversion (*entry 1*). In the absence of I263G, conversion rose to 100% and some reactivity diverted to metabolite **3.27** (7%) (*entry 2*). In the absence of A82M, selectivity for metabolite **3.28** dropped drastically to ~20% (*entries 6* and *9*). When F87 was changed from Ala (A) to Val (V), selectivity was

shifted away from metabolites **3.28** and/or **3.29** toward metabolite **3.27** (*entries 10−13*). The S72A/A82M/F87A/A184I combination also gave full conversion and high selectivity for metabolite **3.28** (*entry 3*). Wild-type $P450_{BM3}$ gave only 16% conversion (*entry 14*).

The preparative scale reactions of KSK19/A82M/I263G and KSK19/A82M both gave metabolite **3.28** with 70% yield. Because the target metabolite **3.28** was obtained in both high conversion and high selectivity, no further screening studies were performed.

Table 3.13 Selected conversion and selectivity data for initial $P450_{BM3}$ screening of phenalene-derived lactam **1.129**, organised first by decreasing selectivity for metabolite **3.28**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.28**. Selectivity is calculated by peak area ((single metabolite/all metabolite) x 100).

Entry	Variant	Conv. (%)	Selectivity (%)			
			3.27	3.28	3.29	Others
1	KSK19/A82M/I263G	74	-	100	-	-
2	KSK19/A82M	100	7	93	-	-
3	S72A/A82M/F87A/A184I	100	6	83	-	11
4	RK/A184I/T260G/A328G	100	9	43	24	25
5	RK/I263G	100	14	23	44	20
6	KSK19/I263A	71	18	21	61	-
7	RK/A184I	75	14	20	57	9
8	RP/A82M/I263A	23	18	19	-	63
9	KSK19/I263G	90	27	11	62	-
10	K19/F87V	85	50	-	17	33
11	K19/F87V/E267V/V78I	85	74	-	6	20
12	K19/F87V/Q403P	66	54	-	18	28
13	K19/F87V/E267V	21	78	-	-	22
14	WT	16	16	-	-	84

3.1.5 Phenalene-derived *cis*-isomer lactam 2.85

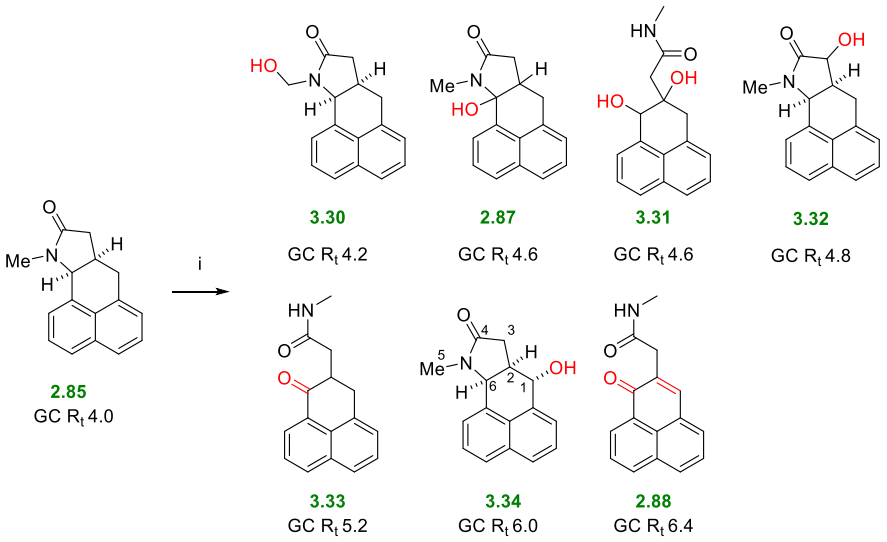
As before, the phenalene-derived lactam **2.85** was screened against the same 36 variants. KSK19/A82M, A74G/F87I/A184I/I263G/A328G and RK/A328I were chosen to perform preparative scale reactions in order to isolate and characterise the seven products identified by GC (*Table 3.14*).

Table 3.14 Enzymes selected for preparative scale reactions with phenalene-derived *cis*-isomer lactam **2.85**. GC retention time for substrate (4.0 min) and metabolites (4.2–6.4 min). Percentages refer to the fractional area of the total GC peak integration.

Variant	Retention time (min)							
	4.0	**4.2**	**4.6**	**4.8**	**5.2**	**6.0**	**6.4**	**Others**
KSK19/A82M	1	-	-	4	5	23	64	3
A74G/F87I/A184I/I263G/A328G	14	41	-	1	2	36	2	4
RK/A328I	21	24	-	11	20	4	9	11

The structures of the seven metabolites are shown in *Scheme 3.5*. Ring-opening was observed with the phenalene-derived *cis*-isomer lactam **2.85**. Metabolite **3.33** is the ring-opened form of hemiaminal **2.87**. Metabolite **2.88** is an over-oxidised product from metabolite **3.33** whereas metabolite **3.31** was formed as a result of carbonyl reduction, followed by further hydroxylation from metabolite **3.33** or *vice versa*.

The NMR data for metabolite **2.87** and **2.88** matched those for compounds synthesised in *Chapter 2* (*Scheme 2.29* and *2.30*). The structures of metabolites **3.33** and **3.31** were confirmed by HRMS, and the presence of additional ^{13}C NMR resonances at 200.3 ppm and 68.9 ppm, corresponding to a ketone and a tertiary alcohol, respectively. The configuration of metabolite **3.32** was not assigned. The *exo*-configuration assigned to the hydroxyl group in metabolite **3.34** was determined by a NOESY correlation between *exo*-situated H-3 and H-6, and between H-1 and *endo*-situated H-3; the large vicinal coupling constant of J = 8.0 Hz between H-1 and H-2 indicated an *exo*-alcohol rather than an *endo*-alcohol (in epimer **3.52** the corresponding coupling constant J = 5.0 Hz, see *section 3.2 Chemical elaboration post-hydroxylation*).



Scheme 3.5 Metabolites isolated from $P450_{BM3}$ preparative scale reactions of phenalene-derived *cis*-isomer lactam **2.85**, with their retention time (R_t) on GC. Reagents and conditions: i) $P450_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C, 2–27 h.

Based on the initial screening studies, the variants that gave high selectivity (30%−43%) for the target metabolite **3.34** were KSK19/A82M/I263G, S72A/A82M/F87A/A184I, and GLQ/I263G/A328G, with the first and second showing near-perfect conversion, while the third exhibited about half the conversion (*Table 3.15*, *entries 1*−*3*). KSK19/A82M gave half the selectivity of that found with KSK19/A82M/I263G (*entry 4*). Without A82M, selectivity dropped as well, from 43% to 12%, and the conversion dropped from 99% to 50% (*entry 6*). Changing of I263 from Gly (G) to Ala (A) improved conversion from 50% to 90% (*entries 5* and *6*). Wild-type $P450_{BM3}$ gave just 15% conversion (*entry 7*). Further screening studies focusing on improving the performance of KSK19/A82M/I263G and S72A/A82M/F87A/A184I were conducted. Full screening data with 158 variants can be found in *Appendices*, *Table 5.5*.

Table 3.15 Selected conversion and selectivity data for $P450_{BM3}$ screening of phenalene-derived *cis*-isomer lactam **2.85**, in the order of decreasing selectivity for metabolite **3.34**. Selectivity is calculated by peak area ((single metabolite/all metabolite) x 100).

Entry	Variant	Conv. (%)	Selectivity (%)						
			3.30	**2.87, 3.31**	**3.32**	**3.33**	**3.34**	**2.88**	**Others**
1	KSK19/A82M/I263G	99	-	-	-	26	43	21	10
2	S72A/A82M/F87A/A184I	98	1	-	7	25	39	21	7
3	GLQ/I263G/A328G	48	36	-	2	12	30	7	14
4	KSK19/A82M	99	-	-	3	5	22	61	9
5	KSK19/I263A	90	1	-	1	50	19	20	9
6	KSK19/I263G	50	1	-	2	66	12	8	11
7	WT	15	28	-	4	11	5	12	39

During further rounds of screening, no variants were found with improved selectivity towards metabolite **3.34**. S72A/A82M/F87A/A184I, from initial screening, gave the highest selectivity (39%) for the target metabolite **3.34** (*Table 3.16*, *entry 3*). Without A184I or F87A, selectivity dropped slightly to ~28% (*entries 1* and *2*). Changing of F87 to Ile (I) favoured demethylation (*entries 6* and *7*) whereas changing to Ala (A) gave rise to selectivity for metabolites **3.33**, **3.34**, and **2.88** in near perfect conversion (*entries 2* and *3*). The addition of A184I/T260G or E435M on S72A/A82M/F87A gave mainly metabolite **3.33** and the conversion dropped from 98% to 30% and 42%, respectively (*entries 4* and *5*).

Table 3.16 Selected conversion and selectivity data for S72A/A82M base variant screening of phenalene-derived *cis*-isomer lactam **2.85**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)						
			3.30	**2.87, 3.31**	**3.32**	**3.33**	**3.34**	**2.88**	**Others**
1	S72A/A82M/A184I	99	-	-	6	7	29	44	14
2	S72A/A82M/F87A	86	1	-	2	39	27	23	8
3	S72A/A82M/F87A/A184I	98	1	-	7	25	39	21	7
4	S72A/A82M/F87A/A184I/T260G	30	19	-	2	43	18	6	13
5	S72A/A82M/F87A/E435M	42	3	-	1	53	25	10	8
6	S72A/A82M/F87I	17	38	-	2	16	21	4	19
7	S72A/A82M/F87I/A184I	29	66	-	1	7	12	3	11

From KSK19 as base variant, the highest selectivity for metabolite **3.34** was achieved by KSK19/A82M/I263G (*Table 3.17*, *entry 7*), followed by KSK19/A82M/F261G (*entry 6*) and KSK19/A82M/I401M (*entry 9*). Changing the F87 from Ala (A) to Ile (I), a residue with

a bulkier side chain, switched the major product from metabolite **3.33** to the hemiaminal **3.30** and target metabolite **3.34** (*entries 10* and *11*), indicating the impact of steric effects on substrate binding. Without A82M, the selectivity for metabolite **3.34** dropped from 43% to 10% (*entries 9* and *10*); without I263G, KS19/A82M gave a selectivity of 22% for metabolite **3.34** (*entry 2*). Further addition of A264G or A264G/A328G or A330W or I263W on KSK19/A82M favoured metabolite **2.88** or **3.33** (*entries 3−5* and *8*). The major product for KSK19 was the over-oxidised metabolite **2.88** (*entry 1*).

Table 3.17 Selected conversion and selectivity data for KSK19 base variant screening phenalene-derived *cis*-isomer lactam **2.85**, in the order of variants.

Entry	Variant	Conv. (%)	Selectivity (%)						
			3.30	**2.87, 3.31**	**3.32**	**3.33**	**3.34**	**2.88**	**Others**
1	KSK19	92	-	-	3	16	10	63	8
2	KSK19/A82M	58	-	-	2	5	22	81	10
3	KSK19/A82M/A264G	87	-	-	2	34	15	45	5
4	KSK19/A82M/A264G/A328G	45	-	-	1	-	-	88	11
5	KSK19/A82M/A330W	73	-	-	1	17	9	56	13
6	KSK19/A82M/F261G	100	-	-	2	21	37	29	11
7	KSK19/A82M/I263G	88	-	-	-	26	43	21	10
8	KSK19/A82M/I263W	23	-	-	2	41	18	10	28
9	KSK19/A82M/I401M	22	-	-	2	32	33	25	8
10	KSK19/I263G	21	1	1	3	63	10	15	8
11	K19/F87I/I263G	56	49	-	2	3	37	2	7

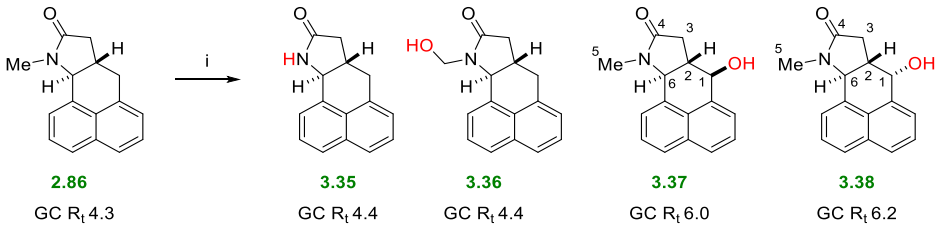
3.1.6 Phenalene-derived *trans*-isomer lactam 2.86

KSK19/A82M and RK/I263G were chosen for preparative scale reactions, from the initial screening of phenalene-derived *trans*-isomer lactam **2.86** against the same 36 variants (*Table 3.18*). Full screening data can be found in *Appendices*, *Table 5.6*.

Table 3.18 Enzymes selected for the scale-up reactions with phenalene-derived *trans*-isomer lactam **2.86**. R_t detected by GC analysis for substrate (4.3 min) and metabolites (4.4–6.2 min). Percentages refer to the fractional area of the total GC peak integration.

Variant	Retention time (min)				
	4.3	**4.4**	**6.0**	**6.2**	**Others**
KSK19/A82M	-	-	52	42	6
RK/I263G	51	30	1	2	16

The structures of four major products were identified and characterised as shown in *Scheme 3.6*. The configurations of diastereomers **3.37** and **3.38** were assigned based on a NOESY correlation between H-1 and H-6 in diastereomer **3.37**, which was not found in the diastereomer **3.38**. Besides that, epimer **3.37** displayed a larger vicinal coupling constant (*J* = 12.0 Hz) between H-1 and H-2 than that found in epimer **3.38** (*J* = 7.0 Hz), which further confirmed the assignment. The diastereomers were barely separated by chromatography, eluting with pentane/ethyl acetate (1:1); only one fraction containing a single diastereomer **3.37** was isolated, the others containing a mixture of diastereomers **3.37** and **3.38** in quantitative overall yield, in a 1:1 ratio.



Scheme 3.6 Metabolites isolated from $P450_{BM3}$ preparative scale reactions of phenalene-derived *trans*-isomer lactam **2.86**, with their retention time (R_t) on GC. Reagents and conditions: i) $P450_{BM3}$ (500:1 substrate:enzyme), $NADP^+$, GDH, glucose, phosphate buffer, 20 °C 3–4 h.

Table *3.19* shows selected data from the initial screening of phenalene-derived *trans*-isomer lactam **2.86**, in order of decreasing selectivity for target metabolite **3.37**. Variants that gave high selectivity (50%−60%) were from K19/F87V and GVQ base variants. In these base variants, F87V favours diastereomer **3.37** over **3.38** (*entries 1*−*5* and *7*−*8*), whereas F87A gave similar selectivity for both diastereomers (*entries 6*, *9* and *11*−*13*). Wild-type enzyme $P450_{BM3}$ gave only a 21% conversion (*entry 10*).

When comparing the enzymatic performance of *cis*- and *trans*-isomer lactams **2.85** and **2.86**, the top two variants, KSK19/A82M/I263G and S72A/A82M/F87A/A184I, which gave reasonable selectivity (39%−43%) for benzylic alcohol **3.34** from *cis*-isomer lactam **2.85**,

also gave similar selectivity for benzylic alcohol **3.37** from *trans*-isomer lactam **2.86** (*entries 9* and *11*). On the other hand, K19/F87V and GVQ, which gave good selectivity for benzylic alcohol **3.37** from *trans*-isomer lactam **2.86**, showed no selectivity at all for the benzylic alcohol **3.34** from *cis*-isomer lactam **2.85**.

The introduction of Q403P to K19/F87V reduced conversion to half, while maintaining the selectivity for metabolite **3.37** (*entries 1* and *4*). The conversion dropped even further, to 22%, when E267V was added, and selectivity dropped slightly from 60% to 41% (*entries 1*, *7* and *8*). On the other hand, with GVQ as base variant, addition of A328G or A330W raised the conversion from 58% to 87% and 73%, respectively, while the selectivity for metabolite **3.37** dropped slightly (*entries 2*, *3* and 5).

Table 3.19 Selected conversion and selectivity data for initial $P450_{BM3}$ screening of phenalene-derived *trans*-isomer lactam **2.86**, in the order of decreasing selectivity for metabolite **3.37**. Selectivity is calculated by peak area ((single metabolite/all metabolite) x 100).

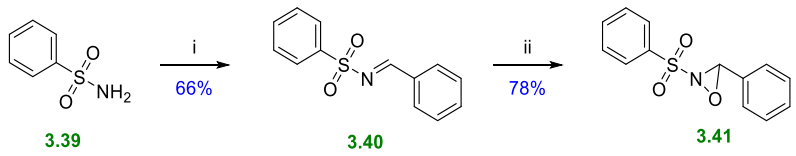
Entry	Variant	Conv. (%)	Selectivity (%)			
			3.35, 3.36	3.37	3.38	Others
1	K19/F87V	92	4	60	17	18
2	GVQ	58	5	57	17	21
3	GVQ/A328G	87	13	55	13	19
4	K19/F87V/Q403P	45	12	54	12	22
5	GVQ/A330W	73	16	48	13	23
6	KSK19/A82M	100	-	44	35	21
7	K19/F87V/E267V/V78I	23	9	41	7	42
8	K19/F87V/E267V	22	7	41	6	46
9	S72A/A82M/F87A/A184I	88	6	34	37	23
10	WT	21	3	34	2	61
11	KSK19/A82M/I263G	56	10	33	26	31
12	KSK19/I263A	73	50	13	7	30
13	KSK19/I263G	54	58	11	1	30

3.2 Chemical elaboration post-hydroxylation

In order to expand the stereoisomer profile of clausenamide analogues, a method to invert the benzylic alcohol stereocentre was sought. From various options available to achieve such an inversion, the method chosen here was an oxidation/reduction sequence, with the

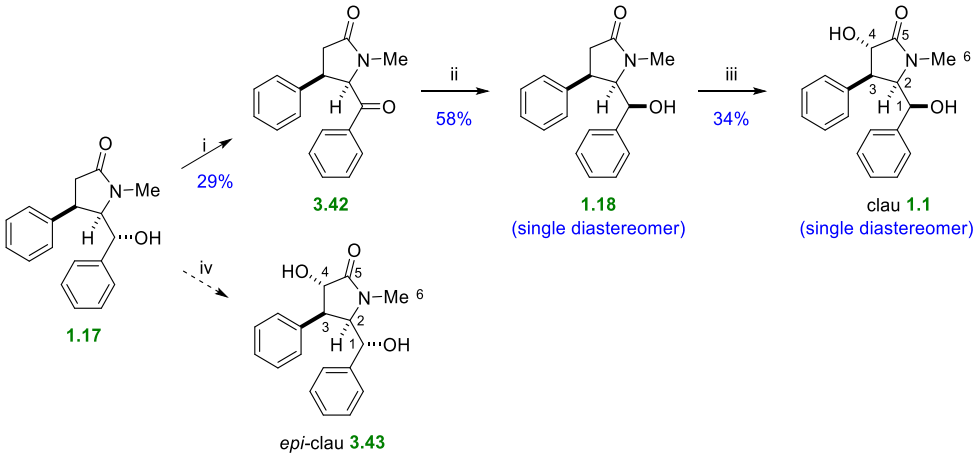
reduction expected to occur from the same, more available, face as the original hydroxylation. Chemical hydroxylation at the carbonyl α-position with Davis oxidant **3.41**, prepared as reported[162] (*Scheme 3.7*), was then expected to give access to the clausenamide analogues.

The carbonyl α-hydroxylation reaction with Davis oxidant is expected to take place with kinetic control on the *exo*-face of the substrate or *anti*- to the bulky aryl substituent position in a *trans*-fused ring and open chain systems.



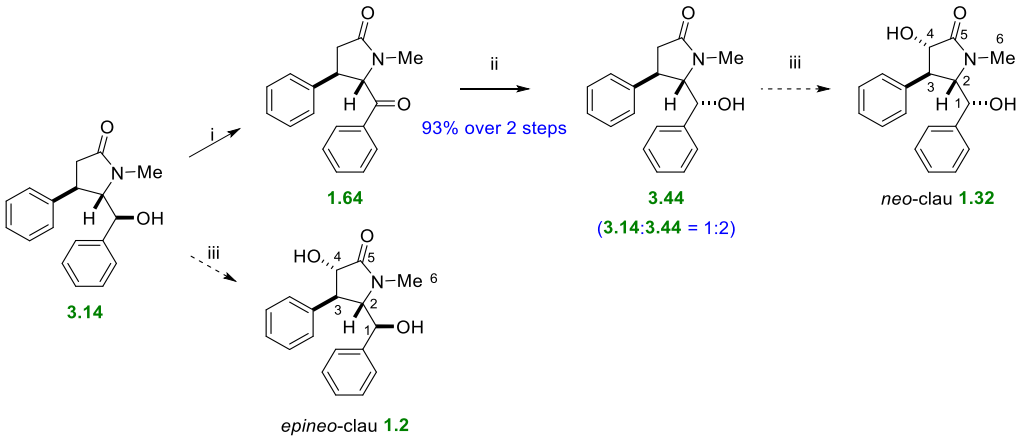
Scheme 3.7 Preparation of Davis oxidant **3.41**. Reagents and conditions: i) benzaldehyde, Amberlyst® 15, 4 Å molecular sieves, toluene, reflux, 9 h; ii) K_2CO_3, Oxone®, toluene, H_2O, RT, 48 h.

Oxidation of alcohol **1.17** followed by reduction, gave alcohol **1.18** as a single stereoisomer, with spectroscopic data matching those reported (*Scheme 3.8*).[37] The benzylic alcohol **1.18** was then entered into the Davis oxidation reaction to give the natural product, (1*S**,2*R**,3*R**,4*S**)-clausenamide **1.1**, with spectroscopic data matching those reported.[39] Carbonyl α-hydroxylation with alcohol **1.17**, which was not undertaken due to the limited amount of alcohol available, is anticipated to give (1*R**,2*R**,3*R**,4*S**)-*epi*-clau **3.43**.



Scheme 3.8 Post-hydroxylation chemical synthesis of clau **1.1** and *epi*-clau **3.43**. Reagents and conditions: i) DMP, $NaHCO_3$, DCM, RT, 21 h; ii) $NaBH_4$, EtOH, RT, 2 h; iii) LiHMDS, Davis oxidant **3.41**, THF, 0 °C, 3 h; iv) LiHMDS, Davis oxidant **3.41**, THF.

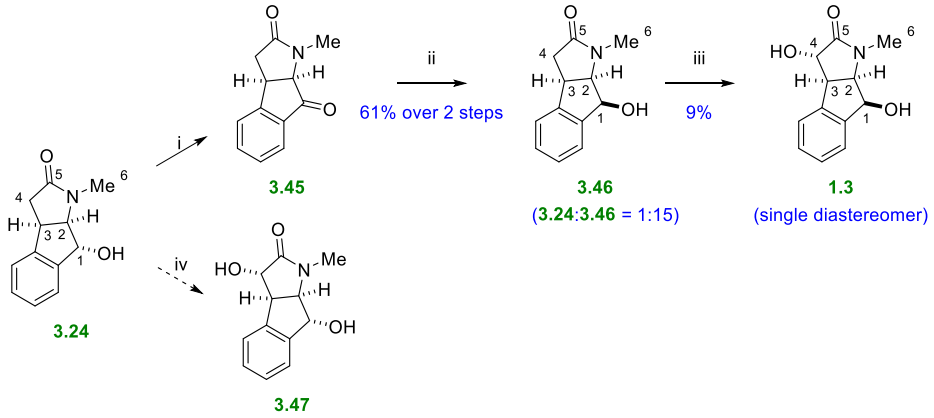
On the other hand, oxidation followed by reduction of alcohol **3.14** (*Scheme 3.9*) gave a pair of alcohols, returned **3.14** and **3.44** in a 1:2 ratio. Carbonyl α-hydroxylation of benzylic alcohols **3.14** and **3.44** is expected to give (1*S**,2*S**,3*R**,4*S**)-*epineo*-clau **1.2** and (1*R**,2*S**,3*R**,4*S**)-*neo*-clau **1.32** respectively.



Scheme 3.9 Post-hydroxylation projected chemical synthesis of *neo*-clau **1.32** and *epineo*-clau **1.2**. Reagents and conditions: i) DMP, $NaHCO_3$, DCM, RT, 24 h; ii) $NaBH_4$, EtOH, RT, 3 h; iii) LiHMDS, Davis oxidant **3.41**, THF.

In the attempt to invert the stereocentre of *exo*-alcohol **3.24** (*Scheme 3.10*), the *exo*- and *endo*-alcohols **3.24** and **3.46** were obtained in a 1:15 ratio, following the oxidation and

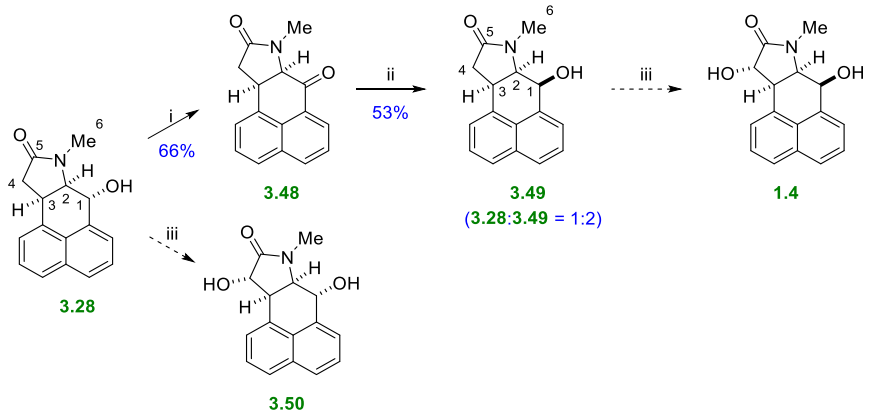
reduction steps. NOESY correlation between H-1 and H-2 in the major product **3.46**, but not the minor product **3.24**, confirmed the assigned configurations. In the Davis oxidation step, only a 9% yield of indene-derived analogue **1.3** was obtained, as a single diastereomer, due to the small scale (0.042 mmol) of the reaction; the minor *exo*-alcohol was returned unreacted in this step (~7%), suggesting that Davis oxidation is faster with the *endo*-alcohol than with the *exo*-alcohol. The configuration of indene-derived analogue **1.3** was determined by NOESY correlations between H-1 and H-2, and between H-2 and H-3, and the absence of NOESY correlations between H-4 and either H-2 or H-3.



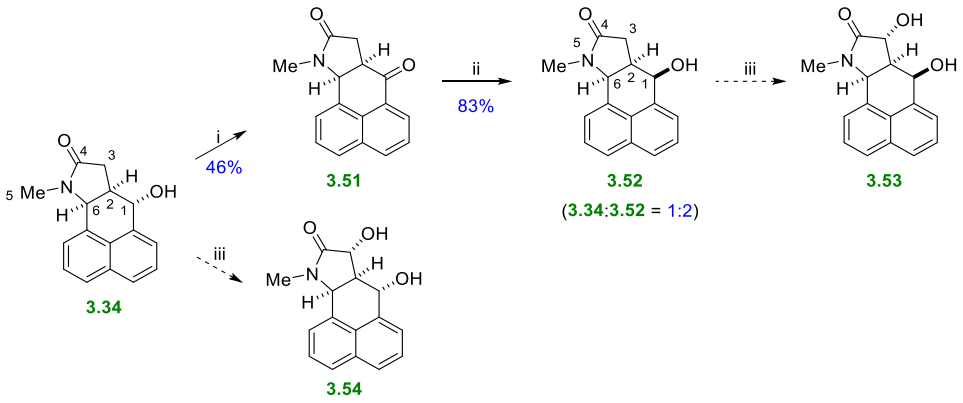
Scheme 3.10 Post-hydroxylation chemical synthesis of indene-derived analogues **1.3** and **3.47**. Reagents and conditions: i) DMP, $NaHCO_3$, DCM, RT, 41 h; ii) $NaBH_4$, EtOH, RT, 4 h; iii) LiHMDS, Davis oxidant **3.41**, THF, 0 °C, 3 h; iv) LiHMDS, Davis oxidant **3.41**, THF.

To access phenalene-derived analogue **1.4** (*Scheme 3.11*) and phenalene-derived *cis*-isomer analogue **3.53** (*Scheme 3.12*), sterecohemical inversion of the *exo*-alcohols **3.28** and **3.34** resulted in a pair of *exo*- and *endo*- alcohols **3.28** and **3.49**, and **3.34** and **3.52**, both in 1:2 ratio. The stereochemical configurations of **3.28** and **3.49** were determined in each case by the vicinal coupling constants typical of a *trans*-diaxial (J = 7.5 Hz) or axial-equatorial (J = 3.0 Hz) relationship between H-1 and H-2, respectively, as mentioned before (see *section 3.1.4 Phenalene-derived lactam **1.129***). The stereochemical configurations of **3.34** and **3.52**

were assigned based on the vicinal coupling constants between H-1 and H-2 of J = 8.0 Hz and J = 5.0 Hz, respectively (see *section 3.1.5 Phenalene-derived cis-isomer lactam* ***2.85***).

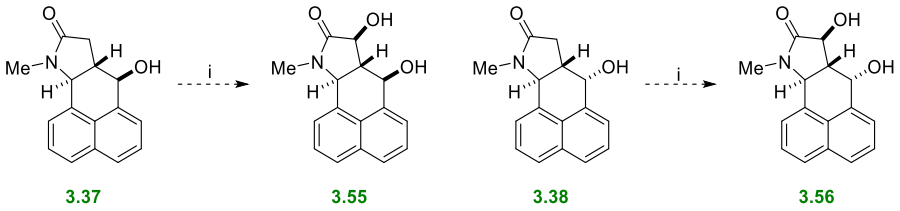


Scheme 3.11 Post-hydroxylation projected chemical synthesis of phenalene-derived analogues **1.4** and **3.50**. Reagents and conditions: i) DMP, $NaHCO_3$, DCM, RT, 20 h; ii) $NaBH_4$, EtOH, RT, 2 h; iii) LiHMDS, Davis oxidant **3.41**, THF.



Scheme 3.12 Post-hydroxylation projected chemical synthesis of phenalene-derived *cis*-isomer analogues **3.53** and **3.54**. Reagents and conditions: i) DMP, $NaHCO_3$, DCM, RT, 24 h; ii) $NaBH_4$, EtOH, RT, 5 h; iii) LiHMDS, Davis oxidant **3.41**, THF.

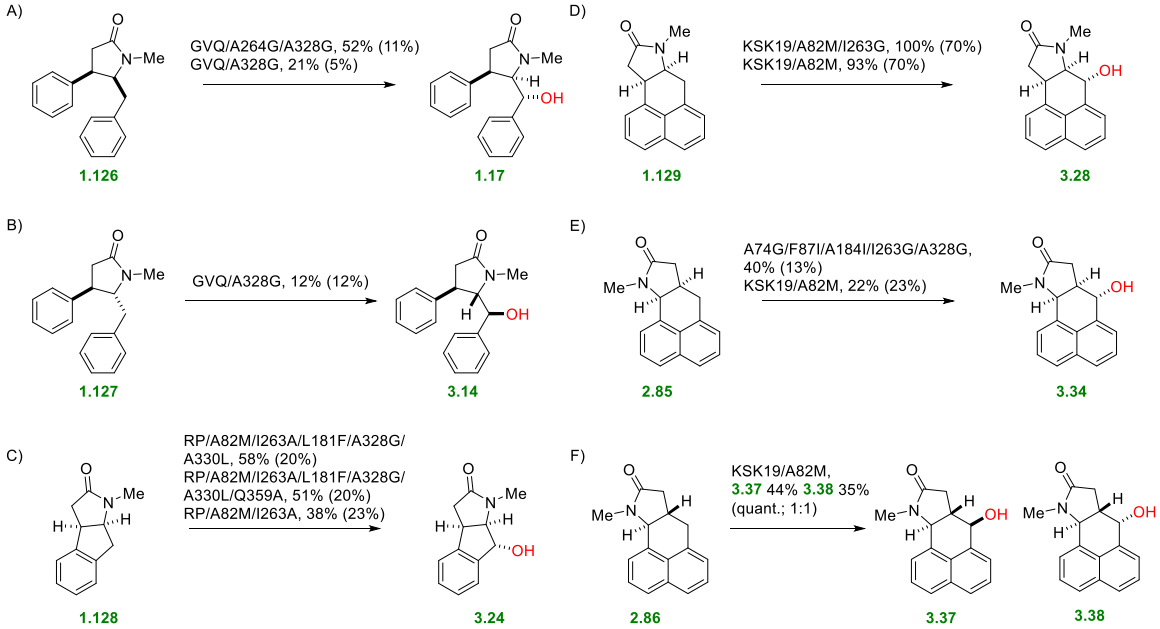
Last but not least, phenalene-derived *trans*-isomer analogues **3.55** and **3.56** are also anticipated to be obtained by performing the Davis oxidation step on benzylic alcohols **3.37** and **3.38** from $P450_{BM3}$ reaction, respectively (*Scheme 3.13*).



Scheme 3.13 Post-hydroxylation projected chemical synthesis of phenalene-derived *trans*-isomer analogues **3.55** and **3.56**. Reagents and conditions: i) LiHMDS, Davis oxidant **3.41**, THF.

3.3 Conclusions and future work

In this study, enzyme engineering has achieved diverse oxidation regioselectivity and diastereoselectivity around the lactam motif, accessing every sp^3 position. Since the goal of the project was to synthesise clausenamide analogues, the benzylic alcohol metabolites were targeted in enzymatic screening. The preparative scale reactions to obtain these alcohols are summarised in *Scheme 3.14*, including selectivity from screening with the isolated yield from scale-up quoted in brackets.



Scheme 3.14 Summary of preparative scale reactions to obtain benzylic alcohols **1.17**, **3.14**, **3.24**, **3.28**, **3.34**, **3.37** and **3.38**.

Screening of $P450_{BM3}$ variants across a wide range of substrates offers a promising foundation for predicting enzyme activity with novel substrates, and hence potentially enabling these variants to serve as general oxidation catalysts. The unpredictable effects of individual residues, which often requiring case-by-case evaluation, remain a significant challenge in controlling the selectivity of these enzymes. This unpredictability arises because even a single replacement can drastically alter the enzyme behaviour, with combined replacements often producing non-additive and complex effects. As a result, insights drawn from the screening results are generally not possible to apply to other systems, and are specific to the particular enzyme and substrate screened.

In future work, larger quantities of the lactam substrates are required so that preparative scale reactions may be performed with the variants showing good conversion and high selectivity for the benzylic alcohols during screening. Reaction engineering such as modifying the substrate-to-enzyme ratio and reaction time could be carried out to investigate whether the activity and selectivity could thereby be improved.

Furthermore, molecular docking (MD) simulations[163, 164] could be employed to rationalise the screening results and to guide on how further mutagenesis could be done to obtain high selectivity for the targeted metabolite. Protein structure prediction tools, such as AlphaFold[165] – developed by 2024 Chemistry Nobel Prize winners and renowned for its high-accuracy prediction of protein structures – could complement MD simulations by aiding in the modelling of enzyme-substrate interactions.

Since enzyme and substrate binding are a complex interaction and there is often not a simple relationship between the introduction of a single replacement and the resulting conversion and selectivity profiles, screening of lactam substrates against a wider library of enzyme with different combinations of replacements could be helpful.

In addition to rational design, enzyme libraries could be generated through random mutagenesis techniques such as error-prone PCR (introducing random replacements),[166] DNA shuffling (recombining sequences for increased diversity)[167] and site-saturation metagenesis (targeting specific residues for systematic variation).[168]

Apart from improving the conversion and selectivity for selected metabolites, the enantiomeric excess data should be determined by establishing a chiral HPLC method for distinguishing the enantiomers of the benzylic alcohol metabolites; being able to access enantiopure clausenamide analogues would provide useful information on their biological activities.

Only clausenamide **1.1** and analogues **1.2** and **1.3** were made, due to the limited amount of benzylic alcohols available for the carbonyl α-hydroxylation step needed to afford clausenamide analogues **1.4**, **1.32**, **3.43**, **3.47**, **3.50**, and **3.53**–**3.56** (*Figure 3.2*). To add to the SAR profile of clausenamide isomers, all the clausenamide analogues should be evaluated for their neuroprotective effect using the MTT method, as described in *Chapter 1*.

While the utility of P450 enzymes in biocatalysis is well-established, their relevance extends far beyond this area. These enzymes play pivotal roles in drug discovery and toxicology, as they are responsible for the oxidative metabolism of approximately 75% of all drugs,[169] including predicting the formation of toxic metabolites that can influence drug safety.[170]

Beyond their catalytic functions, substrates that exhibit poor activity with specific P450 enzymes could have therapeutic potential. Such substrates might act as inhibitors of human P450 enzymes that are overexpressed in certain diseases. For instance, CYP1B1 is often overexpressed in various cancers and is a promising target for anti-cancer therapies.[171]

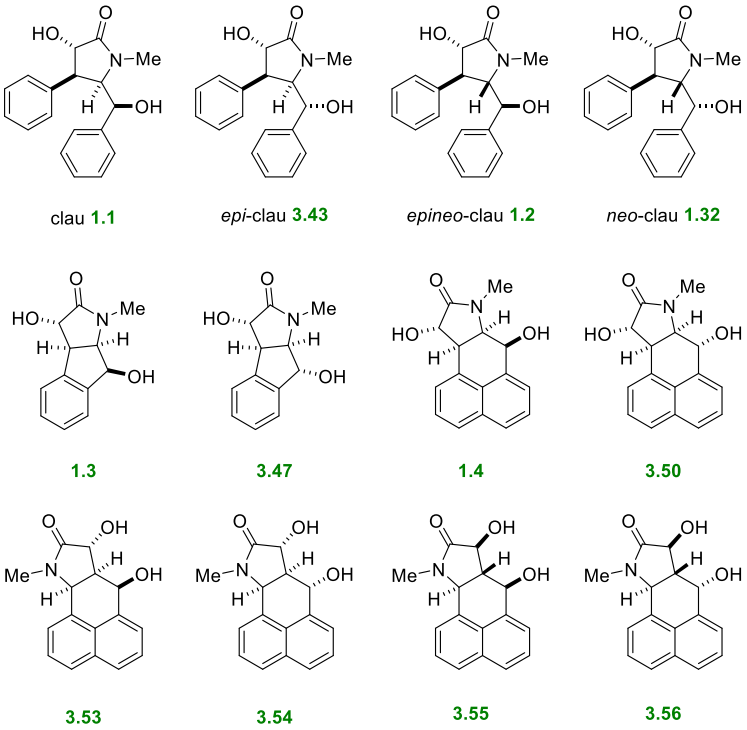


Figure 3.2 The chemical structures of clausenamide (**1.1**) and its analogues (**1.2**, **1.3**, **1.4**, **1.32**, **3.43**, **3.47**, **3.50**, **3.53**–**3.56**) synthesised or anticipated in this project.

Chapter 4: Experimental

4.1 General details

Solvents were used as commercially supplied or obtained from solvent dispenser units of the Chemistry Research Laboratory, University of Oxford. All other reagents were used as commercially supplied, without further purification. Unless specified otherwise, all chemical reactions were performed under nitrogen atmosphere in oven-dried glassware.

Sonication was carried out using a Misonix 3000 sonicator and Fisher Scientific FB-505 ultrasonic processor. Centrifugation was performed by Beckman Coulter Allegra X-22R and Allegra X-30R centrifuges.

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium-backed DC60 F254 0.2 mm pre-coated plates, with visualisation using ultra-violet light (λ_{max} = 254 nm) or TLC staining agents, phosphomolybdic acid (PMA) or vanillin solution, as appropriate. Column chromatography was performed on Merck Geduran® 60 SiO_2 (40–63 μm). Retention factors (R_f) of each novel compound are quoted with the solvent system used for TLC.

Enzymatic reactions were monitored by gas chromatography (GC) using a ThermoFisher Scientific TRACE 1300 gas chromatograph equipped with a flame ionisation detector (FID) and an AS3100 autosampler on a DB-1 capillary GC column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with hydrogen as carrier gas (3.0 mL/min). The injector temperature was set at 300 °C and the FID was set at 250 °C.

Compound names are generated by ChemDraw 21.0.0. The procedures for compounds are organised in numerical order based on how the compounds appear in each chapter. Proton (^1H) and carbon-13 (^{13}C) NMR spectra were recorded in deuterated solvents, with

tetramethylsilane (TMS) as the internal standard, at 295.2 K, on a Bruker 400 MHz, 500 MHz, 600 MHz or 700 MHz spectrometer as specified. Proton and carbon chemical shifts (δ_H, δ_C) are quoted in parts per million (ppm) to the nearest 0.01 ppm and 0.1 ppm, respectively; coupling constants have been rounded to the nearest 0.5 Hz. ^{1}H and ^{13}C NMR spectra were internally referenced (in MestReNova) to the appropriate solvent peak: $CDCl_3$, 7.26/77.16; MeOD, 3.31/49.00; DMSO-d_6, 2.50/39.52, with signal multiplicities described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br) or multiplet (m), and combinations thereof COSY, HSQC, HMBC, 1D TOCSY and NOESY experiments were carried out, where necessary, to aid the assignment of peaks and to confirm the proposed structure. (*) is appended to peak assignments to distinguish resonances attributed to minor diastereomers. Arbitrary numbering, independent from IUPAC numbering, was used for all NMR assignments.

Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and are described as strong (s), medium (m), weak (w), and broad (br) relative to the most intense peak. High resolution mass spectra (HRMS) were recorded on a Bruker Daltronics MicroTOF spectrometer (ESI). Mass to charge ratios *(m/z)* are reported in Daltons. Specific rotations were measured using a Perkin-Elmer 241 polarimeter at a wavelength of 589 nm at 25 °C, and are quoted in units of 10^{-1} deg cm^2 g^{-1}, with concentrations (*c*) expressed in g/100 mL.

4.2 Protein Synthesis

4.2.1 Preparation of growth media and buffers

All growth media and buffers were prepared (*Table 4.1*) with deionised water from a Millipore Elix Essential 3 system ($\rho \geq 15$ MΩ cm^{-1}). Being adding the media for bacterial culture growths, the media were first sterilised *via* a 0.22 μm syringe filter.

Table 4.1 Components for media and buffers.

Media/Buffers	Components
LB_{kan}[a]	10 g tryptone, 5 g yeast extract, 10 g NaCl, 50 mg kanamycin
pre-$FB_{glycerol}$[a]	147 g K_2HPO_4, 31 g NaH_2PO_4, 25 g $(NH_4)_2SO_4$, 20 g Na_2SO_4, 12 g Na_3citrate·$2H_2O$, 5.0 g NH_4Cl, 50 mL glycerol
Trace elements[a]	0.74 g $CaCl_2·H_2O$, 0.25 g $CoCl_2·6H_2O$, 0.10 g $CuSO_4·5H_2O$, 16.7 g $FeCl_3·6H_2O$, 0.132 g $MnSO_4·4H_2O$, 20.1 g Na_2EDTA, 0.18 g $ZnSO_4·7H_2O$
SOC[a]	20 g tryptone, 5 g yeast extract, 0.18 g KCl, 0.5 g NaCl (after autoclaving, add 10 mL 1.0 M $MgCl_2$, 10 mL 1.0 M $MgSO_4$, 2 mL 20% w/v glucose)
Inoculation solution	15 mg kanamycin, 122 mg $MgSO_4·7H_2O$, 50 mg thiamine·HCl, to 1.5 mL trace elements solution
Induction solution[b]	0.476 g IPTG, 0.84 g δ-ALA·HCl, 0.14 g $FeSO_4·7H_2O$, 0.75 g kanamycin
Phosphate buffer[a] (200mM, pH 7.9)	2.4 g KH_2PO_4, 31.7 g K_2HPO_4

a, components per 1L of final aqueous solution; b, components per 50 mL of final aqueous solution

4.2.2 *E. coli* transformation

The pET-28 plasmid bearing the variant CYP102A1 gene was transformed into BL21 (DE3) cells by adding 5 μL of PCR mixture to 40 μL of BL21 (DE3) cells and incubating for 30 min on ice. The mixture was then heat-shocked at 42 °C for 60 s and then placed on ice again for 2 min. 800 μL of SOC media was added to the mixture above and shaken at 220 rpm for 1 h at 37 °C. The cells were spread onto a LB_{kan} agar plate and incubated in an oven overnight at 37 °C.

4.2.3 Protein expression, harvest, purification and quantification

A single colony of transformed *E. coli* BL21 (DE3) from the agar plate was inoculated into a 6 mL LB_{kan} media and was shaken at 180 rpm, 37 °C for 6 h. A 2 L conical flask containing 50 mL of pre-$FB_{glycerol}$ and 450 mL deionised water was autoclaved. Following cooling, 1.5 mL of inoculation solution and 500 μL of the starter culture were added to the flask. The flask was shaken at 120 rpm and 37 °C for 24 h until OD_{600} = 0.4–0.6 was reached. The growth media was then cooled to 20 °C. Then, 1 mL of induction solution and 1 g of tryptone were added to induce protein expression. The flask was shaken at 120 rpm for 24 h at 20 °C after which another 1 mL of induction solution and 1 g of tryptone were added and shaking continued for 24 h.

The cells were harvested by centrifuging the growth media at 4500 rpm for 10 min at 4 °C. After discarding supernatant, the pellet was resuspended in 35 mL of 50 mM phosphate buffer. A spatula tip of lysozyme was added to the mixture and shaken at 160 rpm for 1 h at 20 °C. The suspension was then transferred to a 50 mL Falcon tube, put on ice, and the cells were lysed by 20 cycles of sonication with 15-second pulses followed by a 30-second cooling period, for a total run-time of 15 min. Centrifugation of the lysed suspension was carried out at 9500 rpm for 20 min at 4 °C to remove the cell debris.

The enzyme present in the supernatant was purified using ammonium sulfate fractionation. Ammonium sulfate was added to achieve 40% saturation, calculated using the EnCorbio Ammonium Sulfate Calculator.[i] Once the salt was completely dissolved, the mixture was centrifuged at 9500 rpm for 20 min at 4 °C. The supernatant was transferred to another Falcon tube, and additional ammonium sulfate was then added to it to achieve 60% saturation, again using the EnCorbio Ammonium Sulfate Calculator. After complete

[i] https://www.encorbio.com/protocols/AM-SO4.htm

dissolution of the salt, the mixture was centrifuged at 9500 rpm for 30 min at 4 °C. The supernatant was discarded and the resulting pellet was resuspended in 5 mL of 200 mM phosphate buffer overnight at 0 °C.

The concentration of the harvested P450 enzyme was determined by CO-difference assay. 50 µL of purified P450 enzyme solution and 950 µL of 200 mM phosphate buffer were added to a quartz UV cuvette. Sodium hydrosulphite was added to reduce the haem iron, after which the UV-Vis spectrum (200–700 nm) was recorded and used as the baseline. CO was slowly bubbled into the solution (~30 bubbles). The spectrum of the solution was recorded, indicating the protein absorbance at 450 nm. The absorbance at 450 nm and 490 nm, A_{450} and A_{490}, were recorded and the concentration of the protein was calculated using the following equation:

$$[\text{P450}]\ (\mu\text{M}) = \frac{20 \times (A_{450} - A_{490})}{\varepsilon} \times 10^6$$

where ε, the extinction coefficient is 91,000 $M^{-1}\ cm^{-1}$.[172]

4.2.4 Substrate screening

All the screening reactions of the substrates were conducted on a 500 µL scale in a 24-well plate containing 24 $P450_{BM3}$ variants (one per well), at a substrate-to-enzyme ratio of 500:1. To each well containing 100 µL of enzyme, 400 µL of the mixture detailed in *Table 4.2* was added. 200 mM phosphate buffer was used to make the stock solutions, except for the substrate in which it was in methanol.

Table 4.2 Components for screening reactions.

Substance	Stock concentration	Volume per well (µL)	Final concentration
Glucose	1 M	50	100 mM
Substrate	200 mM (in MeOH)	2.5	1 mM
GDH	400 U/mL	5	4 U/mL
$NADP^+$	4 mM	5	40 µM
Enzyme	10 µM	100	2 µM
Phosphate buffer	200 mM	337.5	-

The reactions were shaken at 120 rpm for 16 h at 20 °C, after which each 500 µL reaction mixture was transferred into a 1.5 mL microcentrifuge tube and extracted with 300 µL of ethyl acetate. The biphasic solution was then vortexed and centrifugated at 13300 rpm for 1 min. 180 µL of the organic layer was transferred into a 250 µL glass GC insert to perform GC analysis. In the GC, the oven temperatures were set according to the physical properties of the substrates.

For diphenyl-derived *cis*-lactam **1.126**, the oven temperature was held at 190.0 °C for 1 min, then increased to 225.0 °C at a rate of 3.0 °C/min, and further increased to 240.0 °C at a rate of 8.0 °C/min, and held at this temperature for 2 min, with a total run time of 16.54 min.

For diphenyl-derived *trans*-lactam **1.127**, the oven temperature was held at 220.0 °C for 1 min, then increased to 238.0 °C at a rate of 10.0 °C/min, followed by an increase to 238.4 °C at a rate of 0.1 °C/min, and further increased to 255.0 °C at a rate of 20.0 °C/min, and held at this temperature for 2 min, with a total run time of 9.63 min.

