

Evolved P450 Mutants

as General Oxidation Catalysts

A thesis submitted to the

Board of the Faculty of the Physical Sciences

in partial fulfilment of the requirements for the degree of

Doctor of Philosophy of the University of Oxford

Jack O'Hanlon

St Hugh's College

Hilary Term 2017

ASLIB Abstract

Evolved P450 Mutants as General Oxidation Catalysts

Jack O'Hanlon D.Phil.

St Hugh's College Hilary Term 2017

The thesis describes the use of a library of mutant $P450_{BM3}$ variants for the biocatalytic oxidation of a range of structurally diverse substrates and the efforts toward the total synthesis of trigoxyphin K and L, employing biocatalytic C-H oxidation as a key step.

Chapter 1 explores the $P450_{BM3}$-catalysed oxidative cyclisation of α-amino (thio)acetamides for the synthesis of bicyclic imidazolidin-4-(thi)ones with $P450_{BM3}$ variant-controlled regio- and diastereoselectivity attainable in some cases. A method was developed for the Lewis acid-mediated ring-opening functionalisation of these compounds employing a variety of nucleophiles.

Chapter 2 discusses the $P450_{BM3}$-catalysed *para*-hydroxylation of three sulfonanilide-derived herbicides and the expansion of this transformation to a number of substituted anilides. Regioselectivity of hydroxylation was possible for certain substrates, with selectivity possible for *para*-hydroxylation or benzylic hydroxylation.

Chapter 3 details the successful approach to the core structures of the natural products trigoxyphin K and L and an investigation into the use of the library of mutant $P450_{BM3}$ variants for late-stage oxidation of intermediates to enable synthesis of the natural products themselves.

Finally, the $P450_{BM3}$-mediated oxidation of a structurally diverse collection of small molecules and commercially available herbicides was examined with transformations including benzylic hydroxylation, acetal formation, *S*-oxidation, and nitro-group reduction.

Contents

Acknowledgements i

Abbreviations iii

Introduction 1

Biocatalysis 1

C–H Oxidation 2

Oxidation at Activated Positions 3

Directed C–H Oxidation 4

C–H Oxidation at Unactivated Positions 6

Enzymatic C–H Oxidation 8

Cytochrome P450 10

Catalytic Cycle 12

Cytochrome $P450_{BM3}$ (CYP102A1) 18

Structure 20

Engineering 22

Applications of mutant $P450_{BM3}$ 31

Research in the Wong Group 36

Project Aims 41

Chapter 1: Synthesis of Imidazolidin-4-ones 45

Aims 45

Background 45

Structure elucidation 48

Substrate Synthesis 49

Substrate Screening 50

Contents

Mechanism 59

Imidazolidin-4-one Ring-opening Functionalisation 63

Chapter 2: Oxidation of Anilides 68

Background/ Aims 68

Oxidation of Trifluoromethanesulfonanilide Containing Herbicides 71

Substrate Synthesis 73

Substrate Screening 75

Investigation into *meta*-Hydroxylation of Herbicides 85

Mechanism 86

Conclusions 88

Chapter 3: Efforts Toward the Synthesis of Trigoxyphin K and L 90

Aims 90

Isolation 90

Synthesis of Similar Structures 91

Retrosynthetic Plan 93

Synthesis of 3-(2-bromo-4-methylphenyl)furan 94

Plan A: Suzuki coupling 94

Plan B: Alkynyl epoxide rearrangement 95

Prenylation of 3-Arylfuran **145** 99

Formation of the Seven-Membered Ring 101

Synthesis of the Core Structure of Trigoxyphin K and L 103

Screening against library of $P450_{BM3}$ variants 107

Conclusions 109

Chapter 4: Screening of Miscellaneous Compounds 112

Background and Aims....112

Substrate Synthesis....112

Substrate Screening....114

Small Molecules....114

Commercially-Available Herbicides....120

Studies on Deuterated Clomazone....129

Conclusion....132

Chapter 5: Conclusions and Future Work....133

Experimental Section....137

General Experimental....137

Enzymes and Molecular Biology....138

General Procedure for Screening of Compounds in Chapter 1....139

General Procedure for Screening of Compounds from Chapter 2, 3, and 4....139

$P450_{BM3}$ Variants and the Mutations in Each....140

Experimental Procedures and Characterisation....142

Summary of Screening Data....244

References....272

Appendix A – Synthesis of monocyclic imidazolidin-4-ones....298

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant....299

Acknowledgements

Firstly, I would like to thank my supervisors Jeremy Robertson and Luet Wong who gave me this opportunity and for the next three years were always available to provide support and advice. Secondly, I would like to thank Mell, Dianne, and Steve from Syngenta for offering a different perspective on the work and supplying many interesting ideas. I want to thank Syngenta for supplying the funding that made this work possible.