For indene-derived lactam **1.128**, the oven temperature was held at 150.0 °C for 1 min, and increased to 220.0 °C at a rate of 10.0 °C/min, and held at this temperature for 5 min, with a total run time of 13.00 min.

For phenalene-derived lactam **1.129**, the oven temperature was held at 220.0 °C for 1 min, then increased to 230.0 °C at a rate of 10.0 °C/min, and further increased to 234.0 °C at a rate of 1.0 °C/min, with a total run time of 6.00 min.

For phenalene-derived *cis*-isomer lactam **2.85**, the oven temperature was held at 220.0 °C for 1 min, then increased to 230.0 °C at a rate of 10.0 °C/min, and further increased to 235.5 °C at a rate of 1.0 °C/min, with a total run time of 7.50 min.

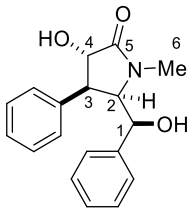
For phenalene-derived *trans*-isomer lactam **2.86**, the oven temperature was held at 220.0 °C for 1 min, then increased to 240.0 °C at a rate of 4.0 °C/min, and further increased to 241.0 °C at a rate of 1.0 °C/min, and held at this temperature for 1 min, with a total run time of 8.00 min.

4.2.5 Preparative scale reactions

The preparative scale reactions were performed using the same ratios of reagents as in the screening procedure (*Table 4.2*). In the conical flask, the mixture was shaken at 120 rpm at 20 °C. The reaction progress was monitored *via* GC, overall reaction times ranging from 2–28 h. When deemed appropriate to work up, the reaction mixture was transferred to Falcon tubes with approximately 25 mL in each tube and ethyl acetate was added to approximately 50 mL. After balancing the Falcon tubes, the tubes were shaken and then centrifuged for 3 min at 9500 rpm. The separated organic layer was pipetted off and the process was repeated twice more. The combined organic layers were concentrated *in vacuo* to give a crude residue, which was then purified by column chromatography. Column fractions were combined and analysed based on GC analysis. TLC was not helpful in this case, as most of the metabolites have similar polarities and are difficult to resolve on TLC.

4.3 Synthesis of compounds

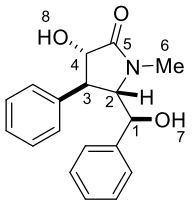
(3*S**,4*R**,5*R**)-3-Hydroxy-5-((*S**)-hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**1.1**)[39]



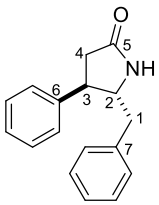
To a solution of lactam **1.18** (2.3 mg, 0.008 mmol) in dry THF (0.2 mL), was added LiHMDS (0.01 mL, 1.0 M solution in THF, 0.01 mmol) dropwise at –10 °C. The mixture was stirred at –10 °C for 1 h. A solution of Davis oxidant (**3.41**, 4 mg, 0.01 mmol) in dry THF (0.03 mL) was then added to the reaction mixture at –10 °C. The reaction was allowed to warm to 0 °C and stirring was continued for 3 h. Since the reaction was not complete, the reaction was thawed to RT and stirring was continued for 48 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL) and extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (DCM/methanol, 100:3) gave clausenamide **1.1** (0.5 mg, 34%) as a yellow oil and as a single diastereomer.

R_f 0.10 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41 – 7.18 (m, 8H, H-Ar), 6.80 (d, *J* = 7.5 Hz, 2H, H-Ar), 4.81 (d, *J* = 4.0, 3.0 Hz, 1H, H-1), 4.23 (dd, *J* = 8.5, 4.0 Hz, 1H, H-2), 3.99 (d, *J* = 11.0 Hz, 1H, H-4), 3.75 (dd, *J* = 11.0, 8.5 Hz, 1H, H-3), 2.99 (s, 3H, H-6), 2.59 (d, *J* = 3.0 Hz, 1H, OH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.8 (C-5), 139.1 (C-Ar), 135.0 (C-Ar), 129.0 (C-Ar), 128.8 (C-Ar), 128.6 (C-Ar), 128.4 (C-Ar), 127.7 (C-Ar), 127.5 (C-Ar), 74.4 (C-1), 69.3 (C-4), 66.2 (C-2), 50.5 (C-3), 31.8 (C-6).

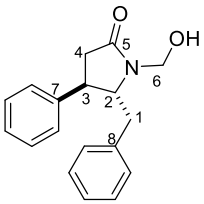
(3*S**,4*R**,5*S**)-3-Hydroxy-5-((*S**)-hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**1.2**),[29, 160] *trans*-5-benzyl-4-phenylpyrrolidin-2-one (**3.10**),[173] *trans*-5-benzyl-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.11**), *trans*-5-benzyl-3-hydroxy-1-methyl-4-phenylpyrrolidin-2-one (**3.12**), *trans*-5-benzyl-3-hydroxy-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.13**), (4*R**,5*S**)-5-((*S**)-hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.14**), (4*R**,5*S**)-5-((*S**)-hydroxy(phenyl)methyl)-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.15**), (4*R**,5*S**)-5-((*S**)-hydroxy(phenyl)methyl)-4-phenylpyrrolidin-2-one (**3.16**),[161] *trans*-2-benzyl-5-oxo-3-phenylpyrrolidine-1-carbaldehyde (**3.17**), *trans*-5-(2-hydroxybenzyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.18**), 5-(2,4-dihydroxybenzyl)-5-hydroxy-4-phenylpyrrolidin-2-one (**3.19**), *trans*-5-(2-hydroxybenzyl)-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.20**), and *trans*-5-(2,4-dihydroxybenzyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.21**)



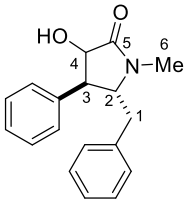
1.2



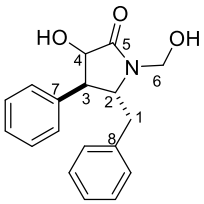
3.10



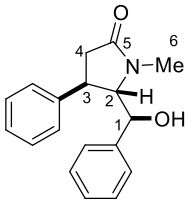
3.11



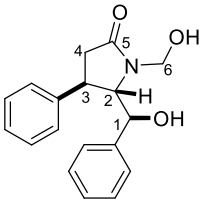
3.12



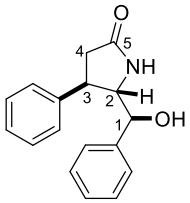
3.13



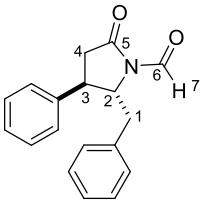
3.14



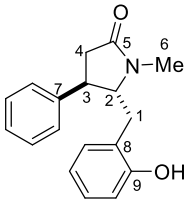
3.15



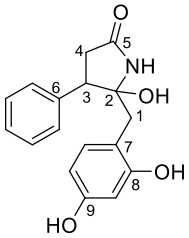
3.16



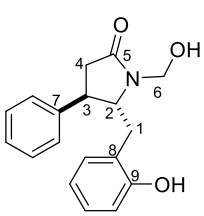
3.17



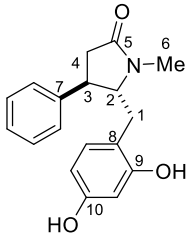
3.18



3.19



3.20



3.21

To a solution of lactam **1.127** (39.4 mg in 740 µL MeOH, 0.15 mmol), glucose (14.8 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (1.5 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (1.5 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 125 mL), was added $P450_{BM3}$ variant GVQ/A328G (4.1 mL of a 72 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 3 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *section* 4.*2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 3:1) gave lactam **3.17** (1.1 mg, 3%) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2876s, 1749s, 1703s, 1290s, 742m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 9.24 (s, 1H, H-7), 7.38 – 7.33 (m, 2H, H-Ar), 7.31 – 7.16 (m, 6H, H-Ar), 6.91 (dd, *J* = 7.0, 2.0 Hz, 2H, H-Ar), 4.45 (ddd, *J* = 8.5, 3.5, 2.5 Hz, 1H, H-2), 3.37 (ddd, *J* = 9.5, 2.5, 2.5 Hz, 1H, H-3), 3.24 (dd, *J* = 13.5, 3.5 Hz, 1H, H-1), 2.95 (dd, *J* = 13.5, 8.5 Hz, 1H, H-1), 2.71 (dd, *J* = 18.5, 9.5 Hz, 1H, H-4), 2.53 (dd, *J* = 18.5, 2.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.9 (C-5), 160.6 (C-6), 143.0 (C-Ar), 136.2 (C-Ar), 132.6 (C-Ar), 129.8 (C-Ar), 129.0 (C-Ar), 127.5 (C-Ar), 127.4 (C-Ar), 126.2 (C-Ar), 63.4 (C-2), 39.9 (C-3), 38.6 (C-1), 38.1 (C-4); **HRMS** (ESI$^+$) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{18}NO_2$ 280.1332, found, 280.1329.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.18** (2.5 mg, 6%) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2992s, 1669s, 1422m, 1242m, 762m, 704m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.22 – 7.13 (m, 3H, H-Ar), 7.12 – 7.05 (m, 2H, H-Ar), 6.96 – 6.92 (m, 2H, H-Ar), 6.88 (t, *J* = 7.5 Hz, 1H, H-Ar), 6.68 (d, *J* = 8.0 Hz, 1H, H-Ar), 3.83 (ddd, *J* = 8.0, 5.0, 3.5 Hz, 1H, H-2), 3.25 (ddd, *J* = 9.5, 3.5, 3.5 Hz, 1H, H-3), 3.18 (dd, *J* = 13.5, 5.0 Hz, 1H, H-1), 2.92 (s, 3H, H-6), 2.79 – 2.72 (m, 3H, H-1, H-4), 2.39 (dd, *J* = 17.5, 3.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.2 (C-5), 153.8 (C-9), 144.6 (C-7), 131.7 (C-Ar), 128.9 (C-Ar), 128.4 (C-Ar), 126.8 (C-Ar), 126.6 (C-Ar), 123.8 (C-8), 121.2 (C-Ar), 115.5 (C-Ar),

68.2 (C-2), 41.8 (C-3), 38.1 (C-4), 33.9 (C-1), 28.6 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1486.

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactams **3.10** and **3.11** (4.0 mg, 6%) in 1:2 ratio, as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2883m, 1687s, 1496m, 1045m, 761s, 702s.

3.10: **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.37 – 7.15 (m, 10H, H-Ar), 5.51 (br s, 1H, NH), 3.88 – 3.84 (m, 1H, H-2), 3.30 – 3.26 (m, 1H, H-3), 3.01 (dd, *J* = 13.5, 4.0 Hz, 1H, H-1), 2.78 (dd, *J* = 18.0, 9.5 Hz, 1H, H-4), 2.64 (dd, *J* = 13.5, 9.5 Hz, 1H, H-1), 2.56 (dd, *J* = 18.0, 9.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.0 (C-5), 141.51 (C-6), 137.5 (C-7), 129.1 (C-Ar), 127.5 (C-Ar × 2), 127.2 (C-Ar × 2), 127.1 (C-Ar), 63.44 (C-2), 47.32 (C-3), 41.88 (C-1), 39.16 (C-4).

3.11: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.15 (m, 8H, H-Ar), 7.05 – 7.01 (m, 2H, H-Ar), 4.99 (d, *J* = 11.0 Hz, 1H, H-6), 4.70 (d, *J* = 11.0 Hz, 1H, H-6), 4.06 (ddd, *J* = 7.0, 5.5, 3.5 Hz, 1H, H-2), 3.22 (ddd, *J* = 9.5, 4.5, 3.5 Hz, 1H, H-3), 3.08 (dd, *J* = 14.0, 5.5 Hz, 1H, H-1), 2.93 (dd, *J* = 14.0, 7.0 Hz, 1H, H-1), 2.72 (dd, *J* = 17.5, 9.5 Hz, 1H, H-4), 2.42 (dd, *J* = 17.5, 4.5 Hz, 1H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.7 (C-5), 143.5 (C-7), 136.8 (C-8), 129.5 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar × 2), 127.2 (C-Ar), 126.8 (C-Ar), 66.6 (C-2), 66.3 (C-6), 42.2 (C-3), 40.2 (C-1), 38.4 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1487.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.14** (5.0 mg, 12%) as a single diastereomer and as a yellow oil. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.40 – 7.30 (m, 6H, H-Ar), 7.25 – 7.17 (m, 2H, H-Ar), 6.96 – 6.93 (m, 2H, H-Ar), 4.92 (d, *J* = 5.5 Hz, 1H, H-1), 3.76 (dd, *J* = 5.5, 3.0 Hz, 1H, H-2), 3.24 (ddd, *J* = 9.0, 3.0, 3.0 Hz, 1H, H-3), 2.98 (s, 3H, H-6), 2.40 (dd, *J* = 17.0, 9.0 Hz, 1H, H-4), 2.26 (dd, *J* = 17.0, 3.0 Hz, 1H, H-4); **^{13}C NMR** (151

MHz, $CDCl_3$) δ 175.1 (C-5), 144.9 (C-Ar), 140.3 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 128.7 (C-Ar), 127.0 (C-Ar), 126.4 (C-Ar × 2), 75.7 (C-1), 73.8 (C-2), 39.2 (C-3), 37.9 (C-4), 30.8 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1486.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.12** as a single diastereomer (0.7 mg, 2%, unassigned) and as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2880m, 1681s, 1495m, 1454m, 1091m, 766m, 702s; **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 4.31 (dd, *J* = 8.5, 2.5 Hz, 1H, H-4), 3.91 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, H-2), 3.00 – 2.97 (m, 3H, H-1, H-3), 2.91 (s, 3H, H-6), 2.70 (d, *J* = 2.5 Hz, 1H, OH); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.5 (C-5), 142.3 (C-Ar), 139.1 (C-Ar), 129.4 (C-Ar), 129.0 (C-Ar), 128.7 (C-Ar), 127.9 (C-Ar), 126.4 (C-Ar × 2), 76.8 (C-4), 63.9 (C-2), 52.9 (C-3), 38.7 (C-1), 29.0 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1487.

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactams **3.7a** and **3.19** as single diastereomers (0.6 mg, 1%, unassigned). **IR** (film) ν_{max}/cm^{-1}: 2928s, 2856m, 1708s, 1464m, 1209m, 769m.

For **3.7a** characterisation data, see below.

3.19: **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, 3H, H-Ar), 7.24 – 7.17 (m, 2H, H-Ar), 6.68 – 6.64 (m, 1H, H-Ar), 6.63 – 6.60 (m, 1H H-Ar), 6.56 – 6.54 (m, 1H, H-Ar), 4.45 (br s, 1H, OH), 3.82 (dd, *J* = 8.5, 2.0 Hz, 1H, H-3), 3.23 (dd, *J* = 17.5, 8.5 Hz, 1H, H-4), 2.97 (d, *J* = 17.0 Hz, 1H, H-1), 2.70 (d, *J* = 17.0 Hz, 1H, H-1), 2.58 (dd, *J* = 17.5, 2.0 Hz, 1H, H-4); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 177.2 (C-5), 150.8 (C-8), 149.9 (C-9), 140.4 (C-6), 129.2 (C-Ar), 127.9 (C-Ar), 127.8 (C-Ar), 125.7 (C-7), 115.1 (C-Ar), 112.2 (C-Ar), 110.0 (C-Ar), 104.1 (C-2), 50.3 (C-3), 37.7 (C-4), 37.5 (C-1); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{18}NO_4$ 300.1214, not found.

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactams **3.15** and **3.20** (9.2 mg, 21%) in 1:1 ratio, as a yellow oil and lactam **3.15** as a single diastereomer. **IR** (film) ν_{max}/cm^{-1}:2885m, 1676s, 1456m, 1274m, 762m, 702s; **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_3$ 298.1438, found, 298.1435.

3.15: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.25 – 7.12 (m, 8H, H-Ar), 6.73 – 6.69 (m, 2H, H-Ar), 5.37 (d, *J* = 11.0 Hz, 1H, H-6), 4.69 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 11.0 Hz, 1H, H-6), 3.83 (dd, *J* = 8.0, 3.0 Hz, 1H, H-2), 3.10 (ddd, *J* = 9.5, 3.0, 3.0 Hz, 1H, H-3), 2.89 (dd, *J* = 17.5, 9.5 Hz, 1H, H-4), 2.47 (dd, *J* = 17.5, 3.0 Hz, 1H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.8 (C-5), 143.6 (C-Ar), 140.0 (C-Ar), 129.0 (C-Ar), 128.6 (C-Ar), 127.4 (C-Ar), 127.1 (C-Ar), 126.3 (C-Ar), one C-Ar resonance not seen, 77.3 (C-1), 74.0 (C-2), 67.4 (C-6), 39.6 (C-3), 37.9 (C-4).

3.20: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.27 – 7.14 (m, 5H, H-Ar), 7.13 – 7.09 (m, 1H, H-Ar), 7.03 (d, *J* = 8.0 Hz, 1H, H-Ar), 6.86 (t, *J* = 8.0 Hz, 1H, H-Ar), 6.75 (d, *J* = 8.5 Hz, 1H, H-Ar), 4.91 (d, *J* = 11.0 Hz, 1H, H-6), 4.85 (d, *J* = 11.0 Hz, 1H, H-6), 4.12 (ddd, *J* = 7.0, 5.0, 4.0, Hz, 1H, H-2), 3.30 (ddd, *J* = 9.0, 4.0, 4.0 Hz, 1H, H-3), 3.16 (dd, *J* = 14.0, 5.0 Hz, 1H, H-1), 2.94 (dd, *J* = 14.0, 7.0 Hz, 1H, H-1), 2.75 (dd, *J* = 17.5, 9.0 Hz, 1H, H-4), 2.39 (dd, *J* = 17.5, 4.0 Hz, 1H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.0 (C-5), 154.3 (C-9), 143.8 (C-7), 131.7 (C-Ar), 129.0 (C-Ar × 2), 127.0 (C-Ar), 126.7 (C-Ar), 123.5 (C-8), 121.0 (C-Ar), 116.1 (C-Ar), 66.4 (C-6), 65.9 (C-2), 42.3 (C-3), 38.4 (C-4), 35.1 (C-1).

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactams **1.2**, **3.13**, and **3.21** (4.6 mg, 10%) in 1.5:1:1 ratio, as a yellow oil, and lactams **3.13** and **1.2** as single diastereomers. **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_3$ 298.1438, found, 298.1434.

1.2: **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.25– 7.07 (m, 10H, H-Ar), 5.80 (d, *J* = 5.0 Hz, 1H, OH-7), 5.72 (d, *J* = 6.0 Hz, 1H, OH-8), 4.57 (dd, *J* = 5.0, 4.5 Hz, 1H, H-1), 4.01 (dd, *J* =

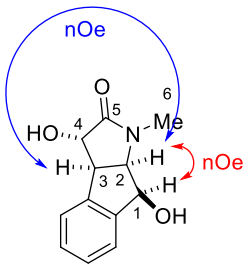
7.5, 6.0 Hz, 1H, H-4), 3.85 (dd, *J* = 7.5, 4.5 Hz, 1H, H-2), 2.92 (dd, *J* = 7.5, 7.5 Hz, 1H, H-3), 2.69 (s, 3H, H-6); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.2 (C-5), 142.7 (C-Ar), 140.9 (C-Ar), 128.7 (C-Ar), 127.9 (C-Ar), 127.0 (C-Ar), 126.4 (C-Ar), 126.3 (C-Ar), one C-Ar resonance not seen, 76.1 (C-4), 73.1 (C-1), 67.6 (C-2), 50.3 (C-3), 30.0 (C-6); **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 4.88 (d, *J* = 5.0 Hz, 1H, H-1), 4.22 (d, *J* = 4.5 Hz, 1H, H-4), 3.93 (t, *J* = 5.0 Hz, 1H, H-2), 3.74 (br s, 1H, OH-8), 3.12 – 3.08 (m, 1H, H-3), 3.00 (br s, 1H, OH-7), 2.85 (s, 3H, H-6); **^{13}C NMR** (176 MHz, $CDCl_3$)) [only selected peaks reported] δ 174.3 (C-5), 140.7 (C-8), 140.5 (C-Ar), 77.0 (C-4), 76.2 (C-1), 70.7 (C-2), 50.6 (C-3), 31.1 (C-6).

3.13: **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 5.79 (br s, 1H, OH), 4.45 (d, *J* = 10.0 Hz, 1H, H-4), 4.13 (d, *J* = 7.0 Hz, 1H, H-6), 4.11 (d, *J* = 7.0 Hz, 1H, H-6), 3.86 (ddd, *J* = 9.5, 9.5, 3.5 Hz, 1H, H-2), 3.12 (dd, *J* = 10.0, 9.5 Hz, 1H, H-3), 3.06 (dd, *J* = 14.0, 3.5 Hz, 1H, H-1), 2.57 (dd, *J* = 14.0, 9.5 Hz, 1H, H-1); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 171.4 (C-5), 137.7 (C-7), 137.0 (C-8), 129.3 (C-Ar), 129.2 (C-Ar), 128.1 (C-Ar), 128.0 (C-Ar), 127.4 (C-Ar), one C-Ar resonance not seen, 76.4 (C-4), 60.6 (C-6), 58.2 (C-2), 56.7 (C-3), 40.9 (C-1).

3.21: **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 6.63 – 6.58 (m, 3H, H-Ar), 3.82 (ddd, *J* = 6.5, 5.0, 3.5 Hz, 1H, H-2), 3.27 (ddd, *J* = 9.5, 3.5, 3.5 Hz, 1H, H-3), 3.09 (dd, *J* = 13.5, 5.0 Hz, 1H, H-1), 2.92 (s, 3H, H-6), 2.75 (dd, *J* = 13.5, 6.5 Hz, 1H, H-1), 2.63 (dd, *J* = 17.5, 9.5 Hz, 1H, H-4), 2.34 (dd, *J* = 17.5, 3.5 Hz, 1H, H-4); **^{13}C NMR** (176 MHz, $CDCl_3$) [only selected peaks reported] δ 174.9 (C-5), 149.9 (C-10), 147.9 (C-9), 144.6 (C-7), 124.4 (C-8), 118.2 (C-Ar), 116.5 (C-Ar), 115.0 (C-Ar), 68.6 (C-2), 41.5 (C-3), 38.2 (C-4), 33.2 (C-1), 28.8 (C-6).

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.16** (2.4 mg, 6%) as a single diastereomer and as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2924s, 2854s, 1679s, 1260s, 1072s, 806m, 746m, 703m; **^{1}H NMR** (600 MHz, MeOD) δ 7.35 – 7.10 (m, 8H, H-Ar), 7.04 – 6.93 (m, 2H, H-Ar), 4.62 (d, *J* = 6.5 Hz, 1H, H-1), 3.87 (dd, *J* = 6.5, 4.0 Hz, 1H, H-2), 3.38 (ddd, *J* = 10.0, 5.0, 4.0 Hz, 1H, H-3), 2.64 (dd, *J* = 17.5, 10.0 Hz, 1H, H-4), 2.28 (dd, *J* = 17.5, 5.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, MeOD) δ 180.0 (C-5), 129.7 (C-Ar), 129.4 (C-Ar), 129.0 (C-Ar), 128.1 (C-Ar), 127.7 (C-Ar), 127.5 (C-Ar), two C-Ar resonance not seen, 77.5 (C-1), 69.7 (C-2), 42.7 (C-3), 39.9 (C-4); **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.32 – 7.15 (m, 8H, H-Ar), 7.00 – 6.97 (m, 2H, H-Ar), 4.65 (t, *J* = 5.0 Hz, 1H, H-1), 3.86 (dd, *J* = 5.0, 6.0 Hz, 1H, H-2), 3.42 (dt, *J* = 10.0, 6.0 Hz, 1H, H-3), 2.87 (dd, *J* = 17.5, 10.0 Hz, 1H, H-4), 2.48 (dd, *J* = 17.5, 6.0 Hz, 1H, H-4), 2.14 (d, *J* = 5.0 Hz, 1H, OH); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 176.8 (C-5), 143.0 (C-Ar), 140.2 (C-Ar), 129.0 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 127.0 (C-Ar × 2), 126.5 (C-Ar), 76.5 (C-1), 67.6 (C-2), 42.2 (C-3), 39.1 (C-4); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{17}H_{18}NO_2$ 268.1332, found, 268.1330.

(3*S**,3a*R**,8*S**,8a*R**)-3,8-Dihydroxy-1-methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**1.3**)

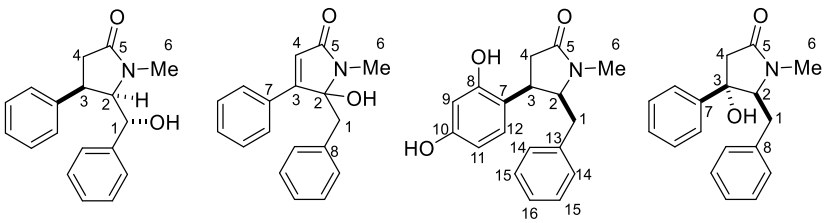


To a solution of lactam **3.46** (8.5 mg, 0.042 mmol) in dry THF (2 mL), was added LiHMDS (0.08 mL, 1.0 M solution in THF, 0.08 mmol) dropwise at –10 °C. The mixture was stirred at –10 °C for 1 h. A solution of Davis oxidant (**3.41**, 20 mg, 0.08 mmol) in dry THF (0.2

mL) was then added to the reaction mixture at –10 °C. The reaction was allowed to warm to 0 °C and stirring was continued for 3 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (DCM/methanol, 25:1) gave indene-derive analogue **1.3** (0.8 mg, 9%) as a yellow oil and as a single diastereomer.

R_f 0.07 (DCM/methanol, 20:1); **IR** (film) ν_{max}/cm^{-1}: 2883s, 1699s, 1399m, 1246m, 912s, 739m; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.51 – 7.46 (m, 2H, H-Ar), 7.45 – 7.40 (m, 1H, H-Ar), 7.40 – 7.35 (m, 1H, H-Ar), 5.22 (d, *J* = 6.0 Hz, 1H, H-1), 4.30 (dd, *J* = 8.5, 6.0 Hz, 1H, H-2), 4.27 (d, *J* = 6.5 Hz, 1H, H-4), 3.75 (dd, *J* = 8.5, 6.5 Hz, 1H, H-3), 3.09 (s, 3H, H-6); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.5 (C-5), 143.1 (C-Ar), 130.5 (C-Ar), 128.9 (C-Ar), 127.8 (C-Ar), 126.1 (C-Ar), 125.6 (C-Ar), 76.0 (C-4), 73.6 (C-1), 64.9 (C-2), 50.9 (C-3), 30.3 (C-6); configuration assigned by NOESY correlations (blue and red arrows); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO_3$ 220.0968, found, 220.0969.

(4*R**,5*R**)-5-((*R**)-Hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**1.17**),[159] 5-benzyl-5-hydroxy-1-methyl-4-phenyl-1,5-dihydro-2*H*-pyrrol-2-one (**3.3**), *cis*-5-benzyl-4-(2,4-dihydroxyphenyl)-1-methylpyrrolidin-2-one (**3.4**), and (4*S**,5*S**)-5-benzyl-4-hydroxy-1-methyl-4-phenylpyrrolidin-2-one (**3.5**)



To a solution of lactam **1.126** (12.6 mg in 238 µL MeOH, 0.048 mmol), glucose (4.8 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (475 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (475 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 37 mL), was added $P450_{BM3}$ variant GVQ/A264G/A328G (5 mL of a 19 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 24 h at 20 °C. When the reaction was complete (TLC), the mixture was extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave lactams **1.17** (1.6 mg, 11%, yellow oil), **3.3** (0.5 mg, 4%, yellow oil), and a mixture of lactams **3.4** and **3.5** (0.4 mg, yellow oil) in 1:1 ratio.

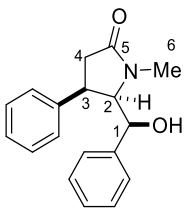
1.17: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.38 (d, *J* = 4.5 Hz, 4H, H-Ar), 7.34 – 7.18 (m, 6H, H-Ar), 4.51 (dd, *J* = 5.5, 2.0 Hz, 1H, H-1), 4.07 (dd, *J* = 8.0, 2.0 Hz, 1H, H-2), 3.86 (ddd, *J* = 12.0, 8.0, 8.0 Hz, 1H, H-3), 3.24 (dd, *J* = 15.5, 12.0 Hz, 1H, H-4), 2.52 (dd, *J* = 15.5, 8.0 Hz, 1H, H-4), 2.45 (s, 3H, H-6), 1.72 (d, *J* = 5.5 Hz, 1H, OH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.9 (C-5), 142.3 (C-Ar), 138.2 (C-Ar), 129.0 (C-Ar), 128.6 (C-Ar), 128.2 (C-Ar), 127.6 (C-Ar), 127.5 (C-Ar), 125.3 (C-Ar), 72.0 (C-1), 70.5 (C-2), 43.5 (C-3), 35.1 (C-4), 29.9 (C-6).

3.3: **IR** (film) ν_{max}/cm^{-1}: 2883s, 1684s, 1496m, 1400m, 993m, 703m; **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 6.10 (s, 1H, H-4), 3.33 (d, *J* = 14.0 Hz, 1H, H-1), 3.29 (d, *J* = 14.0 Hz, 1H, H-1), 3.07 (s, 3H, H-6); **^{13}C NMR** (176 MHz, CDCl3) [only selected peaks reported] δ 168.9 (C-5), 157.8 (C-3), 133.9 (C-8), 131.6 (C-7), 122.0 (C-4), 93.5 (C-2), 40.9 (C-1), 24.2 (C-6); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{18}H_{18}NO_2$ 280.1332, found, 280.1328.

3.4: **IR** (film) ν_{max}/cm^{-1}: 2950s, 2880m, 1664s, 1496m, 1209m, 775m, 702m; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.22 – 7.12 (m, 3H, H-15, H-16), 6.93 (d, *J* = 7.5 Hz, 2H, H-14), 6.65 – 6.59 (m, 2H, H-9, H-11), 6.56 (d, *J* = 8.5 Hz, 1H, H-12), 4.87 (br s, 1H, OH), 4.50 (br s, 1H, OH), 4.38 – 4.33 (m, 1H, H-2), 3.97 (ddd, *J* = 11.5, 8.0, 8.0 Hz, 1H, H-3), 2.77 – 2.66 (m, 1H, H-4), 2.61 (s, 3H, H-6), 2.54 (dd, *J* = 14.0, 5.0 Hz, 1H, H-1), 2.49 – 2.44 (m, 2H, H-1, H-4); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 174.1 (C-5), 149.9 (C-10), 147.7 (C-8), 138.5 (C-13), 129.3 (C-14), 128.5 (C-15), 126.5 (C-16), 126.4 (C-7), 116.2 (C-9), 115.6 (C-11), 114.7 (C-12), 64.5 (C-2), 38.2 (C-3), 36.5 (C-1), 33.8 (C-4), 29.9 (C-6); **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_3$ 298.1438, found, 298.1434.

3.5: **IR** (film) ν_{max}/cm^{-1}: 2948s, 2880m, 1670s, 1496m, 1210m, 702m; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.41 – 7.39 (m, 3H, H-Ar), 7.22 – 7.14 (m, 5H, H-Ar), 6.82 (dd, *J* = 7.0, 2.5 Hz, 2H, H-Ar), 4.00 (dd, *J* = 8.5, 5.0 Hz, 1H, H-2), 3.11 (d, *J* = 16.0 Hz, 1H, H-4), 2.66 (s, 3H, H-6), 2.55 (dd, *J* = 13.5, 5.0 Hz, 1H, H-1), 2.47 (d, *J* = 16.0 Hz, 1H, H-4), 2.37 (dd, *J* = 13.5, 8.5 Hz, 1H, H-1); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.0 (C-5), 140.4 (C-7), 137.1 (C-8), 129.2 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 128.6 (C-Ar), 126.8 (C-Ar), 126.6 (C-Ar), 79.7 (C-3), 73.3 (C-2), 42.8 (C-4), 37.8 (C-1), 30.2 (C-6); **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1485.

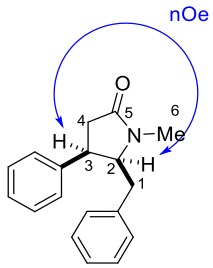
(4*R**,5*R**)-5-((*S**)-Hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**1.18**)[37]



To a solution of crude lactam **3.42** (8.8 mg, 0.032 mmol) in ethanol (1 mL), was added $NaBH_4$ (2 mg, 0.06 mmol) at 0 °C. The mixture was then stirred at RT for 2 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) yielded lactam **1.18** (5.1 mg, 58%) as a colourless oil and as a single diastereomer.

R_f 0.15 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.38 – 7.17 (m, 8H, H-Ar), 6.95 – 6.89 (m, 2H, H-Ar), 4.73 (d, *J* = 5.0 Hz, 1H, H-1), 4.15 (dd, *J* = 8.0, 5.0 Hz, 1H, H-2), 3.95 – 3.84 (m, 1H, H-3), 2.75 (s, 3H, H-6), 2.34 (d, *J* = 10.0 Hz, 2H, H-4), 1.65 (br s, 1H, OH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.9 (C-5), 139.9 (C-Ar), 137.1 (C-Ar), 128.9 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 127.6 (C-Ar), 127.4 (C-Ar), one C-Ar resonance not seen, 73.8 (C-1), 68.9 (C-2), 42.1 (C-3), 33.8 (C-4), 31.1 (C-6).

cis-5-Benzyl-1-methyl-4-phenylpyrrolidin-2-one (**1.126**)[143]

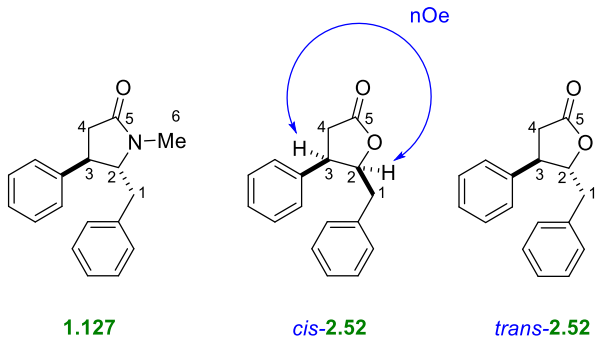


A mixture of **2.53**, **2.54** and **2.55** (19 mg, 0.070 mmol) was dissolved in dry ethyl acetate (1 mL). Pd (7.0 mg, 10% Pd on activated carbon, 0.007 mmol) was added and the mixture was purged with hydrogen gas (balloon) for 5 min. The needle was then removed from the solution and left in the reaction headspace while stirring at RT for 23 h. When the reaction

was complete (TLC), the crude reaction mixture was filtered through Celite® to afford *cis*-lactam **1.126** (22 mg, quant.) as a yellow oil, which was used without further purification.

cis-**1.126**: $\mathbf{R_f}$ 0.30 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.36 – 7.30 (m, 2H, H-Ar), 7.30 – 7.25 (m, 1H, H-Ar), 7.20 – 7.11 (m, 5H, H-Ar), 6.90 – 6.83 (m, 2H, H-Ar), 4.15 – 4.05 (m, 1H, H-2), 3.81 – 3.69 (m, 1H, H-3), 2.71 (dd, *J* = 16.5, 9.5 Hz, 1H, H-4), 2.64 (s, 3H, H-6), 2.62 – 2.52 (m, 2H, H-1, H-4), 2.48 (dd, *J* = 14.0, 5.5 Hz, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (C-5), 138.5 (C-Ar), 138.0 (C-Ar), 129.2 (C-Ar), 128.7 (C-Ar), 128.5 (C-Ar), 128.3 (C-Ar), 127.4 (C-Ar), 126.6 (C-Ar), 65.6 (C-2), 42.8 (C-3), 36.3 (C-1), 35.1 (C-4), 29.7 (C-6); configuration assigned by NOESY correlations (blue arrow). Analytical data in agreement with literature; however, in the literature,[143] it was wrongly assigned to *trans*-lactam.

trans-5-Benzyl-1-methyl-4-phenylpyrrolidin-2-one (**1.127**)[143] and *cis/trans*[144]-5-benzyl-4-phenyldihydrofuran-2(3*H*)-one (**2.52**)



Sodium cyanoborohydride (24.5 mg, 0.390 mmol) was added to a solution of ketone **2.51** (88.8 mg, 0.300 mmol), methylamine (56.0 µL, 8.03 M solution in absolute ethanol, 0.450 mmol) and glacial acetic acid (26.0 µL, 0.450 mmol) in dry methanol (6 mL). The reaction mixture was heated at reflux for 21 h. When the reaction was complete (TLC), the solvent

was removed under reduced pressure. The residue was dissolved in aq. HCl (6.0 mL, 4.0 M) and extracted with DCM (3 × 2 mL). The combined organic extracts were washed sequentially with water (2 mL) and brine (2 mL), then dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 10:1 → 0:1) gave a mixture of lactam diastereomers **1.126** and **1.127** (11.9 mg, 15%, yellow oil) in 62:38 ratio, and lactone diastereomers *cis*-**2.52** (1.7 mg, 2%, colourless oil) and *trans*-**2.52** (7.3 mg, 10%, colourless oil), with 21% of recovered starting material.

For *cis*-**1.126** characterisation data, see above.

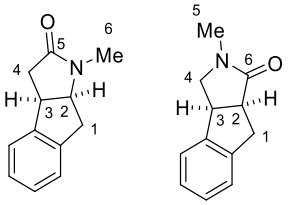
trans-**1.127**: $\mathbf{R_f}$ 0.34 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.11 (m, 8H, H-Ar), 7.03 – 6.97 (m, 2H, H-Ar), 3.77 (ddd, *J* = 7.0, 5.0, 3.5 Hz, 1H, H-2), 3.18 (ddd, *J* = 8.0, 4.5, 3.5 Hz, 1H, H-3), 3.03 (dd, *J* = 14.0, 5.0 Hz, 1H, H-1), 2.93 (s, 3H, H-6), 2.87 (dd, *J* = 14.0, 7.0 Hz, 1H, H-1), 2.64 (dd, *J* = 17.5, 8.0 Hz, 1H, H-4), 2.39 (dd, *J* = 17.5, 4.5 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.2 (C-5), 144.1 (C-Ar), 136.8 (C-Ar), 129.5 (C-Ar), 129.0 (C-Ar), 128.8 (C-Ar), 127.0 (C-Ar), 127.0 (C-Ar), 126.7 (C-Ar), 69.3 (C-2), 41.7 (C-3), 39.0 (C-1), 38.2 (C-4), 28.7 (C-6). Analytical data in agreement with literature; however, in the literature,[143] it was wrongly assigned to the *cis*-lactam.

trans-**2.52**: $\mathbf{R_f}$ 0.25 (pentane/ether, 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.22 (m, 10H, H-Ar), 4.77 – 4.71 (m, 1H, H-2), 3.40 – 3.32 (m, 1H, H-3), 3.08 (dd, *J* = 14.5, 4.0 Hz, 1H, H-1), 2.94 (dd, *J* = 14.5, 7.0 Hz, 1H, H-1), 2.82 (dd, *J* = 17.5, 9.0 Hz, 1H, H-4), 2.71 (dd, *J* = 17.5, 10.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 175.6 (C-5), 139.1 (C-Ar), 136.1 (C-Ar), 129.8 (C-Ar), 129.3 (C-Ar), 128.7 (C-Ar), 127.9 (C-Ar), 127.4 (C-Ar), 127.2 (C-Ar), 86.9 (C-2), 46.1 (C-3), 39.5 (C-1), 37.5 (C-4).

cis-**2.52**: $\mathbf{R_f}$ 0.18 (pentane/ether, 4:1); **IR** (film) ν_{max}/cm^{-1}: 2963s, 2850s, 1780m, 1462m, 1382m, 1261m, 1158m, 950m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 3H, H-Ar),

7.24 – 7.14 (m, 5H, H-Ar), 7.05 – 7.01 (m, 2H, H-Ar), 4.98 (ddd, *J* = 9.0, 6.5, 4.5 Hz, 1H, H-2), 3.81 – 3.74 (m, 1H, H-3), 3.00 (dd, *J* = 17.5, 8.5 Hz, 1H, H-4), 2.77 (dd, *J* = 17.5, 4.0 Hz, 1H, H-4), 2.65 (dd, *J* = 14.5, 9.0 Hz, 1H, H-1), 2.52 (dd, *J* = 14.5, 4.5 Hz, 1H, H-1); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.7 (C-5), 138.2 (C-Ar), 137.0 (C-Ar), 129.3 (C-Ar), 129.1 (C-Ar), 128.6 (C-Ar), 128.1 (C-Ar), 128.0 (C-Ar), 126.9 (C-Ar), 84.7 (C-2), 44.8 (C-3), 37.7 (C-1), 36.4 (C-4); configuration assigned by NOESY correlations (blue arrow); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{17}H_{16}O_2Na$ 275.1043, found, 275.1042.

cis-1-Methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**1**.**128**)[116] and *cis*-2-methyl-3,3a,8,8a-tetrahydroindeno[1,2-*c*]pyrrol-1(2*H*)-one (**2**.**30**)[119]



1.128 **2.30**

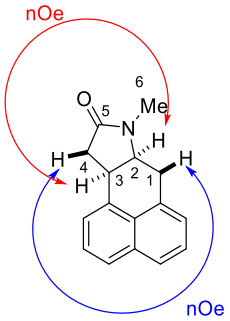
To a mixture of regioisomers **2**.**28** and **2**.**29** (14.2 mg, 0.0820 mmol) in dry THF (0.5 mL), was added sodium hydride (5.0 mg, 60% in mineral oil, 0.123 mmol) at –5 ℃, then methyl iodide (7.25 µL, 0.115 mmol) was added. The reaction mixture was stirred at RT for 2 h. When the reaction was complete (TLC), the reaction was quenched with ice water (1 mL) and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL) and dried over $MgSO_4$. After removal of solvent, the residue was purified by silica gel chromatography (pentane/ethyl acetate, 1:4 → 0:1) to give a mixture of lactam regioisomers **1**.**128** and **2**.**30** (6.3 mg, 41%) in 1:1 ratio, as a yellow oil.

1.**128**: $\mathbf{R_f}$ 0.13 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2981s, 2891m, 1653m, 1388m, 1260m, 1072m, 956m, 774s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 – 7.20 (m, 4H, H-

Ar), 4.39 (ddd, *J* = 7.0, 4.5, 3.5 Hz, 1H, H-2), 3.89 (ddd, *J* = 7.0, 7.0, 3.5 Hz, 1H, H-3), 3.21 – 3.15 (m, 2H, H-1), 2.98 – 2.89 (m, 1H, H-4), 2.88 (s, 3H, H-6), 2.59 (dd, *J* = 17.0, 3.5 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.0 (C-5), 144.5 (C-Ar), 140.1 (C-Ar), 127.8 (C-Ar), 127.6 (C-Ar), 125.2 (C-Ar), 124.7 (C-Ar), 64.4 (C-2), 42.1 (C-3), 37.6 (C-4), 36.3 (C-1), 28.1 (C-6); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO$ 188.1070, found, 188.1068.

2.30: **R_f** 0.13 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.17 (m, 4H, H-Ar), 3.95 – 3.86 (m, 2H, H-3, H-4), 3.48 (d, *J* = 9.0 Hz, 1H, H-4), 3.43 (d, *J* = 14.5 Hz, 1H, H-1), 3.34 – 3.25 (m, 2H, H-1, H-2), 2.83 (s, 3H, H-5); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.3 (C-5), 144.4 (C-Ar), 142.5 (C-Ar), 127.9 (C-Ar), 127.1 (C-Ar), 125.1 (C-Ar), 124.0 (C-Ar), 55.4 (C-4), 45.6 (C-2), 41.9 (C-3), 35.4 (C-1), 30.0 (C-5).

cis-8-Methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**1.129**)

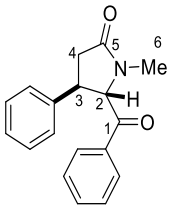


A suspension of sodium hydride (0.168 g, 43% in mineral oil, 3.03 mmol) was added batchwise to a solution of crude lactam **2.68** (0.226 g, <1.01 mmol) in dry DMF (7 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min. Methyl iodide (0.08 m, 1 mmol) was then added and the mixture was stirred at RT for a further 3 h. When the reaction was complete (TLC), the mixture was poured into iced water (3 mL) and the product was extracted with DCM (3 × 3 mL). The combined organic layers were washed sequentially with sat. aq.

NH_4Cl (3 mL) and brine (3 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4→ 0:1) yielded the title compound (**1.129**, 0.024 g, 72% over three steps) as a yellow oil.

R_f 0.10 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2362m, 1688s, 1438m, 1399m, 1261m, 1120m, 815m, 776m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.78 – 7.72 (m, 2H, H-Ar), 7.48 (dd, *J* = 8.5, 7.5 Hz, 1H, H-Ar), 7.42 (dd, *J* = 8.5, 7.0 Hz, 1H, H-Ar), 7.39 – 7.37 (m, 1H, H-Ar), 7.30 (d, *J* = 7.0 Hz, 1H, H-Ar), 4.02 (ddd, *J* = 9.5, 7.0, 5.0 Hz, 1H, H-2), 3.93 (ddd, *J* = 9.5, 9.0, 7.0 Hz, 1H, H-3), 3.47 (dd, *J* = 16.0, 5.0 Hz, 1H, H-1), 3.03 (dd, *J* = 16.0, 9.5 Hz, 1H, H-1), 2.98 (s, 3H, H-6), 2.94 (dd, *J* = 16.5, 9.0 Hz, 1H, H-4), 2.59 (dd, *J* = 16.5, 9.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.1 (C-5), 134.6 (C-Ar), 133.5 (C-Ar), 131.1 (C-Ar), 129.0 (C-Ar), 127.0 (C-Ar × 2), 126.1 (C-Ar), 126.0 (C-Ar), 125.8 (C-Ar), 125.0 (C-Ar), 57.7 (C-2), 39.2 (C-4), 37.0 (C-3), 27.9 (C-1); configuration assigned by NOESY correlations (blue arrow and red arrows); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO$ 238.1226, found, 238.1223.

trans-5-Benzoyl-1-methyl-4-phenylpyrrolidin-2-one (**1.64**)[31]

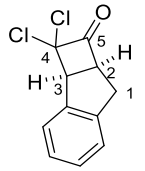


To a solution of lactam **3.14** (1.4 mg, 0.005 mmol) in dry DCM (1 mL), was added $NaHCO_3$ (1.5 mg, 0.018 mmol), DMP (5.0 mg, 0.013 mmol) was then added to the mixture at 0 °C. The reaction mixture was stirred at RT for 24 h. When the reaction was complete (TLC), the mixture was quenched with aq. NaOH (2 × 1 mL, 1.0 M). The mixture was then extracted

with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give crude lactam **1.64** (2.2 mg), which was used without further purification.

R_f 0.27 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.84 – 7.81 (m, 2H, H-Ar), 7.64 – 7.59 (m, 1H, H-Ar), 7.47 – 7.43 (m, 2H, H-Ar), 7.39 – 7.30 (m, 3H, H-Ar), 7.23 – 7.20 (m, 2H, H-Ar), 5.05 (d, *J* = 3.0 Hz, 1H, H-2), 3.37 (ddd, *J* = 9.5, 3.0, 3.0 Hz, 1H, H-3), 3.01 – 2.84 (m, 4H, H-4, H-6), 2.49 (dd, *J* = 17.5, 3.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 195.8 (C-1), 174.7 (C-5), 143.5 (C-Ar), 134.3 (C-Ar) 134.2 (C-Ar), 129.5 (C-Ar), 129.1 (C-Ar), 128.8 (C-Ar), 127.9 (C-Ar), 126.7 (C-Ar), 71.9 (C-2), 41.4 (C-3), 38.3 (C-4), 29.3 (C-6).

cis-2,2-Dichloro-2,2a,7,7a-tetrahydro-1*H*-cyclobuta[*a*]inden-1-one (**2.2**)[114]



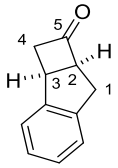
Method A: Zn dust (1.00 g, 15.3 mmol) was suspended in dry ether (40 mL), then indene (**2.1**, 0.90 mL, 7.7 mmol) was added. A solution of trichloroacetyl chloride (1.3 mL, 12 mmol) in dry ether (20 mL) was then added dropwise over a period of 40–45 min during which the mixture was sonicated (ultrasonic bath), while maintaining the bath temperature below 25 °C. Once the addition was complete, the mixture was sonicated for another 4 h. When the reaction was complete (TLC), the mixture was diluted with ether (20 mL) and the solids were filtered off through a plug of Celite® and washed with ether (60 mL). The filtrate was then washed sequentially with water (2 × 20 mL), sat. aq. $NaHCO_3$ solution (4 × 20 mL), and brine (20 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give the title

compound (**2.2**, 1.00 g, 57%) as a colourless solid, which was used without further purification.

Method B: To a solution of indene (**2.1**, 0.90 mL, 7.7 mmol) in dry hexane (25 mL) was added dichloroacetyl chloride (0.90 mL, 9.2 mmol). The reaction mixture was heated at reflux for 20 min then a solution of TEA (1.3 mL, 9.2 mmol) in dry hexane (15 mL) was added dropwise. The mixture was heated for a further 14 h. When the reaction was complete (TLC), the solid material was removed by filtration and washed with hexane (60 mL). The filtrate was washed with sat. aq. $NaHCO_3$ (2 × 25 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 20:1 → 10:1) yielded the title compound (**2.2**, 0.809 g, 46 %) as a colourless solid and as a single diastereomer.

R_f 0.70 (pentane/ethyl acetate, 8:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45 – 7.42 (m, 1H, H-Ar), 7.35 – 7.30 (m, 2H, H-Ar), 7.29 – 7.26 (m, 1H, H-Ar), 4.55 – 4.46 (m, 2H, H-2, H-3), 3.40 (d, *J* = 17.0 Hz, 1H, H-1), 3.20 (dd, *J* = 17.0, 8.5 Hz, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 197.5 (C-5), 143.5 (C-Ar), 137.6 (C-Ar), 129.3 (C-Ar), 128.5 (C-Ar), 127.6 (C-Ar), 125.5 (C-Ar), 88.3 (C-4), 59.3 (C-2), 58.9 (C-3), 34.3 (C-1).

cis-2,2a,7,7a-Tetrahydro-1*H*-cyclobuta[*a*]inden-1-one (**2.3**)[174]



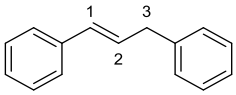
Method A: Zn dust (0.505 g, 7.72 mmol) and ammonium chloride (0.206 g, 3.85 mmol) were suspended in dry methanol (2 mL). A solution of adduct **2.2** (0.357 g, 1.54 mmol) in

dry methanol (8 mL) was added to the suspension at such a rate to prevent it from boiling. Once the addition was complete, the mixture was heated at reflux for 2 h. The mixture was then allowed to cool to RT and the solids were filtered off through a pad of Celite®. The filtrate was concentrated under reduced pressure, diluted with aq. HCl (10 mL, 1.0 M) and extracted with ether (3 × 4 mL). The combined organic layers were washed with brine (4 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (**2.3**, 0.144 g, 59%) as a yellow oil, which was used without further purification.

Method B: Tf_2O (1.01 mL, 6.02 mmol) was added dropwise to a stirred solution of DMA (0.50 mL, 5.2 mmol) in 1,2-DCE (6 mL) at 5 °C. The mixture was stirred at 5 °C for 30 min, then a mixture of indene **2.1** (0.50 mL, 4.3 mmol) and 2,4,6-collidine (0.80 mL, 6.0 mmol) in 1,2-DCE (1 mL) was added dropwise. The reaction mixture was heated at reflux for 2 h. The solvent was then removed under reduced pressure and the residue was treated with H_2O/DCM (4 mL, 1:1). The obtained mixture was heated at reflux for 24 h and extracted with DCM (3 × 1 ml). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 20:1) afforded the title compound (**2.3**, 0.243 g, 36%) as a yellow oil.

R_f 0.36 (pentane/ethyl acetate, 19:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 4H, H-Ar), 4.13 – 4.07 (m, 2H, H-2, H-3), 3.72 – 3.60 (m, 1H, H-4), 3.39 – 3.32 (m, 1H, H-1), 3.19 – 3.10 (m, 1H, H-1), 2.97 – 2.90 (m, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.7 (C-5), 144.7 (C-Ar), 142.8 (C-Ar), 127.2 (C-Ar × 2), 125.2 (C-Ar), 124.9 (C-Ar), 62.3 (C-2), 55.2 (C-4), 36.8 (C-3), 33.4 (C-1).

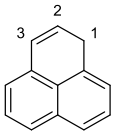
trans-Prop-1-ene-1,3-diyldibenzene (**2.5**)[122]



A solution of phenylacetaldehyde **2.7** (4.9 mL, 42 mmol) and KOH (2.50 g, 44.6 mmol) in absolute ethanol (20 mL) were heated at reflux for 23 h. After cooling, the solvent was removed under reduced pressure and the residue was dissolved in water (20 mL). The mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed sequentially with water (10 mL) and brine (10 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (100% pentane) yielded the title compound (**2.5**, 3.55 g, 87%) as a colourless oil.

R_f 0.79 (DCM/pentane, 1:3); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.10 (m, 10H, H-Ar), 6.40 (d, *J* = 15.5 Hz, 1H, H-1), 6.31 (dt, *J* = 15.5, 6.5 Hz, 1H, H-2), 3.50 (d, *J* = 6.5 Hz, 2H, H-3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.3 (C-Ar), 137.6 (C-Ar), 131.2 (C-1), 129.4 (C-Ar), 128.8 (C-Ar), 128.6 (C-2), 127.2 (C-Ar), 126.3 (C-Ar), 126.3 (C-Ar), 39.5 (C-3), one C-Ar resonance not seen.

1*H*-Phenalene (**2.6**)[175]



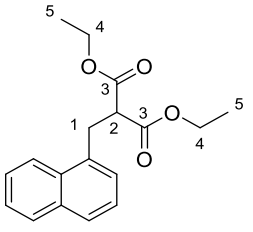
Method A: The alcohol **2.13** (0.302 g, 1.64 mmol) was dissolved in a mixture of THF/H_2O (6 mL, 1:1). Aq. HCl (3.0 mL, 4.0 M, 12 mmol) was then added and the reaction mixture was heated at reflux for 6 h. The mixture was cooled to RT and THF was removed under reduced pressure. The mixture was then extracted with ethyl acetate (3 × 3 mL). The combined organic layers were washed with brine (3 mL), then dried over $MgSO_4$ and

concentrated *in vacuo*. Purification by silica gel chromatography (100% pentane) yielded the title compound (**2.6**, 95 mg, 35%) as a yellow oil.

Method B: A solution of ketone **2.12** (0.10 g, 0.55 mmol), neopentyl glycol (0.234 g, 2.25 mmol) and *p*-toluenesulfonic acid monohydrate (0.016 g, 0.083 mmol) in dry toluene (1 mL) was heated at reflux for 29 h. When the reaction was complete (TLC), the mixture was quenched with Na_2CO_3 and extracted with ether (3 × 1 ml). The combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give crude phenalene **2.6** (34 mg).

R_f 0.69 (pentane/ethyl acetate, 12:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.61 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.43 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.38 – 7.27 (m, 2H, H-Ar), 7.07 (d, *J* = 6.5 Hz, 1H, H-Ar), 6.68 (dd, *J* = 10.0, 2.0 Hz, 1H, H-3), 6.10 (dt, *J* = 10.0, 4.0 Hz, 1H, H-2), 4.17 – 4.09 (m, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 134.3 (C-Ar), 133.7 (C-Ar), 132.1 (C-Ar), 129.6 (C-Ar), 127.8 (C-2/3), 127.7 (C-2/3), 126.8 (C-Ar), 126.3 (C-Ar), 126.1 (C-Ar), 125.1 (C-Ar), 125.0 (C-Ar), 122.2 (C-Ar), 32.1 (C-1).

Diethyl 2-(naphthalen-1-ylmethyl)malonate (**2.10**)[176]

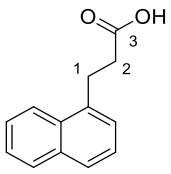


Na (0.551 g, 24.0 mmol) was added in portions to stirred absolute ethanol (11 mL) at RT over 20 min. Until all the Na had dissolved, the resulting solution was cooled to 0 °C. Diethyl malonate (6.1 mL, 40 mmol) was then added dropwise, followed by 1-chloromethylnaphthalene (**2.9**, 2.50 g, 14.2 mmol). After heating at reflux for 1 h, the

mixture was diluted with 10 mL water and neutralised with aq. HCl (5.0 mL, 1.0 M) and then extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed sequentially with sat. aq. $NaHCO_3$ (5 mL) and brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 4:1 → 4:1.5) yielded the title compound (**2.10**, 3.95 g, 93%) as a light-yellow oil.

R_f 0.81 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.86 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.76 – 7.71 (m, 1H, H-Ar), 7.54 (dd, *J* = 8.5, 6.5 Hz, 1H, H-Ar), 7.49 (dd, *J* = 8.0, 6.5 Hz, 1H, H-Ar), 7.38 – 7.34 (m, 2H, H-Ar), 4.17 – 4.09 (m, 4H, H-4), 3.83 (t, *J* = 8.0 Hz, 1H, H-2), 3.70 (d, *J* = 8.0 Hz, 2H, H-1), 1.18 (t, *J* = 7.0 Hz, 6H, H-5); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.2 (C-3), 134.1 (C-Ar), 133.9 (C-Ar), 131.7 (C-Ar), 129.1 (C-Ar), 127.8 (C-Ar), 127.3 (C-Ar), 126.4 (C-Ar), 125.8 (C-Ar), 125.5 (C-Ar), 123.4 (C-Ar), 61.6 (C-4), 53.0 (C-2), 31.9 (C-1), 14.1 (C-5).

3-(Naphthalen-1-yl)propanoic acid (**2.11**)[177]

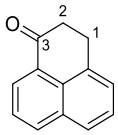


A solution of aq. NaOH (15.0 mL, 15.0 M, 225 mmol), absolute ethanol (60 mL) and ester **2.10** (5.00 g, 16.6 mmol) was heated at reflux for 4 h. The solvent was then removed *in vacuo* and the residue was dissolved in water (75 mL). The mixture was extracted with ether (3 × 30 mL). The aqueous phase was treated with concentrated aq. HCl (6.00 mL, 12.0 M) to adjust the pH to about 1 and extracted with chloroform (3 × 30 mL). The combined chloroform extracts were washed with brine (30 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. The resulted white powder was heated in a round-bottom flask at 175

°C (oil bath temperature) for 30 min (evolution of carbon dioxide was observed) to give the title compound (**2.11**, 1.46 g, 44% over two steps) as a dark brown solid, which was used without further purification.

R_f 0.63 (pentane/ethyl acetate, 1:1); **^{1}H NMR** (400 MHz, MeOD) δ 8.07 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.86 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.76 – 7.70 (m, 1H, H-Ar), 7.56 – 7.44 (m, 2H, H-Ar), 7.41 – 7.36 (m, 2H, H-Ar), 3.42 – 3.37 (m, 2H, H-2), 2.76 – 2.69 (m, 2H, H-1); **^{13}C NMR** (101 MHz, MeOD) δ 176.8 (C-3), 138.0 (C-Ar), 135.5 (C-Ar), 133.0 (C-Ar), 129.9 (C-Ar), 128.1 (C-Ar), 127.1 (C-Ar), 126.9 (C-Ar), 126.6 (C-Ar), 124.4 (C-Ar), one C-Ar resonance not seen, 36.1 (C-2), 29.1 (C-1).

2,3-Dihydro-1*H*-phenalen-1-one (**2.12**)[177]

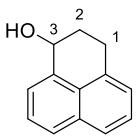


Method A: Compound **2.11** (1.46 g, 7.29 mmol) was dissolved in $SOCl_2$ (29.2 mL), and the solution was heated at reflux for 3 h. The $SOCl_2$ was then distilled off under reduced pressure. The resulting acid chloride was dissolved in dry DCM (5 mL) and then added dropwise to a cold (–10°C) solution of $AlCl_3$ (2.34 g, 17.5 mmol) in dry DCM (50 mL). After stirring for 30 min at 0 °C, the mixture was poured into ice water (50 mL) and the resulting solution was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (20 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (**2.12**, 0.765 g, 58% over two steps) as a yellow solid, which was used without further purification.

Method B: Ketone **2.12** was also obtained during an attempt to prepare keto-ester **2.77**. To a solution of enone **2.17** (0.014 g, 0.076 mmol) in dry THF (2 mL) at –78 °C, was added L-Selectride® (0.08 mL, 1.0 M solution in THF, 0.08 mmol) dropwise. The reaction mixture was stirred at –78 °C for 15 min, before adding ethyl bromoacetate (0.02 mL, 0.2 mmol). The mixture was gradually warmed to RT and then stirred for 53 h. The reaction was quenched with sat. aq. NH_4Cl solution (2 mL) and extracted with DCM (3 × 2 mL). The combined organic layers were washed sequentially with water (2 mL) and brine (2 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (**2.12**, 0.012 mg, 83%), as a yellow oil, which was used without further purification.

R_f 0.34 (pentane/ether, 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.08 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.79 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.59 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.52 – 7.43 (m, 2H, H-Ar), 3.43 (t, *J* = 7.0 Hz, 2H, H-1), 3.00 – 2.95 (m, 2H, H-2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 198.6 (C-3), 134.1 (C-Ar), 133.5 (C-Ar), 133.3 (C-Ar), 131.7 (C-Ar), 129.9 (C-Ar), 126.4 (C-Ar), 125.8 (C-Ar), 125.6 (C-Ar), 125.1 (C-Ar), one C-Ar resonance not seen, 38.6 (C-2), 28.6 (C-1).

2,3-Dihydro-1*H*-phenalen-1-ol (**2.13**)[178]

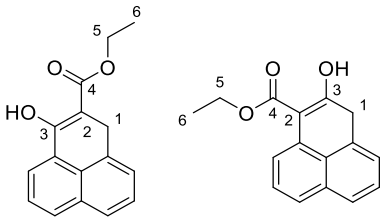


$NaBH_4$ (0.363 g, 9.60 mmol) was added portion-wise to a stirred solution of ketone **2.12** (0.880 g, 4.83 mmol) in dry methanol/THF (20 mL, 2:1) at 0 °C. After 5 min, the reaction mixture was allowed to warm to RT and stirring was continued for 1.5 h. Water (10 mL) was then added to quench the reaction. The mixture was concentrated under reduced

pressure and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 4:1 → 3:2) yielded the title compound (**2.13**, 0.739 g, 84%) as a colourless solid.

R_f 0.60 (pentane/ethyl acetate, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.73 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.54 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.49 – 7.41 (m, 2H, H-Ar), 7.32 – 7.29 (m, 1H, H-Ar), 5.06 (dd, *J* = 6.5, 3.5 Hz, 1H, H-3), 3.31 (ddd, *J* = 16.0, 8.5, 4.5 Hz, 1H, H-1), 3.07 (dt, *J* = 16.0, 7.0 Hz, 1H, H-1), 2.32 (br s, 1H, OH), 2.24 – 2.10 (m, 2H, H-2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.5 (C-Ar), 135.2 (C-Ar), 133.7 (C-Ar), 128.8 (C-Ar), 128.0 (C-Ar), 125.9 (C-Ar), 125.7 (C-Ar), 125.6 (C-Ar), 124.4 (C-Ar), 123.5 (C-Ar), 69.3 (C-3), 31.3 (C-2), 26.4 (C-1).

Ethyl 3-hydroxy-1*H*-phenalene-2-carboxylate (**2.15**) and ethyl 2-hydroxy-1*H*-phenalene-3-carboxylate (**2.16**)



2.15 **2.16**

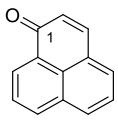
$BF_3{\cdot}OEt_2$ (2.8 mL, 22 mmol) was added to a solution of 1-acenaphthenone **2.14** (2.51 g, 14.9 mmol) in dry ether (30 mL) at 0 °C. A solution of ethyl diazoacetate (2.4 mL, 22 mmol) in dry ether (7 mL) was then added over a period of 25 min and the resulting solution was stirred at RT for 4 h. When the reaction was complete (TLC), the mixture was diluted with water (15 mL). The resulting mixture was extracted with ethyl acetate (3 × 15 mL). The

combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 15:1) yielded a mixture of regioisomers **2.15** and **2.16** (1.78 g, 47%) in 5:1 ratio as an orange oil. $\mathbf{R}_f$ 0.66 (pentane/ethyl acetate, 10:1).

2.15: **IR** (film) ν_{max}/cm^{-1}: 3733m, 3696m, 1693m, 1276m, 761s, 653s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 12.57 (s, 1H, OH), 7.94 – 7.90 (m, 1H, H-Ar), 7.78 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.66 – 7.61 (m, 1H, H-Ar), 7.48 – 7.35 (m, 3H, H-Ar), 4.35 (q, *J* = 7.0 Hz, 2H, H-5), 4.19 (s, 2H, H-1), 1.41 (t, *J* = 7.0 Hz, 3H, H-6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.0 (C-4), 163.0 (C-3), 132.9 (C-Ar), 132.1 (C-Ar), 130.2 (C-Ar), 129.7 (C-Ar), 126.9 (C-Ar), 125.9 (C-Ar), 125.6 (C-Ar), 125.2 (C-Ar), 124.8 (C-Ar), 121.2 (C-Ar), 96.2 (C-2), 60.6 (C-5), 28.6 (C-1), 14.3 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{15}O_3$ 255.1016, found, 255.1016.

2.16: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 13.91 (s, 1H, OH), 8.11 – 7.99 (m, 1H, H-Ar), 7.48 (d, *J* = 8.5, 1H, H-Ar), 7.39 – 7.28 (m, 3H, H-Ar), 7.16 – 7.05 (m, 1H, H-Ar), 4.43 (q, *J* = 7.0 Hz, 2H, H-5), 4.05 (s, 2H, H-1), 1.49 (t, *J* = 7.0 Hz, 3H, H-6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (C-4), 172.3 (C-3), 133.5 (C-Ar), 129.8 (C-Ar), 128.9 (C-Ar), 126.2 (C-Ar), 126.1 (C-Ar), 125.3 (C-Ar), 124.1 (C-Ar), 121.6 (C-Ar), two C-Ar peaks overlapped with those of **2.15**, 99.9 (C-2), 61.4 (C-5), 35.2 (C-1), 14.1 (C-6).

1*H*-Phenalen-1-one (**2.17**)[137]



Method A: A solution of ketone **2.12** (11 mg, 0.060 mmol), aq. HCl (1 mL, 1 M) in isopropanol (1 mL) was heated at reflux for 19 h. When the reaction was complete (TLC), the mixture was extracted with DCM (3 × 1 ml). The combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give enone **2.17** (11 mg, quant.) as a yellow oil, which was used without further purification.

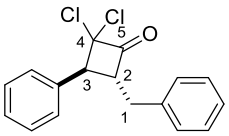
Method B: Enone **2.17** was obtained in an attempt to prepare cyclobutanone **2.44**. The triflic anhydride (0.10 mL, 0.57 mmol) was added dropwise to a stirred solution of DMA (0.05 mL, 0.5 mmol) in 1,2-DCE (1 mL) at 5 ℃. The mixture was stirred at 5 ℃ for 30 min, then a mixture of phenalene **2.6** (68 mg, 0.41 mmol) and 2,4,6-collidine (0.08 mL, 0.6 mmol) in 1,2-DCE (1 mL) was added dropwise. The reaction mixture was heated at reflux for 23 h. 1,2-DCE was then removed under reduced pressure and the residue was treated with H_2O/DCM (1 mL, 1:1). The obtained mixture was heated at reflux for 43 h and extracted with DCM (3 × 1 ml). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give enone (**2.17**, 90 mg, quant.) as a yellow oil, which was used without further purification.

Method C: To a solution of a mixture of regioisomers **2.15** and **2.16** (0.625 g, 2.46 mmol) in isopropanol (30 mL) was added aq. HCl (31 mL, 1.0 M). The reaction mixture was heated at reflux for 26 h. The solvent was removed under reduced pressure and the residue was dissolved in water (30 mL). The mixture was extracted with DCM (3 × 10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 10:1 → 5:1) yielded the title compound (**2.12**, 0.146 g, 48%, yellow oil) and enone **2.17** (2.8 mg, 3%, yellow oil).

For **2.12** characterisation data, see above.

2.17: **R_f** 0.28 (pentane/ethyl acetate, 10:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 7.5 Hz, 1H, H-Ar), 8.21 (d, *J* = 8.0 Hz, 1H, H-Ar), 8.03 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.82 – 7.73 (m, 3H, H-Ar), 7.64 – 7.59 (m, 1H, H-Ar), 6.74 (d, *J* = 10.0 Hz, 1H, H-Ar); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 185.9 (C-1), 142.0 (C-Ar), 135.1 (C-Ar), 132.3 (C-Ar), 132.1 (C-Ar), 131.5 (C-Ar), 130.6 (C-Ar), 129.7 (C-Ar), 129.4 (C-Ar), 128.0 (C-Ar), 127.7 (C-Ar), 127.3 (C-Ar), 126.8 (C-Ar).

trans-4-Benzyl-2,2-dichloro-3-phenylcyclobutan-1-one (**2.19**)



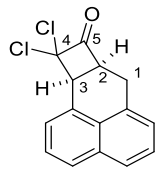
Activation of Zn: To a stirred suspension of Zn dust (1.0 g, 15 mmol) in water (4 mL), was degassed by bubbling N_2 through it for 15 min. Then, $CuSO_4$ (75 mg, 0.47 mmol) was added at once. The suspension was stirred while bubbling with N_2 for an additional 45 min. The Zn-Cu couple was collected on a sintered glass funnel under a stream of N_2 and washed successively with water (10 mL) and acetone (10 mL). The Zn-Cu couple was then transferred to a small flask under a stream of N_2 and dried *in vacuo* for 2 h. The Zn-Cu couple was stored under N_2 in a tightly stoppered flask.

Zn-Cu couple (66.7 mg, 1.02 mmol) was suspended in dry ether (3 mL), then *trans*-diphenylpropene (**2.5**, 0.10 g, 0.51 mmol) was added. A solution of trichloroacetyl chloride (86 µL, 0.77 mmol) in dry ether (2 mL) was then added dropwise during which the mixture was sonicated (ultrasonic bath), while maintaining the bath temperature below 25 °C. Once the addition was complete, the mixture was kept under sonication for another 17 h. The

mixture was then diluted with ether (3 mL) and the solids were filtered off through a plug of Celite® and washed with ether (9 mL). The filtrate was then washed sequentially with water (2 × 3 mL), sat. aq. $NaHCO_3$ solution (4 × 3 mL), and brine (3 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 20:1) gave the title compound (**2.19**, 21 mg, 14%) as a yellow oil and as a single diastereomer, with 42% of recovered starting material **2.5**.

R_f 0.31 (pentane/ether, 6:1); **IR** (film) ν_{max}/cm^{-1}: 2919s, 2850s, 1808m, 1456m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37 – 7.08 (m, 10H, H-Ar), 4.16 – 4.09 (m, 1H, H-2), 3.83 (d, *J* = 11.5 Hz, 1H, H-3), 3.13 (dd, *J* = 14.5, 7.0 Hz, 1H, H-1), 3.04 (dd, *J* = 14.5, 6.0 Hz, 1H, H-1); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 194.6 (C-5), 137.2 (C-Ar), 133.7 (C-Ar), 129.2 (C-Ar), 129.0 (C-Ar), 128.7 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 127.2 (C-Ar), 87.6 (C-4), 59.3 (C-2), 56.0 (C-3), 34.8 (C-1); **HRMS** (APCI) *m/z* $[M+H]^+$ calcd. for $C_{17}H_{15}Cl_2O$ 305.0494, found, 305.0494.

cis-9,9-Dichloro-7,7a,9,9a-tetrahydro-8*H*-cyclobuta[*a*]phenalen-8-one (**2.20**)

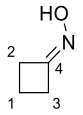


Zn dust (36.6 mg, 0.560 mmol) was suspended in dry ether (1 mL), then phenalene **2.6** (46.7 mg, 0.280 mmol) was added. A solution of trichloroacetyl chloride (47.0 µL, 0.420 mmol) in dry ether (1 mL) was then added dropwise over a period of 40–45 min during which the mixture was sonicated (ultrasonic bath), while maintaining the bath temperature below 25 °C. Once the addition was complete, the mixture was kept under sonication for another 4 h. The mixture was then diluted with ether (2 mL) and the solids were filtered off through a

plug of Celite® and washed with ether (10 mL). The filtrate was then washed sequentially with water (2 × 5 mL), sat. aq. $NaHCO_3$ solution (4 × 5 mL), and brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 8:1) afforded the title compound (**2.20**, 8.2 mg, 11%) as a yellow oil, with 50% of recovered starting material.

R_f 0.43 (pentane/ether, 8:1); **IR** (film) ν_{max}/cm^{-1}: 2981s, 2899s, 1806s, 1382m, 1261m, 773m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.85 – 7.69 (m, 3H, H-Ar), 7.49 – 7.44 (m, 3H, H-Ar), 4.62 – 4.55 (m, 2H, H-2, H-3), 3.75 – 3.67 (m, 1H, H-1), 3.42 – 3.34 (m, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 197.6 (C-5), 140.5 (C-Ar), 135.1 (C-Ar), 133.8 (C-Ar), 130.5 (C-Ar), 128.7 (C-Ar), 128.5 (C-Ar), 126.9 (C-Ar), 126.8 (C-Ar), 125.3 (C-Ar), 124.8 (C-Ar), 93.6 (C-4), 59.9 (C-2), 58.9 (C-3), 33.3 (C-1); **HRMS** (APCI) *m/z* $[M+H]^+$ calcd. for $C_{15}H_{11}Cl_2O$ 277.0181, found, 277.0181.

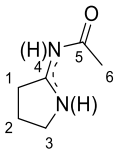
Cyclobutanone oxime (**2.22**)[179]



A mixture of cyclobutanone **2.21** (50.0 µL, 0.670 mmol), $NH_2OH{\cdot}HCl$ (0.140 g, 2.01 mmol), NaOAc (0.165 g, 2.01 mmol) in dry methanol (20 mL) was heated at reflux for 18 h. The solvent was removed under reduced pressure and the residue was dissolved in water (10 mL) and extracted with ether (3 × 5 ml). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford oxime **2.22** (0.493 g, 86%) as a colourless solid, which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (br s, 1H, OH), 2.94 (t, *J* = 8.0 Hz, 2H, H-2/3), 2.89 (t, *J* = 8.0 Hz, 2H, H-2/3), 2.00 (p, *J* = 8.0 Hz, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.9 (C-4), 31.5 (C-2/3), 30.7 (C-2/3), 14.6 (C-1).

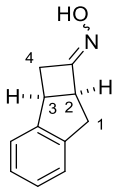
N-(Pyrrolidin-2-ylidene)acetamide (**2.24**)



To a solution of cyclobutanone oxime **2.22** (16 mg, 0.18 mmol), was added $HgCl_2$ (15 mg, 0.054 mmol). The reaction mixture was heated at reflux for 20 h. After allowing it to cool to RT, dry CH_3CN (3 mL) was added to dissolve the solid formed. The solvent was removed under reduced pressure. The residue was then dissolved in water (5 mL) and extracted with DCM (3 × 5 ml). The combined organic layers were washed with brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to afford *N*-acetylamidine **2.24** (21 mg, 92%) as a yellow oil.

R_f 0.09 (pentane/ethyl acetate, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.36 – 3.29 (m, 2H, H-3), 2.37 – 2.31 (m, 2H, H-1), 1.94 (s, 3H, H-6), 1.88 – 1.79 (m, 2H, H-2); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_6H_{11}N_2O$ 127.0866, found, 127.0867.

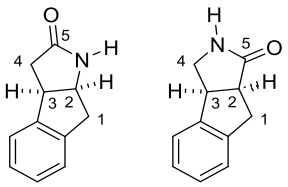
A pair of diastereomers, *cis*-2,2a,7,7a-Tetrahydro-1*H*-cyclobuta[*a*]inden-1-one oxime (**2.27**)



To a stirred solution of cyclobutanone **2.3** (100 mg, 0.632 mmol) in pyridine (1 mL), was added $NH_2OH{\cdot}HCl$ (876 mg, 1.26 mmol). After stirring at RT for 4 h, pyridine was removed under reduced pressure. The residue was diluted with water and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$, and concentrated *in vacuo* to give a mixture of oxime diastereomers **2.27** (91 mg, 84%, *dr* = 64:36, unassigned) as a dark brown oil which was used without further purification.

R_f 0.28 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 3250m, 2918m, 1478m, 1458m, 942m, 750s; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.36 (s, 0.56H, OH*), 10.30 (s, 1H, OH), 7.28 – 7.12 (m, 7H, H-Ar), 3.91 – 3.79 (m, 3.58H, H-2, H-3), 3.23 (dd, *J* = 16.5, 8.5 Hz, 1H, H-4*), 3.13 – 3.01 (m, 3.26H, H-1), 2.56 (ddd, *J* = 16.5, 3.0, 3.0 Hz, 1H, H-4), 2.43 (dt, *J* = 16.0, 3.0 Hz, 1H, H-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{12}NO$ 174.0913, found, 174.0915.

cis-3,3a,8,8a-Tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**2.28**)[129, 174] and *cis*-3,3a,8,8a-tetrahydroindeno[1,2-*c*]pyrrol-1(2*H*)-one (**2.29**)[119]



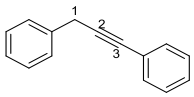
2.28 **2.29**

Thionyl chloride (49.0 µL, 0.672 mmol) was added to a solution of oxime diastereomers **2.27** (38.8 mg, 0.224 mmol) in 1,4-dioxane (1 mL), and stirred for 22 h at RT. The reaction was quenched with sat. aq. $NaHCO_3$ (3 mL) and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4 → 0:1) yielded a mixture of regioisomers **2.28** and **2.29** (7.9 mg, 21%) in 1:1 ratio, as a light-yellow solid and as single diastereomers. $\mathbf{R_f}$ 0.16 (DCM/methanol, 100:3).

2.28: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.20 (m, 4H, H-Ar), 6.67 (s, 1H, NH), 4.51 (t, *J* = 6.5 Hz, 1H, H-2), 4.01 – 3.92 (m, 1H, H-3), 3.30 – 3.20 (m, 1H, H-1), 2.99 (d, *J* = 17.0 Hz, 1H, H-1), 2.88 (dd, *J* = 17.0, 9.5 Hz, 1H, H-4), 2.51 (dd, *J* = 17.0, 2.0 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.3 (C-5), 144.0 (C-Ar), 140.4 (C-Ar), 127.9 (C-Ar), 127.5 (C-Ar), 125.4 (C-Ar), 124.8 (C-Ar), 58.0 (C-2), 45.0 (C-3), 39.7 (C-1), 37.2 (C-4).

2.29: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.20 (m, 4H, H-Ar) , 6.45 (s, 1H, NH), 4.01 – 3.92 (m, 1H, H-3), 3.89 (dd, *J* = 9.5, 7.5 Hz, 1H, H-4), 3.54 (dd, *J* = 9.5, 1.5 Hz, 1H, H-4), 3.44 – 3.37 (m, 1H, H-1), 3.30 – 3.20 (m, 2H, H-1, H-2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 180.3 (C-5), 171.3 (C-Ar), 144.2 (C-Ar), 142.2 (C-Ar), 127.2 (C-Ar), 125.0 (C-Ar), 124.0 (C-Ar), 47.8 (C-4), 44.8 (C-2), 44.7 (C-3), 35.0 (C-1).

1,3-Diphenyl-1-propyne (**2.32**)[180]

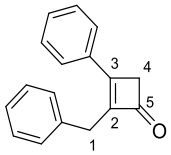


To a solution of CuI (10 mg, 0.05 mmol) or CuCl (0.005 g, 0.05 mmol), LiHMDS (1.2 mL, 1.2 mmol) in dry toluene (4 mL), was added the phenylacetylene (0.16 mL, 1.5 mmol) and benzyl bromide **2.31** (0.17 g, 1.0 mmol). The resultant mixture was heated at reflux for 3 h.

The mixture was then concentrated *in vacuo* to give diphenylpropyne **2.32** (0.22 g, quant.) as a yellow oil, which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.24 (m, 10H, H-Ar), 3.80 (s, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.8 (C-Ar), 131.7 (C-Ar), 128.6 (C-Ar), 128.3 (C-Ar), 128.0 (C-Ar), 127.9 (C-Ar), 126.7 (C-Ar), 123.8 (C-Ar), 87.7 (C-2/3), 82.8 (C-2/3), 25.7 (C-1).

2-Benzyl-3-phenylcyclobut-2-en-1-one (**2.33**)[181]

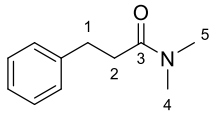


Amide **2.35** (0.500 g, 0.282 mmol) was dissolved in dry DCM (1 mL). 2-Fluoropyridine (29.0 µL, 0.338 mmol) and phenylacetylene (37.0 µL, 0.338 mmol) were sequentially added to the reaction mixture, followed by triflic anhydride (52.0 µL, 0.310 mmol) dropwise. The reaction was heated at reflux for 18 h before cooling to 0 °C. Aq. NaOH (0.6 mL, 1.0 M, 0.6 mmol) was added and the reaction was stirred for 5 h at 0 °C. The reaction mixture was quenched with aq. HCl (0.5 mL, 1.0 M) and the aqueous layer was extracted with DCM (3 × 1 mL). The combined organic layers were washed sequentially with sat. aq. $NaHCO_3$ (1 mL), water (1 mL), brine (1 mL) and then dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was filtered through a short silica plug and washed with eluent (pentane/ethyl acetate, 17:3), and the fractions were combined and re-concentrated *in vacuo*, providing a mixture of cyclobutenone regioisomers **2.36**. The cyclobutenone regioisomers **2.36** were dissolved in ethanol (1 mL). KO*t*-Bu (85 µL, 1.0 M in THF, 0.085 mmol) was added dropwise to the solution and the reaction was left to stir at RT for 16 h. The reaction was quenched with sat. aq. NH_4Cl (1 mL) and the aqueous layer was extracted with ethyl acetate

(3 × 1 ml). The combined organic layers were washed sequentially with water (1 mL), brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 19:1) afforded cyclobutenone **2.33** (12 mg, 18% over two steps) as a yellow oil.

R_f 0.50 (pentane/ethyl acetate, 10:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 – 7.55 (m, 2H, H-Ar), 7.47 – 7.44 (m, 3H, H-Ar), 7.30 – 7.27 (m, 4H, H-Ar), 7.23 – 7.17 (m, 1H, H-Ar), 3.79 (s, 2H, H-4), 3.50 (d, *J* = 1.5 Hz, 1H, H-1), 3.49 (d, *J* = 1.5 Hz, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.7 (C-5), 164.3 (C-3), 143.0 (C-2), 137.2 (C-Ar), 132.5 (C-Ar), 131.4 (C-Ar), 129.5 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 128.6 (C-Ar), 126.7 (C-Ar), 48.7 (C-1), 30.5 (C-4).

N,*N*-Dimethyl-3-phenylpropanamide (**2.35**)[182]

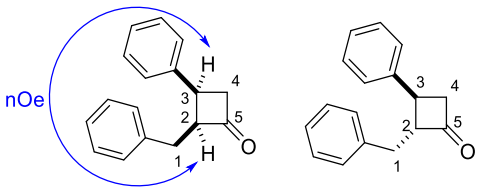


To a solution of the dimethylamine (4.5 mL, 2.0 M solution in THF, 9.0 mmol) was added benzenepropanonyl chloride **2.34** (0.40 mL, 3.0 mmol) dropwise. The resulting reaction mixture was stirred at RT for 3 h. The reaction was quenched with sat. aq. $NaHCO_3$ (2 mL). The resulting reaction mixture was extracted with DCM (3 × 2 mL). The combined organic layers were washed with brine (2 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the amide **2.35** (0.419 g, 80%) as an orange oil, which was used without further purification.

R_f 0.14 (pentane/ethyl acetate, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31 – 7.25 (m, 2H, H-Ar), 7.24 – 7.17 (m, 3H, H-Ar), 2.99 – 2.92 (m, 8H, H-1, H-4, H-5), 2.66 – 2.58 (m, 2H, H-

2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.3 (C-3), 141.6 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 126.2 (C-Ar), 37.3 (C-4/5), 35.5 (C-4/5), 35.4 (C-2), 31.5 (C-1).

cis/*trans*-2-Benzyl-3-phenylcyclobutan-1-one (**2.37**)

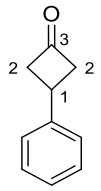


Cyclobutenone **2.33** (5.1 mg, 0.022 mmol) and Pd (2.4 mg, 10% Pd on activated carbon, 0.0022 mmol) were dissolved in dry ethyl acetate (1 mL) and purged with hydrogen gas (balloon) for 5 min. The needle was then removed from the solution and left in the reaction headspace while stirring at RT for 19 h. The crude reaction mixture was filtered through Celite® to afford a pair of diastereomers **2.37** (4.4 mg, 89%, *cis*:*trans* = 93:7) as a colourless oil, which was used without further purification.

A pair of diastereomers **2.37**: **R_f** 0.42 (pentane/ether, 4:1); **IR** (film) ν_{max}/cm^{-1}: 2918s, 2850s, 1779s, 1456m, 1261m, 799m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.35 – 7.32 (m, 2H, H-Ar), 7.30 – 7.27 (m, 1H, H-Ar), 7.21 – 7.11 (m, 5H, H-Ar), 6.86 – 6.84 (m, 2H, H-Ar), 3.99 – 3.91 (m, 1H, H-2), 3.83 – 3.78 (m, 1H, H-3), 3.70 – 3.66 (m, 0.07H, H-2*), 3.57 (ddd, *J* =17.5, 10.0, 3.5 Hz, 1H, H-4), 3.37 – 3.32 (m, 0.08H, H-3*), 3.29 (ddd, *J* = 16.0, 8.5, 2.0 Hz, 0.06H, H-4*), 3.23 (ddd, *J* = 16.0, 8.0, 2.5 Hz, 0.06H, H-4*), 3.17 (ddd, *J* =17.5, 4.5, 2.0 Hz, 1H, H-4), 3.13 (dd, *J* = 14.5, 7.0 Hz, 0.07H, H-1*), 3.02 (dd, *J* = 14.5, 7.0 Hz, 0.08H, H-1*), 2.82 (dd, *J* = 15.0, 6.5 Hz, 1H, H-1), 2.35 (dd, *J* = 15.0, 8.5 Hz, 1H, H-1); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 209.5/208.6 (C-5), 142.9/140.0 (C-Ar), 139.1/138.5 (C-Ar), 129.3 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 128.7 (C-Ar), 128.4 (C-Ar), 128.3 (C-Ar), 127.1 (C-Ar),

126.8 (C-Ar), 126.7 (C-Ar), 126.2 (C-Ar), 68.7/65.7 (C-2), 51.7/51.3 (C-4), 35.2 (C-3*), 35.0 (C-1*), 33.6 (C-3), 31.5 (C-1); configuration assigned by NOESY correlations (blue arrow); **HRMS** (ESI^+) *m*/*z* $[M+MeOH+H]^+$ calcd. for $C_{18}H_{21}O_2$ 269.1172, found, 269.1173.

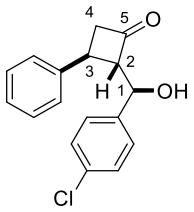
3-Phenylcyclobutan-1-one (**2**.**39**)[133]



The triflic anhydride (11.8 mL, 70.1 mmol) was added dropwise to a solution of DMA (5.6 mL, 60 mmol) in 1,2-DCE (70 mL) at 5 °C. The mixture was stirred at 5 °C for 30 min. A mixture of styrene **2**.**38** (5.8 mL, 50 mmol) and 2,4,6-collidine (9.3 mL, 70 mmol) in 1,2-DCE (10 mL) was then added dropwise. The reaction mixture was heated at reflux for 17 h. The solvent was then removed under reduced pressure and the residue was treated with H_2O/DCM (40 mL, 1:1). The obtained mixture was heated at reflux for 27 h. The reaction was quenched with aq. HCl (5.0 mL, 1.0 M) and extracted with DCM (3 × 5 ml). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 20:1) afforded ketone **2**.**39** (6.21 g, 85%) as a yellow oil.

R_f 0.29 (pentane/ether, 9:1); **^{1}H NMR** (400 MHz, CDCl3) δ 7.44 – 7.27 (m, 5H, H-Ar), 3.75 – 3.67 (m, 1H, H-1), 3.57 – 3.47 (m, 2H, H-2), 3.32 – 3.23 (m, 2H, H-2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.5 (C-3), 143.7 (C-Ar), 128.7 (C-Ar), 126.6 (C-Ar), 126.5 (C-Ar), 54.8 (C-2), 28.5 (C-1).

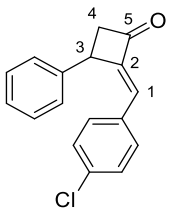
(2*S**,3*S**)-2-((*R**)-(4-Chlorophenyl)(hydroxy)methyl)-3-phenylcyclobutan-1-one (**2.40**)[134]



To a solution of chlorobenzaldehyde (10.3 mg, 0.0682 mmol) and ketone **2.39** (0.200 g, 1.37 mmol) in dry DMSO (1 mL) was added L-proline (0.8 mg, 0.007 mmol). The resulting mixture was stirred at RT for 45 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL) and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 5:1 → 1:1) gave the corresponding aldol adduct **2.40** (12 mg, 59%) as a single diastereomer and as an orange solid.

R_f 0.18 (pentane/ethyl acetate, 10:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 – 7.12 (m, 7H, H-Ar), 7.05 – 6.99 (m, 2H, H-Ar), 4.94 (d, *J* = 6.5 Hz, 1H, H-1), 3.71 – 3.65 (m, 1H, H-2), 3.50 – 3.42 (m, 1H, H-3), 3.32 (ddd, *J* = 17.5, 9.0, 2.0 Hz, 1H, H-4), 3.19 (ddd, *J* = 17.5, 8.5, 2.5 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 207.6 (C-5), 142.1 (C-Ar), 139.4 (C-Ar), 134.1 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 128.1 (C-Ar), 126.9 (C-Ar), 126.6 (C-Ar), 73.5 (C-2), 72.9 (C-1), 52.1 (C-4), 32.8 (C-3).

(*E*)-2-(4-Chlorobenzylidene)-3-phenylcyclobutan-1-one (**2.41**)

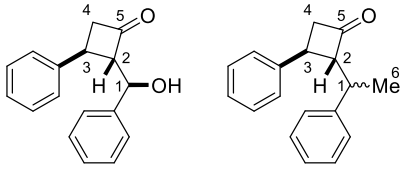


Aldol adduct **2.40** (20.4 mg, 0.0713 mmol), $Pd(OAc)_2$ (1.6 mg, 0.0071 mmol) and KO*t*-Bu (14 µL, 1.0 M solution in THF, 0.014 mmol) were dissolved in chlorobenzene (1 mL). The reaction mixture was purged with hydrogen gas (balloon) for 5 min, before removing the needle from the solution and leaving it in the reaction headspace. The reaction mixture was stirred at RT for 5 h. The mixture was concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 5:1) afforded ketone **2.39** (>11%, yellow oil), and a mixture of *cis*-cyclobutanone **2.37** (~1.2 mg, ~7%) and enone **2.41** (~2.1 mg, ~11%), as a colourless oil.

For *cis*-**2.37** and **2.39** characterisation data, see above.

2.41: $\mathbf{R_f}$ 0.36 (pentane/ether, 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 – 7.11 (m, 9H, H-Ar), 6.78 – 6.74 (m, 1H, H-1), 4.51 (ddd, *J* = 9.0, 5.5, 2.5 Hz, 1H, H-3), 3.71 (dd, *J* = 17.5, 9.0 Hz, 1H, H-4), 3.05 (dd, *J* = 17.5, 5.5 Hz, 1H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 198.9 (C-5), 148.1 (C-Ar), 141.5 (C-Ar), 136.5 (C-Ar), 132.3 (C-Ar), 130.0 (C-Ar), 129.2 (C-Ar × 2), 128.7 (C-Ar), 128.5 (C-Ar), 128.3 (C-Ar), 127.4 (C-Ar), 127.3 (C-Ar), 127.2 (C-Ar × 2), 57.0 (C-4), 41.3 (C-3); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{14}ClO$ 269.0728, found, 269.0724.

(2*S**,3*S**)-2-((*R**)-Hydroxy(phenyl)methyl)-3-phenylcyclobutan-1-one (**2.42**) and a pair of diastereomers, *trans*-3-phenyl-2-(1-phenylethyl)cyclobutan-1-one (**2.43**)

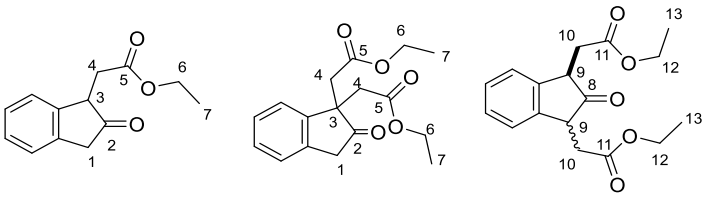


2.42 **2.43**

To a solution of benzaldehyde (13.8 µL, 0.137 mmol) and ketone **2.39** (0.400 g, 2.74 mmol) in dry DMSO (1 mL), was added L-proline (1.6 mg, 0.0137 mmol). The resulting mixture was stirred at RT for 29 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL) and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 2:1) gave aldol adduct **2.43** as a yellow oil and as a pair of diastereomers (23 mg, 68%, *dr* = 45:55, unassigned). $\mathbf{R_f}$ 0.88 (pentane/ether, 1:1); **IR** (film) ν_{max}/cm^{-1}: 3028m, 2968m, 1779s, 1497m, 1454m, 701s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 – 7.01 (m, 18H, H-Ar), 6.99 – 6.89 (m, 2H, H-Ar), 3.72 – 3.64 (m, 0.85H, H-2*), 3.55 – 3.46 (m, 1H, H-2), 3.44 – 3.32 (m, 0.85H, H-3*), 3.30 – 3.03 (m, 7H, H-1, H-3, H-4), 1.45 – 1.37 (m, 6H, H-6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.1/208.8 (C-5), 143.8/143.6 (C-Ar), 143.4/143.2 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 127.9 (C-Ar), 127.8 (C-Ar), 126.8 (C-Ar), 126.8 (C-Ar), 126.8 (C-Ar), 126.6 (C-Ar), 126.5 (C-Ar), 74.6/74.0 (C-2), 52.2/51.5 (C-4), 41.0/40.0 (C-1), 34.7/33.6 (C-3), 20.1/18.9 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{19}O$ 251.1430, found, 251.1427.

Further elution (pentane/ether, 2:1) gave aldol product **2.42** as a yellow oil and as a single diastereomer (5.5 mg, 14%). $\mathbf{R_f}$ 0.43 (pentane/ether, 1:1); **IR** (film) ν_{max}/cm^{-1}: 3471br, 2924s, 1782s, 1455m, 912m, 736s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 9H, H-Ar), 7.01 – 7.00 (m, 1H, H-Ar), 5.04 – 4.97 (m, 1H, H-1), 3.79 – 3.72 (m, 1H, H-2), 3.54 – 3.48 (m, 1H, H-3), 3.42 – 3.33 (m, 1H, H-4), 3.28 – 3.20 (m, 1H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 208.0 (C-5), 142.3 (C-Ar), 140.8 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 128.4 (C-Ar), 126.8 (C-Ar × 2), 126.7 (C-Ar), 73.8 (C-1/2), 73.7 (C-1/2), 51.9 (C-4), 32.8 (C-3); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{17}H_{16}O_2Na$ 275.1043, found, 275.1042.

Ethyl 2-(2-oxo-2,3-dihydro-1*H*-inden-1-yl)acetate (**2.46**),[183] diethyl 2,2'-(2-oxo-2,3-dihydro-1*H*-indene-1,1-diyl)diacetate (**2.47**), and a pair of diastereomers, diethyl 2,2'-((*cis*/*trans*)-2-oxo-2,3-dihydro-1*H*-indene-1,3-diyl)diacetate (**2.48**)



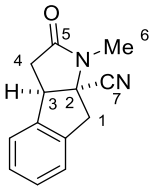
2.46 **2.47** **2.48**

To a solution of LiHMDS (2.4 mL, 1.0 M solution in THF, 2.4 mmol) in dry THF (10 mL) at –78 °C, was added a solution of indan-2-one **2.45** (0.250 g, 1.89 mmol) in dry THF (2 mL) dropwise. After 1 h, the reaction mixture was gradually warmed to –20 °C over 30 min. The mixture was then cooled back to –78 °C and a solution of ethyl bromoacetate (0.30 mL, 2.3 mmol) in dry THF (2 mL) was added dropwise. The mixture was stirred at RT for 17 h. The reaction was quenched with sat. aq. NH_4Cl solution (5 mL) and extracted with ether (3 × 5 mL). The combined organic layers were washed sequentially with water (5 mL) and brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 9:1) gave the targeted compound **2.46** (0.101 g, 24%), as a yellow oil. $\mathbf{R_f}$ 0.22 (pentane/ether, 4:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 – 7.17 (m, 4H, H-Ar), 4.04 – 3.96 (m, 2H, H-6), 3.70 (t, *J* = 5.5 Hz, 1H, H-3), 3.56 (s, 2H, H-1), 2.93 – 2.91 (m, 2H, H-4), 1.09 (t, *J* = 7.0 Hz, 3H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 216.1 (C-2), 171.3 (C-5), 140.8 (C-Ar), 137.4 (C-Ar), 127.9 (C-Ar), 127.7 (C-Ar), 125.0 (C-Ar), 124.1 (C-Ar), 61.0 (C-6), 49.1 (C-3), 43.3 (C-1), 35.6 (C-4), 14.1 (C-7).