I want to thank the NMR and MS staff in the CRL for making compound characterisation so much easier; special thanks go to Barbara Odell and Tim Claridge for their help in solving a few mysteries.

I have to mention past and present members of the Robertson and Wong groups for being there to bounce ideas off and for keeping the lab an entertaining place to work. A special mention must go to Tony for the discussions and the collaboration which led to a publication. Special mention must also go to John, Leonidas, Patri, and Wilf for making sure we could learn while we learnt, listen to goats that scream like a man, and the many delicious pints and pub lunches.

For all the support they offered, for the last three years and all my other years, I'd like to thank my parents, my brother Chris and sister Becky, and my Grandma.

For all the good times had at beeriokarts and nights in the uni club I'd like to thank Rob, Bryony, Hali, Ebba, Marius, and Christian who offered plenty of friendship and chemistry advice over the time I've known them.

Finally, I'd like to thank Niamh, for giving me so much support, especially during my destitute writing time, and for keeping me smiling in the tough times. For all of our de-stressing trips away and for taking such good care of me, I cannot thank you enough.

Abbreviations

°C	degrees Celcius
1°	primary
2°	secondary
3°	tertiary
Ac	acetyl
Ala	alanine
aq	aqueous
Ar	aryl
Arg	arginine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br	broad
Bu	butyl
Bz	benzoyl
Cbz	carboxybenzyl
cm	centimetre
conv.	conversion
COSY	correlation spectroscopy
Cys	cysteine
d	day(s)
d	doublet
D	dextro
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCE	1,2-dichloroethane
DEAD	diethylazodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DMAP	4-(dimethylamino)pyridine
DME	dimethoxyethane
DMEDA	*N*,*N*'-dimethylethylenediamine
DMF	dimethylformamide
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
EPR	electron paramagnetic resonance
EPTC	*S*-ethyl-*N*,*N*-dipropylthiocarbamate
eq	equivalents
Et	ethyl
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
Fur	furyl
g	gram
GC	gas chromatography
GDH	glucose dehydrogenase
Glu	glutamine
Gly	glycine
h	hours
His	histidine
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMPA	hexamethylphosphoramide
HSQC	heteronuclear single-quantum correlation spectroscopy
Hz	Hertz (s^{-1})

hv	irradiation
i	iso
Ile	isoleucine
IR	infra-red
J	coupling constant
K_a	acid dissociation constant
kcal	kilocalories
k_{cat}	turnover number
L	levo
L	litre
LA	Lewis acid
LCMS	liquid chromatography-mass spectrometry
LDA	lithium diisopropylamide
Leu	leucine
lit.	literature
Lys	lysine
m	medium
m	meta
mg	milligram
mL	millilitre
mmol	millimole
m	multiplet
M	mega
M	molar
m-CPBA	3-chloroperbenzoic acid
Me	Methyl
min	minute
mol	mole
mp	melting point
MS	mass spectrometry
Ms	methanesulfonyl
n	normal
NADH	nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
NPG	*N*-palmitoyl glycine
NSAID	non-steroidal anti-inflammatory drug
Nu	nucleophile
o	ortho
p	para
PCC	pyridinium chlorochromate
PCR	polymerase chain reaction
PDP	1,1’-bis(2- pyridinylmethyl)-2,2’-bipyrrolidine
Ph	phenyl
Phe	phenylalanine
ppm	parts per million
ppy	2-phenylpyridyl
Pr	propyl
Pro	proline
Pyr	pyridine
q	quartet

quant.	quantitative
quin	quintet
R	generic group
R_f	retention factor
Red-Al	sodium bis(2-methoxyethoxy)aluminium hydride
RNA	ribonucleic acid
ROESY	rotating-frame nuclear Overhauser effect spectroscopy
rt	retention time
RT	room temperature
S	spin quantum number
s	second
s	secondary
s	singlet
sept	septet
Ser	serine
sex	sextet
SM	starting material
t	triplet
t	tertiary
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TCDI	1,1'-thiocarbonyldiimidazole
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
Tf	trifluoromethanesulfonyl
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Thr	threonine
TMS	trimethylsilyl
TOF	turnover frequency
TON	turnover number
Ts	4-toluenesulfonyl
TTN	total turnover number
Tyr	tyrosine
UV	ultraviolet
Val	valine
WT	wild type
µ	micro
ν_{max}	absorption maximum
v/v	volume-to-volume ratio
w/v	weight-to-volume ratio