Further elution (pentane/ether, 9:1) gave a mixture of dialkylated product **2.47** and dialkylated diastereomers **2.48** (*dr* = 74:26, unassigned) in 61:39 ratio (0.11 g, 34%, yellow oil). $\mathbf{R_f}$ 0.21 (pentane/ether, 4:1); **IR** (film) ν_{max}/cm^{-1}: 2985m, 1754s, 1735s, 1374m, 1185m, 1038m, 750m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.16 (m, 4.57H, H-Ar), 4.19 – 4.06

(m, 1.15H, H-12), 4.04 – 3.96 (m, 0.27H, H-12*), 3.95 – 3.81 (m, 5.65H, H-6, H-9), 3.71 (s, 2H, H-1), 3.06 – 3.00 (m, 0.31H, H-10*), 2.97 (d, *J* = 16.0 Hz, 2H, H-4), 2.88 (dd, *J* = 17.0, 6.5 Hz, 0.88H, H-10), 2.79 (d, *J* = 16.0 Hz, 2H, H-4), 1.20 (t, *J* = 7.0 Hz, 2.78H, H-13), 1.11 (t, *J* = 7.0 Hz, 1.32H, H-13*), 0.99 (t, *J* = 7.0 Hz, 6H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 217.7 (C-2), 216.3 (C-8), 171.6/171.0 (C-11), 169.6 (C-5), 141.9 (C-Ar), 140.5 (C-Ar), 140.1 (C-Ar), 138.8 (C-Ar), 128.1 (C-Ar), 127.9 (C-Ar), 127.3 (C-Ar), 124.7 (C-Ar), 123.9 (C-Ar), 123.7 (C-Ar), 123.2 (C-Ar), 60.85/60.81 (C-12), 60.7 (C-6), 52.4 (C-3), 48.4/48.1 (C-9), 43.8 (C-4), 43.4 (C-1), 36.1/35.1 (C-10), 14.1/14.0 (C-13), 13.8 (C-7); **HRMS** (ESI$^+$) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{21}O_5$ 305.1384, found, 305.1386.

cis-1-Methyl-2-oxo-2,3,3a,8-tetrahydroindeno[2,1-*b*]pyrrole-8a(1*H*)-carbonitrile (**2.49**)

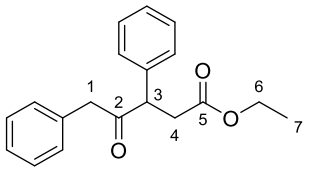


Sodium cyanoborohydride (0.533 g, 8.49 mmol) was added to a solution of keto-ester **2.46** (1.42 g, 6.53 mmol), methylamine (4.02 mL, 4.87 M in absolute ethanol, 19.6 mmol) and glacial acetic acid (0.56 mL, 9.8 mmol) in dry methanol (65 mL). The reaction mixture was heated at reflux for 4 h. The solvent was then removed under reduced pressure. The residue was dissolved in aq. HCl (30 mL, 4.0 M) and extracted with DCM (3 × 30 mL). The combined organic extracts were washed sequentially with water (30 mL) and brine (30 mL), then dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4 → 0:1) gave lactams **1.128** (0.33 g, 27%, yellow oil) and **2.49** (0.50 g, 36%, dark brown oil), as single diastereomers.

For **1.128** characterisation data, see above.

2.49: $\mathbf{R_f}$ 0.26 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2237m, 1704s, 1378m, 1113m, 757m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 2H, H-Ar), 7.24 – 7.19 (m, 2H, H-Ar), 4.26 (dd, *J* = 9.0 Hz, 1.0 Hz, 1H, H-3), 3.53 (s, 2H, H-1), 3.07 (dd, *J* = 17.0, 9.0 Hz, 1H, H-4), 2.94 (s, 3H, H-6), 2.67 (dd, *J* = 17.0, 1.0 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 173.0 (C-5), 141.3 (C-Ar), 137.1 (C-Ar), 128.9 (C-Ar), 128.6 (C-Ar), 125.1 (C-Ar), 124.5 (C-Ar), 119.0 (C-7), 65.2 (C-2), 48.9 (C-3), 41.5 (C-1), 36.0 (C-4), 26.5 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_{13}N_2O$ 213.1022, found, 213.1019.

Ethyl 4-oxo-3,5-diphenylpentanoate (**2.51**)[141, 184]

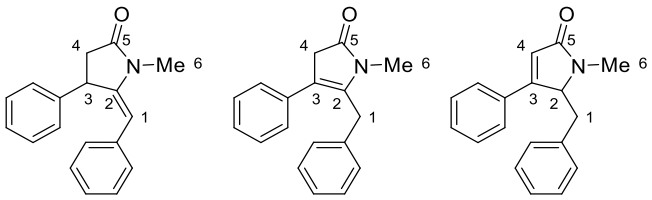


To a solution of LiHMDS (24 mL, 1.0 M solution in THF, 24 mmol) in dry THF (100 mL) at –78 °C, was added a solution of diphenylpropanone (**2.50**, 4.0 g, 19 mmol) in dry THF (20 mL) dropwise. After 1 h, the reaction mixture was gradually warmed to –20 °C over 30 min. The mixture was cooled back to –78 °C and a solution of ethyl bromoacetate (2.5 mL, 23 mmol) in dry THF (20 mL) was added dropwise. The mixture was then stirred at RT for 22 h. The reaction was quenched with sat. aq. NH_4Cl solution (70 mL) and extracted with ether (3 × 70 mL). The combined organic layers were washed with water (70 mL) and brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (**2.51**, 3.65 g, 65%), as a yellow oil, which was used without further purification.

$\mathbf{R_f}$ 0.37 (pentane/ether, 5:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37 – 7.14 (m, 8H, H-Ar), 7.07 – 6.99 (m, 2H, H-Ar), 4.30 (dd, *J* = 9.5, 5.0 Hz, 1H, H-3), 4.12 – 4.03 (m, 2H, H-6), 3.72 (s, 2H, H-1), 3.21 (dd, *J* = 17.0, 9.5 Hz, 1H, H-4), 2.55 (dd, *J* = 17.0, 5.0 Hz, 1H, H-4), 1.18

(t, *J* = 7.0 Hz, 3H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.3 (C-2), 171.9 (C-5), 137.2 (C-Ar), 134.0 (C-Ar), 129.6 (C-Ar), 129.2 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 127.8 (C-Ar), 126.9 (C-Ar), 60.6 (C-6), 53.6 (C-3), 48.3 (C-1), 37.4 (C-4), 14.1 (C-7).

(*E*)-5-Benzylidene-1-methyl-4-phenylpyrrolidin-2-one (**2.53**), 5-benzyl-1-methyl-4-phenyl-1,3-dihydro-2*H*-pyrrol-2-one (**2.54**), and 5-benzyl-1-methyl-4-phenyl-1,5-dihydro-2*H*-pyrrol-2-one (**2.55**)



2.53 **2.54** **2.55**

Sodium cyanoborohydride (25.0 mg, 0.390 mmol) was added to a solution of ketone **2.51** (88.8 mg, 0.300 mmol), methylamine (0.11 mL, 8.03 M solution in absolute ethanol, 0.88 mmol) and glacial acetic acid (26.0 µL, 0.450 mmol) in dry methanol (6 mL). The reaction mixture was heated at reflux for 6 h. When the reaction was complete (TLC), the solvent was removed under reduced pressure. The residue was dissolved in aq. HCl (6.0 mL, 4.0 M) and extracted with DCM (3 × 2 mL). The combined organic extracts were washed sequentially with water (2 mL) and brine (2 mL), then dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave *cis*-lactam **1.126** (3.6 mg, 4%, yellow oil), *trans*-**1.127** (8.9 mg, 10%, yellow oil), **2.53** (9.7 mg, 13%, yellow oil), **2.54** (11 mg, 14%, yellow oil), and **2.55** (32.1 mg, 45%, yellow oil).

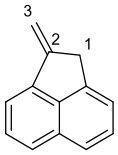
For *cis*-**1.126** and *trans*-**1.127** characterisation data, see above.

2.53: **R_f** 0.75 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 – 7.04 (m, 10H, H-Ar), 5.96 (s, 1H, H-1), 4.50 – 4.43 (m, 1H, H-3), 3.21 (s, 3H, H-6), 3.08 (dd, *J* = 17.5, 9.5 Hz, 1H, H-4), 2.49 (dd, *J* = 17.5, 2.5 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 173.9 (C-5), 145.3 (C-Ar), 142.8 (C-Ar), 135.7 (C-Ar), 129.1 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 127.1 (C-Ar), 126.7 (C-Ar), 126.0 (C-Ar), 105.2 (C-1), 41.2 (C-3), 40.4 (C-4), 27.2 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{18}NO$ 264.1383, found, 264.1379.

2.54: **R_f** 0.63 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 – 7.11 (m, 10H, H-Ar), 3.93 (s, 2H, H-1), 3.46 (s, 2H, H-4), 2.83 (s, 3H, H-6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.5 (C-5), 137.7 (C-Ar), 136.7 (C-Ar), 129.2 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 127.9 (C-Ar), 127.2 (C-Ar), 126.9 (C-Ar), 39.8 (C-4), 31.6 (C-1), 26.9 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{18}NO$ 264.1383, found, 264.1378.

2.55: **R_f** 0.22 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H, H-Ar), 7.40 – 7.37 (m, 2H, H-Ar), 7.16 – 7.12 (m, 2H, H-Ar), 6.80 – 6.76 (m, 2H, H-Ar), 6.17 (s, 1H, H-4), 4.78 (t, *J* = 4.5 Hz, 1H, H-2), 3.23 (dd, *J* = 14.5, 4.5 Hz, 1H, H-1), 3.05 (s, 3H, H-6), 2.93 (dd, *J* = 14.5, 4.5 Hz, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.2 (C-5), 158.4 (C-Ar), 134.9 (C-Ar), 132.3 (C-Ar), 129.9 (C-Ar), 129.2 (C-Ar), 129.1 (C-Ar), 128.2 (C-Ar), 127.0 (C-Ar), 122.1 (C-4), 105.2 (C-3), 64.3 (C-2), 36.7 (C-1), 28.3 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{18}NO$ 264.1383, found, 264.1376.

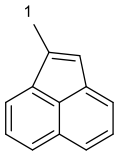
1-Methylene-1,2-dihydroacenaphthylene (**2.56**)



KO*t*-Bu (1.00 g, 8.93 mmol) was added to mixture of methyltripheylphosphonium bromide (3.19 g, 8.93 mmol) in dry ether (12 mL). The resulting mixture was stirred at RT for 1 h, followed by addition of a solution of 1-acenaphthenone **2.14** (1.00 g, 5.95 mmol) in dry THF (3 mL) dropwise. The resulting mixture was stirred at RT for 72 h. The crude reaction mixture was filtered through Celite® and washed with ether (10 mL). The solvent was removed under reduced pressure, and the crude was diluted with pentane (10 mL) before passing it again through a pad of Celite®. Purification by silica gel chromatography (pentane/ether, 100:1) afforded alkene **2.56** (0.317 g, 32%) as a yellow oil, with 57% recovered starting material.

R*f* 0.68 (pentane/ether, 5:1); **IR** (film) ν_{max}/cm^{-1}: 3042m, 2904m, 1717s, 1617m, 1463s, 1361s, 1179m, 880s, 817s, 779s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.83 – 7.68 (m, 3H, H-Ar), 7.66 – 7.56 (m, 2H, H-Ar), 7.39 – 7.36 (m, 1H, H-Ar), 5.97 (t, *J* = 2.0 Hz, 1H, H-3), 5.48 (t, *J* = 2.0 Hz, 1H, H-3), 4.13 – 4.11 (m, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.8 (C-2), 141.6 (C-Ar), 141.5 (C-Ar), 140.3 (C-Ar), 131.6 (C-Ar), 128.0 (C-Ar), 124.5 (C-Ar), 122.6 (C-Ar), 118.9 (C-Ar), 115.9 (C-Ar), one C-Ar resonance not seen, 106.3 (C-3), 37.5 (C-1); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_{11}$ 167.0855, found, 167.0856.

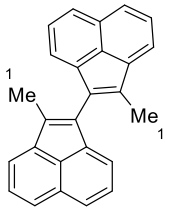
1-Methylacenaphthylene (**2.57**)[147]



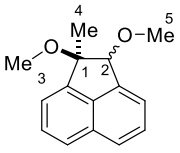
KO*t*-Bu (1.66 g, 14.8 mmol) was added to mixture of methyltripheylphosphonium bromide (5.27 g, 14.8 mmol) in dry ether (12 mL). The resulting mixture was stirred at RT for 1 h, followed by addition of a solution of 1-acenaphthenone **2.14** (0.83 g, 4.9 mmol) in dry THF (4 mL) dropwise. The resulting mixture was stirred at RT for 20 h. The crude reaction mixture was filtered through Celite® and washed with ether (10 mL). The solvent was removed under reduced pressure, and the crude was diluted with pentane (10 mL) before passing it again through a pad of Celite®. Purification by silica gel chromatography (100% pentane) afforded alkene **2.57** (0.21 g, 26%) as a yellow oil.

R_f 0.93 (toluene, 100%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.79 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.71 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.64 – 7.52 (m, 3H, H-Ar), 6.80 (d, *J* = 1.5 Hz, 1H, H-Ar), 2.53 (d, *J* = 1.5 Hz, 3H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 141.1 (C-Ar), 140.4 (C-Ar), 139.9 (C-Ar), 129.1 (C-Ar), 128.0 (C-Ar), 127.9 (C-Ar), 127.5 (C-Ar), 127.2 (C-Ar), 126.0 (C-Ar), 125.4 (C-Ar), 122.5 (C-Ar), 121.8 (C-Ar), 13.2 (C-1).

2,2'-Dimethyl-1,1'-biacenaphthylene (**2.58**) and a pair of diastereomers, 1,2-dimethoxy-1-methyl-1,2-dihydroacenaphthylene (**2.59**)



2.58



2.59

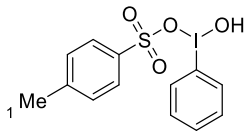
To a solution of alkene **2.57** (100 mg, 0.62 mmol) in dry methanol (3 mL), was added crystalline HTIB **2.61** (120 mg, 0.31 mmol). The reaction mixture was stirred at RT for 3 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL) and extracted with DCM (3

× 1 mL). The combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (100% pentane) yielded dimer **2.58** (0.0037 g, 3%, orangish-yellow oil) and a pair of diastereomers **2.59** (70 mg, 74%, *dr* = 7:3, unassigned, yellow oil), with 34% of recovered starting material.

2.58: $\mathbf{R_f}$ 0.52 (100% pentane); **IR** (film) ν_{max}/cm^{-1}: 2981s, 2886s, 1636m, 1375m, 1264m, 1150m, 1074m, 958m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.0 Hz, 2H, H-Ar), 7.77 – 7.73 (m, 2H, H-Ar), 7.71 (d, *J* = 7.0 Hz, 2H, H-Ar), 7.60 (dd, *J* = 8.0, 7.0 Hz, 2H, H-Ar), 7.49 – 7.45 (m, 4H, H-Ar), 2.41 (s, 6H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 141.8 (C-Ar), 141.3 (C-Ar), 137.3 (C-Ar), 132.6 (C-Ar), 128.9 (C-Ar), 128.1 (C-Ar), 127.9 (C-Ar), 127.8 (C-Ar), 127.1 (C-Ar), 126.3 (C-Ar), 123.2 (C-Ar), 121.6 (C-Ar), 12.6 (C-1); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{26}H_{19}$ 331.1481, found, 331.1483.

A pair of diastereomers **2.59**: $\mathbf{R_f}$ 0.18 (100% pentane); **IR** (film) ν_{max}/cm^{-1}: 2981m, 2825m, 1373m, 1105s, 780s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 2.80H, H-Ar), 7.63 – 7.51 (m, 4.45H, H-Ar), 7.45 (d, *J* = 7.0 Hz, 0.39H, H-Ar*), 7.39 (d, *J* = 7.0 Hz, 1H, H-Ar), 5.21 (s, 1H, H-2), 4.91 (s, 0.41H, H-2*), 3.86 (s, 1.28H, H-3*/5*), 3.78 (s, 3H, H-3/5), 3.22 (s, 3H, H-3/5), 3.08 (s, 1.23H, H-3*/5*), 1.80 (s, 1.45H, H-4*), 1.64 (s, 3H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.6/143.3 (C-Ar), 141.5/140.7 (C-Ar), 136.0/135.9 (C-Ar), 131.2/130.9 (C-Ar), 128.3/128.2 (C-Ar), 128.1/128.0 (C-Ar), 125.0/124.8 (C-Ar), 120.8/120.6 (C-Ar), 120.0/119.6 (C-Ar), 89.8 (C-1), 89.7/87.8 (C-2), 84.6 (C-1*), 59.6/59.5 (C-3/5), 53.7/51.8 (C-3/5), 28.3/22.0 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{15}H_{16}O_2Na$ 251.1043, found, 251.1043.

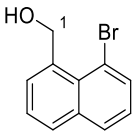
Hydroxy(phenyl)- λ^3-iodaneyl 4-methylbenzenesulfonate (HTIB, **2.61**)[185, 186]



To a solution of *p*-toluenesulfonic acid monohydrate **2.60** (0.61 g, 3.2 mmol) in boiling dry CH_3CN (2 mL) was added, was added (diacetoxyiodo)benzene (1.0 g, 3.1 mmol) in boiling dry CH_3CN (3 mL). The solution was allowed to cool to RT. The crystalline HTIB was then isolated by vacuum filtration and washed with CH_3CN (3 mL) and ether (3 mL) to give the title compound (**2.61**, 0.99 g, 81%) as a yellowish-colourless solid, which was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J* = 7.5 Hz, 2H, H-Ar), 7.71 (t, *J* = 7.0 Hz, 1H, H-Ar), 7.61 (t, *J* = 7.5 Hz, 2H, H-Ar), 7.47 (d, *J* = 8.0 Hz, 2H, H-Ar), 7.11 (d, *J* = 8.5 Hz, 2H, H-Ar), 2.29 (s, 3H, H-1); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 137.1 (C-Ar), 134.5 (C-Ar), 132.4 (C-Ar), 131.2 (C-Ar), 130.7 (C-Ar), 128.1 (C-Ar), 127.7 (C-Ar), 125.5 (C-Ar), 20.8 (C-1).

(8-Bromonaphthalen-1-yl)methanol (**2.63**)[187]

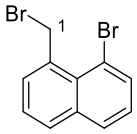


A solution of 8-bromo-1-naphthoic acid **2.62** (0.70 g, 2.8 mmol) in dry THF (4 mL) and trimethyl borate (1.9 mL, 17 mmol) was heated at reflux. After 20 min, $BH_3{\cdot}Me_2S$ (3.4 mL, 2.0 M solution in THF, 6.7 mmol) was added. The mixture was heated further at reflux for 4 h before it was quenched with methanol (5 mL). The residue was diluted with water (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with

brine (5 mL), then dried over $MgSO_4$, and concentrated *in vacuo* to give the title compound (**2.63**, 0.522 g, 79%) as a colourless solid, which was used without further purification.

R_f 0.71 (pentane/ether, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.89 – 7.80 (m, 3H, H-Ar), 7.69 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.47 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.30 – 7.24 (m, 1H, H-Ar), 5.46 (s, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.9 (C-Ar × 2), 133.8 (C-Ar), 130.3 (C-Ar), 130.2 (C-Ar × 2), 129.8 (C-Ar), 126.2 (C-Ar), 126.0 (C-Ar), 118.2 (C-Ar), 65.1 (C-1).

1-Bromo-8-(bromomethyl)naphthalene (**2.64**)[188]

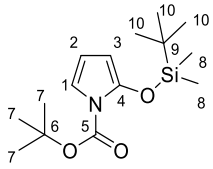


To a solution of bromo-alcohol **2.63** (0.22 g, 0.92 mmol) in dry DCM (10 mL), was added triethylamine (0.13 mL, 0.92 mmol) and mesyl bromide (0.08 mL, 1.0 mmol). The solution was stirred for 23 h at RT. The reaction was quenched with sat. aq. $NaHCO_3$ (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed sequentially with water (5 mL) and brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. The residue was filtered through a short silica plug and washed with eluent (100% pentane) to give the title compound (**2.64**, 0.181 g, 65%) as a yellow oil, which was used without further purification.

R_f 0.78 (pentane/ether, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 7.5, 1.5 Hz, 1H, H-Ar), 7.84 (ddd, *J* = 9.5, 8.0, 1.5 Hz, 2H, H-Ar), 7.63 (dd, *J* = 7.0, 1.5 Hz, 1H, H-Ar), 7.43 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.32 – 7.25 (m, 1H, H-Ar), 5.52 (s, 2H, H-1); **^{13}C NMR**

(101 MHz, $CDCl_3$) δ 136.8 (C-Ar), 134.8 (C-Ar), 134.3 (C-Ar), 132.9 (C-Ar), 131.5 (C-Ar), 129.8 (C-Ar), 129.3 (C-Ar), 126.3 (C-Ar), 125.9 (C-Ar), 118.2 (C-Ar), 35.6 (C-1).

tert-Butyl 2-((*tert*-butyldimethylsilyl)oxy)-1*H*-pyrrole-1-carboxylate (TBSOP, **2.65**)[189]

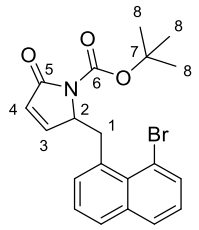


Method A: Crude compound **2.69** (mass not measured) was dissolved in dry DCM (2 mL), followed by adding Celite® (0.12 g), heptane (1.1 mL), and TEA (0.23 mL) sequentially. The reaction mixture was cooled to 0 °C. TBSOTf (0.15 mL, 0.66 mmol) was added dropwise to the reaction mixture and stirred at 0 °C for 1 h. The mixture was quenched with methanol (1 mL). The reaction mixture was then stirred at 0 °C for another 20 min, followed by removing the solvent under reduced pressure. The residue was redissolved in heptane (5 mL) and filtered to remove the Celite®. The filtrate was concentrated *in vacuo* to give TBSOP **2.65** (0.05 g, 28% over five steps) as a yellow oil, which was used without further purification.

Method B: 2,6-Lutidine (0.35 mL, 3.0 mmol) and TBSOTf (0.23 mL, 1.0 mmol) were added to a solution of carboxylate **2.69** (0.18 g, 1 mmol) in dry DCM (2 mL). The mixture was stirred at RT for 1 h. When the reaction was complete (TLC), the mixture was quenched with sat. aq. $NaHCO_3$ (1 mL). The biphasic mixture was stirred vigorously. The resulting reaction mixture was extracted with petroleum ether (3 × 2 mL). The combined organic layers were washed with brine (2 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give TBOSP **2.65** (0.24 g, 79%) as a yellow oil, which was used without further purification.

R$_f$ 0.86 (DCM/methanol, 20:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.69 (dd, *J* = 4.0, 2.0 Hz, 1H, H-1), 5.89 (dd, *J* = 4.0, 3.5 Hz, 1H, H-2), 5.23 (dd, *J* = 3.5, 2.0 Hz, 1H, H-3), 1.56 (s, 9H, H-7), 0.99 (s, 9H, H-10), 0.23 (s, 6H. H-8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 148.4 (C-5), 143.7 (C-4), 113.2 (C-1), 108.2 (C-2), 92.5 (C-3), 82.9 (C-6), 28.2 (C-7), 25.8 (C-10), 18.5 (C-9), -4.7 (C-8).

tert-Butyl 2-((8-bromonaphthalen-1-yl)methyl)-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**2.66**)

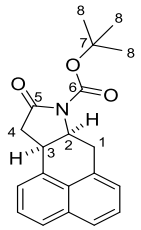


To a solution of TBSOP **2.65** (0.12 g, 0.39 mmol) in dry DCM (2 mL), was added compound **2.64** (0.23 g, 0.78 mmol). The mixture was cooled to 0 ℃. Silver trifluoroacetate (0.17 g, 0.78 mmol) was then added to the mixture. The mixture was stirred at 0 ℃ for 3 h. When the reaction was complete (TLC), the mixture was allowed to warm to RT before being quenched with sat. aq. $NaHCO_3$ (1 mL). The mixture was extracted with DCM (3 × 1 mL) and the combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 15:1→ 5:1) yielded the target adduct **2.66** (0.057 g, 18%) as a yellow oil.

R$_f$ 0.61 (pentane/ethyl acetate, 1:1); **IR** (film) ν_{max}/cm^{-1}: 2961m, 1771m, 1263s, 1105s, 1038s, 812s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.92 – 7.78 (m, 3H, H-Ar), 7.45 – 7.35 (m, 2H, H-Ar), 7.27 (dd, *J* = 8.0, 7.5 Hz, 1H, H-Ar), 6.90 (dd, *J* = 6.0, 2.0 Hz, 1H, H-3), 5.94 (dd, *J* = 6.0, 2.0 Hz, 1H, H-4), 5.15 (tdd *J* = 7.0, 2.0, 2.0 Hz, 1H, H-2), 4.32 (dd, *J* = 14.0, 7.0 Hz,

1H, H-1), 4.05 (dd, *J* = 14.0, 7.0 Hz, 1H, H-1), 1.47 (s, 9H, H-8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.7 (C-3), 136.9 (C-Ar), 134.7 (C-Ar), 133.5 (C-Ar), 132.7 (C-Ar), 130.2 (C-Ar), 129.8 (C-Ar), 126.3 (C-4), 126.1 (C-Ar), 126.0 (C-Ar), 83.2 (C-7), 64.3 (C-2), 38.6 (C-1), 28.2 (C-8) [weak sample, C-Ar × 2 and C=O × 2 not seen]; **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{20}H_{20}O_3NBrNa$ 424.0519, found, 424.0515.

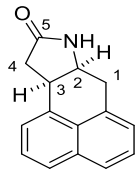
cis-*tert*-Butyl 9-oxo-7a,9,10,10a-tetrahydronaphtho[1,8-*ef*]indole-8(7*H*)-carboxylate (**2.67**)



TEA (0.08 mL, 0.56 mmol) was added to a solution of adduct **2.66** (0.057 g, 0.14 mmol), $Pd(OAc)_2$ (0.006 g, 0.03 mmol), PPh_3 (0.008 g, 0.03 mmol) and formic acid (0.01 mL, 0.3 mmol) in dry THF (3 mL). The mixture was heated at reflux for 4 h. The reaction mixture was filtered on Celite® and concentrated *in vacuo* to give crude lactam **2.67** (0.17 g), as a single diastereomer, which was used without further purification.

R_f 0.13 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2954s, 2926s, 1781m, 1466m, 1261s, 1099m, 810s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 – 7.27 (m, 6H, H-Ar), 4.61 (ddd, *J* = 12.0, 7.0, 5.5 Hz, 1H, H-2), 3.89 (ddd, *J* = 13.0, 8.5, 7.0 Hz, 1H, H-3), 3.62 (dd, *J* = 15.5, 5.5 Hz, 1H, H-1), 3.06 (dd, *J* = 15.5, 12.0 Hz, 1H, H-1), 2.90 (dd, *J* = 17.0, 8.5 Hz, 1H, H-4), 2.64 (dd, *J* = 17.0, 13.0 Hz, 1H, H-4), 1.53 (s, 9H, H-8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.9 (C-5), 150.1 (C-6), 133.6 (C-Ar), 133.3 (C-Ar), 131.7 (C-Ar), 128.4 (C-Ar), 127.4 (C-Ar), 127.0 (C-Ar), 126.1 (C-Ar), 126.0 (C-Ar), 125.8 (C-Ar), 125.2 (C-Ar), 83.4 (C-7), 55.7 (C-2), 40.2 (C-4), 36.4 (C-3), 32.3 (C-1), 28.3 (C-8); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{20}H_{21}NO_3Na$ 346.1414, found, 346.1408.

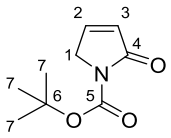
cis-7a,8,10,10a-Tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**2.68**)



To a solution of crude lactam **2.67** (0.17 g, <0.53 mmol) in dry DCM (2 mL), was added TFA (0.72 mL, 9.62 mmol) at 0 °C. The mixture was allowed to warm to RT and stirred at RT for 2 h. The mixture was concentrated *in vacuo* to give the title compound **2.68** (0.226 g), which was used without further purification.

R_f 0.06 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.87 (br s, 1H, NH), 7.80 – 7.36 (m, 6H, H-Ar), 4.32 – 4.23 (m, 1H, H-2), 4.11 – 4.02 (m, 1H, H-3), 3.41 (dd, *J* = 16.0, 5.0 Hz, 1H, H-1), 3.15 – 3.09 (dd, *J* = 16.0, 9.0 Hz, 1H, H-1), 3.03 (dd, *J* = 17.0, 8.5 Hz, 1H, H-4), 2.68 (dd, *J* = 17.0, 9.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 180.1 (C-5), 133.5 (C-Ar), 133.4 (C-Ar), 130.7 (C-Ar), 128.6 (C-Ar), 127.4 (C-Ar), 127.2 (C-Ar), 126.1 (C-Ar), 125.9 (C-Ar), 125.3 (C-Ar), 114.6 (C-Ar), 53.1 (C-2), 38.9 (C-4), 38.6 (C-3), 33.8 (C-1); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{15}H_{14}NO$ 224.1070, found, 224.1066.

tert-Butyl 2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**2.69**)[189]



Method A: To a solution of crude compound **2.74** (0.12 g, <0.6 mmol) in dry THF (2 mL), was added TEA (0.23 mL, 1.7 mmol). The reaction mixture was cooled to 0 °C.

Methanesulfonyl chloride (0.06 mL, 0.8 mmol) was added to the reaction mixture dropwise. The reaction mixture was stirred at 0 °C for 30 min and at RT for 2 h. When the reaction was complete (TLC), ethyl acetate (2 mL) was added to the reaction mixture and stirred for another 30 min. The mixture was poured into water (1 mL) and the resulting solution was extracted with ethyl acetate (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give crude title compound **2.69** (mass not measured), which was used which was used without further purification.

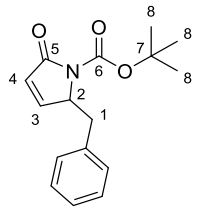
Method B: To a solution of crude residue **2.76** (1.57 g, <18.9 mmol) and di-*tert*-butyl dicarbonate (4.13 g, 18.9 mmol) in dry CH_3CN (9 mL), was added DMAP (0.115 g, 0.945 mmol). The reaction mixture was stirred at RT for 20 h. The solvent was then removed under reduced pressure. The resulting residue was dissolved in ethyl acetate (25 mL). Silica gel (1.89 g) was added, and the resulting mixture was stirred at RT for 20 min. It was then filtered through filter paper and the filtrate was concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 1:1.5) yielded the title compound (**2.69**, 1.45 g, 32% over two steps) as a yellow solid.

Method C: To a solution of TBSOP **2.65** (0.024 g, 0.083 mmol) in dry DCM (1 mL) was added silver trifluoroacetate (0.027 g, 0.12 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h. When the reaction was complete (TLC). The mixture was extracted with DCM (3 × 1 mL). The combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (**2.69**, mass not measured) as a yellow oil, which was used without further purification.

$\mathbf{R_f}$ 0.16 (pentane/ether, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.25 (dt, J = 6.0, 2.0 Hz, 1H, H-2), 6.10 (dt, J = 6.0, 2.0 Hz, 1H, H-3), 4.34 (dd, J = 2.0, 2.0 Hz, 2H, H-1), 1.51 (s, 9H,

H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.9 (C-4), 149.0 (C-5), 145.3 (C-2), 126.8 (C-3), 82.1 (C-6), 51.3 (C-1), 27.5 (C-7).

tert-Butyl 2-benzyl-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**2.70**)[190]

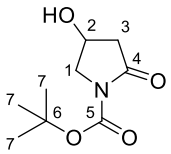


To a solution of TBSOP **2.65** (20 mg, 0.07 mmol) in dry DCM (0.3 mL), was added benzyl bromide **2.31** (16 μL, 0.13 mmol). The mixture was cooled to 0 ℃. Silver trifluoroacetate (29 mg, 0.13 mmol) was added to the mixture and stirred at 0 ℃ for 2 h. When the reaction was complete (TLC), the mixture was warmed to RT before quenching with sat. aq. $NaHCO_3$ (1 mL). The mixture was extracted with DCM (3 × 1 mL). The combined organic layers were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 5:1) yielded the title compound (**2.70**, 4.0 mg, 22%, colourless solid) and side product **2.69** (2.7 mg, 24%, yellow solid).

For **2.69** characterisation data, see above.

2.70: **R_f** 0.64 (pentane/ethyl acetate, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.25 (m, 3H, H-Ar), 7.17 – 7.13 (m, 2H, H-Ar), 7.02 (dd, *J* = 6.0, 2.0 Hz, 1H, H-3), 6.02 (dd, *J* = 6.0, 2.0 Hz, 1H, H-4), 4.75 (dddd, *J* = 9.5, 4.0, 2.0, 2.0 Hz, 1H, H-2), 3.54 (dd, *J* = 13.0, 4.0 Hz, 1H, H-1), 2.74 (dd, *J* = 13.0, 9.5 Hz, 1H, H-1), 1.63 (s, 9H, H-8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.2 (C-5), 150.0 (C-3), 149.6 (C-6), 135.8 (C-Ar), 129.5 (C-Ar), 128.8 (C-Ar), 127.3 (C-Ar), 126.8 (C-4), 83.3 (C-7), 63.5 (C-2), 38.7 (C-1), 28.4 (C-8).

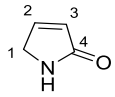
tert-Butyl 4-hydroxy-2-oxopyrrolidine-1-carboxylate (**2.74**)[150]



A solution of acid **2.71** (0.06 g, 0.5 mmol), py (0.30 mL, 3.7 mmol), HMDS (0.17 mL, 0.80 mmol) and *o*-xylenes (0.6 mL) was heated at reflux for 5 h. The reaction mixture was then cooled to RT and concentrated *in vacuo*. The residue was dissolved in dry CH_3CN (0.40 mL) and was cooled to 0 °C. A solution of DMAP (0.003 g, 0.03 mmol) and di-*tert*-butyl dicarbonate (0.13 g, 0.60 mmol) in dry CH_3CN (0.13 mL) was added dropwise to the residue solution. The reaction mixture was stirred for 30 min at 0 °C, followed by stirring at RT for 18 h. Triethylamine trihydrofluoride (33 µL, 0.20 mmol) was then added to the reaction mixture dropwise. The reaction mixture was stirred at RT for 1 h to cleave the TMS group. The stirring speed was slowly increased while heptane (0.97 mL) was added dropwise. Reaction mixture was cooled to 0 °C and stirred for 3 h. The mixture was filtered by vacuum filtration and washed with heptane (1 mL). The solvent was then removed under reduced pressure to give the crude title compound (**2.74**, 0.23 g), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.16 (br s, 1H, OH), 4.22 – 4.17 (m, 1H, H-2), 3.63 (dd, *J* = 11.5, 5.0 Hz, 1H, H-1), 3.55 – 3.46 (m, 1H, H-1), 2.50 (dd, *J* = 17.5, 6.0 Hz, 1H, H-3), 2.25 (dd, *J* = 17.5, 2.5 Hz, 1H, H-3), 1.27 (s, 9H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 173.6 (C-4), 149.8 (C-5), 82.4 (C-6), 62.0 (C-2), 55.2 (C-1), 42.2 (C-3), 27.4 (C-7).

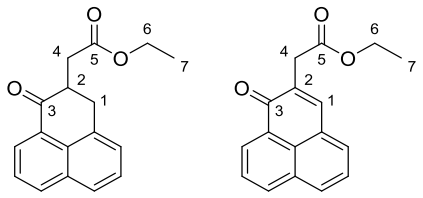
1,5-Dihydro-2*H*-pyrrol-2-one (**2.76**)[191]



To a solution of $BaCO_3$ (0.50 g, 2.5 mmol) and pyrrole **2.75** (1.8 mL, 25 mmol) in water (105 mL), was added 30% hydrogen peroxide (3 mL, 31.9 mmol). The reaction mixture was heated at reflux for 5 h. When the reaction was complete (TLC), the mixture was allowed to cool to RT before adding tiny amount of MnO_2 to quench the remaining hydrogen peroxide (till the effervescence cease). The reaction mixture was then filtered through filter paper and concentrated *in vacuo* to obtain the solid residue. Dioxane (15 mL) was added and stirred vigorously for 10 min and filtered again. The filtrate was dried over $MgSO_4$ and concentrated *in vacuo* to obtain reddish residue **2.76** (1.57 g), which was used without further purification.

R_f 0.05 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.26 (s, 1H, NH), 7.16 (dt *J* = 6.0, 2.0 Hz, 1H, H-2/3), 6.17 (dt, *J* = 6.0, 2.0 Hz 1H, H-2/3), 4.07 (dd, *J* = 2.0, 2.0 Hz, 2H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.2 (C-4), 143.9 (C-2/3), 127.7 (C-2/3), 47.9 (C-1).

Ethyl 2-(1-oxo-2,3-dihydro-1*H*-phenalen-2-yl)acetate (**2.77**) and ethyl 2-(1-oxo-1*H*-phenalen-2-yl)acetate (**2.78**)



2.77 **2.78**

To a solution of LiHMDS (1.4 mL, 1.0 M solution in THF, 1.4 mmol) in dry THF (5 mL) at –78 °C, was added a solution of ketone **2.12** (0.198 g, 1.08 mmol) in dry THF (1 mL)

dropwise. After 1 h, the reaction mixture was gradually warmed to –20 °C over 30 min. The mixture was cooled back to –78 °C and a solution of ethyl bromoacetate (0.20 mL, 1.3 mmol) in dry THF (1 mL) was added dropwise. The mixture was then stirred at RT for 4 d. The reaction was quenched with sat. aq. NH_4Cl solution (7 mL) and extracted with ether (3 × 5 mL). The combined organic layers were washed sequentially with water (5 mL) and brine (5 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 8:1) yielded the keto-ester **2.77** (0.072 g, 25%, yellow oil), enone **2.17** (0.0022 g, 1%, yellow oil) and enone-ester **2.78** (0.013 g, 5%, yellow oil), with 28% of recovered starting material.

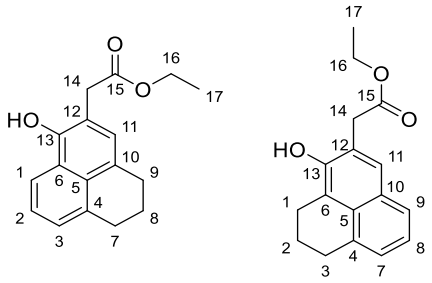
For **2.17** characterisation data, see above.

2.77: $\mathbf{R_f}$ 0.36 (pentane/ether, 4:1); **IR** (film) ν_{max}/cm^{-1}: 2923s , 2854s, 1732s, 1687s, 1464m , 1269m, 825s, 772s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 7.0, 1.5 Hz, 1H, H-Ar), 8.07 (dd, *J* = 8.0, 1.5 Hz, 1H, H-Ar), 7.80 (dt, *J* = 8.0, 1.5 Hz, 1H, H-Ar), 7.59 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.52 – 7.41 (m, 2H, H-Ar), 4.22 (q, *J* = 7.0 Hz, 2H, H-6), 3.52 – 3.44 (m, 2H, H-1, H-2), 3.33 – 3.22 (m, 1H, H-1), 3.13 (dd, *J* = 16.5, 5.5 Hz, 1H, H-4), 2.53 (dd, *J* = 16.5, 6.5 Hz, 1H, H-4), 1.30 (t, *J* = 7.0 Hz, 3H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 198.6 (C-3), 172.4 (C-5), 134.1 (C-Ar), 133.3 (C-Ar), 132.6 (C-Ar), 131.5 (C-Ar), 129.7 (C-Ar), 126.5 (C-Ar), 126.4 (C-Ar), 125.9 (C-Ar), 125.7 (C-Ar), 125.5 (C-Ar), 60.8 (C-6), 44.0 (C-2), 34.7 (C-4), 34.6 (C-1), 14.4 (C-7); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{17}O_3$ 269.1172, found, 269.1173.

2.78: $\mathbf{R_f}$ 0.24 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 2925m, 2854m, 1736s, 1641s, 1573s, 1261m, 1176m, 1023m, 791m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 7.5 Hz, 1H, H-Ar), 8.18 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.99 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.80 – 7.70 (m, 3H, H-Ar, H-1), 7.61 – 7.52 (m, 1H, H-Ar), 4.21 (q, *J* = 7.0 Hz, 2H, H-6), 3.65 (s, 2H, H-4), 1.28

(t, J = 7.0 Hz, 3H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 184.5 (C-3), 171.4 (C-5), 140.5 (C-1), 135.0 (C-Ar), 133.8 (C-2), 132.1 (C-Ar), 131.6 (C-Ar), 131.3 (C-Ar), 130.9 (C-Ar), 129.1 (C-Ar), 127.8 (C-Ar), 127.4 (C-Ar), 127.3 (C-Ar), 126.8 (C-Ar), 61.1 (C-6), 35.9 (C-4), 14.3 (C-7); **HRMS** (ESI^+) m/z $[M+H]^+$ calcd. for $C_{17}H_{15}O_3$ 267.1016, found, 267.1016.

Ethyl 2-(6-hydroxy-2,3-dihydro-1*H*-phenalen-5-yl)acetate (**2.79**) and ethyl 2-(4-hydroxy-2,3-dihydro-1*H*-phenalen-5-yl)acetate (**2.80**)



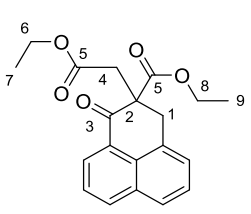
Enone-ester **2.78** (29 mg, 0.11 mmol) and Pd (0.011g, 10% Pd on activated carbon, 0.011 mmol) were dissolved in dry ethyl acetate (2 mL). The reaction mixture was purged with hydrogen gas (balloon) for 5 min. The needle was then removed from the solution and left in the reaction headspace while stirring at RT for 4 h. The crude reaction mixture was filtered through Celite® and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 10:1) yielded isomers **2.79** (8.2 mg, 28%, orange oil) and **2.80** (8.1 mg, 28%, yellow oil).

2.79: **R_f** 0.50 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 3392br, 2933s, 1736s, 1701s, 1401s, 1375s, 1335s, 1240s, 1029m, 767s; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.22 (br s, 1H, OH), 8.18 – 8.13 (m, 1H, H-2), 7.39 (dd, J = 8.5, 7.0 Hz, 1H, H-1), 7.22 (d, J = 7.0 Hz, 1H, H-3), 6.94 (s, 1H, H-11), 4.21 (q, J = 7.0 Hz, 2H, H-16), 3.78 (s, 2H, H-14), 3.07 (t, J = 6.0

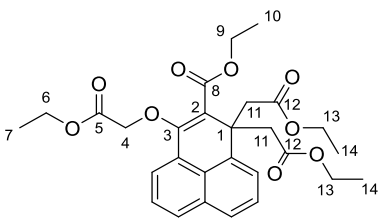
Hz, 2H, H-7), 3.01 (t, *J* = 6.0 Hz, 2H, H-9), 2.07 – 2.02 (m, 2H, H-8), 1.30 (t, *J* = 7.0 Hz, 3H, H-17); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C-15), 149.5 (C-6), 136.1 (C-4), 130.6 (C-5), 128.7 (C-10), 126.3 (C-13), 126.2 (C-11), 125.2 (C-1), 124.5 (C-3), 120.4 (C-2), 113.3 (C-12), 62.3 (C-16), 38.6 (C-14), 31.3 (C-7), 30.8 (C-9), 23.5 (C-8), 14.2 (C-17); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{19}O_3$ 271.1329, found, 271.1327.

2.80: **R_f** 0.41 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 3451m, 2943m, 1715s, 1626m, 1450s, 1335s, 1213s, 1027m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.59 (br s, 1H, OH), 7.54 (d, *J* = 8.0 Hz, 1H, H-9), 7.47 (s, 1H, H-11), 7.22 (dd, *J* = 8.0, 7.0 Hz, 1H, H-8), 7.16 (d, *J* = 7.0 Hz, 1H, H-7), 4.22 (q, *J* = 7.0 Hz, 2H, H-16), 3.84 (s, 2H, H-14), 3.08 (t, *J* = 6.0 Hz, 2H, H-1), 3.04 (t, *J* = 6.0 Hz, 2H, H-3), 2.08 – 2.02 (m, 2H, H-2), 1.30 (t, *J* = 7.0 Hz, 3H, H-17); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.4 (C-15), 148.6 (C-13), 135.4 (C-4), 131.0 (C-5), 129.0 (C-10), 127.5 (C-11), 125.5 (C-9), 124.2 (C-7), 123.6 (C-8), 122.6 (C-12), 121.0 (C-6), 62.1 (C-16), 39.1 (C-14), 30.9 (C-3), 24.5 (C-1), 22.5 (C-2), 14.3 (C-17); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{17}H_{19}O_3$ 271.1329, found, 271.1326.

Ethyl 2-(2-ethoxy-2-oxoethyl)-1-oxo-2,3-dihydro-1*H*-phenalene-2-carboxylate (**2.81**) and diethyl 2,2'-(3-(2-ethoxy-2-oxoethoxy)-2-(ethoxycarbonyl)-1*H*-phenalene-1,1-diyl)diacetate (**2.82**)



2.81



2.82

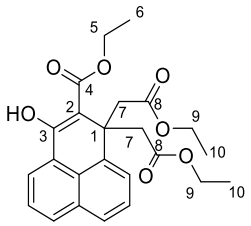
To a solution of mixture of regioisomers **2.15** and **2.16** (0.48 g, 1.9 mmol) in dry DMF (19 mL), was added ethyl bromoacetate (0.25 mL, 2.3 mmol) and potassium carbonate (0.66 g, 4.8 mmol). The reaction mixture was stirred at RT for 2 h. The mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (10 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 10:1 → 100% DCM) yielded keto-ester **2.81** (0.44 g, 69%) and side product **2.82** (0.11 g, 11%) as a yellow oil and colourless oil, respectively.

2.81: $\mathbf{R_f}$ 0.28 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 2987m, 1739s, 1694s, 1249m, 1210m , 772m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.04 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.78 – 7.71 (m, 1H, H-Ar), 7.56 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.48 – 7.40 (m, 2H, H-Ar), 4.16 (q, *J* = 7.0 Hz, 2H, H-8), 3.97 (q, *J* = 7.0 Hz, 2H, H-6), 3.84 (d, *J* = 16.0 Hz, 1H, H-1), 3.67 (d, *J* = 16.0 Hz, 1H, H-1), 3.06 (d, *J* = 16.5 Hz, 1H, H-4), 2.91 (d, *J* = 16.5 Hz, 1H, H-4), 1.24 (t, *J* = 7.0 Hz, 3H, H-9), 0.89 (t, *J* = 7.0 Hz, 3H, H-7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 194.3 (C-3), 170.6 (C-5), 134.2 (C-Ar), 132.9 (C-Ar), 130.7 (C-Ar), 130.6 (C-Ar), 128.9 (C-Ar), 126.5 (C-Ar), 126.5 (C-Ar), 126.3 (C-Ar), 126.2 (C-Ar), 125.9 (C-Ar), 61.5 (C-6), 60.7 (C-8), 56.8 (C-2), 38.2 (C-4), 37.2 (C-1), 14.1 (C-9), 13.6 (C-7); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{20}H_{21}O_5$ 341.1384, found, 341.1378.

2.82: $\mathbf{R_f}$ 0.06 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 2984m, 1727s, 1650m, 1271m, 1186s, 1034m, 831m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.60 – 7.56 (m, 1H, H-Ar), 7.52 – 7.39 (m, 2H, H-Ar), 7.35 – 7.25 (m, 1H, H-Ar), 7.14 (d, *J* = 7.5 Hz, 1H, H-Ar), 4.73 (s, 2H, H-4), 4.46 (q, *J* = 7.0 Hz, 2H, H-6/9), 4.25 (q, *J* = 7.0 Hz, 2H, H-6/9), 3.78 – 3.63 (m, 4H, H-13), 3.52 (d, *J* = 14.5 Hz, 2H, H-11), 2.82 (d, *J* = 14.5 Hz, 2H, H-11), 1.43 (t, *J* = 7.0 Hz, 3H, H-7/10), 1.29 (t, *J* = 7.0 Hz, 3H, H-7/10), 0.71 (t, *J*

= 7.0 Hz, 6H, H-14); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.8 (C-12), 168.6 (C-5), 168.1 (C-8), 157.6 (C-3), 136.7 (C-Ar), 133.1 (C-Ar), 128.4 (C-Ar), 127.1 (C-Ar), 126.9 (C-Ar), 126.2 (C-Ar), 126.1 (C-Ar × 2), 123.4 (C-Ar), 120.6 (C-Ar), 116.5 (C-2), 70.0 (C-4), 61.7 (C-6/9), 61.3 (C-6/9), 60.4 (C-13), 47.2 (C-11), 46.8 (C-1), 14.3 (C-7, -10), 13.5 (C-14); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{28}H_{32}O_9Na$ 535.1939, found, 535.1936.

Diethyl 2,2'-(2-(ethoxycarbonyl)-3-hydroxy-1*H*-phenalene-1,1-diyl)diacetate (**2.83**)



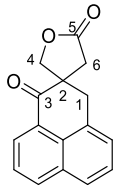
To a solution of mixture of regioisomers **2.15** and **2.16** (0.48 g, 1.9 mmol) in dry DMF (19 mL), was added ethyl bromoacetate (0.25 mL, 2.3 mmol) and lithium carbonate (0.35 g, 4.8 mmol). The reaction mixture was stirred at RT for 3 h. The mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (10 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 10:1 → 1:1 → 100% DCM) yielded keto-esters **2.81** (0.019 g, 3%, yellow oil) and **2.83** (0.061 g, 8%, yellow oil) with 65% of recovered starting material.

For **2.81** characterisation data, see above.

2.83: **R_f** 0.40 (pentane/ether, 2:1); **IR** (film) ν_{max}/cm^{-1}: 2984m, 1737s, 1634m, 1590m, 1415m, 1313m, 1235m, 775m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 14.43 (s, 1H, OH), 8.22 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.67 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.56 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.46 – 7.34 (m, 3H, H-Ar), 4.50 (q, *J* = 7.0 Hz, 2H, H-5), 3.78 (q, *J* = 7.0 Hz, 4H, H-9), 3.37 (d,

J = 15.0 Hz, 2H, H-7), 2.96 (d, *J* = 15.0 Hz, 2H, H-7), 1.50 (t, *J* = 7.0 Hz, 3H, H-6), 0.82 (t, *J* = 7.0 Hz, 6H, H-10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.2 (C-3), 173.1 (C-4), 169.6 (C-8), 135.3 (C-Ar), 133.5 (C-Ar), 127.5 (C-Ar × 2), 126.3 (C-Ar), 125.5 (C-Ar × 2), 122.2 (C-Ar × 2), one C-Ar resonance not seen, 101.1 (C-2), 61.9 (C-5), 60.5 (C-9), 46.8 (C-7), 46.2 (C-1), 14.4 (C-6), 13.7 (C-10); **HRMS** (ESI^+) *m*/*z* $[M+Na]^+$ calcd. for $C_{24}H_{26}O_7Na$ 449.1571, found, 449.1570.

1'*H*,2*H*,3'-Spiro[furan-3,2'-phenalene]-1',5(4*H*)-dione (**2.84**)



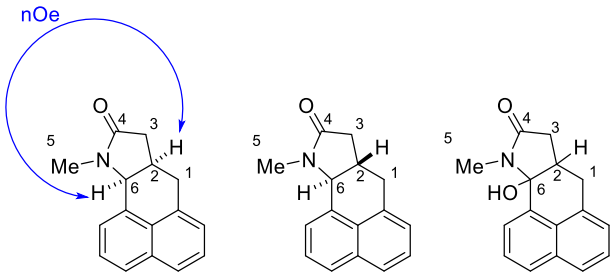
A solution of the keto-ester **2.81** (0.052 g, 0.15 mmol), NaCl (0.018 g, 0.32 mmol), and H_2O (5.7 µL, 0.32 mmol) in DMSO (1 mL) was heated at reflux for 6 h. The reaction was then cooled to RT before diluting with aq. HCl (1 mL, 4.0 M). The reaction mixture was extracted with chloroform (3 × 1 mL). The combined organic layers were washed sequentially with water (5 × 1 mL) and brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ether, 7:1 → 3:1) yielded keto-ester **2.77** (0.017 g, 42%, yellow oil), enone-ester **2.78** (5.5 mg, 14%, yellow oil) and the title compound (**2.84**, 6.4 mg, 17%, yellow oil).

For **2.77** and **2.78** characterisation data, see above.

2.84: $\mathbf{R}_f$ 0.16 (pentane/ether, 3:1); **IR** (film) ν_{max}/cm^{-1}: 1785s, 1689s, 1377m, 1239m, 979m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.30 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.17 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.88 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.68 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.60 – 7.51 (m,

2H, H-Ar), 4.50 (d, *J* = 9.5 Hz, 1H, H-4), 4.18 (d, *J* = 9.5 Hz, 1H, H-4), 3.53 (s, 2H, H-1), 3.28 (d, *J* = 17.5 Hz, 1H, H-6), 2.49 (d, *J* = 17.5 Hz, 1H, H-6); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 195.8 (C-3), 175.0 (C-5), 135.3 (C-Ar), 133.4 (C-Ar), 131.0 (C-Ar), 129.5 (C-Ar), 127.7 (C-Ar), 127.5 (C-Ar × 2), 127.4 (C-Ar), 126.9 (C-Ar), 126.4 (C-Ar), 73.5 (C-4), 51.1 (C-2), 38.8 (C-1), 36.7 (C-6); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{16}H_{13}O_3$ 253.0859, found, 253.0859.

cis-10-Methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**2.85**), *trans*-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**2.86**), and 10a-hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**2.87**)



cis-**2.85** *trans*-**2.86** **2.87**

Sodium cyanoborohydride (21.2 mg, 0.337 mmol) was added to a solution of keto-ester **2.77** (69.6 mg, 0.259 mmol), methylamine (98.0 µL, 8.03 M solution in absolute ethanol, 0.787 mmol) and glacial acetic acid (22.0 µL, 0.389 mmol) in dry methanol (5 mL). The reaction mixture was heated at reflux for 53 h. When the reaction was complete (TLC), the solution was cooled to RT and the solvent was removed under reduced pressure. The residue was dissolved in aq. HCl (4.0 mL, 4.0 M) and extracted with DCM (3 × 2 mL). The combined organic extracts were washed sequentially with water (2 mL) and brine (2 mL), then dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave lactam **2.87** as a greenish-yellow oil and as a single

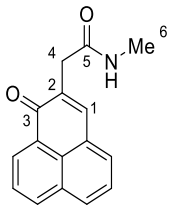
diastereomer (3.6 mg, 5%, unassigned). $\mathbf{R_f}$ 0.64 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2922s, 2851m, 1714s, 1465m, 1359m, 1120m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.94 – 7.91 (m, 1H, H-Ar), 7.82 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.63 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.51 (dd, *J* = 7.5, 7.0 Hz, 1H, H-Ar), 7.38 (d, *J* = 7.0 Hz, 1H, H-Ar), 3.28 – 3.17 (m, 2H, H-1, H-2), 3.08 (dd, *J* = 16.5, 6.0 Hz, 1H, H-3), 3.01 – 2.95 (m, 1H, H-1), 2.88 (s, 3H, H-5), 2.48 (d, *J* = 16.5 Hz, 1H, H-3); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.0 (C-4), 133.7 (C-Ar), 131.2 (C-Ar), 130.8 (C-Ar), 128.9 (C-Ar), 127.9 (C-Ar), 127.2 (C-Ar), 126.7 (C-Ar), 125.8 (C-Ar), 125.5 (C-Ar), 125.2 (C-Ar), 63.7 (C-6), 37.8 (C-3), 37.4 (C-2), 31.9 (C-1), 26.6 (C-5); **HRMS** (APCI) *m/z* $[M-H_2O+H]^+$ calcd. for $C_{16}H_{15}NO_2$ 236.1070, found, 236.1070.

Further elution (pentane/ethyl acetate, 1:1) gave *trans*-lactam **2.86** (16.7 mg, 27%) as a brown oil. $\mathbf{R_f}$ 0.32 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2923m, 2852m, 1698s, 1322m, 1262m, 1017m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 – 7.71 (m, 2H, H-Ar), 7.59 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.47 – 7.39 (m, 2H, H-Ar), 7.36 – 7.33 (m, 1H, H-Ar), 4.50 (d, *J* = 10.5 Hz, 1H, H-6), 3.39 (s, 3H, H-5), 3.34 (dd, *J* = 16.0, 4.0 Hz, 1H, H-1), 3.19 (dd, *J* = 16.0, 12.0 Hz, 1H, H-1), 2.75 (dd, *J* = 15.5, 7.0 Hz, 1H, H-3), 2.52 (ddddd, *J* = 12.0, 12.0, 10.5, 7.0, 4.0 Hz, 1H, H-2), 2.33 (dd, *J* = 15.5, 12.0 Hz, 1H, H-3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.0 (C-4), 136.3 (C-Ar), 134.6 (C-Ar), 134.2 (C-Ar), 129.0 (C-Ar), 127.4 (C-Ar), 126.7 (C-Ar), 125.9 (C-Ar), 125.9 (C-Ar), 125.0 (C-Ar), 118.6 (C-Ar), 66.7 (C-6), 38.3 (C-2), 36.9 (C-3), 35.5 (C-1), 32.6 (C-5); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO$ 238.1226, found, 238.1228.

Further elution (pentane/ethyl acetate, 1:1) gave *cis*-lactam **2.85** (26.2 mg, 42%) as a green oil. $\mathbf{R_f}$ 0.18 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2923m, 2852m, 1688s, 1418m, 1398m, 1309m, 1119m, 800m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.0 Hz, 1H, H-

Ar), 7.76 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.53 (t, *J* = 8.0 Hz, 1H, H-Ar), 7.49 – 7.43 (m, 2H, H-Ar), 7.32 (d, *J* = 6.5 Hz, 1H, H-Ar), 4.77 (d, *J* = 4.5 Hz, 1H, H-6), 3.08 (dd, *J* = 16.1, 5.5 Hz, 1H, H-1), 3.02 – 2.89 (m, 1H, H-1), 2.88 – 2.79 (m, 1H, H-2), 2.79 (s, 3H, H-5), 2.77 – 2.72 (m, 1H, H-3), 2.38 – 2.33 (m, 1H, H-3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.8 (C-4), 133.7 (C-Ar), 133.4 (C-Ar), 129.4 (C-Ar), 129.3 (C-Ar), 129.1 (C-Ar), 128.8 (C-Ar), 126.5 (C-Ar), 126.2 (C-Ar), 125.3 (C-Ar), 124.9 (C-Ar), 61.5 (C-6), 39.3 (C-3), 32.2 (C-1), 31.6 (C-2), 27.8 (C-5); configuration assigned by NOESY correlations (blue arrow); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO$ 238.1226, found, 238.1227.

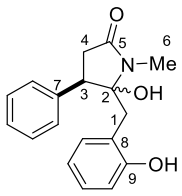
N-Methyl-2-(1-oxo-1*H*-phenalen-2-yl)acetamide (**2.88**)



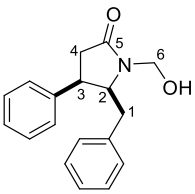
Enone-amide **2.88** was obtained in an attempt to prepare lactam **2.85**. Sodium cyanoborohydride (2.0 mg, 0.037 mmol) was added to a solution of enone-ester **2.78** (5.0 mg, 0.019 mmol), methylamine (0.08 mL, 4.87 M solution in absolute ethanol, 0.39 mmol) and glacial acetic acid (0.01 mL, 0.2 mmol) in dry methanol (0.20 mL). The reaction mixture was stirred at reflux for 43 h. When the reaction was complete (TLC), the solvent was removed under reduced pressure. The residue was dissolved in aq. HCl (0.1 mL, 4.0 M) and extracted with DCM (3 × 1 mL). The combined organic extracts were washed sequentially with water (1 mL) and brine (1 mL), then dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (100% ethyl acetate) gave enone-amide **2.88** (1.3 mg, 28%) as a yellow oil, with 60% recovered starting material.

R_f 0.11 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2956s, 2926s, 2855s, 1717m, 1665m, 1465m, 1378m; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.68 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.25 (d, *J* = 8.0 Hz, 1H, H-Ar), 8.04 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.90 (s, 1H, H-1), 7.85 – 7.77 (m, 2H, H-Ar), 7.62 (dd, *J* = 8.5, 7.0 Hz, 1H, H-Ar), 6.65 (br s, 1H, NH), 3.58 (s, 2H, H-4), 2.77 (d, *J* = 5.0 Hz, 3H, H-6); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 185.9 (C-3), 171.4 (C-5), 141.5 (C-1), 135.7 (C-Ar), 134.1 (C-2), 132.3 (C-Ar), 132.2 (C-Ar), 132.1 (C-Ar), 131.4 (C-Ar), 129.1 (C-Ar), 127.7 (C-Ar), 127.4 (C-Ar), 127.2 (C-Ar), 127.1 (C-Ar), 39.1 (C-4), 26.7 (C-6); **HRMS** (ESI^+) *m/z* $[M+H]^+$ calcd. for $C_{16}H_{14}NO_2$ 252.1019, found, 252.1016.

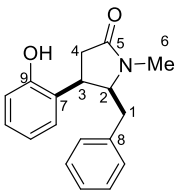
A pair of diastereomers, (4*R**)-5-hydroxy-5-(2-hydroxybenzyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.1a**/**b**), *cis*-5-benzyl-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.2**), *cis*-5-benzyl-4-(2-hydroxyphenyl)-1-methylpyrrolidin-2-one (**3.6**), and a pair of diastereomers, (4*R**)-5-(2,4-dihydroxybenzyl)-5-hydroxy-1-methyl-4-phenylpyrrolidin-2-one (**3.7a/b**)



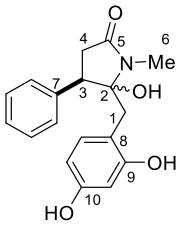
3.1a/b



3.2



3.6



3.7a/b

To a solution of lactam **1.126** (93.4 mg in 1.80 mL MeOH, 0.35 mmol), glucose (35.0 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (3.5 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (3.5 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 300 mL), was added $P450_{BM3}$ variant GVQ/A328G (8.3 mL of a 85 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 6 h at 20 °C. Upon completion, the mixture was

extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave lactam **2.53** (1.5 mg, 2%) as a yellow oil.

For **2.53** characterisation data, see above.

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactam diastereomers **3.1a** and **3.1b** (7.2 mg, 7%, *dr* = 56:44, unassigned) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 1712s, 1481s, 1385s, 1279s, 990m, 870m, 754m; **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_3$ 298.1438, found, 298.1434.

Major diastereomer **3.1a**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 3H, H-Ar), 7.25 – 7.17 (m, 1H, H-Ar), 7.16 – 7.13 (m, 2H, H-Ar), 7.03 (t, *J* = 8.0 Hz, 1H, H-Ar), 6.85 (t, *J* = 8.0 Hz, 1H, H-Ar), 6.81 (d, *J* = 8.0 Hz, 1H, H-Ar), 3.78 (dd, *J* = 9.0, 3.5 Hz, 1H, H-3), 3.16 (dd, *J* = 17.0, 9.0 Hz, 1H, H-4), 3.00 (d, *J* = 17.5 Hz, 1H, H-1), 2.88 (d, *J* = 17.5 Hz, 1H, H-1), 2.74 (s, 3H, H-6), 2.64 (dd, *J* = 17.0, 3.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.9 (C-5), 158.0 (C-9), 140.2 (C-7), 129.2 (C-Ar), 128.7 (C-Ar), 128.4 (C-Ar), 127.9 (C-Ar × 2), 124.7 (C-8), 124.5 (C-Ar), 121.2 (C-Ar), 108.9 (C-Ar), one C-Ar resonance not seen, 107.1 (C-2), 48.7 (C-3), 37.3 (C-4), 34.5 (C-1), 25.4 (C-6).

Minor diastereomer **3.1b**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 3H, H-Ar), 7.25 – 7.17 (m, 1H, H-Ar), 7.16 – 7.13 (m, 1H, H-Ar), 7.04 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.00 (t, *J* = 8.0 Hz, 1H, H-Ar), 6.75 (t, *J* = 8.0 Hz, 1H, H-Ar), 6.60 (d, *J* = 8.0 Hz, 1H, H-Ar), 3.54 (dd, *J* = 11.0, 8.5 Hz, 1H, H-3), 3.44 (d, *J* = 17.0 Hz, 1H, H-1), 3.39 (d, *J* = 17.0 Hz, 1H, H-1), 2.97 (dd, *J* = 17.0, 11.0 Hz, 1H, H-4), 2.80 (dd, *J* = 17.0, 8.5 Hz, 1H, H-4), 2.77 (s, 3H, H-6); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.7 (C-5), 158.5 (C-9), 135.2 (C-7), 129.8 (C-Ar), 129.4 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 127.9 (C-Ar), 127.5 (C-Ar), 124.3 (C-Ar), 124.2

(C-8), 120.8 (C-Ar), 108.9 (C-Ar), 104.2 (C-2), 50.4 (C-3), 36.8 (C-4), 36.4 (C-1), 25.4 (C-6).

Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactam diastereomers **3.7a** and **3.7b** (2.2 mg, 2%, *dr* = 57:43, unassigned) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2880m, 1685s, 1496s, 1476s, 1396s, 1206s, 994m, 738m; **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_4$ 314.1387, not found.

Major diastereomer **3.7a**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 3H, H-Ar), 7.15 – 7.12 (m, 2H, H-Ar), 6.66 (d, *J* = 8.5 Hz, 1H, H-Ar), 6.61 (dd, *J* = 8.5, 2.5 Hz, 1H, H-Ar), 6.55 (d, *J* = 2.5 Hz, 1H, H-Ar), 4.63 (br s, 1H, OH), 3.77 (dd, *J* = 8.5, 3.5 Hz, 1H, H-3), 3.13 (dd, *J* = 17.5, 8.5 Hz, 1H, H-4), 2.96 (d, *J* = 17.0 Hz, 1H, H-1), 2.82 (d, *J* = 17.0 Hz, 1H, H-1), 2.75 (s, 3H, H-6), 2.63 (dd, *J* = 17.5, 3.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.9 (C-5), 152.2 (C-9), 150.2 (C-10), 140.2 (C-7), 129.2 (C-Ar), 127.9 (C-Ar), 127.8 (C-Ar), 125.8 (C-8), 114.9 (C-Ar), 111.9 (C-Ar), 109.0 (C-Ar), 107.2 (C-2), 48.7 (C-3), 37.1 (C-4), 34.9 (C-1), 25.4 (C-6).

Minor diastereomer **3.7b**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.36 – 7.26 (m, 3H, H-Ar), 7.25 – 7.18 (m, 2H, H-Ar), 6.57 (d, *J* = 2.5 Hz, 1H, H-Ar), 6.45 (dd, *J* = 8.5, 2.5 Hz, 1H, H-Ar), 6.43 (d, *J* = 8.5 Hz, 1H, H-Ar), 4.52 (br s, 1H, OH), 3.50 (dd, *J* = 11.0, 8.0 Hz, 1H, H-3), 3.41 (d, *J* = 17.0 Hz, 1H, H-1), 3.35 (d, *J* = 17.0 Hz, 1H, H-1), 2.95 – 2.91 (m, 1H, H-4), 2.80 – 2.76 (m, 4H, H-4, H-6); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.8 (C-5), 152.7 (C-9), 149.8 (C-10), 135.2 (C-7), 129.8 (C-Ar), 128.4 (C-Ar), 127.9 (C-Ar), 125.3 (C-8), 114.9 (C-Ar), 111.6 (C-Ar), 108.9 (C-Ar), 104.3 (C-2), 50.4 (C-3), 36.9 (C-1/4), 36.8 (C-1/4), 25.5 (C-6).

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.3** (1.9 mg, 2%) as a yellow oil.

For **3.3** characterisation data, see above.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.6** (8.3 mg, 8%) as a yellow oil. **^{1}H NMR** (700 MHz, $CDCl_3$) [only selected peaks reported] δ 6.88 (t, *J* = 7.5 Hz, 1H, H-Ar), 6.83 (d, *J* = 7.5 Hz, 2H, H-Ar), 6.62 (d, *J* = 7.5 Hz, 1H, H-Ar), 4.30 (ddd, *J* = 8.0, 8.0, 5.0 Hz, 1H, H-2), 3.95 (ddd, *J* = 12.0, 8.0, 8.0 Hz, 1H, H-3), 2.75 – 2.71 (m, 1H, H-4), 2.53 (s, 3H, H-6), 2.46 – 2.39 (m, 3H, H-1, -4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ l74.2 (C-5), 154.7 (C-9), 138.6 (C-8), 129.3 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar × 2), 127.6 (C-Ar), 127.5 (C-Ar), 126.4 (C-7), 120.5 (C-Ar), 115.3 (C-Ar), one C-Ar resonance not seen, 64.8 (C-2), 38.6 (C-3), 36.6 (C-1), 33.4 (C-4), 29.8 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1485.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **1.17** (5.1 mg, 5%) as a yellow oil and as a single diastereomer. **$[\alpha]_D^{25}$** –66.0 (*c* 0.51, DCM) {lit.,[159] **$[\alpha]_D^{20}$** –139.2 (*c* 0.50, MeOH).

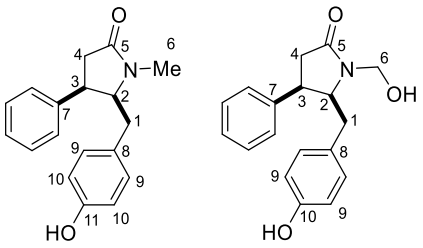
For **1.17** characterisation data, see above.

Further elution (pentane/ethyl acetate, 1:4) gave lactam **3.2** (5.9 mg, 6%) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2886m, 1698s, 1454s, 1045m, 755s, 701s; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H, H-Ar), 7.22 – 7.16 (m, 4H, H-Ar), 6.94 – 6.92 (m, 1H, H-Ar), 4.90 (d, *J* = 11.0 Hz, 1H, H-6), 4.40 (ddd, *J* = 8.5, 7.5, 5.5 Hz, 1H, H-2), 4.00 (d, *J* = 11.0 Hz, 1H, H-6), 3.80 (ddd, *J* = 10.0, 7.5, 7.5 Hz, 1H, H-3), 2.81 (dd, *J* = 16.5, 10.0 Hz, 1H, H-4), 2.63 (dd, *J* = 16.5, 7.5 Hz, 1H, H-4), 2.56 (dd, *J* = 14.0, 8.5 Hz, 1H, H-1), 2.47 (dd, *J* = 14.0, 5.5 Hz, 1H, H-1); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.0 (C-5), 138.2 (C-Ar), 137.7 (C-Ar), 129.2 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 128.2 (C-Ar), 127.5 (C-Ar), 126.9 (C-Ar), 66.6 (C-6), 62.9 (C-2), 43.3 (C-3), 36.8 (C-1), 35.3 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1491.

Further elution (100% ethyl acetate) gave lactams **3.4** (7.4 mg, 7%, yellow oil) and **3.5** (1.4 mg, 1%, yellow oil).

For **3.4** and **3.5** characterisation data, see above.

cis-5-(4-Hydroxybenzyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.8**) and *cis*-5-(4-hydroxybenzyl)-1-(hydroxymethyl)-4-phenylpyrrolidin-2-one (**3.9**)



3.8 **3.9**

To a solution of lactam **1.126** (19.0 mg in 0.36 mL MeOH, 0.072 mmol), glucose (7.2 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (715 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (715 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 57 mL), was added $P450_{BM3}$ variant K19/F87V (5 mL of a 16 µM solution in phosphate buffer, pH 7.9 + 1 mL of a 63 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 6 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 3:1) gave lactams **2.53** (1.0 mg, 2%) as a yellow oil.

For **2.53** characterisation data, see above.

Further elution (pentane/ethyl acetate, 2:1) gave a mixture of lactams **3.2** and **3.6** (5.2 mg, 26%) in 1:2.5 ratio, as a yellow oil.

For **3.2** and **3.6** characterisation data, see above.

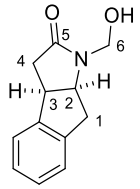
Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.8** (5.9 mg, 26%) as a yellow oil. **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.40 – 7.28 (m, 4H, H-Ar), 7.23 – 7.12 (m, 3H, H-Ar), 6.69 – 6.63 (m, 2H, H-9), 4.09 – 4.05 (m, 1H, H-2), 3.78 – 3.74 (m, 1H, H-3), 2.70 (s, 3H, H-6), 2.66 – 2.56 (m, 2H, H-4), 2.52 – 2.46 (m, 2H, H-1); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 174.7 (C-5), 154.6 (C-10), 138.3 (C-7), 130.4 (C-Ar), 128.7 (C-Ar), 128.3 (C-Ar), one C-Ar resonance not seen, 127.3 (C-8), 115.4 (C-9), 65.6 (C-2), 42.7 (C-3), 35.1 (C-4), 34.9 (C-1), 29.9 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1485.

Further elution (pentane/ethyl acetate, 1:1) gave lactam **3.4** and **3.9** (2.0 mg, 9%) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2883m, 1672s, 1516m, 1455m, 1251m, 702m.

For **3.4** characterisation data, see above.

3.9: **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.20 – 7.13 (m, 5H, H-Ar), 6.79 – 6.76 (m, 2H, H-Ar), 6.68 (d, *J* = 8.0 Hz, 2H, H-9), 4.93 – 4.89 (m, 1H, H-6), 4.37 – 4.31 (m, 1H, H-2), 4.10 – 4.06 (m, 1H, H-6), 3.82 – 3.77 (m, 1H, H-3), 2.78 – 2.74 (m, 1H, H-4), 2.67 – 2.62 (m, 1H, H-4), 2.50 – 2.45 (m, 1H, H-1), 2.44 – 2.40 (m, 1H, H-1); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 176.1 (C-5), 154.5 (C-10), 138.2 (C-7), 130.4 (C-Ar × 2), 128.8 (C-Ar), 128.2 (C-Ar), 127.5 (C-8), 115.6 (C-9), 66.7 (C-6), 63.0 (C-2), 43.3 (C-3), 35.9 (C-1), 35.2 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{20}NO_3$ 298.1438, found, 298.1435.

cis-1-(Hydroxymethyl)-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.22**)

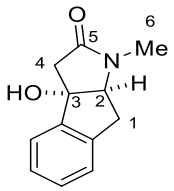


To a solution of lactam **1.128** (9.4 mg in 250 µL MeOH, 0.05 mmol), glucose (5.0 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (500 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (500 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 38 mL), was added $P450_{BM3}$ variant R19/A328L/I263A (10 mL of a 100 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 20 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (DCM/methanol, 100:1) gave a mixture of lactams **2.28** and **3.22** (5.9 mg, 62%) in 1:3.5 ratio, as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2882s, 1687m, 1418m, 1312m, 1236m, 755m.

For **2.28** characterisation data, see above.

3.22: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.26 – 7.20 (m, 4H, H-Ar), 4.96 (d, *J* = 11.0 Hz, 1H, H-6), 4.75 (d, *J* = 11.0 Hz, 1H, H-6), 4.64 (ddd, *J* = 6.5, 4.0, 4.0 Hz, 1H, H-2), 3.90 (ddd, *J* = 9.0, 6.5, 2.0 Hz, 1H, H-3), 3.23 (d, *J* = 4.0 Hz, 2H, H-1), 2.88 (dd, *J* = 17.0, 9.0 Hz, 1H, H-4), 2.51 (dd, *J* = 17.0, 2.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) [only selected peaks reported based on HSQC] δ 128.7 (C-Ar), 126.0 (C-Ar), 67.1 (C-6), 62.7 (C-2), 43.1 (C-3), 38.1 (C-1), 37.6 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{13}NO_2Na$ 226.0839, found, 226.0836.

(3a*S**,8a*S**)-3a-Hydroxy-1-methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.23**)

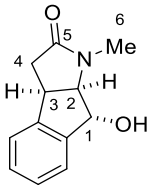


To a solution of lactam **1.128** (70.0 mg in 1.88 mL MeOH, 0.38 mmol), glucose (75 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (7.5 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (7.5 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 266 mL), was added $P450_{BM3}$ variant RP/A82M/I263A (17.2 mL of a 87 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 24 h at 20 °C. An additional amount of variant and cofactors (6 mL of 84 µM variant, 4 mL of $NADP^+$, 4 mL of GDH) was added at 19 h to bring reaction to completion. The mixture was then extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (DCM/methanol, 25:1) gave lactams **3.22** (1.3 mg, 2%, yellow oil), **3.23** (17.5 mg, 23%, colourless oil), and **3.24** (17.7 mg, 23%, colourless oil, single diastereomer, $[\alpha]_D^{25}$ +99.3 (*c* 0.06, DCM)).

For **3.22** and **3.24** characterisation data, see above and below, respectively.

3.23: **IR** (film) ν_{max}/cm^{-1}: 2884s, 2807m, 1671s, 1484m, 1419m 1283m, 1058m; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44 – 7.41 (m, 1H, H-Ar), 7.33 – 7.29 (m, 2H, H-Ar), 7.24 – 7.20 (m, 1H, H-Ar), 4.13 (dd, *J* = 7.0, 2.5 Hz, 1H, H-2), 3.40 – 3.31 (m, 1H, H-1), 2.97 – 2.84 (m, 6H, H-1, H-4, H-6); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.3 (C-5), 144.6 (C-Ar), 140.2 (C-Ar), 130.0 (C-Ar), 128.3 (C-Ar), 125.8 (C-Ar), 124.0 (C-Ar), 84.2 (C-3), 72.0 (C-2), 44.6 (C-1), 34.8 (C-4), 28.2 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO_2$ 204.1019, found, 204.1017.

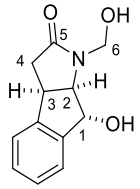
(3a*R**,8*R**,8a*R**)-8-Hydroxy-1-methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.24**)



To a solution of lactam **1.128** (27.5 mg in 740 µL MeOH, 0.15 mmol), glucose (29.6 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (3.0 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (3.0 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 101 mL), was added $P450_{BM3}$ variant RP/A82M/I263A/L181F/A328G/A330L/Q359A (10.5 mL of a 37 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 24 h at 20 °C. An additional amount of variant and cofactors (6 mL of 37 µM variant, 1.5 mL of $NADP^+$, 1.5 mL of GDH) was added at 20 h to bring reaction to completion. The mixture was then extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (DCM/methanol, 25:1) gave lactam **3.24** (6.1 mg, 20%) as a single diastereomer and as a colourless oil.

IR (film) ν_{max}/cm^{-1}: 2924m, 1670s, 1483m, 1253m, 1051m, 767m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.38 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.33 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.27 (d, *J* = 7.5 Hz, 1H, H-Ar), 5.18 (s, 1H, H-1), 4.16 (d, *J* = 7.0 Hz, 1H, H-2), 4.07 – 4.00 (m, 1H, H-3), 2.95 (s, 3H, H-6), 2.88 (dd, *J* = 17.5, 10.0 Hz, 1H, H-4), 2.44 (dd, *J* = 17.5, 3.0 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 173.7 (C-5), 145.3 (C-Ar), 141.9 (C-Ar), 130.4 (C-Ar), 128.6 (C-Ar), 125.8 (C-Ar), 125.2 (C-Ar), 77.5 (C-1), 73.2 (C-2), 39.8 (C-3), 37.2 (C-4), 28.8 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO_2$ 204.1019, found, 204.1016.

(3a*R**,8*R**,8a*R**)-8-Hydroxy-1-(hydroxymethyl)-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.25**)



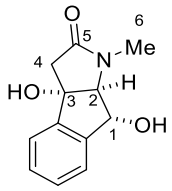
To a solution of lactam **1.128** (29.0 mg in 780 µL MeOH, 0.16 mmol), glucose (31.2 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (3.1 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (3.1 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 99 mL), was added $P450_{BM3}$ variant RP/A82M/I263A/L181F/A328G/A330L (18.4 mL of a 17 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 30 h at 20 °C. An additional amount of variant and cofactors (21.0 mL of 37 µM variant, 1.5 mL of $NADP^+$, 1.5 mL of GDH) was added at 22 h to bring reaction to completion. The mixture was then extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (DCM/methanol, 25:1) gave lactams **3.24** (6.5 mg, 20%, colourless oil, $[\alpha]_D^{25}$ –10.4 (*c* 0.46, DCM)) and **3.25** (1.1 mg, 3%, colourless oil) as single diastereomers.

For **3.24** characterisation data, see above.

3.25: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.38 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.34 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.28 (d, *J* = 7.5 Hz, 1H, H-Ar), 5.20 (s, 1H, H-1), 5.05 (d, *J* = 11.0 Hz, 1H, H-6), 4.88 (d, *J* = 11.0 Hz, 1H, H-6), 4.40 (d, *J* = 7.0 Hz, 1H, H-2), 4.07 (ddd, *J* = 10.0, 7.0, 3.5 Hz, 1H, H-3), 2.91 (dd, *J* = 17.5, 10.0 Hz, 1H, H-4), 2.48 (dd, *J* = 17.5, 3.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 175.2 (C-5), 144.5 (C-Ar), 141.8 (C-Ar), 130.2 (C-Ar), 128.6 (C-Ar), 125.8 (C-Ar), 125.1 (C-Ar), 79.0 (C-1), 71.2 (C-

2), 66.7 (C-6), 40.2 (C-3), 37.2 (C-4); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO_3$ 220.0968, found, 220.0971.

(8*R**,8a*S**)-3a,8-Dihydroxy-1-methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.26**)



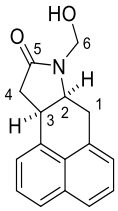
To a solution of lactam **1.128** (29.0 mg in 780 µL MeOH, 0.16 mmol), glucose (31.2 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (3.1 mL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (3.1 mL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 109 mL), was added $P450_{BM3}$ variant RP/A82M/I263A/L181F/A328G/A330L/Q359A (8.4 mL of a 37 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 28 h at 20 °C. An additional amount of variant and cofactors (4.0 mL of 37 µM variant, 1.6 mL of $NADP^+$, 1.6 mL of GDH) was added at 25 h to bring reaction to completion. The mixture was then extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (DCM/methanol, 25:1) gave lactam **3.24** (6.1 mg, 10%, colourless oil, single diastereomer), and a mixture of lactams **3.25** and **3.26** (1.1 mg, 3%, colourless oil, single diastereomers) in 1:1 ratio.

For **3.24** and **3.25** characterisation data, see above.

3.26: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.47 – 7.30 (m, 4H, H-Ar), 4.96 (d, *J* = 2.0 Hz, 1H, H-1), 3.96 (d, *J* = 2.0 Hz, 1H, H-2), 3.01 (s, 3H, H-6), 2.93 (d, *J* = 17.5 Hz, 1H, H-4), 2.76 (d,

J = 17.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.0 (C-5), 144.8 (C-Ar), 141.6 (C-Ar), 130.7 (C-Ar), 130.5 (C-Ar), 125.8 (C-Ar), 124.2 (C-Ar), 81.8 (C-3), 81.3 (C-2), 76.3 (C-1), 44.6 (C-4), 28.7 (C-6).

cis-8-(Hydroxymethyl)-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**3.27**)

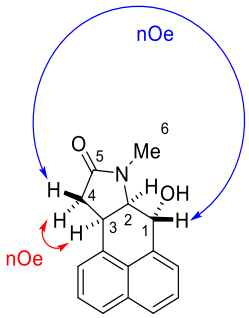


To a solution of lactam **1.129** (5.0 mg in 105 µL MeOH, 0.021 mmol), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (210 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (210 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 18 mL), was added $P450_{BM3}$ variant K19/F87V/E267V/V78I (1.0 mL of a 67 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 3 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) gave lactam **3.27** (3.9 mg, 73%) as a yellow oil.

IR (film) ν_{max}/cm^{-1}: 2942s, 2862m, 1685s, 1438s, 725s; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.76 (t, *J* = 8.5 Hz, 2H, H-Ar), 7.50 – 7.41 (m, 2H, H-Ar), 7.39 – 7.35 (m, 1H, H-Ar), 7.31 (d, *J* = 6.5 Hz, 1H, H-Ar), 4.99 (d, *J* = 11.0 Hz, 1H, H-6), 4.93 (d, *J* = 11.0 Hz, 1H, H-6), 4.29 – 4.24 (m, 1H, H-2), 3.99 – 3.92 (m, 1H, H-3), 3.55 (dd, *J* = 16.0, 5.0 Hz, 1H, H-1), 3.14 (dd, *J* = 16.0, 5.5 Hz, 1H, H-1), 2.96 (dd, *J* = 16.5, 8.5 Hz, 1H, H-4), 2.65 (dd, *J* = 16.5, 9.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 175.4 (C-5), 134.1 (C-Ar), 133.0 (C-Ar), 131.3

(C-Ar), 128.8 (C-Ar), 127.1 (C-Ar), 127.0 (C-Ar), 126.1 (C-Ar × 2), 125.8 (C-Ar), 125.1 (C-Ar), 66.2 (C-6), 56.0 (C-2), 39.4 (C-4), 37.3 (C-3), 33.0 (C-1); **HRMS** (ESI^+) *m*/*z* [M-+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1172.

(7*R**,7a*R**,10a*R**)-7-Hydroxy-8-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**3.28**)

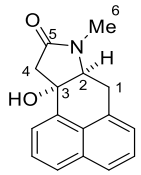


To a solution of lactam **1.129** (5.0 mg in 105 µL MeOH, 0.021 mmol), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (210 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (210 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 18 mL), was added $P450_{BM3}$ variant KSK19/A82M (0.29 mL of a 145 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 2 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) gave lactam **3.28** (3.7 mg, 70%) as a single diastereomer and as a yellow oil.

IR (film) ν_{max}/cm^{-1}: 2945m, 1748s, 1671m, 1240s, 674m; $[\alpha]_D^{25}$ +4.0 (*c* 0.37, DCM); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.79 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.67 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.54 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.51 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.39 (d, *J* = 7.0 Hz, 1H, H-Ar), 5.06 (d, *J* = 7.5 Hz, 1H, H-1), 4.03 (ddd, *J* =

9.0, 8.5, 7.5 Hz, 1H, H-3), 3.87 (dd, *J* = 7.5, 7.5 Hz, 1H, H-2), 3.15 (s, 3H, H-6), 2.93 (dd, *J* = 16.5, 8.5 Hz, 1H, H-4), 2.56 (dd, *J* = 16.5, 9.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.3 (C-5), 134.7 (C-Ar), 133.5 (C-Ar × 2), 128.3 (C-Ar), 127.3 (C-Ar), 127.0 (C-Ar), 126.3 (C-Ar), 126.1 (C-Ar), 125.9 (C-Ar), 122.6 (C-Ar), 71.9 (C-1), 64.3 (C-2), 39.5 (C-4), 36.9 (C-3), 30.5 (C-6); configuration assigned by NOESY correlations (blue and red arrows); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1171.

(7a*S**,10a*S**)-10a-Hydroxy-8-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**3.29**)

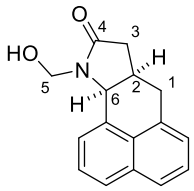


To a solution of lactam **1.129** (5.0 mg in 105 µL MeOH, 0.021 mmol), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (210 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (210 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 18 mL), was added $P450_{BM3}$ variant KSK19/A82M/I263G (1.0 mL of a 67 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 3 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) gave a mixture of lactams **3.28** and **3.29** (4.5 mg, 85%) in 1:5 ratio, as a yellow oil.

For **3.28** characterisation data, see above.

3.29: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.80 – 7.74 (m, 2H, H-Ar), 7.57 – 7.50 (m, 2H, H-Ar), 7.45 – 7.41 (m, 1H, H-Ar), 7.31 (d, *J* = 7.0 Hz, 1H, H-Ar), 3.91 (dd, *J* = 9.5, 5.5 Hz, 1H, H-2), 3.56 (dd, *J* = 16.0, 5.5 Hz, 1H, H-1), 3.02 (dd, *J* = 16.0, 9.5 Hz 1H, H-1), 2.97 (s, 3H, H-6), 2.93 – 2.87 (m, 2H, H-4); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.5 (C-5), 136.9 (C-Ar), 133.1 (C-Ar), 129.9 (C-Ar), 128.5 (C-Ar), 127.3 (C-Ar), 127.1 (C-Ar), 126.5 (C-Ar), 126.2 (C-Ar), 125.3 (C-Ar), 124.8 (C-Ar), 74.5 (C-3), 65.6 (C-2), 46.5 (C-4), 32.3 (C-1), 27.9 (C-6).

cis-10-(Hydroxymethyl)-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.30**)

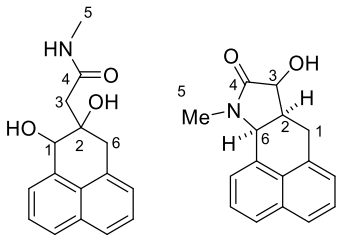


To a solution of lactam **2.85** (3.5 mg in 72.5 µL MeOH, 0.015 mmol), glucose (1.5 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (145 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (145 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 10 mL), was added $P450_{BM3}$ variant A74G/F87I/A184I/I263G/A328G (2.9 mL of a 10 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 26 h at 20 °C. An additional amount of variant and cofactors (11 mL of 10 µM variant, 73 µL of $NADP^+$, 73 µL of GDH) was added at 21 h to bring reaction to completion. The mixture was then extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave lactams **3.7** (0.5 mg, 13%, yellow oil) and **3.30** (1.0 mg, 26%, yellow oil).

For **3.7** characterisation data, see above.

3.30: **IR** (film) ν_{max}/cm^{-1}: 2949s, 2825m, 1686s, 1402m, 777s; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.77 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.66 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.55 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.45 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.32 (d, *J* = 7.0 Hz, 1H, H-Ar), 5.26 (dd, *J* = 11.0, 3.0 Hz, 1H, H-5), 5.13 (d, *J* = 4.5 Hz, 1H, H-6), 4.26 (dd, *J* = 11.0, 11.0 Hz, 1H, H-5), 3.09 (dd, *J* = 16.0, 5.0 Hz, 1H, H-1), 2.99 – 2.91 (m, 1H, H-1), 2.87 – 2.82 (m, 1H, H-2), 2.81 – 2.72 (m, 1H, H-3), 2.39 (dd, *J* = 16.0, 4.0 Hz, 1H, H-3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.2 (C-4), 133.7 (C-Ar), 133.2 (C-Ar), 129.4 (C-Ar), 129.3 (C-Ar), 129.2 (C-Ar), 128.4 (C-Ar), 126.6 (C-Ar), 126.1 (C-Ar), 125.7 (C-Ar), 124.9 (C-Ar), 64.1 (C-5), 58.3 (C-6), 39.5 (C-3), 32.0 (C-1), 31.4 (C-2); **HRMS** (ESI^+) *m*/*z* [M-+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1174.

2-(1,2-Dihydroxy-2,3-dihydro-1*H*-phenalen-2-yl)-*N*-methylacetamide (**3.31**) and *cis*-8-hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.32**)



3.31 **3.32**

To a solution of lactam **2.85** (6.2 mg in 129 µL MeOH, 0.026 mmol), glucose (2.6 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (258 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (258 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 17 mL), was added $P450_{BM3}$ variant RK/A328I (5.2 mL of a 10 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 27 h at 20 °C. An additional amount of variant and cofactors (7.8 mL of 10 µM variant, 388 µL of $NADP^+$, 388 µL of GDH) was added at 24 h to bring

reaction to completion. The mixture was then extracted following the general procedure (see *section 4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave a mixture of lactams **2.87** and **3.31** as single diastereomers (0.5 mg, yellow oil, unassigned) in a 62:38 ratio. **IR** (film) ν_{max}/cm^{-1}: 2926s, 2854m, 1735m, 1459m, 1377m.

For **2.87** characterisation data, see above.

3.31: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.02 (d, *J* = 6.5 Hz, 1H, H-Ar), 7.96 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.81 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.66 – 7.61 (m, 1H, H-Ar), 7.51 – 7.47 (m, 1H, H-Ar), 7.35 (d, *J* = 7.0 Hz, 1H, H-Ar), 4.04 (s, 1H, H-1), 3.66 (d, *J* = 15.5 Hz, 1H, H-6), 3.60 (d, *J* = 15.5 Hz, 1H, H-6), 3.31 (d, *J* = 16.0 Hz, 1H, H-3), 2.86 – 2.81 (m, 4H, H-3, -5); **^{13}C NMR** (151 MHz, $CDCl_3$) [only selected peaks reported based on HSQC and HMBC] δ 171.9 (C-4), 130.2 (C-Ar), 127.3 (C-Ar), 126.6 (C-Ar), 126.5 (C-Ar), 125.8 (C-Ar), 125.7 (C-Ar), 68.9 (C-2), 65.6 (C-1), 45.5 (C-3), 42.6 (C-6), 25.7 (C-5); **HRMS** (ESI^+) *m*/*z* $[M+NH_4]^+$ calcd. for $C_{16}H_{21}N_2O_3$ 289.1547, found, 289.1540.

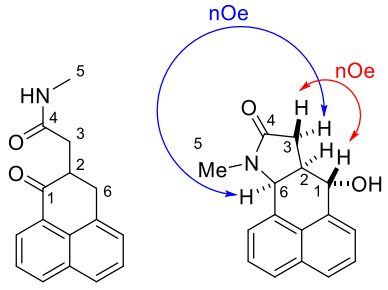
Further elution (pentane/ethyl acetate, 1:1) gave a mixture of lactams **2.88** and **3.32** (1.0 mg, yellow oil) in a 1:1 ratio with lactam **3.32** as a single diastereomer (unassigned). **IR** (film) ν_{max}/cm^{-1}: 2922s, 2852s, 1708s, 1468m, 1303m, 976m.

For **2.88** characterisation data, see above.

3.32: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.74 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.54 – 7.49 (m, 2H, H-Ar), 7.47 – 7.42 (m, 1H, H-Ar), 7.37 – 7.30 (m, 1H, H-Ar), 5.05 (d, *J* = 6.5 Hz, 1H, H-6), 4.10 (d, *J* = 6.0 Hz, 1H, H-3), 3.24 (dd, *J* = 16.0, 5.5 Hz, 1H, H-1), 3.16 (dd, *J* = 16.0, 7.0 Hz, 1H, H-1), 3.05 (s, 3H, H-5), 2.98 – 2.90 (m, 1H, H-2); **^{13}C NMR** (126 MHz, $CDCl_3$) [only selected peaks reported based on HSQC] δ 73.9 (C-3), 59.0

(C-6), 40.6 (C-2), 29.8 (C-5), 28.6 (C-1); **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1174.

N-Methyl-2-(1-oxo-2,3-dihydro-1*H*-phenalen-2-yl)acetamide (**3.33**) and (7*S**,7a*R**,10a*S**)-7-hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.34**)



To a solution of lactam **2.85** (6.2 mg in 129 µL MeOH, 0.026 mmol), glucose (2.6 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (258 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), NADP$^+$ monosodium salt (258 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 22 mL), was added $P450_{BM3}$ variant KSK19/A82M (0.36 mL of a 145 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 2 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:2) gave lactams **2.88** (2.2 mg, 34%, yellow oil), **3.33** (1.0 mg, 15%, yellow oil), and **3.34** (1.5 mg, 23%, single diastereomer, yellow oil).

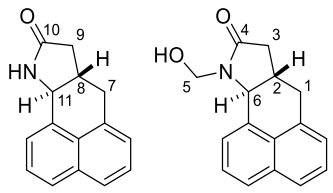
For **2.88** characterisation data, see above.

3.33: **IR** (film) ν_{max}/cm^{-1}: 3480br, 2945s, 2880m, 1657s, 1571m, 1409m, 1276m, 750s; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.10 (d, *J* = 8.0 Hz, 1H, H-Ar),

7.81 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.61 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.52 – 7.44 (m, 2H, H-Ar), 6.06 (br s, 1H, NH), 3.58 – 3.46 (m, 2H, H-2, H-6), 3.26 (dd, *J* = 13.0, 13.0 Hz, 1H, H-6), 2.94 (dd, *J* = 14.5, 6.5 Hz, 1H, H-3), 2.85 (d, *J* = 5.0 Hz, 3H, H-5), 2.51 (dd, *J* = 14.5, 5.0 Hz, 1H, H-3); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 200.3 (C-1), 172.3 (C-4), 134.4 (C-Ar), 133.4 (C-Ar), 132.8 (C-Ar), 131.5 (C-Ar), 129.9 (C-Ar), 126.6 (C-Ar), 126.5 (C-Ar), 125.9 (C-Ar × 2), 125.6 (C-Ar), 44.9 (C-2), 36.8 (C-3), 34.9 (C-6), 26.6 (C-5); **HRMS** (ESI^+) *m*/*z* [M+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1174.