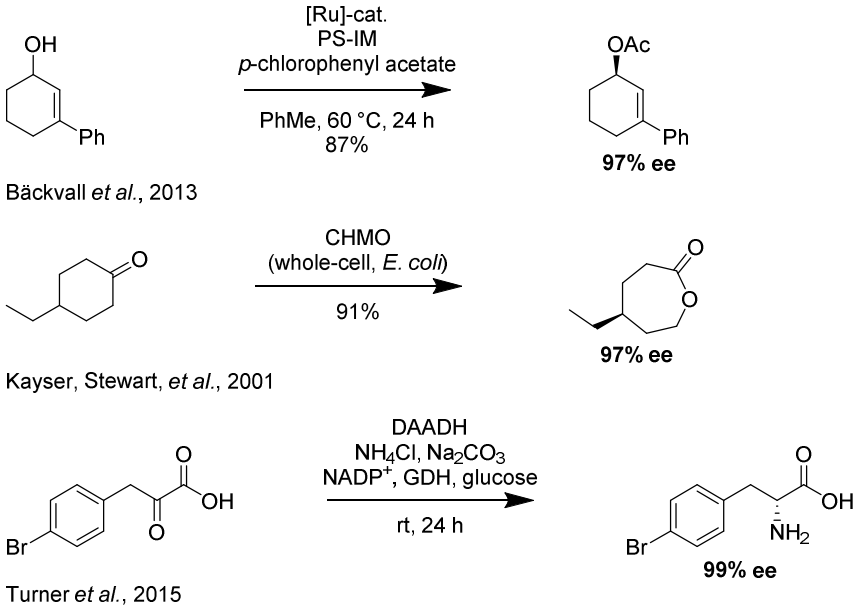
Introduction

Biocatalysis

Biocatalysis refers to the use of enzymes to carry out synthetic transformations. It can offer a number of advantages over traditional chemical synthesis, with reactions typically being carried out in aqueous media at room temperature and exhibiting excellent selectivity (chemo-, regio-, and stereoselectivity) with high catalyst total turnover number. Extensive work has been undertaken towards the discovery and development of enzymes, both wild-type and genetically modified, for catalysis of a variety of synthetically useful transformations; this has been thoroughly reviewed.[1–14] Despite the aforementioned advantages, biocatalysis is relatively underemployed in organic synthesis, due, in part, to a perception of technical difficulty associated with the techniques of biocatalysis, and because wild-type enzymes can be extremely specific for substrate and transformation, with limited scalability and general utility. In the case that a specific reaction is required, chemical methods with greater generality will be more appropriate.[15]

The International Union of Biochemistry classifies enzymatic reaction types in six groups: oxidoreductases (redox reactions), transferases (functional group transfer), hydrolases (hydrolysis), lyases (addition to double bonds), isomerases (isomerisations), and ligases (bond formations accompanied by cleavage of a nucleotide triphosphate).[16] Enzymes are commonly used in synthesis when high levels of enantiopurity are required, and are inaccessible by synthetic methods. The group of hydrolases are most commonly employed by synthetic chemists, with lipases and amidases demonstrating particular utility in resolutions for the synthesis of enantiomerically pure alcohols and amines.[17–19] For example, dynamic kinetic resolution of allylic alcohols with a lipase (lipase PS, isolated from *Burkholderia cepacia*, immobilised on Celite (PS-IM)) and a ruthenium catalyst is an excellent method for the synthesis of enantioenriched esters (Scheme 1).[20] The reductases have been widely used to catalyse asymmetric reductions in tandem with a cofactor such as NADH or NADPH.[21–23] Use of oxidases for asymmetric transformations is possible; Baeyer–Villiger monooxygenases are of particular interest, catalysing the enantioselective synthesis of lactones

from, typically, cyclic ketones.[24,25] The example shown in Scheme 1 illustrates an asymmetric Baeyer–Villiger oxidation catalysed by a cyclohexanone monooxygenase (CHMO).[26] Different oxidases can be used for the enantioselective oxidation of C–O and C–N bonds, with resolutions and desymmetrisations featuring prominently.[27] The synthesis of enantioenriched amines has been developed, and widely applied, by biocatalytic reductive amination of ketones with transaminases (transferases).[28–30] This method is especially valuable for the enantioselective synthesis of unnatural amino acids and has been applied for the synthesis of 4-bromophenylalanine catalysed by engineered D-amino acid dehydrogenase (DAADH) (Scheme 1).[31]