3.34: **IR** (film) ν_{max}/cm^{-1}: 2960m, 2851m, 1742m, 1263s, 1100s, 1036s, 806s; **[α]$_D^{25}$** +26.0 (*c* 0.35, DCM)); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.85 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.77 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.59 – 7.41 (m, 3H, H-Ar), 4.89 (d, *J* = 5.0 Hz, 1H, H-6), 4.79 (d, *J* = 8.0 Hz, 1H, H-1), 2.85 (s, 3H, H-5), 2.83 – 2.77 (m, 1H, H-3), 2.75 – 2.70 (m, 2H, H-2, H-3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.6 (C-4), 135.8 (C-Ar), 133.7 (C-Ar), 129.2 (C-Ar), 128.5 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 127.8 (C-Ar), 126.4 (C-Ar), 125.6 (C-Ar), 123.0 (C-Ar), 69.4 (C-1), 61.1 (C-6), 40.3 (C-2), 36.2 (C-3), 28.2 (C-5); configuration assigned by NOESY correlations (blue and red arrows); **HRMS** (ESI^+) *m*/*z* [M+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1172.

trans-7a,8,10,10a-Tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.35**) and *trans*-10-(hydroxymethyl)-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.36**)

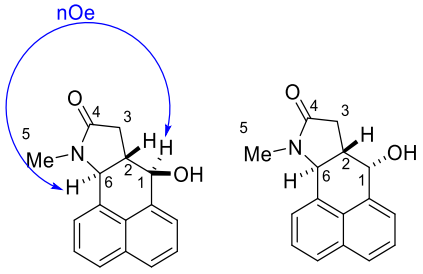


3.35 **3.36**

To a solution of lactam **2.86** (4.1 mg in 87 µL MeOH, 0.017 mmol), glucose (1.7 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (174 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (174 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 15 mL), was added $P450_{BM3}$ variant RK/I263G (0.4 mL of a 171 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 3 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) gave a mixture of a mixture of lactams **3.35** and **3.36** (1.9 mg, 47%) in 1:1 ratio, as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2954s, 2929s, 1699s, 1466m, 1377m, 1326m, 968m; **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1174; *m*/*z* $[M+H]^+$ calcd. for $C_{15}H_{14}NO$ 224.1070, found, 224.1069.

A mixture of lactams **3.35** and **3.36**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.79 – 7.74 (m, 2H, H-Ar), 7.74 – 7.70 (m, 1H, H-Ar), 7.47 – 7.41 (m, 6H, H-Ar), 7.37 – 7.33 (m, 2H, H-Ar), 7.14 – 7.09 (m, 1H, H-Ar), 5.42 (dd, *J* = 11.0, 6.0 Hz, 1H, H-5), 5.09 (dd, *J* = 11.0, 8.9 Hz, 1H, H-5), 4.70 (d, *J* = 10.5 Hz, 1H, H-6), 4.64 (d, *J* = 10.5 Hz, 1H, H-11), 3.41 – 3.33 (m, 2H, H-1, H-7), 3.25 – 3.20 (m, 2H, H-1, H-7), 2.75 (dd, *J* = 16.5, 7.5 Hz, 1H, H-3), 2.69 (dd, *J* = 15.5, 6.5 Hz, 1H, H-9), 2.62 – 2.48 (m, 2H, H-2, H-8), 2.43 (dd, *J* = 15.5, 12.5 Hz, 1H, H-9), 2.37 (dd, *J* = 16.5, 12.5 Hz, 1H, H-3); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 179.4 (C-4), 178.3 (C-10), 135.1 (C-Ar), 134.5 (C-Ar), 134.4 (C-Ar × 2), 134.2 (C-Ar), 130.2 (C-Ar), 129.9 (C-Ar), 129.1 (C-Ar), 128.5 (C-Ar), 127.6 (C-Ar), 126.9 (C-Ar), 126.8 (C-Ar), 126.2 (C-Ar), 126.1 (C-Ar), 126.0 (C-Ar), 125.9 (C-Ar), 125.5 (C-Ar), 125.2 (C-Ar), 119.0 (C-Ar), 118.4 (C-Ar), 68.9 (C-5), 64.6 (C-6), 60.8 (C-11), 41.3 (C-8), 38.6 (C-2), 38.0 (C-9), 37.2 (C-3), 35.7 (C-1, C-7).

(7*R**,7a*S**,10a*S**)-7-Hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.37**) and (7*S**,7a*S**,10a*S**)-7-hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.38**)



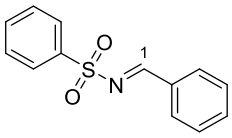
To a solution of lactam **2.86** (4.1 mg in 87 µL MeOH, 0.017 mmol), glucose (1.7 mL of a 1.0 M solution in phosphate buffer, pH 7.9), GDH (174 µL of a 400 U/mL solution in phosphate buffer, pH 7.9), $NADP^+$ monosodium salt (174 µL of a 4.0 mM solution in phosphate buffer, pH 7.9) in 200 mM phosphate buffer (pH 7.9, 15 mL), was added $P450_{BM3}$ variant KSK19/A82M (0.24 mL of a 145 µM solution in phosphate buffer, pH 7.9). The solution was shaken at 120 rpm for 4 h at 20 °C. Upon completion, the mixture was extracted following the general procedure (see *4.2.5 Preparative scale reactions*). Purification by silica gel chromatography (pentane/ethyl acetate, 1:1) gave a mixture of lactam diastereomers **3.37** and **3.38** (5.1 mg, quant., *dr* = 1:1) as a yellow oil. **IR** (film) ν_{max}/cm^{-1}: 2927s, 2857m, 1684s, 1095m, 806m; **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1173.

3.37: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.85 – 7.78 (m, 1H, H-Ar), 7.77 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.60 – 7.53 (m, 3H, H-Ar), 7.43 (dd, *J* = 8.5, 7.0 Hz, 1H, H-Ar), 5.15 (d, *J* = 12.0 Hz, 1H, H-1), 4.58 (d, *J* = 10.5 Hz, 1H, H-6), 3.37 (s, 3H, H-5), 2.89 – 2.83 (m, 1H, H-3), 2.53 – 2.45 (m, 2H, H-2, H-3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.7 (C-4), 136.1 (C-Ar), 134.1 (C-Ar), 128.3 (C-Ar), 127.5 (C-Ar), 126.4 (C-Ar), 125.3 (C-Ar), 123.6 (C-Ar), 118.9 (C-

Ar), two C-Ar resonances not seen, 73.9 (C-1), 63.5 (C-6), 46.1 (C-2), 35.4 (C-3), 32.6 (C-5); configuration assigned by NOESY correlations (blue arrow).

3.38: $[\alpha]_D^{25}$ – 10.0 (*c* 0.35, DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.91 – 7.88 (m, 1H, H-Ar), 7.82 – 7.79 (m, 1H, H-Ar), 7.70 (d, *J* = 7.0 Hz, 1H), 7.60 – 7.53 (m, 2H, H-Ar), 7.49 (dd, *J* = 8.5, 7.0 Hz, 1H, H-Ar), 5.13 (d, *J* = 7.0 Hz, 1H, H-1), 4.94 (d, *J* = 10.5 Hz, 1H, H-6), 3.40 (s, 3H, H-5), 2.79 – 2.75 (m, 1H, H-3), 2.60 – 2.53 (m, 2H, H-2, H-3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.7 (C-4), 136.1 (C-Ar), 134.2 (C-Ar), 129.7 (C-Ar), 127.9 (C-Ar), 127.4 (C-Ar), 126.2 (C-Ar), 125.5 (C-Ar), 119.6 (C-Ar), two C-Ar resonances not seen, 68.0 (C-1), 59.0 (C-6), 43.7 (C-2), 32.9 (C-5), 32.1 (C-3).

(*E*)-*N*-Benzylidenebenzenesulfonamide (**3.40**)[192]

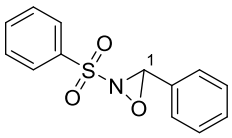


To a solution of benzenesulfonamide (15.7g, 100 mmol), 4 Å molecular sieves (15 g) and Amberlyst® 15 (0.2 g) in toluene (165 mL), was added benzaldehyde (10.7 mL, 105 mmol). The reaction mixture was heated at reflux for 9 h. The reaction mixture was then filtered through a sintered funnel and the residue was washed with toluene (3 × 70 mL). The filtrate was then concentrated *in vacuo*. The resulting yellow oil was triturated with pentane (80 mL), filtered, washed with pentane (10 mL) and air dried to give the title compound (**3.40**, 16.9 g, 69%) as a colourless solid, which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H, H-1), 8.04 – 7.99 (m, 2H, H-Ar), 7.96 – 7.90 (m, 2H, H-Ar), 7.68 – 7.59 (m, 2H, H-Ar), 7.58 – 7.53 (m, 2H, H-Ar), 7.52 – 7.45 (m, 2H,

H-Ar); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.8 (C-1), 138.4 (C-Ar), 135.2 (C-Ar), 133.7 (C-Ar), 132.5 (C-Ar), 131.5 (C-Ar), 129.3 (C-Ar × 2), 128.2 (C-Ar).

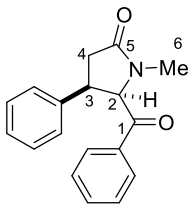
3-Phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (Davis oxidant, **3.41**)[193]



To a solution of (*E*)-*N*-Benzylidenebenzenesulfonamide (**3.40**, 0.5 g, 2 mmol) in toluene (20 mL) was added K_2CO_3 (2.24 g, 16.8 mmol) in water (11 mL). The reaction mixture was stirred vigorously at RT and a solution of Oxone® (1.4 g, 4.8 mmol) in water (11 mL) was added. If, after 1 h, the reaction had not reached completion (NMR), the aqueous phase was removed from the reaction mixture and a fresh solution of K_2CO_3 (2.24 g, 16.8 mmol) in water (11 mL) was added, followed by a solution of Oxone® (1.4 g, 4.8 mmol) in water (11 mL). This process was repeated a further three times and, upon completion, the phases were separated and the aqueous phase was extracted with toluene (3 x 20 mL). The combined organic extracts were washed with aq. Na_2SO_3 (10%, 20 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting yellow oil was triturated with a few mL of pentane to afford the title compound (**3.41**, 0.405 g, 78%) as a light-yellow solid, which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.04 (m, 2H, H-Ar), 7.77 – 7.71 (m, 1H, H-Ar), 7.65 – 7.59 (m, 2H, H-Ar), 7.49 – 7.34 (m, 5H, H-Ar), 5.51 (s, 1H, H-1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 135.2 (C-Ar), 134.7 (C-Ar), 131.5 (C-Ar), 130.5 (C-Ar), 129.5 (C-Ar), 129.4 (C-Ar), 128.8 (C-Ar), 128.3 (C-Ar), 76.3 (C-1).

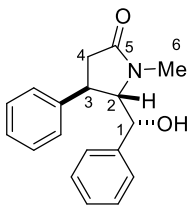
cis-5-Benzoyl-1-methyl-4-phenylpyrrolidin-2-one (**3.42**)[12]



To a solution of lactam **1.17** (30 mg, 0.11 mmol) in dry DCM (20 mL), was added $NaHCO_3$ (26 mg, 0.31 mmol). DMP (93 mg, 0.22 mmol) was then added to the mixture at 0 °C. The reaction mixture was stirred at RT for 21 h. When the reaction was complete (TLC), the mixture was quenched with aq. NaOH (2 × 10 mL, 1.0 M). The mixture was then extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (10 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) yielded lactam **3.42** (8.8 mg, 29%) as a yellow oil.

R_f 0.20 (pentane/ethyl acetate, 1:4); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H, H-Ar), 7.47 – 7.35 (m, 1H, H-Ar), 7.28 – 7.19 (m, 3H, H-Ar), 7.03 – 6.94 (m, 4H, H-Ar), 5.42 (d, *J* = 9.0 Hz, 1H, H-2), 4.02 (q, *J* = 9.0 Hz, 1H, H-3), 2.93 (dd, *J* = 16.0, 9.0 Hz, 1H, H-4), 2.88 (s, 3H, H-6), 2.79 (dd, *J* = 16.0, 9.0 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 197.0 (C-1), 175.4 (C-5), 136.8 (C-Ar), 136.4 (C-Ar), 133.4 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 127.7 (C-Ar), one C-Ar resonance not seen, 68.3 (C-2), 42.6 (C-3), 36.4 (C-4), 29.5 (C-6).

(4*R**,5*S**)-5-((*S**)-Hydroxy(phenyl)methyl)-1-methyl-4-phenylpyrrolidin-2-one (**3.44**)

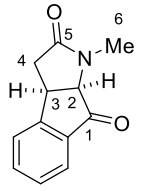


To a solution of crude lactam **1.64** (2.2 mg, <0.0079 mmol) in ethanol (1 mL), was added $NaBH_4$ (0.6 mg, 0.016 mmol) at 0 °C. The mixture was then stirred at RT for 3 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo.* Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) yielded to give a mixture of lactam diastereomers **3.14** and **3.44** (1.3 mg, 93% over 2 steps, *dr* = 1:2) as a colourless oil. $\mathbf{R}_f$ 0.10 (pentane/ethyl acetate, 1:4).

For **3.14** characterisation data, see above.

3.44: **IR** (film) ν_{max}/cm^{-1}:2962m, 2925m, 1263s, 1100s, 1033s, 809s; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H, H-Ar), 7.20 – 7.08 (m, 3H, H-Ar), 6.76 – 6.74 (m, 2H, H-Ar), 5.10 (d, *J* = 3.5 Hz, 1H, H-1), 3.65 (dd, *J* = 3.5, 2.0 Hz, 1H, H-2), 3.42 (dt, *J* = 10.0, 2.0, 2.0 Hz, 1H, H-3), 2.96 (s, 3H, H-6), 2.88 (dd, *J* = 17.0, 10.0 Hz, 1H, H-4), 2.37 (dd, *J* = 17.0, 2.0 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 175.6 (C-5), 145.4 (C-Ar), 139.8 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 128.2 (C-Ar), 126.7 (C-Ar), 126.3 (C-Ar), 126.2 (C-Ar), 74.5 (C-2), 72.6 (C-1), 38.5 (C-4), 36.8 (C-3), 29.5 (C-6); **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{18}H_{20}NO_2$ 282.1489, found, 282.1486.

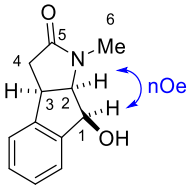
cis-1-Methyl-1,3,3a,8a-tetrahydroindeno[2,1-*b*]pyrrole-2,8-dione (**3.45**)



To a solution of lactam **3.24** (7.5 mg, 0.037 mmol) in dry DCM (1 mL), was added $NaHCO_3$ (17 mg, 0.21 mmol). DMP (63 mg, 0.15 mmol) was then added to the mixture at 0 °C. The reaction mixture was stirred at RT for 41 h. When the reaction was complete (TLC), the mixture was quenched with aq. NaOH (2 × 1 mL, 1.0 M). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give crude lactam **3.45** (7.5 mg), which was used without further purification.

R_f 0.20 (DCM/methanol, 20:1); **IR** (film) ν_{max}/cm^{-1}: 3299m, 2925s, 2854m, 1640s, 1572s, 1466m, 1405m, 1359m, 1259m, 792m, 772m; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.71 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.51 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.47 (t, *J* = 7.5 Hz, 1H, H-Ar), 4.14 (d, *J* = 7.0 Hz, 1H, H-2), 4.01 (ddd, *J* = 11.0, 7.0, 3.5 Hz, 1H, H-3), 3.07 (s, 3H, H-6), 3.03 (dd, *J* = 17.5, 11.0 Hz, 1H, H-4), 2.53 (dd, *J* = 17.5, 3.5 Hz, 1H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 200.8 (C-1), 172.9 (C-5), 156.1 (C-Ar), 136.7 (C-Ar), 134.4 (C-Ar), 129.1 (C-Ar), 126.4 (C-Ar), 125.1 (C-Ar), 66.2 (C-2), 37.0 (C-4), 35.1 (C-3), 28.8 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{12}NO_2$ 202.0863, found, 202.0860.

(3a*R**,8*S**,8a*R**)-8-Hydroxy-1-methyl-3,3a,8,8a-tetrahydroindeno[2,1-*b*]pyrrol-2(1*H*)-one (**3.46**)



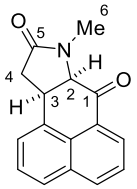
To a solution of crude lactam **3.45** (7.5 mg, <0.037 mmol) in ethanol (2 mL), was added $NaBH_4$ (3 mg, 0.08 mmol) at 0 °C. The mixture was then stirred at RT for 4 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was

then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give a mixture of lactam diastereomers **3.24** and **3.46** (4.6 mg, 61% over two steps, *dr* = 1:15) as a colourless solid, which was used without further purification. $\mathbf{R_f}$ 0.10 (DCM/methanol, 20:1).

For **3.24** characterisation data, see above.

3.46: **IR** (film) ν_{max}/cm^{-1}:1668s, 1483m, 1263s, 1106s, 812s; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.35 (m, 1H, H-Ar), 7.32 – 7.20 (m, 2H, H-Ar), 7.20 – 7.12 (m, 1H, H-Ar), 5.20 (d, *J* = 6.0 Hz, 1H, H-1), 4.22 (dd, *J* = 8.0, 6.0 Hz, 1H, H-2), 3.69 (ddd, *J* = 10.5, 8.0, 5.5 Hz, 1H, H-3), 2.93 (s, 3H, H-6), 2.71 (dd, *J* = 17.5, 10.5 Hz, 1H, H-4), 2.24 (dd, *J* = 17.5, 5.5 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.8 (C-5), 143.5 (C-Ar), 142.8 (C-Ar), 129.8 (C-Ar), 128.5 (C-Ar), 125.7 (C-Ar), 124.9 (C-Ar), 76.4 (C-1), 67.0 (C-2), 40.0 (C-3), 38.0 (C-4), 31.1 (C-6); configuration assigned by NOESY correlations (blue arrow); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}NO_2$ 204.1019, found, 204.1017.

cis-8-Methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indole-7,9-dione (**3.48**)

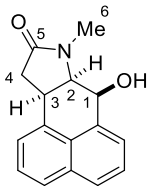


To a solution of lactam **3.28** (4.5 mg, 0.018 mmol) in dry DCM (1 mL), was added $NaHCO_3$ (4 mg, 0.05 mmol). DMP (15 mg, 0.036 mmol) was then added to the mixture at 0 °C. The reaction mixture was stirred at RT for 20 h. When the reaction was complete (TLC), the mixture was quenched with aq. NaOH (2 × 1 mL, 1.0 M). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then

dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (pentane/ethyl acetate, 1:4) yielded lactam **3.48** (3.0 mg, 66%) as a yellow oil.

R_f 0.32 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2955m, 1746s, 1373m, 1239s, 1048m; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 7.0 Hz, 1H, H-Ar), 8.19 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.89 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.67 (dd, *J* = 8.0, 7.0 Hz, 1H, H-Ar), 7.62 – 7.52 (m, 2H, H-Ar), 4.44 (d, *J* = 8.5 Hz, 1H, H-2), 4.32 (ddd, *J* = 12.0, 8.5, 8.5 Hz, 1H, H-3), 3.30 (s, 3H, H-6), 2.81 (dd, *J* = 16.5, 8.5 Hz, 1H, H-4), 2.45 (dd, *J* = 16.5, 12.0 Hz, 1H, H-4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 196.7 (C-1), 173.0 (C-5), 135.3 (C-Ar), 133.7 (C-Ar), 131.8 (C-Ar), 129.9 (C-Ar), 128.2 (C-Ar), 127.5 (C-Ar), 127.4 (C-Ar), 126.8 (C-Ar), 126.7 (C-Ar), 126.4 (C-Ar), 65.4 (C-2), 39.8 (C-3), 39.5 (C-4), 31.3 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{14}NO_2$ 252.1019, not found; **HRMS** (APCI) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{14}NO_2$ 252.1019, not found.

(7*S**,7a*R**,10a*R**)-7-Hydroxy-8-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*ef*]indol-9(7*H*)-one (**3.49**)



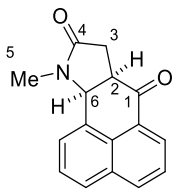
To a solution of lactam **3.48** (3.0 mg, 0.012 mmol) in ethanol (1 mL), was added $NaBH_4$ (1.6 mg, 0.048 mmol) at 0 °C. The mixture was stirred at RT for 2 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography

(pentane/ethyl acetate, 1:4 → 1:10) yielded a mixture of lactam diastereomers **3.28** and **3.49** (1.6 mg, 53%) as a reddish-brown oil in 1:2 ratio. $\mathbf{R}_f$ 0.11 (pentane/ethyl acetate, 1:4).

For **3.28** characterisation data, see above.

3.49: **IR** (film) ν_{max}/cm^{-1}: 2923m, 1674m, 1263s, 1103s, 822s; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.92 – 7.90 (m, 1H, H-Ar), 7.81 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.58 – 7.50 (m, 3H, H-Ar), 7.46 (d, *J* = 7.0 Hz, 1H, H-Ar), 5.31 (d, *J* = 3.0 Hz, 1H, H-1), 4.08 – 4.04 (m, 2H, H-2, H-3), 3.12 (s, 3H, H-6), 2.93 – 2.89 (m, 2H, H-4); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.0 (C-5), 134.2 (C-Ar), 134.0 (C-Ar), 133.9 (C-Ar), 129.7 (C-Ar), 127.4 (C-Ar), 126.8 (C-Ar), 126.7 (C-Ar), 126.5 (C-Ar), 126.2 (C-Ar), 126.1 (C-Ar), 68.3 (C-1), 61.8 (C-2), 39.0 (C-4), 35.4 (C-3), 28.3 (C-6); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1173.

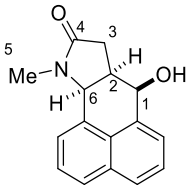
cis-10-Methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indole-7,9-dione (**3.51**)



To a solution of lactam **3.34** (3.5 mg, 0.014 mmol) in dry DCM (1 mL), was added $NaHCO_3$ (3 mg, 0.04 mmol). DMP (12 mg, 0.028 mmol) was then added to the mixture at 0 °C. The reaction mixture was stirred at RT for 24 h. When the reaction was complete (TLC), the mixture was quenched with aq. NaOH (2 × 1 mL, 1.0 M). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give lactam **3.51** (1.6 mg, 46%) as a yellow oil, which was used without further purification.

R_f 0.24 (pentane/ethyl acetate, 1:4); **IR** (film) ν_{max}/cm^{-1}: 2957s, 2927s, 2855m, 1685s, 1459m, 1262s, 1098s, 808s; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.35 (d, *J* = 7.5 Hz, 1H, H-Ar), 8.20 (d, *J* = 8.0 Hz, 1H, H-Ar), 8.02 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.73 – 7.68 (m, 2H, H-Ar), 7.67 – 7.62 (m, 1H, H-Ar), 5.11 (d, *J* = 5.5 Hz, 1H, H-6), 3.48 – 3.40 (m, 2H, H-2, H-3), 2.76 (dd, *J* = 17.0, 8.5 Hz, 1H, H-3), 2.57 (s, 3H, H-5); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 196.0 (C-1), 174.1 (C-4), 135.0 (C-Ar), 133.7 (C-Ar), 130.5 (C-Ar), 130.0 (C-Ar), 129.6 (C-Ar), 127.4 (C-Ar), 127.3 (C-Ar), 126.9 (C-Ar), 126.7 (C-Ar), 126.1 (C-Ar), 62.6 (C-6), 43.9 (C-2), 34.1 (C-3), 27.9 (C-5); **HRMS** (ESI^+) *m*/*z* $[M+H]^+$ calcd. for $C_{16}H_{14}NO_2$ 252.1019, found, 252.1021.

(7*R**,7a*R**,10a*S**)-7-Hydroxy-10-methyl-7a,8,10,10a-tetrahydronaphtho[1,8-*fg*]indol-9(7*H*)-one (**3.52**)



To a solution of lactam **3.51** (1.6 mg, 0.0062 mmol) in ethanol (1 mL), was added $NaBH_4$ (1.0 mg, 0.025 mmol) at 0 °C. The mixture was then stirred at RT for 5 h. When the reaction was complete (TLC), the mixture was diluted with water (1 mL). The mixture was then extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (1 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give a mixture of lactam diastereomers **3.34** and **3.52** (4.8 mg, 83%, *dr* = 1:2) as a yellow oil, which was used without further purification. **R_f** 0.10 (pentane/ethyl acetate, 1:4).

For **3.34** characterisation data, see above.

3.52: **IR** (film) ν_{max}/cm^{-1}:2929m, 2925m, 1669m, 1264s, 1102s, 810s; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.91 – 7.88 (m, 1H, H-Ar), 7.63 (d, *J* = 7.0 Hz, 1H, H-Ar), 7.56 – 7.55 (m, 1H, H-Ar), 7.19 – 7.15 (m, 3H, H-Ar), 5.14 (d, *J* = 5.0 Hz, 1H, H-1), 4.98 (d, *J* = 6.5 Hz, 1H, H-6), 3.19 – 3.15 (m, 1H, H-2), 2.96 (s, 3H, H-5), 2.67 (d, *J* = 6.5 Hz, 2H, H-3); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 175.4 (C-4), 138.0 (C-Ar), 134.5 (C-Ar), 129.2 (C-Ar), 128.9 (C-Ar), 128.4 (C-Ar), 127.8 (C-Ar), 126.4 (C-Ar), 125.7 (C-Ar), 125.4 (C-Ar), 125.3 (C-Ar), 70.2 (C-1), 60.7 (C-6), 37.8 (C-2), 33.8 (C-3), 29.1 (C-5); **HRMS** (ESI$^+$) *m*/*z* [M+H]$^+$ calcd. for $C_{16}H_{16}NO_2$ 254.1176, found, 254.1172.

Appendices

A: Enzyme screening data for diphenyl-derived *cis*-lactam **1.126**.

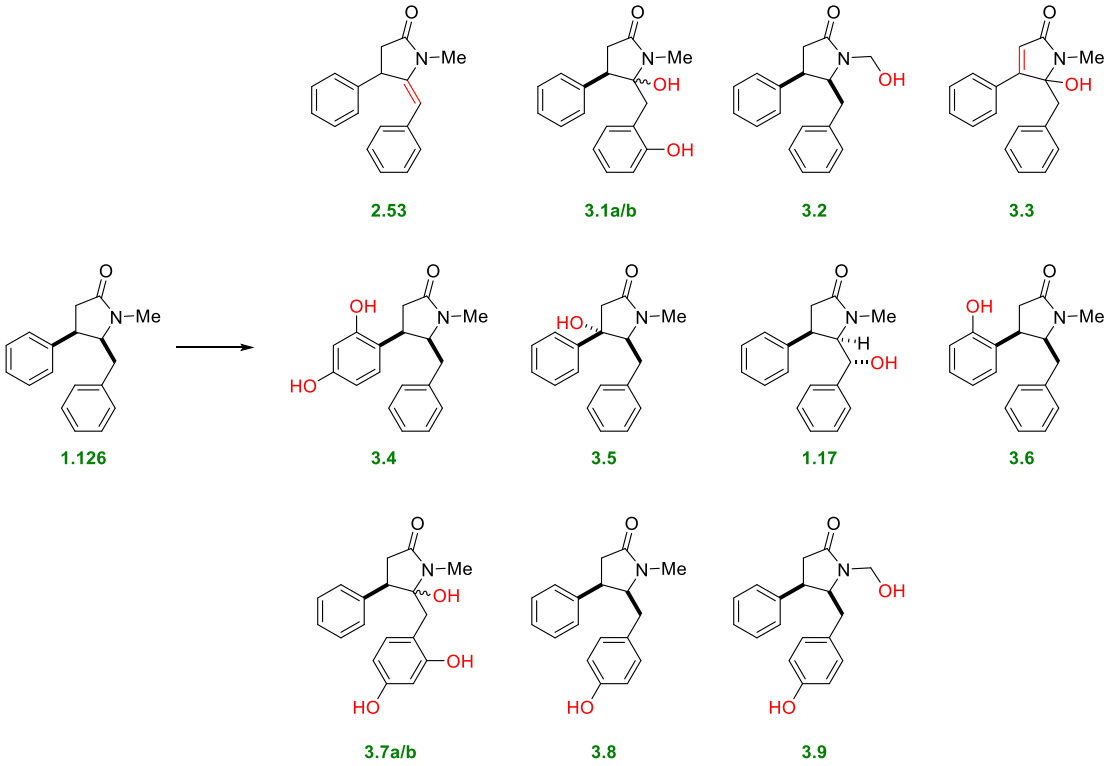


Table 5.1 Conversion and selectivity data for the $P450_{BM3}$ screening of diphenyl-derived *cis*-lactam **1.126**, organised first by decreasing selectivity for metabolite **1.17**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **1.17**.

Variant	Conv. (%)	2.53 (%)	3.1b (%)	3.1a (%)	3.2 (%)	3.3 (%)	3.4, 3.5 (%)	1.17 (%)	3.6 (%)	3.7a (%)	3.8 (%)	3.9 (%)	Others (%)
GVQ/A264G/A328G	94	-	4	5	12	4	6	52	3	2	1	1	10
GV/A184I/A264G	26	1	-	6	40	-	6	27	6	-	5	1	8
GV/A328G	98	-	3	3	10	2	7	24	4	-	3	2	42
GVQ/A328G	87	2	10	10	17	-	9	21	13	-	1	-	18
GVQ/A264G	41	2	-	11	43	-	18	19	4	-	3	-	-
KSK19/A82M/A264G/A328G	26	8	1	32	9	1	14	12	4	-	-	-	19
RK/A328G/T260G/A82M	77	2	-	5	9	-	44	11	-	-	-	-	30
RK/A328I/A264G	20	2	-	7	29	-	37	10	1	-	-	-	13
KSK19/A82M/I263G/A264G/A328G/A330V	55	13	1	31	7	1	20	9	-	-	-	-	18
KSK19/A82M/T260G/A328G	76	4	8	15	8	-	37	9	1	-	-	-	18
K19/F87V/A264G	27	2	-	12	34	-	8	9	10	-	9	1	14
GV/A184I/A328G	62	2	4	20	20	2	6	7	12	1	1	-	25
RK/T260G/A328G/A82M	36	8	6	19	14	-	30	7	1	-	-	-	16
KSK19/A82M/I263G/A264G/A330I/A328G	72	10	2	31	5	2	25	7	-	-	-	-	17
RK/A328G/A184I/T260G/A82M/A330V	81	2	9	7	13	-	32	7	-	-	-	-	32
GLQ/I263G/A328L	2	-	-	4	20	-	-	7	-	-	24	-	46
KSK19/A82M/I263G/A328G	39	7	-	32	16	1	19	6	-	-	-	-	17
RK/A328G/A184I/T260G/A82M/A330I	79	2	10	9	12	-	33	6	-	-	-	-	28
GV/A184I/A264G/A328G	63	4	1	34	19	2	5	6	4	-	-	-	24
RK/A328G/A184I/T260G/A82M/S72W	78	2	8	9	13	-	33	6	-	-	-	-	29
GLQ/I263G/A328L/V26L	2	-	-	3	18	-	3	6	-	-	27	-	43
GVQ/A264G/A330P	39	5	3	20	47	1	5	5	-	-	1	-	13
GVQ/I263A/E435A	2	2	-	4	34	-	2	5	3	-	7	-	43
RK/A328G/I263W	7	19	-	26	18	-	15	5	-	-	-	-	17
RK/A328G/A184I/T260G/A82M/A330L	61	4	6	14	14	-	30	5	-	-	-	-	27
KU3/A330P/I263G	7	-	-	-	80	-	8	5	-	-	-	-	7

GVQ/A264G/P329G/A330W	12	4	-	20	34	-	10	5	4	-	1	-	21
RP/A264G	5	-	-	9	51	5	15	4	-	-	-	-	16
GLQ/I263G/A328L/V26F	3	-	-	-	25	-	-	4	-	-	47	-	24
KSK19/A82M/I263G/A264G/A330F/A328G	83	12	2	34	14	1	14	3	-	-	-	-	20
RK/A328G/A184I/T260G/A82M	35	9	5	23	16	-	23	3	-	-	-	-	21
RK/A184I/A264G	18	4	1	16	31	1	21	3	1	-	-	-	22
RK/A264G	12	5	-	20	34	1	17	3	1	-	-	-	20
A82M/F87A/A184I/I263G/A264G/A328G	69	10	3	26	16	1	24	3	-	-	-	-	17
KSK19/A82M/I263G/A264G/A328G	13	9	3	27	9	1	29	3	1	-	-	-	18
GLQ/I263G/A328L/L75F	5	-	-	-	13	-	2	3	-	-	65	-	18
RK/A328G/A184I/T260G/A82M/P329F	4	28	-	21	8	-	20	3	-	-	-	-	20
RP/A82M/I263A/A328G/A330L	9	-	-	4	65	4	17	3	-	-	-	-	7
GLQ/I263G/A264G	4	-	-	3	17	-	-	3	2	-	-	-	76
A82M/F87A/I263G/A264G/A328G	28	12	1	26	12	-	23	3	-	-	-	-	23
GLQ/I263G/A328L/L437F	6	-	-	-	27	-	1	3	3	-	53	-	13
RP/A82M/I263A/A328G/A330V	18	-	-	5	67	5	16	2	-	-	-	-	5
A82M/F87A/A184I/T260G/A328G	62	7	8	23	14	-	29	2	1	-	-	-	16
GV/A184I/I263G/A264G	39	-	-	1	28	-	2	2	-	-	57	4	7
GVQ/A264G/I263G	19	7	-	23	40	1	1	2	-	-	12	-	13
RK/I263G/A328G	9	8	2	28	31	1	4	2	-	-	-	-	25
RP/A82M/I263A/L181F/A328G/A330V	82	4	3	15	61	-	11	2	-	-	-	-	4
RK/A328L	10	-	-	-	86	-	6	2	-	-	-	-	6
A82M/F87A/A184I/T260G/A328G	61	7	7	25	15	-	29	2	1	-	-	-	15
RK/A328G/A184I/T260G/A82M/A330F	4	25	-	22	20	-	14	2	-	-	-	-	16
RK/A184I/I263G/A264G/A328G	9	6	2	19	33	2	4	2	2	-	2	-	27
RK/A328G/A184I/T260G/A82M/S72F	17	11	-	17	42	-	20	2	-	-	-	-	7
RK/A184I/A264G/A328G	20	8	3	31	15	3	6	2	1	-	-	-	31
GVQ/A264G/A328G /I263G	17	14	-	5	33	1	2	2	3	-	3	-	36
RK/A328G/P329G/A330G	10	7	2	33	14	3	7	2	-	-	-	-	34
RK/A328G/T260G/F81W	7	23	-	38	12	-	17	2	-	-	-	-	9
RK/I263G/A264G/A330P	58	5	3	17	40	-	14	2	-	-	8	-	10

RK/I263G/A264G/L181F	29	5	1	11	51	-	22	2	-	-	1	-	8
KSK19/A82M/I263G/A264G	27	5	2	20	33	1	23	1	1	-	-	-	14
GLQ/I263G/A328L/A330L	11	-	-	-	22	-	1	1	-	-	67	-	9
GV/A184I/I263G/A328G	32	6	1	16	49	1	1	1	-	-	14	2	10
GLQ/I263G/A328L/V26M	9	-	-	-	22	-	-	1	-	-	38	-	38
RK/A184I/I263G/A264G	24	6	1	19	45	-	9	1	-	-	4	-	14
RK/A184I/I263G/A328G	22	5	4	23	29	2	2	1	-	-	1	-	33
RK/I263G/A264G/A330V	33	7	1	20	39	-	15	1	-	-	7	-	9
GI/A184I/I263G/A328G/S72F	35	6	1	16	62	1	1	1	-	-	3	-	9
KSK19/I263G/A264G	26	8	1	23	37	-	16	1	-	-	4	-	11
RK/A328G/L262G	34	15	-	42	18	-	13	1	-	-	-	-	11
RK/A328G/T260G/A184I	24	14	-	38	13	-	18	1	-	-	-	-	16
GL/A184I/I263G/A328G/S72F	17	4	-	10	61	-	1	1	1	-	13	-	10
R19/F87I/A184I/I263G/A264G	24	2	-	2	37	-	2	1	1	-	51	-	5
RK/A328I/S72W	16	1	-	3	94	-	1	1	-	-	-	-	1
GV/A184I/I263G/A328G/L75F	38	6	1	21	49	1	1	1	-	-	3	-	18
R19/F87I/L437LV	18	-	-	1	81	-	1	1	1	-	3	-	13
RK/A328G/I263G	17	13	4	31	32	-	3	1	-	-	-	-	18
GVQ/I263G/L437LM	36	-	-	2	51	-	1	1	5	-	34	3	4
KSK19/A82M/I263G	26	4	1	21	44	1	10	1	1	-	1	-	17
RK/A328G	32	16	-	43	10	-	6	1	-	-	-	-	25
RK/A328G/A184I/I263G/A264G	43	14	6	34	23	-	4	1	-	-	-	-	18
R19/F87I	21	-	-	2	80	-	1	1	-	-	5	-	11
RK/A184I	74	6	27	24	27	-	2	1	-	-	1	-	11
A82M/F87A/A184I/I263G	29	12	1	27	28	-	18	1	-	-	-	-	14
A82M/F87A/A184I/A264G	59	6	4	20	42	-	12	1	1	-	-	-	14
GVQ/I263G/S72M	31	1	-	3	53	-	2	1	2	-	28	2	8
GVQ/I263G/S72W	90	-	1	1	37	-	-	1	-	-	21	30	8
GVQ/S72W	94	-	3	3	31	-	1	-	13	-	16	-	32
GVQ/I263G/A330I	90	-	-	1	47	-	-	-	-	-	35	13	4
KSK19/A82M/I263G	90	5	10	21	35	-	12	-	-	-	-	-	16

GVQ/I263G/S72F	88	-	1	1	42	-	-	-	1	-	21	28	7
GVQ/I263G/L437F	88	1	1	2	47	-	-	-	-	-	27	20	3
GVQ/I263G/V26L	88	1	1	2	41	-	-	-	-	-	37	14	3
GVQ/I263G/L437M	87	-	1	1	41	-	-	-	-	-	29	16	10
R19/I263G/A328G	87	2	-	1	76	-	13	-	-	-	4	-	3
KSK19/A82M/S270G	86	1	39	-	16	5	9	-	1	-	2	1	27
GVQ/I263G/V26F	84	1	2	6	44	-	-	-	1	-	29	11	6
K19/F87V	81	1	6	8	37	-	2	-	14	-	16	9	7
GVQ/I263G/V26M	81	1	1	3	43	-	-	-	1	-	39	9	3
GVQ/A330W	78	2	8	10	32	-	2	-	13	-	7	4	19
KSK19/A82M/I263G/A330W	74	2	10	-	45	5	5	-	-	-	1	1	32
S72A/A82M/F87A/A184I	73	4	25	18	45	-	3	-	-	-	1	1	2
GVQ/I263G/Q307H	68	1	1	5	47	-	1	-	-	-	36	-	8
GVQ/I263G/A330F	68	1	1	3	45	-	1	-	1	-	40	5	4
K19/F87V/Q403P	67	2	5	11	42	-	3	-	14	-	15	3	4
I263G/A328G	65	1	-	1	82	-	8	-	-	-	6	-	3
A74G/F87I/A184I/I263G/A328G	63	7	-	20	47	-	-	-	-	-	17	-	8
KU3/A330P/F87V	63	2	10	-	46	8	1	-	-	-	-	-	33
GVQ/I263G	62	1	-	3	41	-	1	-	2	-	46	4	3
GVQ/I263G/L75M	60	4	1	13	40	1	2	-	-	-	25	-	16
KSK19/A82M	60	7	7	20	40	-	5	-	5	-	1	-	16
GVQ/I263G/A330W	59	1	1	5	38	-	1	-	1	-	37	6	9
R19/A330W/F87V	57	1	7	-	58	2	1	-	7	-	6	4	15
GVQ/I263G/V78L	55	-	-	1	51	-	1	-	2	-	35	5	4
KT2/L188Q/I263G	55	1	-	1	67	1	7	-	-	-	23	-	1
KSK19/A82M/L262G	55	1	9	-	38	6	13	-	4	-	1	-	28
KSK19/A82M/T268S	55	3	11	-	42	6	3	-	4	-	1	-	31
GVQ	52	1	2	7	57	-	1	-	20	-	3	1	6
GI/A184I/I263G/A328G/L75M	52	7	1	21	59	-	-	-	-	-	2	-	10
K19/F87V/I263G	50	1	-	2	47	-	-	-	2	-	36	5	5
GVQ/I263G/S332F	48	-	-	1	74	-	1	-	1	-	16	4	2

KSK19/I263G	46	3	1	17	53	1	2	-	-	-	9	1	12
GL/A184I/I263G/A328G/L75M	45	6	-	22	44	1	1	-	-	-	14	-	12
RK/A184I/T260G/A328G	45	8	10	30	30	-	2	-	-	-	1	-	19
KSK19/I263A	43	7	3	29	35	-	2	-	-	-	14	-	11
GV/A184I/I263G/A328G/A330F	43	11	-	27	50	-	-	-	-	-	7	-	4
GLQ/I263G/A328G	40	12	-	35	39	-	1	-	-	-	12	-	-
RK/A264G/A328G	39	16	4	40	11	1	9	-	-	-	-	-	20
RK/A328I	39	1	-	4	90	-	1	-	-	-	1	-	1
RP/F87V/I263G/L188G	39	1	-	-	42	-	3	-	-	-	45	5	4
GVQ/A330P	37	5	3	24	53	1	2	-	-	-	1	-	11
K19/F87V/E267V/V78I	36	6	1	19	50	-	5	-	9	-	6	-	3
RK/A328G/A184I/I263G/T260G	34	16	1	39	24	-	5	-	-	-	-	-	14
RK/A328G/S270G	33	14	-	42	16	-	6	-	-	-	-	-	23
RK/A328G/T268S	32	15	2	41	8	1	15	-	-	-	-	-	18
GVQ/I263G/L437LH	31	-	-	2	42	-	2	-	3	-	43	2	5
RP/A82M/I263A/L181F/A328G/A330L	31	2	-	-	74	3	10	-	-	-	-	-	10
KSK19/I263G	30	5	1	20	59	-	2	-	-	-	8	-	4
GV/A184I/I263G/P329F	28	4	-	4	64	-	-	-	-	-	26	-	2
KSK19/A82M/T260G/I263G	28	4	3	-	53	5	8	-	-	-	-	-	27
GV/A330F	26	2	1	7	64	-	2	-	7	-	4	-	13
RK/A328G	26	13	5	43	10	-	6	-	1	-	-	-	22
K19/F87V/E267V	29	2	1	8	60	-	4	-	11	-	5	4	5
RK/I263G	22	5	1	18	56	-	1	-	-	-	7	-	10
GV/A184I	20	2	-	6	59	-	1	-	14	-	7	-	10
KSK19/A82M/I263G/A264G/A330W	18	10	1	17	52	-	9	-	-	-	-	-	11
RK/A328G/T268S	18	19	-	49	7	-	14	-	-	-	-	-	10
RK/A328G/T269G	18	21	-	53	3	-	10	-	-	-	-	-	13
GVQ/I263V/E435A	17	4	-	4	58	-	1	-	8	-	8	-	16
KSK19/A141G/A264G/L181F	16	12	2	-	36	-	35	-	-	-	-	-	15
RT2/S72G/A330W	16	12	-	28	45	14	2	-	-	-	-	-	-
KSK19/A82M/T260G/T268S	16	5	2	-	46	4	15	-	-	-	-	-	25

R19/I263G/A264G/A328G	15	4	-	5	58	-	26	-	-	-	1	-	6
RK/A328G/T260G/T268S	15	19	6	43	10	-	4	-	-	-	-	-	18
RP/F87V/E267V/L437LV	15	10	-	-	57	1	5	-	11	-	5	2	9
RP/A82M/I263A/L181F/A328G/A330L/Q359A	15	4	-	-	76	3	10	-	-	-	-	-	8
RK/A328G/A264G	14	22	-	49	11	-	8	-	-	-	-	-	11
RLYF/H171L/I263G/L437LM/A82L	13	4	-	-	68	-	-	-	-	-	3	-	25
GL/A184I	12	2	-	4	53	-	-	-	6	-	21	-	14
GL/A184I/I263G/A328G/L75M	12	20	-	28	39	-	-	-	-	-	9	-	4
GVQ/I263A/E267G	11	6	-	5	50	-	2	-	5	-	23	-	9
GV/A184I/A328G/T260G	11	8	-	-	25	6	12	-	14	-	-	-	35
RK/A328G/A184I/T260G/A264G	11	14	-	18	14	-	9	-	-	-	-	-	45
A330P	10	-	-	-	80	-	8	-	-	-	-	-	12
GL/A184I/I263G/A328G/A330F	10	14	-	15	59	-	-	-	-	-	10	-	2
GV/A184I/I263G/A328G/S72F/L188F	10	26	-	26	38	-	-	-	-	-	6	-	4
RLYF/H171L/I263G/L437LM/A82I	10	6	-	-	50	-	-	-	-	-	6	-	38
RK/A328G/A184I/T260G/T268S	10	23	-	45	7	-	10	-	-	-	-	-	14
S72A/A82M/F87V/A184I/T260G/E435M	10	7	-	-	79	-	5	-	1	-	-	-	7
RP/A82M/I263A/A328G/A330W	10	-	-	8	72	6	11	-	-	-	-	-	3
R19/A330W	9	-	-	14	27	-	8	-	-	-	-	-	51
RK	9	-	-	-	84	-	8	-	-	-	-	-	8
GL/A184I/I263G/A328G/A330F/S72F	9	20	-	26	45	-	-	-	-	-	5	-	4
RP/H171L/I263G/L437LV/A82L	9	7	-	-	55	-	-	-	-	-	3	-	35
RK/A328G/A184I/T260G/A264G/S72W	9	17	-	21	17	-	14	-	-	-	-	-	31
RP/F87V/E267V/V178F/L437LV	9	7	-	-	63	4	10	-	-	-	5	-	10
GL/A184I/I263G/A328G	8	18	-	19	47	-	-	-	-	-	13	-	3
GL/A184I/I263G/A328G/L75M/V26M	8	17	-	18	50	-	-	-	-	-	7	-	9
RLYF/H171L/I263G/L437LM/A328I	8	7	-	-	18	-	-	-	-	-	50	-	25
R19/F87I/T88M/A82L	8	14	-	-	62	-	-	-	-	-	-	-	24
KSK19/A82M/T260G/I263G/A264G	7	17	-	18	27	-	20	-	-	-	-	-	19
R19/A82M/A264G/A328G	7	10	-	12	57	-	4	-	-	-	-	-	18
GV/A184I/I263G/A328G/S72F	7	22	-	24	51	-	-	-	-	-	-	-	3

GI/A184I/I263G/A328G/S72F	7	22	-	22	54	-	-	-	-	-	-	-	3
GV/A184I/I263G/S332F	7	11	-	6	51	-	-	-	-	-	32	-	-
RP/A82M/I263A/A328G/A330L	7	9	-	-	63	2	15	-	3	-	-	-	8
R19/F87I/T88M/A82I	7	13	-	-	42	-	-	-	-	-	-	-	45
R19/F87I/T88M/A328I	7	10	-	-	10	-	-	-	-	-	-	-	81
RK/A328G/A184I/T260G/A82M/S332M	7	14	-	9	18	-	6	-	-	-	-	-	53
RP/F87V/E267V	7	9	-	-	57	2	8	-	7	-	2	-	15
RP/A82M/I263A/A328G/A330V	7	12	-	-	60	3	15	-	-	-	-	-	11
GV/A184I/T260G/I263G/A264G	6	11	-	6	26	-	2	-	-	-	34	-	22
R19/A328G/I263W	6	29	-	34	17	-	4	-	-	-	-	-	16
R19/A82M/I263G/A328G	6	16	-	10	64	-	3	-	-	-	-	-	8
RK/P329G/A330W/T260G	6	26	-	37	22	-	6	-	-	-	-	-	8
GI/A184I/I263G/A328G/L75F	6	22	-	22	52	-	-	-	-	-	-	-	4
GL/A184I/I263G/A328G/S72F/V26L	6	14	-	6	67	-	-	-	-	-	-	-	13
RLYF/I263R	6	11	-	-	73	-	-	-	-	-	-	-	17
RK/A328G/A184I/T260G/S72G	6	14	-	9	12	-	7	-	-	-	-	-	58
RP/F87V	6	14	-	-	62	-	3	-	10	-	3	-	9
R19/S72A/A330W	5	-	-	-	67	-	16	-	-	-	-	-	17
RK/A328G/T260G/P329G/A330G	5	29	-	42	9	-	10	-	-	-	-	-	11
GV/A184I/I263G/A328G/L437S	5	23	-	15	62	-	-	-	-	-	-	-	-
GL/A184I/I263G/A328G/S72F/L188F	5	19	-	15	58	-	-	-	-	-	5	-	3
F87V/L188Q/S72G/A330W	5	15	-	13	65	-	-	-	-	-	2	-	4
RT2/A330P/V78I/A184I	5	10	-	6	85	-	-	-	-	-	-	-	-
RT2/I263A/A330W	5	14	-	7	14	43	11	-	-	-	11	-	-
A82I/I263R	5	14	-	-	71	-	-	-	-	-	-	-	15
RLYF/H171L/I263G/L437LM/A82M	5	21	-	-	37	-	-	-	-	-	-	-	42
R19/F87I/T88M/A82M	5	17	-	-	10	-	-	-	-	-	-	-	74
RK/A328G/A184I/T260G/S72L	5	23	-	18	3	-	23	-	-	-	-	-	34
RK/A328G/A184I/T260G/A82M/S332F	5	19	-	15	34	-	9	-	-	-	-	-	22
GVQ/T260G	4	16	-	9	39	-	3	-	7	-	2	-	24
RK/F81W/T260G/A328G	4	-	-	-	47	-	22	-	-	-	-	-	31

R19/A328G/P329G/A330G	4	29	-	31	8	-	3	-	11	-	-	-	18
RK/A328G/P329G/A330G	4	33	-	45	9	-	6	-	-	-	-	-	7
GL/A184I/I263G/A328G/S72F	4	23	-	16	54	-	-	-	-	-	7	-	-
KT2/E267L	4	25	-	24	18	25	8	-	-	-	-	-	-
KT2/F87I/H171L/I263F/A276T/A328V	4	18	-	9	46	-	4	-	-	-	22	-	-
RP/A82M/I263A	4	20	-	10	55	-	15	-	-	-	-	-	-
RLYF/KSK19/I263G	4	29	-	-	51	-	-	-	-	-	-	-	20
RP/H171L/I263G/L437LV/A82M	4	21	-	-	29	-	-	-	-	-	-	-	50
RP/H171L/I263G/L437LV/A82I	4	23	-	-	29	-	-	-	-	-	-	-	48
K19/A264G	4	-	-	8	26	8	45	-	-	-	-	-	13
RK/A328G/P329G/A330G	3	-	-	-	45	-	25	-	-	-	-	-	31
RP/A82M/I263A	3	-	-	-	57	-	18	-	-	-	-	-	25
RP/A82W/I263A	3	-	-	-	37	-	28	-	-	-	-	-	35
WT	3	-	-	-	49	-	23	-	-	-	-	-	28
KSK19/A82M/I263G/A264G/S332M	3	29	-	18	22	-	19	-	-	-	-	-	13
RK/T260G/I263G/A264G	3	26	-	14	20	-	18	-	-	-	-	-	22
RK/A328G/T260G	3	34	-	41	8	-	6	-	-	-	-	-	10
F87V/L188Q/S72G/A330W/I263G	3	29	-	12	41	-	-	-	-	-	-	-	19
KT2/L75Q/F87I/I263I/A276T/A328V	3	32	-	13	9	-	18	-	-	-	28	-	-
RP/I263A	3	22	-	-	18	-	-	-	-	-	-	-	60
KSK19/A184I/Y51L/I263A	3	25	-	-	36	-	-	-	-	-	-	-	39
RP/H171L/I263G/A330W	3	23	-	-	51	-	-	-	-	-	-	-	26
A82F/I263R	3	29	-	-	42	-	-	-	-	-	-	-	29
A82I	3	24	-	-	51	-	-	-	-	-	-	-	25
A82I/I263F	3	21	-	-	25	-	-	-	-	-	-	-	54
A330P/I263A	3	23	-	-	19	-	-	-	-	-	-	-	59
A82M	3	33	-	-	28	-	-	-	-	-	-	-	39
A82M/I263R	3	24	-	-	19	-	-	-	-	-	-	-	57
KU3/A330P/F87A	3	25	-	-	28	-	-	-	-	-	-	-	47
RK/A328G/A184I/T260G/A82M/S332F/A330I	3	30	-	10	-	-	4	-	-	-	-	-	56
RK/A328G/A184I/T260G/A82M/S332L	3	31	-	18	9	-	13	-	-	-	-	-	28

RP/F87V/E267V/L437V	3	13	-	-	35	6	-	-	-	-	12	-	34
RP/A264G	3	24	-	-	23	-	31	-	-	-	-	-	22
RP/A82M/I263A/A328G/A330W	3	25	-	-	40	-	9	-	-	-	-	-	26
KSK19/A82M/I263G/A264G/A328G/A330L/S332F	2	51	-	14	-	-	-	-	-	-	-	-	35
KSK19/A82M/I263G/A264G/A328G/P329G	2	49	-	17	4	-	-	-	-	-	-	-	31
KSK19/A82M/I263G/A264G/S332L	2	46	-	21	8	-	25	-	-	-	-	-	-
KSK19/A82M/T260G/A264G	2	51	-	16	15	-	-	-	-	-	-	-	19
A82M/F87A/T260G/A264G	2	35	-	11	2	-	-	-	-	-	-	-	53
R19/F87W/I263G/A264G	2	41	-	21	11	-	22	-	-	-	-	-	5
R19/F81W/A328G/T260G	2	50	-	30	20	-	-	-	-	-	-	-	-
R19/A82M/A328G/I263W	2	41	-	21	25	-	6	-	-	-	-	-	7
A328G	2	47	-	28	6	-	-	-	-	-	-	-	19
RK/A328G/T260G/A264G	2	54	-	32	-	-	9	-	-	-	-	-	6
RK/A328G/T260G/A264G/I263G	2	46	-	32	8	-	14	-	-	-	-	-	-
R19/A330W/S72A/S332F	2	47	-	24	29	-	-	-	-	-	-	-	-
KT2/F87I/L75W	2	64	-	19	17	-	-	-	-	-	-	-	-
KT2/F87I/A328V	2	40	-	17	32	-	12	-	-	-	-	-	-
KT2/F87M	2	41	-	21	15	-	22	-	-	-	-	-	-
RT2/S72G/A330W	2	36	-	-	53	-	-	-	-	-	-	-	11
KSK19/A184I/E267G/F42V	2	46	-	-	26	-	-	-	-	-	-	-	28
A330P/A82M	2	31	-	-	-	-	-	-	-	-	-	-	69
A82M/I263F	2	35	-	-	37	-	-	-	-	-	-	-	28
KU3/A330P	2	35	-	-	18	-	-	-	-	-	-	-	47
KU3/A330P/A328I	2	34	-	-	-	-	-	-	-	-	-	-	66
KU3/A330P/S72W	2	33	-	-	-	-	-	-	-	-	-	-	67
KU3/A330P/I263A	2	28	-	-	-	-	-	-	-	-	-	-	72
RP/H171L/I263G/L437LV/A328I	2	40	-	-	-	-	-	-	-	-	-	-	60
RK/A328G/A184I/T260G/A82M/S332L	2	42	-	23	8	-	19	-	-	-	-	-	7
KSK19/A328I/Y51F	2	35	-	-	44	-	-	-	-	-	-	-	20
KSK19/A82M/I263G/A264G/A328G/A330I/S332F	1	80	-	20	-	-	-	-	-	-	-	-	-
KSK19/A82M/I263G/A264G/S332F	1	55	-	17	-	-	-	-	-	-	-	-	27

R19/A328G/P329G/A330G/T260G	1	66	-	20	-	-	-	-	-	-	-	-	14
RK/T260G/A264G	1	58	-	18	-	-	-	-	-	-	-	-	24
RK/T260G/A328G	1	55	-	16	-	-	-	-	-	-	-	-	29
RK/A328G/T260G/I263G	1	70	-	21	-	-	-	-	-	-	-	-	10
R19/A330W/S72A	1	57	-	23	20	-	-	-	-	-	-	-	-
R19/A330W/S72A/S332L	1	57	-	31	12	-	-	-	-	-	-	-	-
KT2/F87I/I263F/A328I	1	64	-	22	14	-	-	-	-	-	-	-	-
KT2/F87I/I263F/A328V/L353I	1	78	-	22	-	-	-	-	-	-	-	-	-
S72A/A82M/F87A/A184I/T260G/A328G	1	52	-	-	-	-	13	-	-	-	-	-	35
A82T/I263A	1	52	-	-	48	-	-	-	-	-	-	-	-
L322H	1	64	-	-	36	-	-	-	-	-	-	-	-
A82M/I263A	1	59	-	-	-	-	-	-	-	-	-	-	41
K19/A328G	1	-	-	14	40	-	18	-	-	-	-	-	28
GVQ/A328G/T260G	0	-	-	-	-	-	-	-	-	-	-	-	-

B: Enzyme screening data for diphenyl-derived *trans*-lactam **1.127**.

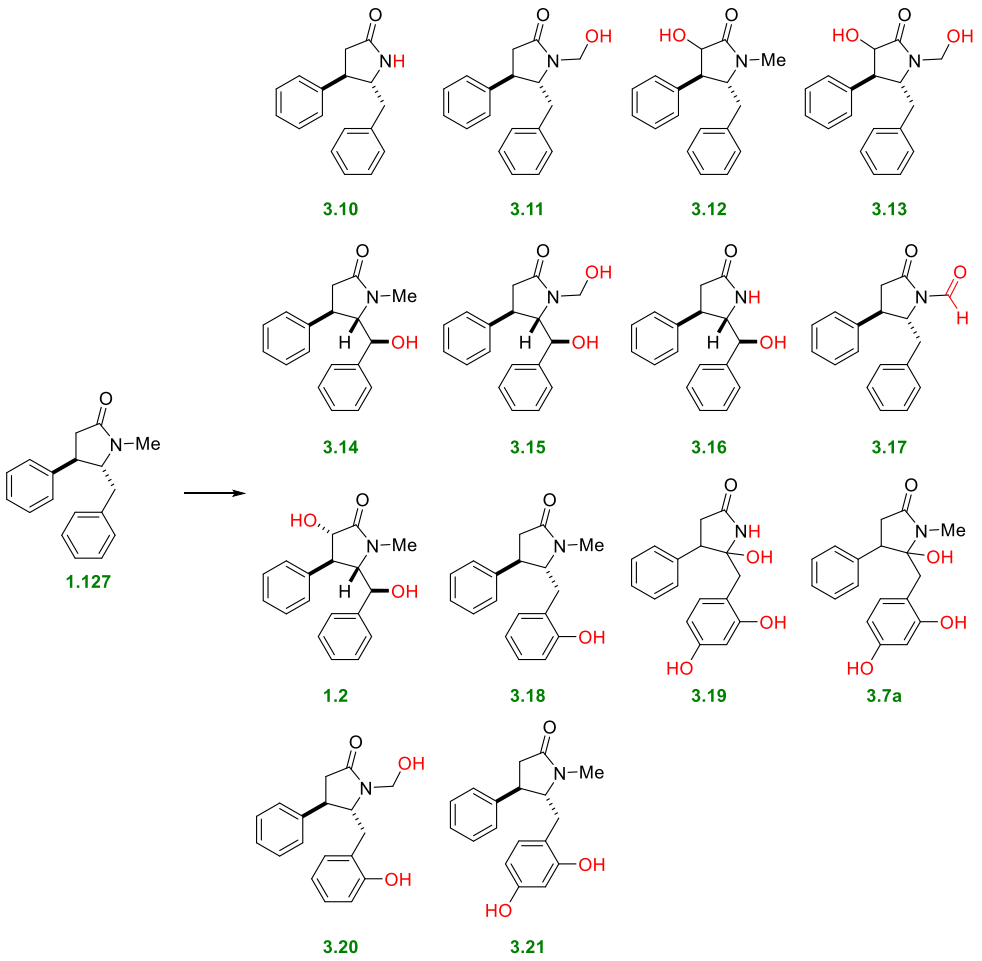


Table 5.2 Conversion and selectivity data for the $P450_{BM3}$ screening of diphenyl-derived *trans*-lactam **1.127**, organised first by decreasing selectivity for metabolite **3.14**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.14**.

Variant	Conv. (%)	3.10, 3.11 (%)	3.12 (%)	3.13 (%)	3.14 (%)	3.15, 3.16 (%)	3.17 (%)	1.2 (%)	3.18 (%)	3.19, 3.7a, 3.20 (%)	3.21 (%)	Others (%)
RK/A328G/T260G/A82M	100	11	1	1	55	15	-	-	6	-	-	10
KSK19/A82M/T260G/A328G	86	22	2	1	53	7	-	-	8	3	-	4
RK/A328G/A184I/T260G/A82M/A330V	100	4	-	4	51	23	-	1	1	2	-	15
RK/A328G/A184I/T260G/A82M/A330L	96	19	-	2	50	15	-	-	3	4	-	6
RK/A328G/A184I/T260G/A82M/P329F	6	52	-	-	48	-	-	-	-	-	-	-
RK/A328G/A184I/T260G/A82M/A330I	98	18	1	3	46	19	-	-	2	3	-	9
KSK19/A82M/A264G/A328G	76	11	3	1	44	14	-	-	-	2	2	23
KSK19/A82M/I263G/A264G	68	43	2	1	42	8	-	-	-	-	-	5
RK/A328I/A264G	19	44	-	-	41	1	-	-	-	-	-	14
RK/A328G/A184I/T260G/A82M/S72W	95	31	1	2	40	17	-	-	1	2	-	5
KSK19/A82M/I263G/A264G/A330I/A328G	96	23	1	4	39	25	-	-	-	1	-	8
KSK19/A82M/I263G/A264G/A328G/A330V	78	40	2	2	38	10	-	-	1	1	-	7
A82M/F87A/A184I/T260G/A328G	90	44	-	1	36	10	-	-	1	2	-	6
A82M/F87A/A184I/I263G	25	56	2	1	35	2	-	-	-	-	-	4
KSK19/A82M/I263G/A264G/S332M	2	65	-	-	35	-	-	-	-	-	-	-
GV/A184I/A328G/T260G	34	40	-	1	34	-	-	-	-	-	-	25
KSK19/A82M/T260G/I263G/A264G	7	62	-	-	33	-	-	-	-	-	-	4
KSK19/A82M/I263G/A264G/A328G	58	49	3	2	33	6	-	-	-	-	-	8
KSK19/A82M/I263G/A264G/S332L	1	67	-	-	33	-	-	-	-	-	-	-
A82M/F87A/A184I/A264G	80	46	-	1	32	14	-	-	1	-	-	6
RK/A328G/A184I/T260G/A82M	74	56	1	1	30	6	-	-	2	1	-	4
RK/A328G/K69V	97	7	15	1	25	14	-	2	-	-	7	30
A82M/F87A/A184I/A264G	26	65	-	-	25	-	-	-	-	-	-	10
A82M/F87A/A184F/T260G/I263G	8	76	-	-	24	-	-	-	-	-	-	-
RK/A328G/A184I/T260G/A82M/S72F	49	65	2	1	24	4	-	-	2	-	-	2

A82M/F87A/I263G/A264G/A328G	46	60	3	1	23	4	-	-	1	-	-	7
KSK19/A82M/I263G/A264G/A330F/A328G	89	37	8	8	22	18	-	1	1	-	-	5
KSK19/A82M/I263G/A264G/A330W	63	61	2	2	20	10	-	-	-	-	-	5
A82M/F87A/A184I/I263G/A264G/A328G	91	48	2	2	20	20	-	-	-	-	-	8
S72A/A82M/F87V/A184I/T260G/E435M	12	72	-	3	20	-	-	-	-	-	-	6
A82M/F87A/A184I/T260G/H171L	100	30	-	4	19	29	-	-	-	-	-	18
GV/A184I/A328G	100	5	-	5	18	34	-	7	-	-	13	11
GV/A184I/A264G/A328G	100	37	3	7	17	20	-	2	-	4	6	5
A82M/F87A/A184I/T260G/A264G	2	74	-	-	17		-	-	-	-	-	9
GVQ/A328G	100	12	3	11	16	22	-	6	1	6	11	12
R19/A82M/F87A/A184I/T260G	100	4	-	-	15	51	-	-	-	-	-	30
A82M/F87A/A184F/T260G/A264G	1	75	-	-	15	-	-	-	-	-	-	10
RK/I263G/A264G/A330P	68	58	-	2	15	13	-	-	-	-	-	12
KSK19/A141G/A264G/L181F	40	80	1	-	14	3	-	-	-	-	-	2
RK/A184I/A264G	93	37	1	8	14	30	-	-	-	-	-	11
KSK19/I263G/A264G	62	69	1	1	13	4	-	-	-	-	-	12
RK/I263G/A264G/A330V	62	65	1	1	13	10	-	-	-	-	-	10
GVQ/A328G	98	6	2	14	12	29	-	10	-	5	11	11
KSK19/A82M/I263G/A328G	78	63	9	3	12	4	-	1	-	1	1	7
GVQ/A264G	69	69	1	1	11	6	-	-	-	-	-	11
K19/F87V/A264G	59	71	1	1	11	4	-	-	-	1	-	12
GV/A184I/A264G	37	76	1	-	10	2	-	-	-	-	-	11
RK/A264G	65	73	2	2	10	6	-	-	-	-	-	7
GVQ/A264G/A330P	24	68	1	-	9	1	-	-	-	-	-	21
RK/A184I/I263G/A264G	67	75	-	1	9	5	-	-	-	-	-	10
R19/A82M/A264G/A328G	14	81	2	-	8	-	-	-	6	-	-	2
RK/A328G/A184I/T260G/A82M/A330F	7	82	4	-	8	-	-	-	6	-	-	-
RK/A328G/T260G/F81W	72	60	6	8	8	12	-	-	-	-	-	6
A82M/F87A/T260G/I263G	37	86	-	-	7	-	-	-	-	-	-	7
R19/A328L/I263A	24	58	5	8	7	8	-	-	-	-	-	13
A82M/F87A/A184I/T260G	69	80	-	-	7	9	-	-	-	-	-	4

RK/I263G/A264G/L181F	64	77	2	1	7	5	-	-	-	-	-	8
A82M/F87A/A184I/T260G/N319Y	76	67	-	-	7	14	-	-	-	-	-	13
A330P	2	34	-	-	7	-	-	-	-	-	-	60
GVQ/I263G/A328G/A264G	67	81	4	-	6	-	-	-	-	-	-	9
KSK19/A82M/S270G	84	71	-	2	6	4	-	-	-	-	-	17
RP/A264G	4	72	6	-	6	-	-	-	-	-	-	17
KSK19/A82M/T260G/I263G	11	80	-	3	6	-	-	-	-	-	-	11
A82M/F87A/A184I/T260G	62	87	-	-	5	8	-	-	-	-	-	-
RK/T260G/I263G/A264G	6	95	-	-	5	-	-	-	-	-	-	-
KSK19/I263A	63	-	-	2	5	-	-	-	-	-	-	26
KSK19/A82M/L262G	78	-	-	2	5	4	-	-	-	-	-	15
RK/A328G/A184I/A264G	100	25	1	30	5	20	-	2	-	1	-	17
RK/A328G/P329G/A330G	4	52	4	3	4	-	-	-	-	-	-	37
A82M/F87A/A184I/T260G	7	94	-	-	4	-	-	-	-	-	-	2
RK/F81W/T260G/A328G	35	81	3	1	4	1	-	-	-	-	-	10
KSK19/A82M/T268S	52	81	3	3	3	1	-	-	-	-	-	9
RK/A264G/A328G	79	49	14	20	3	7	-	-	-	1	-	5
RK/A328G/T438L	97	26	14	40	3	4	-	4	-	-	-	10
R19/F87I/A184I/I263G/A264G	20	84	-	-	3	-	-	-	-	-	-	13
RK/A328G/L181M	97	27	13	41	2	4	-	5	-	-	-	8
RK/A328G/L262G	36	73	14	2	2	1	-	-	1	-	-	7
A82M/F87A	28	87	-	-	2	-	-	-	-	-	-	10
RK/A328G/A184I/I263G/A264G	65	75	6	1	2	1	-	-	-	-	-	15
RK/A328I/S72W	6	79	-	-	2	-	-	-	-	-	-	19
RK/A328G/K69I	50	79	7	5	2	2	-	-	-	-	-	6
GV/A328G	100	-	-	12	2	33	-	13	-	2	15	23
A82M/F87A/A184I	46	78	-	-	2		-	-	-	-	-	20
RK/A184I/A264G/A328G	63	79	3	6	2	3	-	-	-	-	-	8
GVQ/A264G/P329G/A330W	37	77	5	2	2	-	-	-	-	-	-	15
KSK19/A82M/I263G	68	75	3	1	2	-	-	-	-	-	-	19
GVQ/A264G/I263G	44	81	2	-	2	-	-	-	1	-	-	15

RK/A328G	81	58	17	13	2	4	-	-	-	2	-	5
GV/A184I/I263G/A264G	41	79	1	-	2	1	-	-	-	-	-	18
A82M/F87A/A184I/I263G	36	84	-	-	1	-	-	-	-	-	-	14
RK/A328G/A184I/T260G/A264G/S72W	54	83	1	1	1	4	-	-	-	-	-	9
RK/A328G	60	65	10	6	1	-	-	-	-	-	-	17
RK/A328G/A264G	55	78	6	4	1	1	-	-	-	-	-	9
S72A/A82M/F87A/A184I	98	56	-	2	1	8	-	-	-	1	-	31
RK/A328G/A184I/T260G/A264G	42	93	1	-	1	1	-	-	-	-	-	5
RK/A328G/S270G	61	76	9	8	1	1	-	-	-	-	-	5
RP/A82M/I263A/A328G/A330L	44	87	5	1	1	-	-	-	-	-	-	6
RP/A82M/I263A/A328G/A330V	30	88	6	1	1	-	-	-	-	-	-	4
GVQ/I263G/Q307H	58	75	1	-	1	-	-	-	-	-	-	23
RK/A328G/T260G	48	96	2	-	1	-	-	-	-	-	-	1
GVQ	75	86	1	2	1	-	-	1		1	-	10
KSK19/A82M/I263G/A330W	94	76	6	7	1	2	-	-	-	-	-	9
RK/A328G/S72W	100	10	-	42	-	7	-	26	-	-	-	15
RK/A328G/L437F	100	32	16	22	-	11	-	-	-	-	-	19
RP/A82M/I263A/L181F/A328G/A330V	99	80	8	4	-	4	-	-	-	-	-	5
RK/A184I	97	69	1	9	-	4	-	-	-	1	-	17
RK/A328G/S72D	97	65	5	19	-	5	-	-	-	-	-	5
RK/A328G	96	21	13	45	-	6	-	4	-	-	-	10
RK/A328G/L181F	96	30	8	40	-	6	-	4	-	-	-	12
RK/A328G/I263F/L181M	95	29	5	41	-	4	-	2	-	-	-	18
RK/A328G/I263F	95	43	9	31	-	7	-	2	-	-	-	9
RK/A328G/S72V	87	66	14	19	-	1	-	-	-	-	-	1
GV/A184I/I263G/A328G/L75F	85	67	15	6	-	-	-	-	-	-	-	11
K19/F87V	85	67	1	5	-	3	-	-	-	2	-	21
KU3/A330P/F87V	85	88	2	3	-	2	-	-	-	-	-	5
KSK19/I263G	83	80	8	3	-	-	-	-	-	-	-	9
R19/I263G/A328G	80	84	6	4	-	1	-	-	-	-	-	5
A82M/F87A	80	100	-	-	-	-	-	-	-	-	-	-

RP/A82M/I263A/L181F/A328G/A330L	73	87	7	1	-	-	-	-	-	-	-	5
RK/A328G/P329G/A330G	71	79	4	2	-	1	-	-	-	1	-	12
RK/A184I/T260G/A328G	71	74	14	4	-	-	-	-	-	-	-	8
A74G/F87I/A184I/I263G/A328G	71	80	-	-	-	-	-	-	1	2	-	17
RK/P329G/A330W/T260G	71	84	8	2	-	-	5	-	-	-	-	1
GVQ/I263G/L75M	69	84	2	1	-	-	-	-	-	-	-	13
RK/A328G/A184I/I263G	69	81	14	3	-	-	-	-	-	-	-	3
RK/A328G/I263W	69	84	10	4	-	-	-	-	-	-	-	1
K19/F87V/Q403P	67	69	-	10	-	3	-	3	-	-	-	15
RK/A328G/T260G/T268S	67	58	19	6	-	-	-	-	-	-	-	17
R19/A330W/F87V	65	76	2	8	-	1	-	-	-	-	-	13
RK/A328G/T268S	65	63	22	6	-	2	-	-	-	-	-	6
RK/A328G/I263G	64	47	45	3	-	-	-	-	-	-	-	5
GVQ/A330P	63	85	3	1	-	-	-	-	-	-	-	11
RK/A184I/I263G/A328G	63	84	11	2	-	-	-	-	-	-	-	3
K19/F87V/I263G	62	67	1	-	-	-	-	-	-	-	-	32
RK/A328G/I263F/T438L	62	94	2	-	-	-	-	-	-	-	-	4
GI/A184I/I263G/A328G/S72F	59	88	1	-	-	-	3	-	-	-	-	8
KSK19/A82M	58	70	8	3	-	-	-	-	-	-	-	19
RK	57	91	2	1	-	-	-	-	-	-	-	6
R19/I263G/A264G/A328G	56	89	7	3	-	1	-	-	-	-	-	1
RK/A328G/A184I/T260G/T268S	55	59	14	9	-	1	-	-	1	-	-	17
GV/A330F	53	86	1	-	-	-	-	-	1	-	-	12
GL/A184I/I263G/A328G/L75M	53	85	-	-	-	-	-	-	-	-	-	13
R19/F87I	49	85	2	3	-	-	-	-	-	-	-	10
R19/F87I/L437LV	49	54	36	6	-	-	-	-	-	-	-	3
I263G/A328G	49	93	5	1	-	-	-	-	-	-	-	-
GVQ/A330W	48	80	2	2	-	-	-	-	-	1	-	14
GI/A184I/I263G/A328G/L75M	47	87	3	-	-	-	-	-	-	-	-	10
R19/S72A/A330W	46	84	3	6	-	-	-	-	-	-	-	7
KSK19/I263G	46	76	9	2	-	-	-	-	-	-	-	13

A82M/F87A/A184I/T260G/I263G	46	56	-	-	-	-	44	-	-	-	-	-
RK/A328G/T260G/A264G	45	95	-	-	-	-	-	-	-	-	-	5
GL/A184I/I263G/A328G/S72F	44	79	1	-	-	-	-	-	-	-	-	20
RP/A82M/I263A/L181F/A328G/A330L/Q359A	41	93	4	-	-	-	-	-	-	-	-	3
RK/A328G/A184I/I263G/T260G	40	83	10	2	-	1	-	-	-	-	-	4
RK/I263G/A328G	39	88	2	-	-	-	-	-	-	-	-	10
RK/A184I/I263G/A264G/A328G	39	85	3	-	-	-	-	-	-	-	-	12
RK/I263G	37	88	5	-	-	-	-	-	-	-	-	7
A82M/F87A/I263G	37	100	-	-	-	-	-	-	-	-	-	-
A82M/F87A/A184I	37	100	-	-	-	-	-	-	-	-	-	-
RK/A328I	35	98	-	-	-	-	-	-	-	-	-	2
R19/A330W	34	83	8	4	-	-	-	-	-	-	-	5
K19/F87V/E267V	33	94	-	-	-	-	-	-	-	-	-	6
RK/A328G/T269G	33	63	5	2	-	-	-	-	-	-	-	30
RK/A328G/A184I/T260G/S72G	32	98	1	-	-	-	-	-	-	-	-	1
RP/F87V/I263G/L188G	24	77	2	-	-	-	-	-	-	-	-	21
RK/A328G/I263W	24	87	10	-	-	-	-	-	1	-	-	2
GL/A184I	22	48	-	-	-	-	-	-	9	-	6	38
A82M/F87A/A184F/T260G/I263G	22	100	-	-	-	-	-	-	-	-	-	-
RK/A328G/T260G/P329G/A330G	22	100	-	-	-	-	-	-	-	-	-	-
R19/A82M/I263G/A328G	20	97	3	-	-	-	-	-	-	-	-	-
GV/A184I	19	88	3	-	-	-	-	-	-	-	-	9
RK/A328G/T260G/A264G/I263G	18	100	-	-	-	-	-	-	-	-	-	-
A82M/F87A/T260G	17	100	-	-	-	-	-	-	-	-	-	-
RP/F87V	16	56	-	-	-	-	-	-	-	-	-	44
RP/F87V/E267V/V178F/L437LV	15	88	2	-	-	-	-	-	-	-	-	10
GVQ/I263A/E267G	14	49	-	-	-	-	-	-	-	-	-	51
RK/A328G/A264F	13	100	-	-	-	-	-	-	-	-	-	-
RK/A328G/A184I/T260G/S72L	12	100	-	-	-	-	-	-	-	-	-	-
A82M/F87A/A184F	11	54	-	-	-	-	-	-	-	-	-	46
GV/A184I/T260G/I263G/A264G	10	35	-	-	-	-	-	-	-	-	-	65

RP/A82M/I263A	9	93	7	-	-	-	-	-	-	-	-	-
RP/F87V/E267V	9	92	-	-	-	-	-	-	-	-	-	8
R19/A328G/I263W	9	93	7	-	-	-	-	-	-	-	-	-
GVQ/I263A/E435A	8	13	-	-	-	-	14	-	-	-	-	73
RP/F87V/E267V/L437LV	8	75	3	-	-	-	-	-	-	-	-	22
GVQ/T260G	7	25	-	-	-	-	-	-	-	-	-	75
KSK19/A82M/T260G/T268S	7	80	-	4	-	-	-	-	-	-	-	17
RP/A82M/I263A/A328G/A330W	7	82	5	-	-	-	-	-	-	-	-	13
A82M/F87A/T260G	7	16	-	-	-	-	-	-	-	-	-	84
RK/A328G/S72D/T438L	7	100	-	-	-	-	-	-	-	-	-	-
GLQ/I263G/A264G	6	8	-	-	-	-	-	-	-	-	-	92
RK/A328L	6	77	-	-	-	-	-	-	-	-	-	23
KU3/A330P/I263G	5	100	-	-	-	-	-	-	-	-	-	-
K19/F87V/E267V/V78I	5	88	-	-	-	-	-	-	-	-	-	12
GLQ/I263G/A328G	5	58	-	-	-	-	-	-	-	-	-	42
RP/F87V/E267V/L437V	5	79	4	-	-	-	-	-	-	-	-	16
R19/A82M/A328G/I263W	4	100	-	-	-	-	-	-	-	-	-	-
RK/A328G/A184I/T260G/A82M/S332F	4	92	8	-	-	-	-	-	-	-	-	-
WT	3	77	-	-	-	-	-	-	-	-	-	23
A82M/F87A/A184I/T260G/I263G	3	100	-	-	-	-	-	-	-	-	-	-
KSK19/A328I/Y51F	2	37	-	-	-	-	-	-	-	-	-	63
S72A/A82M/F87A/A184I/T260G/A328G	2	36	-	-	-	-	-	-	-	-	-	64
R19/A328G/P329G/A330G	2	68	-	-	-	-	-	-	32	-	-	-
R19/F81W/A328G/T260G	2	100	-	-	-	-	-	-	-	-	-	-
A328G	2	85	15	-	-	-	-	-	-	-	-	-
GV/A184I/I263G/A328G	1	100	-	-	-	-	-	-	-	-	-	-
R19/F87W/I263G/A264G	1	100	-	-	-	-	-	-	-	-	-	-
A82M/F87A/A184F/T260G	1	67	-	-	-	-	-	-	-	-	-	33
RK/A328G/A184I/T260G/A82M/S332L	1	23	33	-	-	-	-	-	-	-	-	45
RK/A328G/A184I/T260G/A82M/S332M	1	100	-	-	-	-	-	-	-	-	-	-
RP/A82W/I263A	0	-	-	-	-	-	-	-	-	-	-	-

KSK19/A82M/I263G/A264G/A328G/A330I/S332F	0	-	-	-	-	-	-	-	-	-	-	-
KSK19/A82M/I263G/A264G/A328G/A330L/S332F	0	-	-	-	-	-	-	-	-	-	-	-
KSK19/A82M/I263G/A264G/A328G/P329G	0	-	-	-	-	-	-	-	-	-	-	-
KSK19/A82M/I263G/A264G/S332F	0	-	-	-	-	-	-	-	-	-	-	-
KSK19/A82M/T260G/A264G	0	-	-	-	-	-	-	-	-	-	-	-
A82M/F87A/T260G/A264G	0	-	-	-	-	-	-	-	-	-	-	-
R19/A328G/P329G/A330G/T260G	0	-	-	-	-	-	-	-	-	-	-	-
RK/T260G/A264G	0	-	-	-	-	-	-	-	-	-	-	-
A82M/F87A/T260G/A264G	0	-	-	-	-	-	-	-	-	-	-	-
RK/A328G/T260G/I263G	0	-	-	-	-	-	-	-	-	-	-	-
RK/A328G/A184I/T260G/A82M/S332F/A330I	0	-	-	-	-	-	-	-	-	-	-	-

C: Enzyme screening data for indene-derived lactam **1.128**.

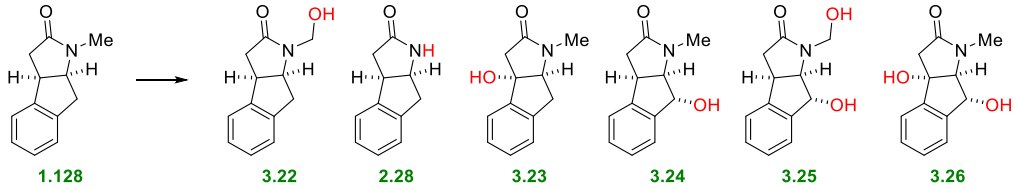


Table 5.3 Conversion and selectivity data for the $P450_{BM3}$ screening of indene-derived lactam **1.128**, organised first by decreasing selectivity for metabolite **3.24**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.24**.

Variant	Conv. (%)	3.22, 2.28 (%)	3.23 (%)	3.24 (%)	3.25 (%)	3.26 (%)	Others (%)
RP/A82M/I263A/L181F/A328G/A330L	90	6	9	58	13	-	14
RP/A82M/I263A/L181F/A328G/A330V	41	13	6	56	4	-	22
RP/A82M/I263A/L181F/A328G/A330L/Q359A	44	11	6	51	4	-	29
RP/A82M/I263A/L181F/A328G/A330L/K440F	42	8	11	49	3	-	29
RP/A82M/I263A/L181F/A328G/A330L/K349L	25	11	10	45	2	-	33
RP/A82M/I263A/S332L/L181F	28	5	40	45	1	-	9
RP/A82M/I263G/L181F	53	7	27	44	1	-	22
RP/A82M/I263A/L181F	27	10	32	42	-	-	16
RP/A82M/I263G/L181F/V26M	29	8	31	41	1	-	19
A82M/I263A/L181F	44	4	27	40	1	-	27
RP/A82M/I263A/L181F/A328G/A330L/V26H	19	10	11	39	1	-	38
RP/A82M/I263A	74	3	34	38	1	-	24
A82M/I263A/L181F/V26F	20	7	31	36	-	-	27
RP/A82M/I263G/L181F/V26F	33	7	30	36	1	-	27
RP/A82M/I263A/L181F/L188F	59	4	37	36	2	-	21
RP/A82M/I263F	68	10	29	35	1	-	25
RP/A82M	49	10	28	35	1	-	26
RP/A82M/I263G	43	8	30	32	-	-	30
S72A/A82M/F87A/A184I/E435M	43	9	47	31	-	-	12

RP/A82M/I263A/L181F/A328G/A330L/E267Q	13	20	11	31	-	-	38
RP/A82M/I263A/L181F/A328G/A330L/M354F	18	9	21	30	-	-	41
S72A/A82M/F87A/A184I	63	9	51	27	1	-	12
RP/A82M/I263A/S332L	21	11	26	27	-	-	36
GVQ/I263G/V78L	24	22	13	27	-	-	37
RP/A82M/I263A/S332F	23	14	22	26	1	-	37
A82M/F87A/A184I/E435M	82	6	51	26	1	-	17
RK/A82M/A184I/T260G	37	20	39	25	-	-	16
RP/A82M/I263A/S72G	19	9	21	25	-	-	45
RP/A82M/I263G/L181F/L188F	20	9	34	24	-	-	33
RP/A82M/I263A/A328G/A330V	7	21	9	24	-	-	46
GVQ/I263G/A330I	33	20	24	23	-	-	34
GV/A184I/I263W	64	24	4	23	2	-	47
GVQ/I263G/S72F	36	16	21	23	-	-	41
RK/A82M/A184I/T260G	38	21	40	22	1	-	15
RP/A82M/I263A/S332M	6	30	22	21	-	-	27
S72A/A82M/F87A/A184I/N239H/T260G/E435M	13	19	31	20	-	-	30
RP/A82M/I263A/S72F/A330W	25	19	33	20	1	-	27
RP/A82M/I263A/S332I	17	13	24	19	-	-	44
RP/A82M/I263A/S72F	8	20	20	19	-	-	42
GVQ/I263G/S72W	45	15	39	18	-	-	28
S72A/A82M/F87A/A184I/T260G/E435M	12	26	28	18	-	-	28
RP/A82M/I263A/S332I/L181F	4	-	48	18	-	-	34
A82M/F87A/A184I/T260G	15	32	27	17	-	-	24
GVQ/I263G/V26L	29	21	23	17	-	-	39
KSK19/A82M	68	6	60	17	-	-	17
GVQ/I263G/V26M	23	24	21	16	-	-	39
S72A/A82M/F87A/A184I/T260G/E435M	15	28	28	16	-	-	29
GVQ/I263G/V26F	24	25	22	16	-	-	37
S72A/A82M/F87A/E435M	33	8	58	15	1	-	19
KT2/L188G/I263G	60	37	28	15	-	-	19

GVQ/I263G/L437M	21	22	28	15	-	-	36
RP/A82M/I263A/A328G/A330L	10	10	5	14	-	-	70
S72A/A82M/F87A/A184I	66	14	19	14	-	-	53
RP/A82M/I263A/S332I/S72G	5	-	19	14	-	-	67
S72A/A82M/F87I/A184I/N239H/T260G/E435I	11	20	27	14	-	-	38
RP/A264G	36	47	10	14	-	-	29
S72A/A82M/F87A	22	11	52	14	-	-	23
WT	94	18	21	13	3	-	45
RP/A82M/I263A/A330W/S72G	3	-	18	13	-	-	68
A82M/F87A/A184I	14	18	39	13	-	-	30
S72A/A82M/F87A/A184I/T260G/V26L/N239H/E435M	13	31	28	13	-	-	28
GVQ/I263G	17	23	14	13	-	-	51
GVQ/I263G/A330W	20	25	23	13	-	-	40
GVQ/I263G/A330F	15	23	16	12	-	-	49
RP/A82M/I263A/A330W	11	12	51	12	-	-	25
A82M/F87A/E435M	27	10	58	12	-	-	20
GVQ/I263G/S72M	11	22	18	11	-	-	48
RP	77	26	20	11	2	-	40
A82M/F87A/A184I/T260G/E435M	13	25	25	11	-	-	39
RP/A82M/I263A/S332I/A330W	10	22	42	11	-	-	25
S72A/A82M/F87A/A184I/T260G/E435I	12	29	29	10	-	-	32
S72A/A82M/F87A/A184I/T260G/E435M/V26M	14	25	20	10	-	-	44
S72A/A82M/F87A/A184I/T260G/E435M/V26H	36	33	12	9	-	-	46
RK/A184I	33	26	48	9	-	-	16
GVQ/I263G/L437LH	12	16	10	9	-	-	65
KSK19/A82M/I263G	28	7	57	9	-	-	27
A82M/F87A/A184I/T260G/M354F	11	32	22	9	-	-	38
S72A/A82M/F87A/A184I/T260G/E435T	10	23	23	9	-	-	45
S72A/A82M/F87A/A184I/T260G	12	23	16	8	-	-	53
S72A/A82M/F87A/A184G/T260G/E435T	14	17	23	8	-	-	51
RK/A328G/L262G	65	8	62	8	1	-	21

A82M/I263G/L181F	11	8	5	8	-	-	79
S72A/A82M/F87A/A184I/T260G	9	35	17	8	-	-	39
A74G/F87A/A184I	28	40	39	8	-	-	13
A82M/F87A/T260G/E435M	17	14	39	7	-	-	40
RP/A82M/I263A/S72W	10	22	6	7	-	-	65
S72A/A82M/F87A/A184I/T260G/E435I	13	18	19	7	-	-	57
KSK19/A82M/I263G/A330W	18	16	29	7	-	-	48
A82M/I263G/L181F/K440F	8	-	6	6	-	-	88
RP/I263A	84	44	17	6	2	-	31
GL/A184I	22	56	3	6	1	-	34
RK/F81W/T260G/A328G	13	24	39	6	-	-	31
I263G	25	34	24	6	-	-	36
R19/A330W	44	56	11	5	-	-	27
RK/A328G	29	12	46	5	1	-	36
S72A/A82M/F87A/T260G	9	20	24	5	-	-	50
RK/A328G/S270G	19	18	49	5	-	-	28
A82M/I263G/L181F/V26M	9	14	4	5	-	-	77
RP/A82M/I263A/A328G/A330W	4	46	13	5	-	-	36
RP/A82M/I263A/L181F/A328G/A330L/K440F/M354F	13	24	8	5	-	-	63
RP/A82M/I263A/L181F/A328G/A330L/V26H/M354F	9	13	11	5	-	-	72
S72A/A82M/F87A/A184I/T260G/E435T/V26L	13	13	24	5	-	-	58
GV/A184I/S72M	31	73	6	4	1	-	15
S72A/A82M/F87A/A184I/T260G/E435I/V26M	11	24	17	4	-	-	54
RK/A328G	18	15	38	4	-	-	43
A82M/I263G/L181F/K394L	10	18	5	4	-	-	73
GVQ/I263G/S332F	12	21	3	4	-	-	73
GLQ/I263G/A328G	8	43	14	4	-	-	39
RLYF/H171L/I263G/L437LM	10	40	1	4	-	-	55
RK/G265GG	17	26	6	4	-	-	64
GVQ/I263G/L437F	12	28	12	3	-	-	57
RP/A82M/I263A/L181F/A328G/A330L/K440F/K349L	9	37	12	3	-	-	48

RK/A328G/P329G/A330G	6	21	26	3	-	-	50
RK/A328G/T269G	22	12	52	3	-	-	33
RLYF/H171L/I263G/L75F	46	68	2	3	-	-	27
RK/A328I/S72W	36	51	31	3	-	-	15
RP/A82M/I263A/L181F/A328G/A330L/V26H/K440F/K349L	10	-	11	3	-	-	87
RP/I263F	17	51	5	3	-	-	41
GG/A184I	20	13	59	3	-	-	26
S72A/A82M/F87A/A184I/T260G/E435I/V26L	12	21	17	3	-	-	59
GV/A184II263G/A328G	10	35	24	3	-	-	39
GLQ/I263G/A328L/L75F	15	52	2	3	-	-	44
RP/A82W/I263A	8	29	6	3	-	-	63
S72A/A82M/F87A/T260G/E435M	14	20	20	3	-	-	58
RP/A82M/F87I/I263A	15	59	11	3	-	-	28
RK/S270G	19	17	42	3	-	-	38
GLQ/I263G/A328L/A330L	19	46	3	2	-	-	48
RP/A82M/I263A/L181F/A328L/A330L	14	47	3	2	-	-	47
R19/S72A/A330W	13	17	15	2	-	-	66
R19/F87A	56	11	63	2	-	-	23
RK/P329G/A330W/T260G	15	8	58	2	2	-	30
GV/A184F	27	74	4	2	1	-	18
RK/A328I	69	41	37	2	1	-	19
RK/T260G	9	35	10	2	-	-	53
S72A/F87A/A184I/T260G/E435M	9	34	12	2	-	-	52
RK/A264G	48	8	62	2	1	-	27
GVQ/I263G/L437LM	10	32	8	2	-	-	58
A82M/I263A/S332M	7	-	5	2	-	-	93
RK/L262G	24	18	44	2	1	-	35
RK/A328G/P329G/A330G	10	29	6	2	-	-	63
GLQ/I263G/A328L/V26M	16	43	1	2	-	-	53
RK	34	11	54	2	-	-	33
S72A/F87A/A184I/T260G/E435T	8	32	12	2	-	-	54

GV/A184I/H171L/L188Q	60	63	10	2	5	-	20
K19/F87V/E267V/V78I	21	69	14	2	-	-	15
A82M/F87A/T260G/A184I/L437V	10	19	13	1	-	-	67
RK/T269G	59	9	65	1	1	-	24
A82M/I263G	9	17	4	1	-	-	78
GLQ/I263G/A328L/L437F	9	36	4	1	-	-	59
GV/A184I/L75F	40	61	11	1	-	-	27
A82M/I263G/L181F/V26H	11	-	4	1	-	-	95
GLQ/I263G/A330L	11	39	4	1	-	-	56
R19/F87I/A330I/A184F/I263G	13	47	4	1	-	-	47
RK/A184I/T260G/A328G	9	15	22	1	-	-	62
I263A	12	23	3	1	-	-	73
GLQ/I263G/A328L/V26F	16	43	1	1	-	-	55
S72A/A82M/F87I/A184I/N239H/T260G/E435M	16	65	9	1	-	-	25
RP/A82M/I263A/L181F/A328G/A330L/V26H/K349L	12	23	12	1	-	-	64
S72W/F87V/A184I	41	78	6	1	1	-	13
RK/A328G/T260G	12	19	8	1	-	-	71
RP/A82M/I263A/L181F/A328G/A330L/V26H/K440F	7	-	14	1	-	-	85
S72A/A82M/A184I/T260G/F87I/E435M	21	56	8	1	-	-	35
RK/P329G/A330W	34	10	60	1	2	-	27
KSK19/I263A	15	7	10	1	-	-	83
GVQ/A328G	49	67	17	1	-	-	15
K19/F87V/E267V	13	62	10	1	-	-	26
GV/A184I/A330W	60	69	7	1	3	-	20
GV/A184I/L437LM	25	52	7	1	-	-	39
S72A/A82M/A184I/T260G/F87I/E435I	11	63	10	1	-	-	26
GV/A184I/V26L	55	74	5	1	2	-	17
S72A/A82M/F87I/A184I/T260G/E435T	8	30	11	1	-	-	59
RP/A82M/I263A/L181F/L437F/T438F	11	33	9	1	-	-	56
GV/A184I/V26M	44	87	4	1	1	-	7
RK/Q403P	49	11	62	1	2	-	24

GV/A184I/A330I	60	65	5	1	3	-	26
RK/T260G/I263G/A264G	10	24	7	1	-	-	69
GV/A184M	69	64	12	1	5	-	18
R19/A328L/I263A	99	55	1	1	-	-	13
S72A/A82M/F87I/A184I/T260G/E435M	11	65	8	1	-	-	26
GV/A184I/V26F	50	81	4	1	1	-	13
GV/A184I/L188Q	54	74	6	1	2	-	17
GV/A184I/L188M	71	60	10	1	6	-	24
GV/A184I/L437M	58	73	6	1	1	-	19
GLQ/I263G/A328L/V26L	12	37	2	1	-	-	60
GV/A184I	58	74	7	1	4	-	15
RP/A74I/F87I/L181F/I263A/A328G/A330L	10	25	10	1	-	-	64
S72A/A82M/F87I/A184I/T260G/E435I	11	33	8	1	-	-	58
RK/T260G/A328G/T268S	11	23	6	1	-	-	71
RK/I263G	12	14	2	1	-	-	83
S72A/A82M/F87A/A184I/T260G/E435I/V26H	12	13	7	1	-	-	80
RK/P329V/A330P	20	17	44	1	1	-	38
RT2/V78I/A184I/A330P	96	36	1	-	6	-	57
RK/T260G/I263G/A328G	84	-	1	-	-	-	99
GV/A184I/A328L	62	87	1	-	-	-	11
GV/A184I/L437F	58	60	4	-	1	-	36
K19/F87V	54	76	7	-	-	-	16
K19/F87V/Q403P	52	81	6	-	-	-	12
GVQ	51	85	6	-	-	-	8
GVQ/A330W	43	84	5	-	-	-	10
GV/A184I/L437LF	39	74	7	-	-	-	19
R19/F87I/A330P	37	67	6	-	-	-	27
R19/F87I/A184I/V26M	31	77	3	-	-	-	20
R19/F87I/L181F	27	77	4	-	-	-	19
R19/F87I/K440W	27	75	-	-	-	-	25
R19/A82M	25	18	35	-	-	-	47

RK/T260G/I263G	22	8	5	-	-	-	87
GV/A184I/L437LH	22	80	4	-	-	-	16
R19/A184I/T260G/A330I	22	56	6	-	-	-	37
R19/F87I/V26M	20	68	6	-	-	-	26
R19/F87I/A184I/L437M	19	77	4	-	-	-	19
R19/F87I/V26L	19	86	4	-	-	-	10
KU3/A330P/I263G	17	60	3	-	-	-	36
R19/F87I/V26M/L71M	17	76	7	-	-	-	17
A330P	16	73	1	-	-	-	26
R19/F87I/T88M	16	64	5	-	-	-	30
R19/F87I/V26L/L71M	15	61	7	-	-	-	32
RK/A328L	14	42	14	-	-	-	45
R19/A82M/A184I/T260G/A330I	14	38	9	-	-	-	53
RK/T260G/A264G	13	15	5	-	-	-	79
R19/F87I/A184I/V26L	13	62	6	-	-	-	32
R19/F87I/A184I/E267F	13	33	6	-	-	-	61
R19/F87I/A184I/L437F	12	64	7	-	-	-	29
R19/F87I/A184I/L437LV	12	47	7	-	-	-	46
S72A/A82M/F87A/A184I/T260G/E435T/V26M	11	24	9	-	-	-	68
S72A/A82M/F87A/A184I/T260G/E435T/V26H	11	16	7	-	-	-	77
KSK19/I263G	11	17	19	-	-	-	64
RK/A328G/I263G/A264G/T260G	11	23	5	-	-	-	71
R19/F87I/A82M/A72L	11	51	8	-	-	-	41
R19/F87I/T88L	10	46	7	-	-	-	47
R19/F87I/L437LV	10	43	6	-	-	-	51
R19/A328L/I263A	9	38	8	-	-	-	54
R19/F87I/A184I/S72L	9	40	8	-	-	-	53
RK/T260G/A264G/A328G	9	14	9	-	-	-	78
RK/I263G/A328G	8	17	9	-	-	-	73
R19/F87I	8	65	9	-	-	-	26
R19/A184I	8	50	9	-	-	-	41

R19/F87I/T88F	6	22	11	-	-	-	66
A82M/I263A	2	-	-	-	-	-	100

D: Enzyme screening data for phenalene-derived lactam **1.129**.