Scheme 1: Examples of biocatalysis in organic synthesis

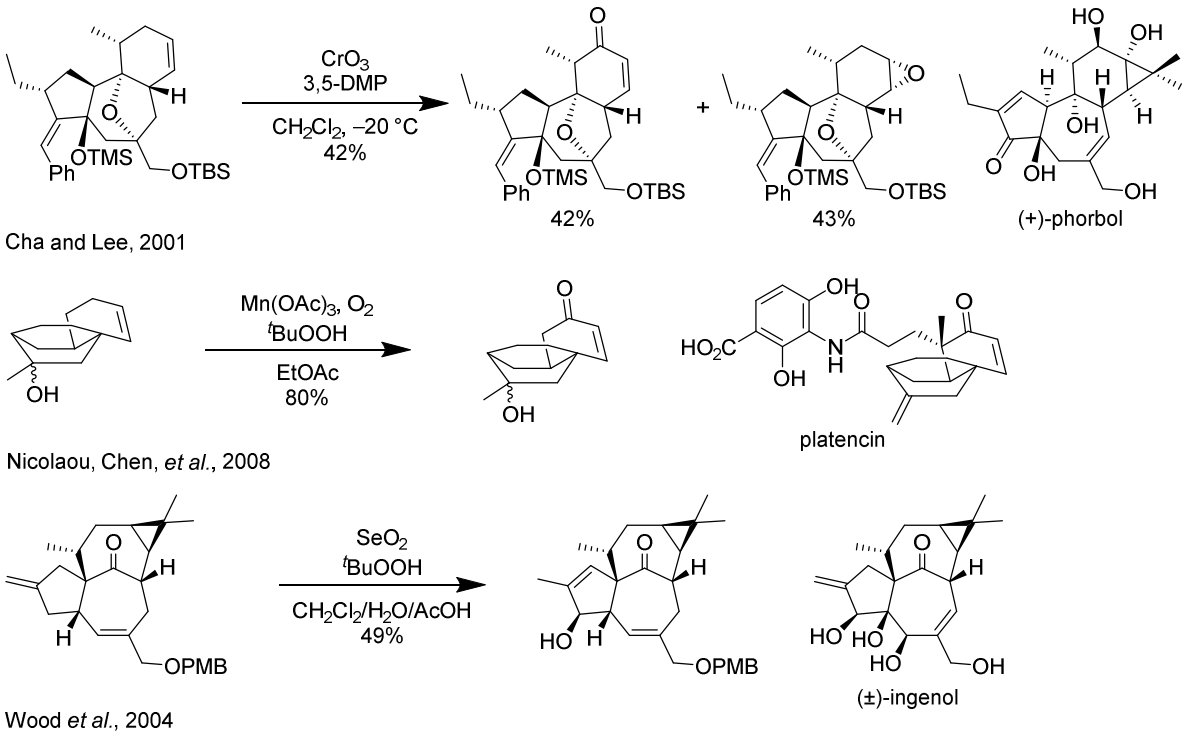
C–H Oxidation

The difficulty of C–H bond oxidation varies depending on the functionality surrounding the targeted C–H bond. In cases where the C–H bond is in a benzylic or allylic position, or adjacent to a heteroatom, it is more active towards oxidation (as radicals formed at these positions are more stable), and methods for carrying out these transformations have been very well established.[32,33] Less-reactive C–H bonds usually require the presence of a directing group in proximity to a C–H bond to allow for oxidation; regioselectivity, in these cases, is controlled by pre-installed functionality.[34] It is more difficult to oxidise C–H bonds which are neither activated towards oxidation nor proximal to

a directing group. In these cases, reagent design is very important for control of regioselectivity; attempting to rely on innate reactivity can result in the formation of mixtures of products.[34]

Oxidation at Activated Positions

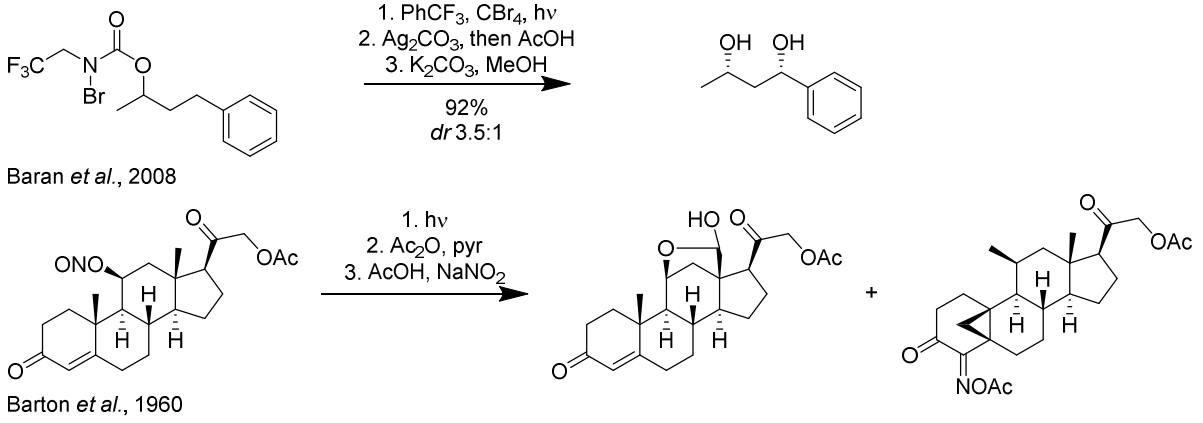
Oxidation at activated positions is typically carried out with strong oxidants such as Cr(VI), Mn(VII), or Se(IV).[35–38] Given the harsh conditions, tolerance of other functional groups, regioselectivity of oxidation, and avoiding over-oxidation can be troublesome; however, this has not precluded their application in total synthesis of complex natural products.[38] Cha and Lee employed the Corey–Fleet reagent [CrO_3-3,5-dimethylpyrazole (3,5-DMP) complex] to construct an enone *via* allylic oxidation in their formal synthesis of (+)-phorbol (Scheme 2).[39] The combination of $Mn(OAc)_3$ and *t*-BuOOH under an O_2 atmosphere was utilised by Nicolaou, Chen, *et al.* (towards a formal synthesis of platencin) for the synthesis of an enone *via* allylic oxidation with good selectivity (Scheme 2).[40] SeO_2 was used by Wood *et al.* in their total synthesis of (±)-ingenol, with hydroxylation occurring selectively to afford only one of the possible allylic alcohols (Scheme 2).[41]