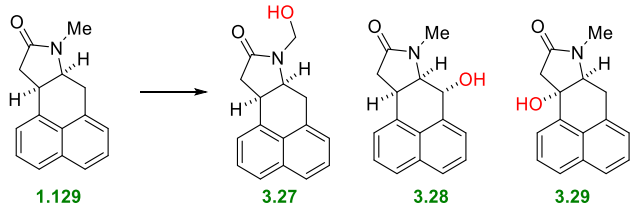


Table 5.4 Conversion and selectivity data for the $P450_{BM3}$ screening of phenalene-derived lactam **1.129**, organised first by decreasing selectivity for metabolite **3.28**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.28**.

Variant	Conv. (%)	3.27 (%)	3.28 (%)	3.29 (%)	Others (%)
KSK19/A82M/I263G	74	-	100	-	-
KSK19/A82M	100	7	93	-	-
S72A/A82M/F87A/A184I	100	6	83	-	11
RK/A184I/T260G/A328G	100	9	43	24	25
RK/I263G	100	14	23	44	20
KSK19/I263A	71	18	21	61	-
RK/A184I	75	14	20	57	9
RP/A82M/I263A	23	18	19	-	63
KSK19/I263G	90	27	11	62	-
RK/A328G/P329G/A330G	71	15	9	48	28
RK/F81W/T260G/A328G	76	17	9	42	32
GVQ/A328G	100	27	9	9	55
RK/A328G	100	24	6	47	23
RK	60	12	6	71	11
RK/A328I	57	28	4	43	25
RP/A82M/I263A/L181F/A328G/A330V	100	-	-	-	-
K19/F87V/E267V/V78I	85	74	-	6	20
K19/F87V	85	50	-	17	33
GVQ/A330W	83	71	-	6	23

K19/F87V/Q403P	66	54	-	18	28
GVQ	65	48	-	16	36
GV/A184I	43	100	-	-	-
R19/F87I	35	71	-	8	21
RK/A328L	25	32	-	24	44
R19/A328L/I263A	22	41	-	16	43
K19/F87V/E267V	21	78	-	-	22
KU3/A330P/I263G	19	49	-	-	51
R19/F87I/L437LV	18	91	-	-	9
GV/A184I/I263G/A328G	18	-	-	-	-
WT	16	16	-	-	84
RK/A328I/S72W	13	49	-	37	14
R19/A330W	11	39	-	-	61
A330P	11	71	-	-	29
RP/A82W/I263A	11	21	-	-	79
R19/S72A/A330W	6	61	-	-	39
GLQ/I263G/A328G	0	-	-	-	-

E: Enzyme screening data for phenalene-derived *cis*-isomer lactam **2.85**.

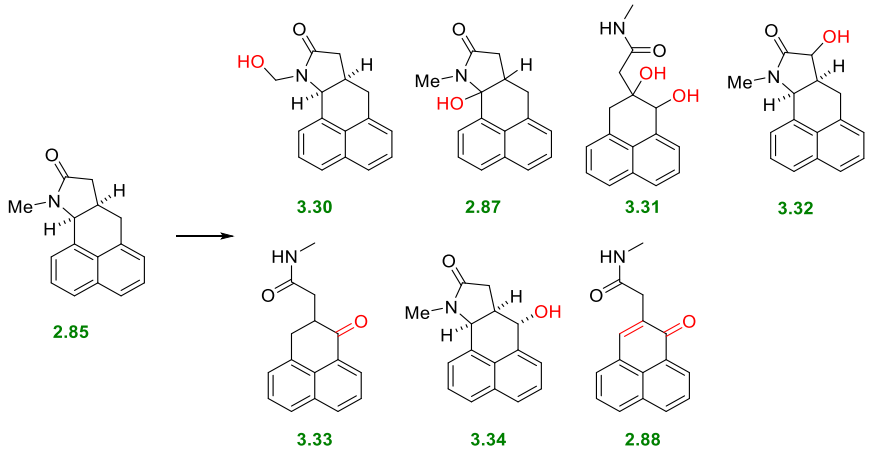


Table 5.5 Conversion and selectivity data for the $P450_{BM3}$ screening of phenalene-derived *cis*-isomer lactam **2.85**, organised first by decreasing selectivity for metabolite **3.34**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.34**.

Variant	Conv. (%)	3.30 (%)	2.87, 3.31 (%)	3.32 (%)	3.33 (%)	3.34 (%)	2.88 (%)	Others (%)
KSK19/A82M/I263G	99	-	-	-	26	43	21	10
S72A/A82M/F87A/A184I	98	1	-	7	25	39	21	7
KU3/A330P/V78I	37	62	-	-	-	38	-	-
K19/F87I/I263G	77	49	-	2	3	37	2	7
KSK19/A82M/F261G	100	-	-	2	21	37	29	11
K19/F87A/A82M/L437M	93	3	-	5	29	37	17	10
GI/A184I/I263G/A328G/S72F	30	27	2	2	16	36	5	12
KSK19/A82M/T260G/I263G	61	1	1	1	50	36	5	6
KSK19/A82M/I263G/A330W	88	1	1	1	44	36	13	6
KU3/A330P/V78I/A184I	17	29	-	2	11	35	4	19

KSK19/A82M/I401M	96	-	-	2	32	33	25	8
KU3/A330P/S72W	78	50	1	1	2	32	3	11
KSK19/A141G/A264G/L181F	90	-	-	1	24	31	26	18
S72A/A82M/F87A/A184I/E435M	98	2	-	5	32	31	21	9
GLQ/I263G/A328G	48	36	-	2	12	30	7	14
KSK19/A82M/I263G/A328G	94	-	1	-	40	29	22	7
S72A/A82M/A184I	99	-	-	6	7	29	44	14
A82M/F87A/A184I	67	4	-	2	44	29	12	9
A82M/F87A/A184I/A264G	74	2	-	2	43	28	17	8
RT2/A330P/L437LA	11	58	-	-	14	28	-	-
K19/F87I/I263A	51	61	-	1	3	28	2	6
K19/F87V/I263G	31	37	2	2	17	27	4	11
RT2/A330P/S72G/L437LA	8	73	-	-	-	27	-	-
S72A/A82M/F87A	86	1	-	2	39	27	23	8
KU3/A330P/I263A	64	55	-	-	2	27	2	15
A82M/F87A/A184I/I263G/A264G/A328G	98	-	-	1	30	27	34	7
RK/A330P/P329G/I263G	78	-	-	3	56	26	-	15
KU3/A330P/A82M	30	74	-	-	-	26	-	-
S72A/A82M/F87A/E435M	42	3	-	1	53	25	10	8
K19/F87A/A82M/S72A	100	-	-	4	8	25	56	7
KSK19/A82M/T260G/I263G/A264G	45	7	-	1	53	25	8	6
A82M/F87A/A184I/I263G	37	-	-	-	63	24	8	5
A82M/F87A/I263G/A264G/A328G	93	-	-	1	46	24	23	6
KU3/A330P/I263S	25	51	3	1	9	23	5	8
S72A/A82M/F87I/E435M	18	35	-	2	16	23	6	17
GI/A184I/I263G/A328G/L75M	18	32	3	3	24	23	6	10
KSK19/A82M/I263G/A264G/A330W	68	-	-	-	52	23	17	8
KSK19/A82M	99	-	-	3	5	22	61	9
A82M/F87A/A184I/T260G	99	2	-	13	32	22	20	12
A82M/F87A/E435M	100	-	-	1	24	22	44	9
KSK19/A82M/I263G/A264G	97	-	-	-	33	22	34	11

A82M/F87A/A184I/T260G/E435M	27	15	-	2	44	21	6	12
S72A/A82M/F87I	17	38	-	2	16	21	4	19
S72A/A82M/F87A/A184I/T260G/N239H/E435M	27	15	-	2	46	21	7	9
A330P/I263A	12	60	-	-	12	20	-	8
S72A/A82M/F87A/A184G/T260G/E435T	28	10	-	2	47	20	8	12
RT2/A330P	43	70	-	-	5	20	-	6
KSK19/I263A	90	1	-	1	50	19	20	9
KU3/A330P	21	75	-	-	7	19	-	-
KSK19/A82M/S270G	99	-	-	3	6	19	48	24
A82M/F87A/A184I/T260G/A328G	94	6	-	9	39	18	20	7
KSK19/A82M/I263W	32	-	-	2	41	18	10	28
R19/A82M/F87A/A184I/T260G	100	-	-	11	12	18	49	10
KU3/A330P/I263G	17	43	-	-	-	18	3	36
A82M/F87A/A184I/T260G/M354F	35	19	-	2	41	18	6	14
S72A/A82M/F87A/A184I/T260G	30	19	-	2	43	18	6	13
S72A/A82M/F87A/T260G/E435M	16	12	-	2	37	17	7	25
S72A/A82M/F87A/A184I/T260G/E435I	16	12	-	-	40	17	7	23
RP/A82M/I263A	27	5	-	2	12	17	11	53
KSK19/A82M/T260G	100	-	-	7	28	17	38	10
A82M/F87A/A184I/E435M	100	-	-	6	15	17	56	6
KU3/A330P/L437LA	18	47	3	2	9	17	6	16
A330P	17	73	-	-	10	17	-	-
KSK19/I263G/A264G	86	1	-	5	60	16	11	7
A82M/F87A/T260G/E435M	13	4	-	3	44	16	9	24
K19/F87A/A82M/E267F	89	4	-	2	32	16	32	14
RK/I263G	83	1	-	3	58	16	13	9
KSK19/A82M/I263W	22	12	3	2	45	16	9	13
GVQ/A330P/A264G	54	55	-	-	30	15	-	-
KSK19/A82M/A264G	97	-	-	1	34	15	45	5
KSK19/A82M/I263G/A264G/A330F/A328G	100	1	-	2	21	15	47	13
KSK19/A82M/L262G	99	-	-	3	7	14	68	8

KU3/A330P/F81W	12	69	-	-	-	14	-	17
S72A/A82M/F87I/A184I/N239H/T260G/E435I	41	72	-	-	5	13	3	7
KSK19/A82M/I263G/A264G/S332M	14	-	-	2	56	13	12	17
GIQ/I263G	9	35	-	4	18	13	8	22
S72A/A82M/F87A/T260G	11	6	-	-	47	13	14	19
KSK19/A82M/I263G/A264G/A328G/A330V	100	-	-	3	28	13	52	4
GI/A184I/I263G/A328G/L75F	12	19	5	3	38	12	10	13
S72A/A82M/F87I/A184I	29	66	-	1	7	12	3	11
KSK19/I263G	50	1	-	2	66	12	8	11
S72A/A82M/F87I/A184I/E435M	35	66	-	1	6	12	4	11
RK/A328G/P329G/A330G	90	15	-	6	34	11	13	22
KU3/A330P/S72G	26	56	3	2	9	11	6	14
KU3/A330S	20	52	3	2	8	11	5	20
KSK19/A82M/I263G/A264G/S332L	9	-	-	4	45	11	14	25
RP/A82M/I263A/L181F/A328G/A330V	92	24	-	1	2	10	12	50
KSK19	100	-	-	3	16	10	63	8
S72A/A82M/F87V/A184I/T260G/E435I	35	70	-	-	5	10	1	13
RK/A184I/T260G/A328G	60	1	-	4	61	10	12	12
S72A/A82M/A184I/T260G/F87I/E435M	16	54	-	2	10	10	5	18
RK/F81W/T260G/A328G	96	23	-	11	29	10	21	7
KSK19/I263G	88	1	1	3	63	10	15	8
KSK19/A82M/T260G/A328G	97	1	-	5	34	10	44	6
R19/A330W	12	36	-	-	-	9	4	51
KU3/A330P/I263G/E267G	13	40	5	3	17	9	8	17
KU3/A330P/I263V	8	30	-	4	21	9	8	27
KSK19/A82M/A330W	99	-	-	4	17	9	56	13
KSK19/A82M/I263G/A264G/S332F	5	-	-	7	32	9	18	34
KSK19/A82M/T268S	96	-	-	3	46	9	36	6
S72A/A82M/A184I/T260G/F87I/E435I	21	66	-	-	8	9	4	13
KSK19/A82M/I263G/A264G/A330I/A328G	100	-	-	2	17	9	67	5
RLYF/KSK19	99	-	-	2	15	9	60	14

K19/F87I/E267V/A82M	7	22	9	5	25	9	13	17
A82M/F87A/T260G/A264G	6	-	-	6	29	9	16	40
K19/F87I/E267V	11	48	6	3	17	8	6	12
S72A/A82M/F87A/T260G/E435M	7	-	-	5	32	8	14	41
K19/F87A/A82M/T438F	17	25	-	2	12	8	9	43
RK/I263G/A264G/A330P	7	-	8	5	37	8	12	29
GIQ/I263G/A264G	6	-	11	6	30	8	16	30
K19/F87V/E267V	28	44	-	3	19	7	8	19
A330P	15	29	-	-	12	7	11	40
K19/F87V/E267V/V78I	40	40	-	2	28	7	6	17
KSK19/A82M	100	-	-	2	1	7	81	10
GV/A184I	62	59	-	1	8	7	7	18
K19/F87V/A264G	92	42	1	1	33	7	11	6
A82M/F87A/T260G/A184I/L437V	8	-	-	4	31	6	13	46
GIQ/I263G/A330F	8	12	-	5	25	6	12	40
KU3/A330P/L437LM	8	28	7	4	21	6	14	20
R19/S72A/A330W	19	42	-	10	10	6	13	19
K19/L75M	65	73	-	-	2	6	4	15
KU3/A330P/I263W	7	6	8	5	17	6	12	47
WT	15	28	-	4	11	5	12	39
K19/F87I	59	69	-	-	11	5	2	12
GIQ/I263G/A330L	11	35	5	3	20	5	11	20
KSK19/A82M/I263G/A264G/A328G/A330I/S332F	6	-	-	6	25	5	14	50
KU3/A330S/L437LA	36	65	2	1	5	5	5	17
RK/A328I/S72W	31	35	-	18	18	5	6	17
RK/A328I	91	27	-	16	30	5	6	16
RP/A82W/I263A	10	6	-	6	14	5	19	50
K19/F87V/A328V	40	78	-	1	5	5	2	9
RK/A328I	80	29	-	14	25	5	11	17
GVQ/A328G	94	38	-	3	27	5	11	16
KU3/A330P/F87V	86	59	-	-	31	4	-	7

K19/F87V	92	32	-	2	36	4	7	20
GVQ/A330W	78	39	-	2	29	4	6	20
RK	86	7	-	1	58	3	8	24
R19/A328L/I263A	14	12	-	5	15	3	18	46
RK/A328G	98	6	-	6	24	3	37	24
R19/F87I	59	64	-	1	7	3	9	16
RK/A330P/P329G	98	-	-	2	51	3	22	22
R19/F87I/L437LV	27	59	-	2	8	2	8	21
RK/A184I	99	-	-	7	13	2	55	23
K19/F87V/Q403P	67	59	-	1	16	2	10	12
GVQ/A330P/A264G/P329G	100	25	-	-	62	-	-	13
RK/A330P/A184I	100	-	-	23	39	-	-	38
KSK19/A82M/A264G A328G	98	-	-	1	-	-	88	11
KU3/A330P/F87A	93	15	-	9	59	-	4	12
GVQ	70	52	-	1	25	-	1	20
GVQ/A330P	61	71	-	-	29	-	-	-
RK/A328L	10	18	-	-	21	-	4	57
GV/A184I/I263G/A328G	10	5	-	6	15	-	18	55
K19/F87A/A82M/L437I	7	19	-	6	22	-	11	43
KSK19/A82M/I263G/A264G/A328G/A330L/S332F	6	-	-	6	27	-	14	53
KSK19/A82M/I263G/A264G/A328G/P329G	6	-	-	6	26	-	16	53
KSK19/A82M/T260G/A264G	6	-	-	6	29	-	16	48
RP/A330P	6	58	-	-	34	-	-	7
KU3/A330P/A184I	2	100	-	-	-	-	-	-
RT2/A330P/V78I	0	-	-	-	-	-	-	-
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F: Enzyme screening data for phenalene-derived *trans*-isomer lactam **2.86**.

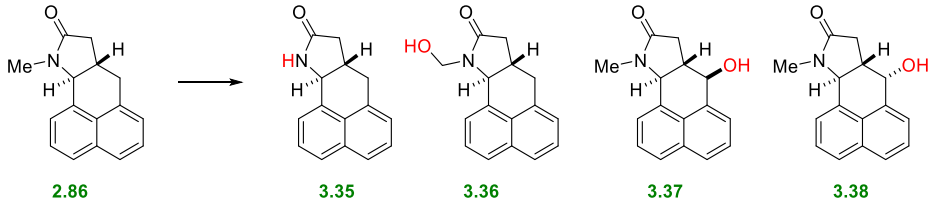

Table 5.6 Conversion and selectivity data for the $P450_{BM3}$ screening of phenalene-derived *trans*-isomer lactam **2.86**, organised first by decreasing selectivity for metabolite **3.37**, followed by decreasing conversion for enzymes with no selectivity toward metabolite **3.37**.

Variant	Conv. (%)	3.35, 3.36 (%)	3.37 (%)	3.38 (%)	Others (%)
K19/F87V	92	4	60	17	18
GVQ	58	5	57	17	21
GVQ/A328G	87	13	55	13	19
K19/F87V/Q403P	45	12	54	12	22
GVQ/A330W	73	16	48	13	23
RK/A328G/P329G/A330G	28	3	46	10	41
KSK19/A82M	100	-	44	35	21
RK/F81W/T260G/A328G	56	11	42	28	18
K19/F87V/E267V/V78I	23	9	41	7	42
RK	89	8	41	30	21
K19/F87V/E267V	22	7	41	6	46
RK/A184I	94	10	39	24	27
GV/A184I	48	24	38	25	13
GV/A184I/I263G/A328G	17	-	38	-	62
R19/A328L/I263A	18	-	37	-	63
R19/F87I/L437LV	18	9	37	-	54
RKA/S72W	17	7	37	-	56

RP/A82W/I263A	19	-	35	-	65
R19/A330W	20	2	34	-	64
WT	21	3	34	2	61
S72A/A82M/F87A/A184I	88	6	34	37	23
KSK19/A82M/I263G	56	10	33	26	31
A330P	20	11	32	-	57
R19/S72A/A330W	19	3	31	-	66
RK/A328G	62	8	30	30	31
GLQ/I263G/A328G	42	20	30	21	29
RP/A82M/I263A	23	14	29	5	52
RK/A328L	23	4	29	-	67
RP/A82M/I263A/L181F/A328G/A330V	72	7	27	46	21
R19/F87I	31	24	23	2	51
RKA	29	24	23	-	53
RK/A184I/T260G/A328G	36	38	23	-	39
KSK19/I263A	73	50	13	7	30
KSK19/I263G	54	58	11	1	30
RK/I263G	53	53	1	4	42
KU3/A330P/I263G	16	-	-	19	81

References

1. R. Xu, Y. Ye and W. Zhao, *Introduction to Natural Products Chemistry*, CRC Press, Boca Raton, 2010.
2. K. C. Nicolaou, *Isr. J. Chem.*, 2018, **58**, 104−113.
3. S.-f. Chu and J.-t. Zhang, *Acta Pharm. Sin. B*, 2014, **4**, 417−423.
4. S. Chu, S. Liu, W. Duan, Y. Cheng, X. Jiang, C. Zhu, K. Tang, R. Wang, L. Xu, X. Wang, X. Yu, K. Wu, Y. Wang, M. Wang, H. Huang and J. Zhang, *Pharmacol. Ther.*, 2016, **162**, 179−187.
5. J.-F. Hu, S.-F. Chu, N. Ning, Y.-H. Yuan, W. Xue, N.-H. Chen and J.-T. Zhang, *Neurosci. Lett.*, 2010, **483**, 78−82.
6. J.-J. Xue, Y.-M. Zhou and X.-M. Yu, *Chin. Chem. Lett.*, 2011, **22**, 1261−1264.
7. X. Li, K. Lai, K. Wu, D. Huang and L. Huang, *Eur. J. Med. Chem.*, 2014, **74**, 736−741.
8. X. Z. Li, C. J. Zhu, C. H. Li, K. M. Wu, D. F. Huang and L. Huang, *Eur. J. Med. Chem.*, 2010, **45**, 5531−5538.
9. G. T. Liu, W.-X. Li, Y.-Y. Chen and H.-L. Wei, *Drug Dev. Res.*, 1996, **39**, 174−178.
10. Y. Ming-He, C. Yan-Yong and H. Liang, *Phytochemistry*, 1988, **27**, 445−450.
11. M. H. Yang, Y. H. Cao, W. X. Li, Y. Q. Yang, Y. Y. Chen and L. Huang, *Acta Pharmaceutica Sinica*, 1987, **22**, 33−40.
12. W. Hartwig and L. Born, *J. Org. Chem.*, 1987, **52**, 4352−4358.
13. M. Wang, C.-Y. Liu, T. Wang, H.-M. Yu, S.-H. Ouyang, Y.-P. Wu, H.-B. Gong, X.-H. Ma, G.-L. Jiao, L.-L. Fu, Q.-S. Wu, H. Kurihara, Y.-F. Li, T. Shen and R.-R. He, *Cell Death Dis.*, 2020, **11**, 781.
14. X. Y. Jiang and J. T. Zhang, *J. Asian Nat. Prod. Res.*, 1998, **1**, 53−58.
15. J.-F. Hu, F. Niu, N. Ning, W.-Z. Duan, S.-F. Chu, W. Xue, Y.-H. Yuan, N.-H. Chen and J.-T. Zhang, *J. Asian Nat. Prod. Res.*, 2012, **14**, 256−262.
16. N. Ning, J.-F. Hu, J.-D. Sun, N. Han, J.-T. Zhang and N.-H. Chen, *Eur. J. Pharmacol.*, 2012, **682**, 50−55.
17. F. Hua, M.-j. Shi, X.-l. Zhu, M. Li, H.-x. Wang, X.-m. Yu, Y. Li and C.-j. Zhu, *Biochem. Pharmacol.*, 2015, **98**, 224−230.
18. Z. Feng, X. Li, G. Zheng and L. Huang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2112−2115.
19. J. Zhang, Y. Cheng and J. Zhang, *Yaoxue Xuebao*, 2007, **42**, 935−942.
20. K. Tang and J.-T. Zhang, *Neurol. Res.*, 2002, **24**, 473−478.
21. W. Duan and J. Zhang, *Chin. Med. J.*, 1998, **111**, 1035−1038.
22. K. Tang and J.-T. Zhang, *Life Sci.*, 2004, **74**, 1427−1434.
23. L. Yang, D.-X. Wang, Q.-Y. Zheng, J. Pan, Z.-T. Huang and M.-X. Wang, *Org. Biomol. Chem.*, 2009, **7**, 2628−2634.
24. M. W. Cappi, W.-P. Chen, R. W. Flood, Y.-W. Liao, S. M. Roberts, J. Skidmore, J. A. Smith and N. M. Williamson, *Chem. Commun.*, 1998, **10**, 1159−1160.
25. J.-Q. Wang and W.-s. Tian, *J. Chem. Soc., Perkin Trans. 1*, 1996, 209−212.
26. T. Yakura, Y. Matsumura and M. Ikeda, *Synlett*, 1991, 343−344.
27. X. Dai and P. Huang, *Chin. J. Chem.*, 2012, **30**, 1953−1956.
28. L. Zhang, Y. Zhou and X. Yu, *Synlett*, 2012, **23**, 1217−1220.
29. Y. Yan, S. Zhu, X. Luo, Y. Rao, J. Su, G. He and H. Lin, *Chirality*, 2021, **33**, 643−651.

30. D. A. Gutierrez, J. Fettinger, K. N. Houk, K. Ando and J. T. Shaw, *Org. Lett.*, 2022, **24**, 1164−1168.
31. Z. Hu, Y. Zhu, Z. Fu and W. Huang, *J. Org. Chem.*, 2019, **84**, 10328−10337.
32. Y. Ming-He, C. Yan-Yong and H. Liang, *Acta Chim. Sinica*, 1987, **5**, 267−272.
33. E. C. Rao, H. Hong, J. C. Cheng, G. Z. Yang, H. S. Lin and L. Huang, *Chin. Chem. Lett.*, 1994, **5**, 267−268.
34. D. F. Huang, H. S. Lin and L. Huang, *Chin. Chem. Lett.*, 1994, **5**, 371−372.
35. *U.S. Pat. Pat.*, US8912345, 2011.
36. M. He, M. Rommel and J. W. Bode, *Heterocycles*, 2012, **86**, 1689−1696.
37. D. Liu, X. Yu and L. Huang, *Chin. J. Chem.*, 2013, **31**, 344−348.
38. H.-J. Gais, H. Eichelmann, N. Spalthoff, F. Gerhards, M. Frank and G. Raabe, *Tetrahedron: Asymmetry*, 1998, **9**, 235−248.
39. Y.-n. Xuan, H.-S. Lin and M. Yan, *Org. Biomol. Chem.*, 2013, **11**, 1815−1817.
40. K. Tanda, A. Toyao, A. Watanabe, M. Sakamoto and T. Yamasaki, *Synlett*, 2014, **25**, 2953−2956.
41. D. H. R. Barton and S. W. McCombie, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1574−1585.
42. W. Hartwig, *Tetrahedron*, 1983, **39**, 2609−2645.
43. S. Zhu, Y. Yan, J. Su, G. He, Y. Rao and H. Lin, *Arkivoc*, 2021, **part viii**, 234−241.
44. H. M. L. Davies and J. R. Manning, *Nature*, 2008, **451**, 417−424.
45. J. C. K. Chu and T. Rovis, *Angew. Chem. Int. Ed.*, 2018, **57**, 62−101.
46. S. Sarkar, K. P. S. Cheung and V. Gevorgyan, *Chem. Sci.*, 2020, **11**, 12974−12993.
47. J. H. Docherty, T. M. Lister, G. McArthur, M. T. Findlay, P. Domingo-Legarda, J. Kenyon, S. Choudhary and I. Larrosa, *Chem. Rev.*, 2023, **123**, 7692−7760.
48. R. G. Bergman, *Nature*, 2007, **446**, 391−393.
49. M. C. White, *Science*, 2012, **335**, 807−809.
50. Y. Aihara and N. Chatani, *J. Am. Chem. Soc.*, 2014, **136**, 898−901.
51. X. Huang, Y. Wang, J. Lan and J. You, *Angew. Chem. Int. Ed.*, 2015, **54**, 9404−9408.
52. N. Barsu, S. K. Bolli and B. Sundararaju, *Chem Sci*, 2017, **8**, 2431−2435.
53. S. Rej, A. Das and N. Chatani, *Coord. Chem. Rev.*, 2021, **431**, 213683.
54. P.-X. Shen, L. Hu, Q. Shao, K. Hong and J.-Q. Yu, *J. Am. Chem. Soc.*, 2018, **140**, 6545−6549.
55. L. V. Desai, K. L. Hull and M. S. Sanford, *J. Am. Chem. Soc.*, 2004, **126**, 9542−9543.
56. W. von E. Doering and A. K. Hoffmann, *J. Am. Chem. Soc.*, 1954, **76**, 6162-6165.
57. M. P. Doyle and W. Hu, *Chirality*, 2002, **14**, 169−172.
58. J. L. Roizen, M. E. Harvey and J. Du Bois, *Acc. Chem. Res.*, 2012, **45**, 911−922.
59. P. M. Wehn and J. Du Bois, *J. Am. Chem. Soc.*, 2002, **124**, 12950−12951.
60. J. J. Fleming, K. W. Fiori and J. Du Bois, *J. Am. Chem. Soc.*, 2003, **125**, 2028−2029.
61. J. J. Fleming and J. Du Bois, *J. Am. Chem. Soc.*, 2006, **128**, 3926−3927.
62. A. Hinman and J. Du Bois, *J. Am. Chem. Soc.*, 2003, **125**, 11510−11511.
63. S. L. Titouani, J. P. Lavergne, P. Viallefont and R. Jacquier, *Tetrahedron*, 1980, **36**, 2961−2965.
64. R. Hernández, A. Rivera, J. A. Salazar and E. Suárez, *J. Chem. Soc., Chem. Commun.*, 1980, 958−959.
65. C. Betancor, J. I. Concepcion, R. Hernandez, J. A. Salazar and E. Suarez, *J. Org. Chem.*, 1983, **48**, 4430−4432.
66. C. G. Francisco, A. J. Herrera and E. Suárez, *J. Org. Chem.*, 2003, **68**, 1012−1017.
67. R. Carrau, R. Hernández, E. Suárez and C. Betancor, *J. Chem. Soc., Perkin Trans. 1*, 1987, 937−943.

68. P. de Armas, R. Carrau, J. I. Concepción, C. G. Francisco, R. Hernández and E. Suárez, *Tetrahedron Lett.*, 1985, **26**, 2493−2496.
69. E. J. Corey, J. F. Arnett and G. N. Widiger, *J. Am. Chem. Soc.*, 1975, **97**, 430-431.
70. R. Fasan, *ACS Catal.*, 2012, **2**, 647−666.
71. A. O'Connell, A. Barry, A. J. Burke, A. E. Hutton, E. L. Bell, A. P. Green and E. O'Reilly, *Chem. Soc. Rev.*, 2024, **53**, 2828−2850.
72. C. J. C. Whitehouse, S. G. Bell and L.-L. Wong, *Chem. Soc. Rev.*, 2012, **41**, 1218−1260.
73. A. W. Munro, D. G. Leys, K. J. McLean, K. R. Marshall, T. W. B. Ost, S. Daff, C. S. Miles, S. K. Chapman, D. A. Lysek, C. C. Moser, C. C. Page and P. L. Dutton, *Trends Biochem. Sci*, 2002, **27**, 250−257.
74. S. Ariyasu, J. K. Stanfield, Y. Aiba and O. Shoji, *Curr. Opin. Chem. Biol.*, 2020, **59**, 155−163.
75. D. R. Nelson, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 2018, **1866**, 141−154.
76. M. R. Waterman and G. I. Lepesheva, *Biochem. Biophys. Res. Commun.*, 2005, **338**, 418−422.
77. M. Lisurek and R. Bernhardt, *Molecular and Cellular Endocrinology*, 2004, **215**, 149−159.
78. I. A. Pikuleva, *Pharmacol. Ther.*, 2006, **112**, 761−773.
79. M. Zhao, J. Ma, M. Li, Y. Zhang, B. Jiang, X. Zhao, C. Huai, L. Shen, N. Zhang, L. He and S. Qin, *Int. J. Mol. Sci.*, 2021, **22**, 12808.
80. W. L. Miller, *Molecular and Cellular Endocrinology*, 2002, **198**, 7−14.
81. T. Omura and R. Sato, *J. Biol. Chem.*, 1964, **239**, 2370−2378.
82. K. D. Dubey and S. Shaik, *Acc. Chem. Res.*, 2019, **52**, 389−399.
83. J. T. Groves and G. A. McClusky, *J. Am. Chem. Soc.*, 1976, **98**, 859−861.
84. J. T. Groves, G. A. McClusky, R. E. White and M. J. Coon, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 154−160.
85. J. H. Dawson and M. Sono, *Chem. Rev.*, 1987, **87**, 1255−1276.
86. J. Rittle and M. T. Green, *Science*, 2010, **330**, 933−937.
87. K. Omura, Y. Aiba, H. Onoda, J. K. Stanfield, S. Ariyasu, H. Sugimoto, Y. Shiro, O. Shoji and Y. Watanabe, *Chem. Commun.*, 2018, **54**, 7892−7895.
88. M. Wang, D. L. Roberts, R. Paschke, T. M. Shea, B. S. S. Masters and J.-J. P. Kim, *Proc. Natl. Acad. Sci.*, 1997, **94**, 8411−8416.
89. P. A. Hubbard, A. L. Shen, R. Paschke, C. B. Kasper and J.-J. P. Kim, *J. Biol. Chem.*, 2001, **276**, 29163−29170.
90. N. Li, S. Yan, P. Wu, J. Li and B. Wang, *ACS Catal.*, 2024, **14**, 7893−7900.
91. M. Sugishima, H. Sato, Y. Higashimoto, J. Harada, K. Wada, K. Fukuyama and M. Noguchi, *Proc. Natl. Acad. Sci.*, 2014, **111**, 2524−2529.
92. P. A. Hubbard, C. Xia, A. L. Shen and J.-J. P. Kim, *Arch. Biochem. Biophys.*, 2021, **701**, 108792.
93. B. D. Fleming, Y. Tian, S. G. Bell, L.-L. Wong, V. Urlacher and H. A. O. Hill, *Eur. J. Biochem.*, 2003, **270**, 4082−4088.
94. X. Ren, J. A. Yorke, E. Taylor, T. Zhang, W. Zhou and L. L. Wong, *Chem. Eur. J.*, 2015, **21**, 15039−15047.
95. I. F. Sevrioukova, H. Li, H. Zhang, J. A. Peterson and T. L. Poulos, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 1863.
96. U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore and K. Robins, *Nature*, 2012, **485**, 185−194.
97. A. Glieder, E. T. Farinas and F. H. Arnold, *Nat. Biotechnol.*, 2002, **20**, 1135−1139.

98. A. Dennig, N. Lülsdorf, H. Liu and U. Schwaneberg, *Angew. Chem. Int. Ed.*, 2013, **52**, 8459−8462.
99. S. Kille, F. E. Zilly, J. P. Acevedo and M. T. Reetz, *Nature Chemistry*, 2011, **3**, 738−743.
100. F. Li, H. Deng and H. Renata, *J. Am. Chem. Soc.*, 2022, **144**, 7616−7621.
101. Y. Peng, C. Gao, Z. Zhang, S. Wu, J. Zhao and A. Li, *ACS Catal.*, 2022, **12**, 2907−2914.
102. Q. Chen, Z. Chao, K. Wang, X. Wang, H. Meng, X. Liu, X. Shan and J. Zhou, *ACS Catal.*, 2024, **14**, 4117−4129.
103. S. A. Loskot, D. K. Romney, F. H. Arnold and B. M. Stoltz, *J. Am. Chem. Soc.*, 2017, **139**, 10196−10199.
104. M. M. H. Miraz, M. A. Ullah, A. A. Nayem, B. Chakrobortty, S. Deb, A. Laskar, N. U. Tithi, N. Saha, A. R. Chowdhury, K. M. K. Alam, T. B. Wahed, M. Khursheed Alam and S. K. Kundu, *Int. J. Clin. Pract.*, 2023, **2023**, 14 pages.
105. A. Li, C. G. Acevedo-Rocha, L. D'Amore, J. Chen, Y. Peng, M. Garcia-Borràs, C. Gao, J. Zhu, H. Rickerby, S. Osuna, J. Zhou and M. T. Reetz, *Angew. Chem. Int. Ed.*, 2020, **59**, 12499−12505.
106. R. Agudo, G. D. Roiban and M. T. Reetz, *ChemBioChem*, 2012, **13**, 1465−1473.
107. Y. Zhang, Z. Xiong, Y. Li, M. Wilson, K. E. Christensen, E. Jaques, P. Hernández-Lladó, J. Robertson and L. L. Wong, *Nature Synthesis*, 2022, **1**, 936−945.
108. L. A. Harwood, Z. Xiong, K. E. Christensen, R. Wang, L. L. Wong and J. Robertson, *J. Am. Chem. Soc.*, 2023, **145**, 27767−27773.
109. L.-D. Syntrivanis, F. Javier del Campo, L.-L. Wong and J. Robertson, *Abstr. Pap. Am. Chem. Soc.*, 2016, **251**, 213.
110. S. Li, J. A. O'Hanlon, A. Mattimoe, H. D. Pickford, L. A. Harwood, L. L. Wong and J. Robertson, *Org. Lett.*, 2023, **25**, 7507−7511.
111. F. Le Vaillant, M. Garreau, S. Nicolai, G. Gryn'Ova, C. Corminboeuf and J. Waser, *Chem. Sci.*, 2018, **9**, 5883−5889.
112. L. R. Krepski and A. Hassner, *J. Org. Chem.*, 1978, **43**, 2879−2882.
113. J. Robertson, W. P. Unsworth and S. G. Lamont, *Tetrahedron*, 2010, **66**, 2363−2372.
114. N. E. Behnke, J. H. Siitonen, S. A. Chamness and L. Kürti, *Org. Lett.*, 2020, **22**, 5715−5720.
115. E. Lee-Ruff and D. Wells, *Nucleosides, Nucleotides and Nucleic Acids*, 2008, **27**, 484−494.
116. P. W. Jeffs, G. Molina, N. A. Cortese, P. R. Hauck and J. Wolfram, *J. Org. Chem.*, 1982, **47**, 3876−3881.
117. D. H. R. Barton, M. J. Day, R. H. Hesse and M. M. Pechet, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1764−1767.
118. C. Ramalingan and Y.-T. Park, *J. Org. Chem.*, 2007, **72**, 4536−4538.
119. *U.S. Pat. Appl. Publ. Pat.*, 20060025601, 2006.
120. *U.S. Pat. Appl. Publ. Pat.*, 20210059251, 2021.
121. Q. Yuan, K. Yao, D. Liu and W. Zhang, *Chem. Commun.*, 2015, **51**, 11834−11836.
122. B. Yao, Y. Liu, M. K. Wang, J. H. Li, R. Y. Tang, X. G. Zhang and C. L. Deng, *Adv. Synth. Catal.*, 2012, **354**, 1069−1076.
123. N. Fujiwara and Y. Yamamoto, *J. Org. Chem.*, 1999, **64**, 4095−4101.
124. L. Zhao, R. I. Kaiser, W. Lu, M. Ahmed, A. D. Oleinikov, V. N. Azyazov, A. M. Mebel, A. H. Howlader and S. F. Wnuk, *Phys. Chem. Chem. Phys.*, 2020, **22**, 15381−15388.
125. A. S. Kende, R. Greenhouse and J. A. Hill, *Tetrahedron Lett.*, 1979, **20**, 2867−2870.
126. D. Mitchell, J. H. Eilert and N. L. Bauld, *Tetrahedron Lett.*, 1979, **20**, 2865-2866.

127. M. Imai, G. R. Morais, B. al-Hindawi, M. A. M. al-Sulaibi, M. Meetani and T. Thiemann, *J. Chem. Res.*, 2010, **34**, 325−329.
128. W. E. Fristad and J. R. Peterson, *J. Org. Chem.*, 1985, **50**, 10−18.
129. G. Newsoroff and S. Sternhell, *Aust. J. Chem.*, 1972, **25**, 1669−1693.
130. Y. Hwang, H. Jung, E. Lee, D. Kim and S. Chang, *J. Am. Chem. Soc.*, 2020, **142**, 8880−8889.
131. H. Zhang, N. Sun, B. Hu, Z. Shen, X. Hu and L. Jin, *Org, Chem. Front.*, 2019, **6**, 1983−1988.
132. Q. Qin, X. Luo, J. Wei, Y. Zhu, X. Wen, S. Song and N. Jiao, *Angew. Chem. Int. Ed.*, 2019, **58**, 4376−4380.
133. J. Guo, X. Xu, Q. Xing, Z. Gao, J. Gou and B. Yu, *Org. Lett.*, 2018, **20**, 7410−7414.
134. D. J. Aitken, A. M. Bernard, F. Capitta, A. Frongia, R. Guillot, J. Ollivier, P. P. Piras, F. Secci and M. Spiga, *Org. Biomol. Chem.*, 2012, **10**, 5045−5048.
135. S. Afewerki, A. Córdova and C. Palo-Nieto, *Synthesis*, 2020, **52**, 2330−2336.
136. N. Iwasawa, T. Matsuo, M. Iwamoto and T. Ikeno, *J. Am. Chem. Soc.*, 1998, **120**, 3903−3914.
137. A. Späth, C. Leibl, F. Cieplik, K. Lehner, J. Regensburger, K.-A. Hiller, W. Bäumler, G. Schmalz and T. Maisch, *J. Med. Chem.*, 2014, **57**, 5157−5168.
138. B.-C. Yan, M. Zhou, J. Li, X.-N. Li, S.-J. He, J.-P. Zuo, H.-D. Sun, A. Li and P.-T. Puno, *Angew. Chem. Int. Ed.*, 2021, **60**, 12859−12867.
139. H. C. Brown and D. H. McDaniel, *J. Am. Chem. Soc.*, 1955, **77**, 3752−3755.
140. K. Clarke and K. Rothwell, *J. Chem. Soc.*, 1960, 1885−1895.
141. G. A. I. Abu Deiab and M. P. Croatt, *Bioorg. Med. Chem.*, 2019, **27**, 338−342.
142. E. P. Peçanha, C. A. M. Fraga, E. J. Barreiro, M. F. M. Braga, E. F. R. Pereira and E. X. Albuquerque, *J. Braz. Chem. Soc.*, 2001, **12**, 408−412.
143. T. Sato, N. Chono, H. Ishibashi and M. Ikeda, *J. Chem. Soc., Perkin Trans. 1*, 1995, 1115−1120.
144. J. A. Griswold and J. S. Johnson, *ACS Catal.*, 2019, **9**, 11614−11618.
145. A. Ahmad, P. Scarassati, N. Jalalian, B. Olofsson and L. F. Silva, *Tetrahedron Lett.*, 2013, **54**, 5818−5820.
146. M. Justik and G. Koser, *Molecules*, 2005, **10**, 217−225.
147. I. Nishiguchi, Y. Yamamoto, S. Kawano and H. Maekawa, *Synlett*, 2004, 30−36.
148. F. Zanardi, L. Battistini, G. Rassu, M. Cornia and G. Casiraghi, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2471−2475.
149. X. Ma, H. Lubin, E. Ioja, O. Kékesi, Á. Simon, Á. Apáti, T. I. Orbán, L. Héja, J. Kardos and I. E. Markó, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 417−423.
150. Z. Tian, M. Rasmussen and S. J. Wittenberger, *Org. Process Res. Dev.*, 2002, **6**, 416−418.
151. J. A. O'Hanlon, X. Ren, M. Morris, L. L. Wong and J. Robertson, *Org. Biomol. Chem.*, 2017, **15**, 8780−8787.
152. X. Ren, J. A. O'Hanlon, M. Morris, J. Robertson and L. L. Wong, *ACS Catal.*, 2016, **6**, 6833−6837.
153. Y. Li and L. L. Wong, *Angew. Chem. Int. Ed.*, 2019, **58**, 9551−9555.
154. W. Chen, M. J. Fisher, A. Leung, Y. Cao and L. L. Wong, *ACS Catal.*, 2020, **10**, 8334−8343.
155. A. B. Carmichael and L. L. Wong, *Eur. J. Biochem.*, 2001, **268**, 3117−3125.
156. C. F. Butler, C. Peet, A. E. Mason, M. W. Voice, D. Leys and A. W. Munro, *J. Biol. Chem.*, 2013, **288**, 25387−25399.
157. M. A. Noble, C. S. Miles, S. K. Chapman, D. A. Lysek, A. C. MacKay, G. A. Reid, R. P. Hanzlik and A. W. Munro, *Biochem. J*, 1999, **339**, 371−376.

158. M. W. Peters, P. Meinhold, A. Glieder and F. H. Arnold, *J. Am. Chem. Soc.*, 2003, **125**, 13442−13450.
159. L. Zhang, Y. Zhou and X. Yu, *Synlett*, 2012, **23**, 1217−1220.
160. L. Yang, Q.-Y. Zheng, D.-X. Wang, Z.-T. Huang and M.-X. Wang, *Org. Lett.*, 2008, **10**, 2461−2464.
161. T. J. Donohoe, C. K. A. Callens and A. L. Thompson, *Org. Lett.*, 2009, **11**, 2305−2307.
162. T. Kobayashi, Y. Teshigahara, M. Sakakibara, K. Murakami, Y. Kawamoto and H. Ito, *Org. Lett.*, 2023, **25**, 4510−4513.
163. X.-Y. Meng, H.-X. Zhang, M. Mezei and M. Cui, *Current Computer Aided-Drug Design*, 2011, **7**, 146−157.
164. R. Norel, D. Fischer, H. J. Wolfson and R. Nussinov, *Protein Engineering, Design and Selection*, 1994, **7**, 39−46.
165. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli and D. Hassabis, *Nature*, 2021, **596**, 583−589.
166. D. S. Wilson and A. D. Keefe, *Current Protocols in Molecular Biology*, 2000, **51**, 8.3.1−8.3.9.
167. A. Crameri, S.-A. Raillard, E. Bermudez and W. P. C. Stemmer, *Nature*, 1998, **391**, 288−291.
168. R. M. P. Siloto and R. J. Weselake, *Biocatal. Agric. Biotechnol.*, 2012, **1**, 181−189.
169. F. P. Guengerich, *Chem. Res. Toxicol.*, 2008, **21**, 70−83.
170. B. Testa and S. D. Krämer, *Chemistry & Biodiversity*, 2006, **3**, 1053−1101.
171. J. A. McKay, W. T. Melvin, A. K. Ah-See, S. W. B. Ewen, W. F. Greenlee, C. B. Marcus, M. D. Burke and G. I. Murray, *FEBS Lett.*, 1995, **374**, 270−272.
172. F. P. Guengerich, M. V. Martin, C. D. Sohl and Q. Cheng, *Nature Protocols*, 2009, **4**, 1245−1251.
173. *Kunming Plant Inst Chinese ACA Pat.*, CN1186071A, 1998.
174. P. Quinodoz, A. Lumbroso, M. Lachia, C. Screpanti, S. Rendine, B. Horoz, M. Bozoflu, S. Catak, R. Fonné-Pfister, K. Hermann and A. De Mesmaeker, *Helv. Chim. Acta*, 2023, **106**, e202200145.
175. E. M. Arnett, K. Amarnath, N. G. Harvey and J. Cheng, *J. Am. Chem. Soc.*, 1990, **112**, 344−355.
176. H. Yan, Z.-F. Li, Z.-F. Guo, Z.-L. Lu, F. Wang and L.-Z. Wu, *Bioorg. Med. Chem.*, 2012, **20**, 801−808.
177. L. F. Fieser and M. D. Gates, Jr., *J. Am. Chem. Soc.*, 1940, **62**, 2335−2341.
178. V. Boekelheide and C. E. Larrabee, *J. Am. Chem. Soc.*, 1950, **72**, 1245−1249.
179. G. Olive, F. Le Moigne, A. Mercier, A. Rockenbauer and P. Tordo, *J. Org. Chem.*, 1998, **63**, 9095−9099.
180. W.-W. Zhang, X.-G. Zhang and J.-H. Li, *J. Org. Chem.*, 2010, **75**, 5259−5264.
181. H. A. Clement, M. Boghi, R. M. McDonald, L. Bernier, J. W. Coe, W. Farrell, C. J. Helal, M. R. Reese, N. W. Sach, J. C. Lee and D. G. Hall, *Angew. Chem. Int. Ed.*, 2019, **58**, 18405−18409.
182. Y. Motoyama, K. Mitsui, T. Ishida and H. Nagashima, *J. Am. Chem. Soc.*, 2005, **127**, 13150−13151.
183. H. F. Ye, G. Dive, D. Dehareng, F. Heymans and J. J. Godfroid, *Bioorg. Med. Chem.*, 2000, **8**, 1861−1869.

184. T. Ohno, M. Sakai, Y. Ishino, T. Shibata, H. Maekawa and I. Nishiguchi, *Org. Lett.*, 2001, **3**, 3439−3442.
185. Z. Gonda and Z. Novák, *Chem. Eur. J.*, 2015, **21**, 16801−16806.
186. Y. Yamamoto and H. Togo, *Synlett*, 2005, 2486−2488.
187. *U.S. Pat. Appl. Publ. Pat.*, 20190144444, 2019.
188. B. J. Anderson, M. A. Guino-o, D. S. Glueck, J. A. Golen, A. G. DiPasquale, L. M. Liable-Sands and A. L. Rheingold, *Org. Lett.*, 2008, **10**, 4425−4428.
189. C. Curti, B. Ranieri, L. Battistini, G. Rassu, V. Zambrano, G. Pelosi, G. Casiraghi and F. Zanardi, *Adv. Synth. Catal.*, 2010, **352**, 2011−2022.
190. M. Singh, M. Kumar, S. A. Nalawade, D. R. Puneeth Kumar and H. N. Gopi, *Org. Biomol. Chem.*, 2023, **21**, 3766−3769.
191. J. T. Baker and S. Sifniades, *J. Org. Chem.*, 1979, **44**, 2798−2800.
192. W. Zhang, S. Ning, Y. Li and X. Wu, *Chem. Commun.*, 2022, **58**, 12843−12846.
193. M. Wegmann and T. Bach, *Synthesis*, 2017, **49**, 209−217.