Scheme 2: Cr, Mn, and Se reagents for oxidation in total syntheses

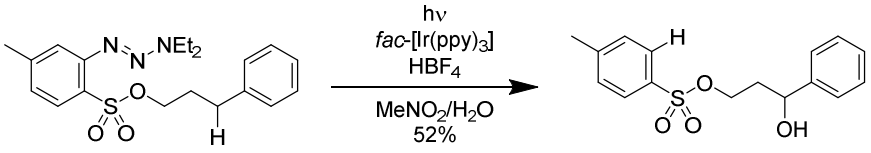
Directed C–H Oxidation

Rather than relying on steric or electronic factors for the activation of C–H bonds or to control regioselectivity of C–H oxidation, directing groups may be used to selectively oxidise certain positions.[34] A classical example of this approach is the Hofmann–Löffler–Freytag reaction, with *N*-halo amines for selective C–H halogenations. Subsequent heterocycle formation *via* intramolecular substitution by the amine is possible. When an *N*-halo amide is used, cyclisation can occur through oxygen or nitrogen and, when desired, the intermediate can be hydrolysed to form an alcohol.[42] A modern example of this approach is in the synthesis of 1,3-diols as developed by Baran (Scheme 3).[43] Another radical-mediated C–H oxidation methodology is use of the Barton nitrite ester photolysis, which enables the direct synthesis of δ-nitroso alcohols from alkyl nitrites; hydrolysis of the oxime (tautomer of the nitroso-group) affords a ketone or an aldehyde, used by Barton for the C–H oxidation of corticosterone acetate (Scheme 3).[44,45]



Scheme 3: Use of Hofmann–Löffler–Freytag and Barton nitrite ester photolysis for directed C–H oxidation

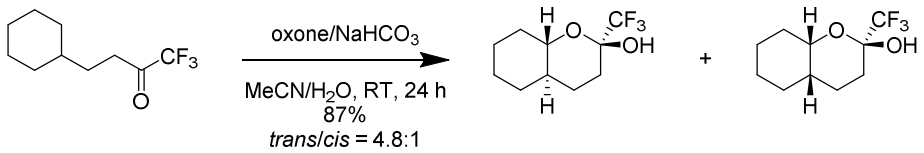
An elegant method utilising photoredox chemistry to effect radical generation from aryldiazonium salts, generated *in-situ*, followed by translocation and radical-polar crossover (with concomitant trapping by water) has been developed by Ragains for hydroxylation at 2° or 3° alkyl centres (Scheme 4).[46]



Ragains *et al.*, 2015

Scheme 4: Remote hydroxylation *via* photoredox catalysis

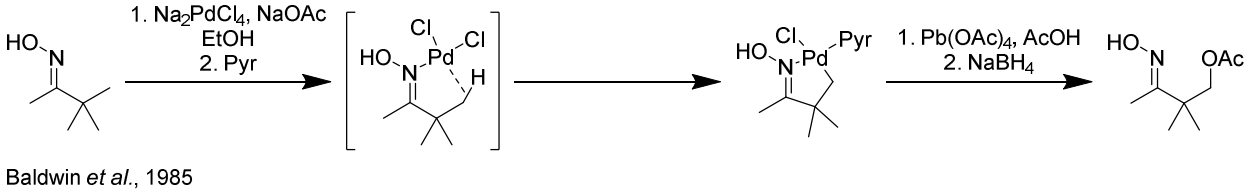
C–H oxidation with intramolecular directing groups is also possible by covalent mechanisms. The synthesis of carefully-designed substrates bearing tethered electron-deficient ketones (α-substituted with esters, trifluoro-, fluoro-, or chloromethyl groups) makes possible regioselective intramolecular C–H oxidation, mediated by a dioxirane generated *in-situ*; in this case, the electronic bias towards 3° alkyl-radical generation is overcome, with hydroxylation occurring exclusively at the less-favoured 2° position (Scheme 5).[47]



Yang *et al*,, 1998

Scheme 5: Intramolecular, dioxirane mediated, regioselective C–H hydroxylation

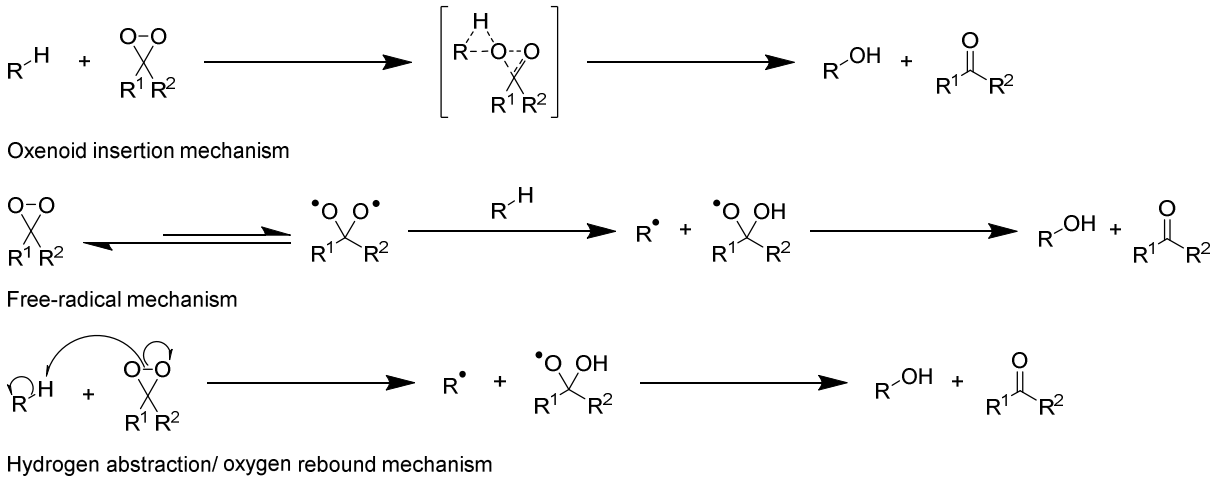
Transition metal catalysed C–H activation is a well-established method for the functionalisation of unactivated C–H bonds (e.g. arylation, amidation, halogenation); chelate control and formation of a metallocycle is vital for these processes, typically with *N*-based chelating groups (e.g. oxime, amide, sulfonamide) or those based on oxygen (e.g. carboxylic acid, alcohol, *N*-oxide).[48–50] Use of different nucleophiles or electrophiles effects different C–H functionalisations (e.g. oxidation, arylation, halogenation, *etc*.). In 1985, Baldwin demonstrated the formation of an oxime-chelated palladacycle with subsequent acetoxylation for the functionalisation of an unactivated methyl group (Scheme 6).[51] C–H acyloxylation has since been rendered catalytic in transition metal and the range of chelating groups has been expanded to include pyridines, carbamates, amides, oxazolines, and sulfoximines.[52–60] When carboxylic acids are used as the directing group, intramolecular acyloxylation is possible; hydrolysis of the product lactones affords free alcohols.[61]



Scheme 6: Palladacycle-mediated sp^3-acetyloxylation

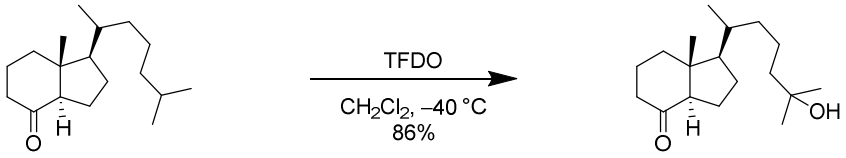
C–H Oxidation at Unactivated Positions

As shown above, selective C–H oxidation can originate in innate activation towards oxidation (e.g. benzylic or allylic C–H bonds) or by proximal functionality (e.g. *via* metallocycle formation or radical translocation). A far more difficult situation is the non-directed oxidation of unactivated C–H bonds where selectivity is much harder to achieve as there is likely to be little reactivity difference between the various C–H bonds.[62] For simple alkyl C–H bonds, the reactivity towards oxidation is 3° > 2° > 1° which is the reverse order of bond dissociation energies (96.5 kcalmol^{-1} < 98.6 kcalmol^{-1} < 101.1 kcalmol^{-1}).[34,63,64] Initial efforts towards oxidation of C–H bonds in alkanes relied on high-energy oxidants such as dioxiranes, commonly dimethyldioxirane (DMDO) or the more reactive methyl(trifluoromethyl)dioxirane (TFDO).[65] The mechanism by which these 3-membered cyclic peroxides react with alkyl C–H bonds has been disputed with three possible mechanisms suggested: concerted oxenoid insertion, a free radical mechanism, or a hydrogen abstraction/oxygen rebound mechanism (Scheme 7).[66] Evidence has been displayed in support of all three mechanisms, and the debate into the true mechanistic pathway followed is ongoing.[66–69]



Scheme 7: Proposed mechanisms for dioxirane-mediated C–H hydroxylation

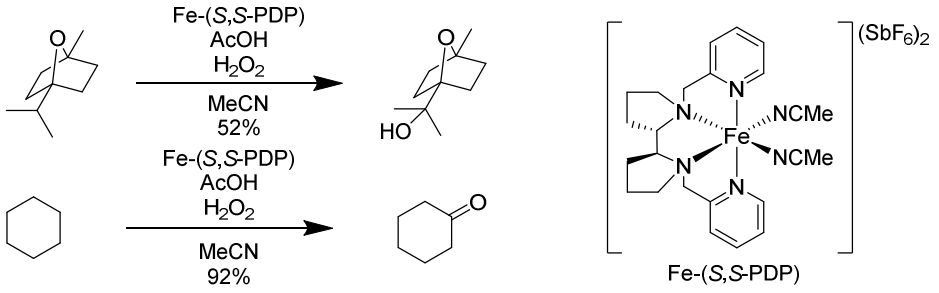
Oxidations with this system demonstrate poor selectivity between 2° alkyl groups; however, regioselective oxidation in complex systems is possible, with oxidation occurring at the least sterically hindered 3° alkyl group. This selectivity was exploited by Mincione and Curci for the C-25 hydroxylation of a Windaus–Grundmann ketone (forming a valuable synthon for the synthesis of 1,25-dihydroxy vitamin D_3) (Scheme 8).[70–72] Similar selectivity is observed when C–H hydroxylation is effected by the more stable oxaziridines, Cr(VI) catalysis, or Ru(VIII) catalysis.[73–75]



Mincione, Curci, *et al*., 1992

Scheme 8: Hydroxylation of a Windaus–Grundmann ketone

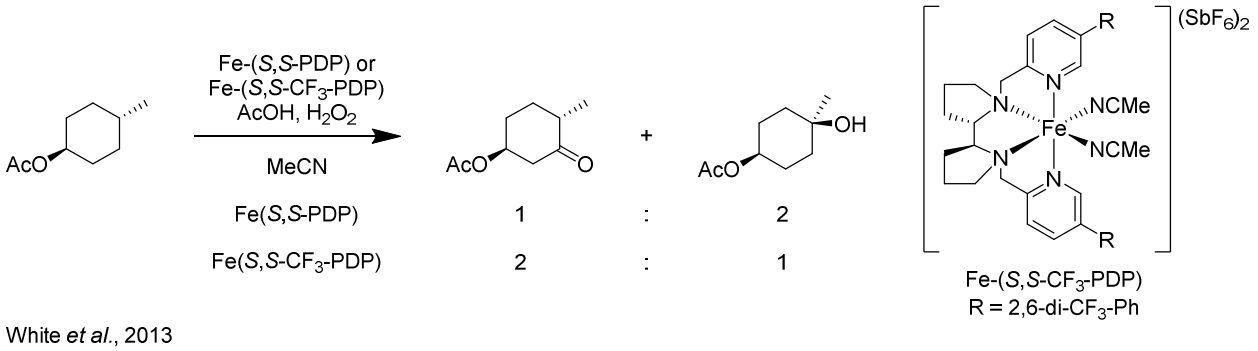
Non-haem iron complexes showed promise as catalysts for oxidation of unactivated C–H bonds (*via* high-valent iron-oxo species); however, turnover numbers were low and regioselectivity was poor.[76–78] The early promise shown by these systems has been investigated and greatly improved upon by the White group; a non-haem iron complex-based catalytic system (Fe-*S*,*S*-PDP/H_2O_2) has been developed which is able to convert 2° alkanes to ketones or 3° alkanes to alcohols (Scheme 9).[79]



White *et al*., 2007

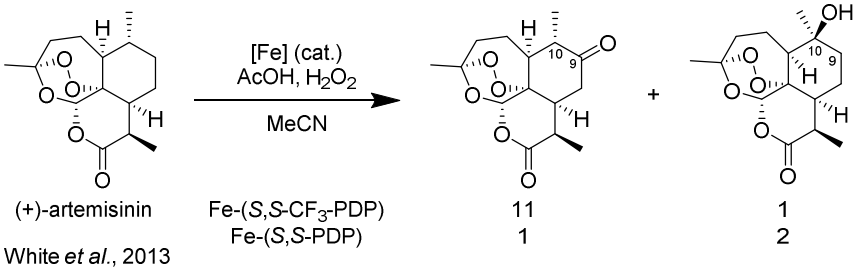
Scheme 9: Fe(*S*,*S*-PDP) catalysed C–H oxidation

Building on these initial results, the catalyst was modified to offer selectivity between 2° and 3° oxidation; by adding bulk to the pyridine groups it was possible to constrict the trajectory cone angle from 145° to 76°, ensuring that steric control dominates (i.e. oxidation at 2° centres in the presence of 3° centres).[80] This was demonstrated in the oxidation of a di-substituted cyclohexane (Scheme 10).[80]



Scheme 10: Catalyst-controlled induction of selectivity in C–H oxidation

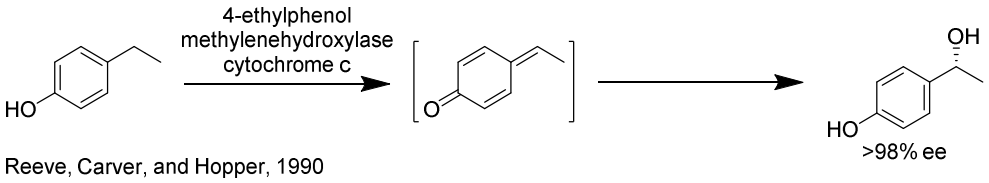
This catalytic system has been applied for the late-stage oxidation of complex natural products; a prime example of this is the oxidation of (+)-artemisinin (the discovery of which led to its award of the 2015 Nobel prize for medicine) selectively at C-9 or C-10 depending on the catalyst used (Scheme 11).[80]



Scheme 11: Oxidation of (+)-arteminisin with Fe-(*S*,*S*-CF_3-PDP) and Fe-(*S*,*S*-PDP)

Enzymatic C–H Oxidation

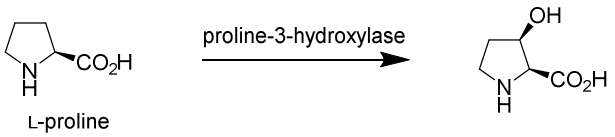
It is well established that enzymes are capable of C–H functionalisation, installing a wide range of functionality with extremely high levels of selectivity.[81] One such example is the group of cytochrome P450s, a superfamily of haem monooxygenases; these will be discussed in more detail later.[82] Flavoprotein monooxygenases are flavin-based monooxygenases which can act as a nucleophilic or electrophilic source of oxygen. They display activity complementary to the cytochrome P450s, catalysing a variety of oxidations, with examples of sulfoxidation, epoxidation, hydroxylation (amongst others) having been observed.[83] Flavoprotein monooxygenases most commonly hydroxylate arenes; alkyl hydroxylation is only seen with alkyl phenols [for example: stereoselective benzylic oxidation of 4-ethylphenol by 4-ethylphenol methylenehydroxylase (4EPMH)]; quinone methide formation is necessary for the hydroxylation (Scheme 12).[84]



Reeve, Carver, and Hopper, 1990

Scheme 12: Hydroxylation of 4-ethylphenol by 4-EPMH

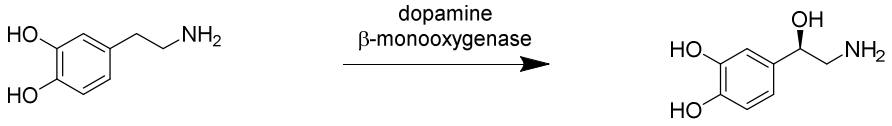
The α-ketoglutarate-dependent oxygenases are the largest subgroup of mononuclear non-haem iron enzymes with the greatest functional diversity.[85,86] α-Ketoglutarate-dependent oxygenases catalyse reactions that effect protein side-chain modification, repair of alkylated DNA/RNA, biosynthesis of plant products and antibiotics, lipid metabolism, and biodegradation (Scheme 13).[85,87] α-Ketoglutarate is required as a co-substrate to assist in the reduction of dioxygen (by its conversion into succinate and CO_2, supplying two electrons) for formation of the active species (an Fe(IV)-oxo complex) which can effect C–H oxidation.[81]



L-proline
Ozaki *et al.*, 1996

Scheme 13: *cis*-Hydroxylation of L-proline

Another class of non-haem iron enzymes is the bacterial multicomponent monooxygenases, which contain a carboxylate-bridged diiron centre in the active site to activate dioxygen for hydroxylation of alkanes, alkene epoxidation, and arene hydroxylation.[88] A dinuclear complex is also found in the active site of the Cu-dependent monooxygenases; however, it is only in the tyrosinases that multiple Cu nuclei are required for binding of dioxygen.[89] These enzymes are mainly found in eukaryotes and the most-studied member is dopamine β-monooxygenase, which catalyses the synthesis of norepinephrine from dopamine *via* a benzylic oxidation (Scheme 14).[89,90] There has been little investigation (apart from one example) of over-expressing copper-dependent monooxygenases in recombinant hosts so the application of these enzymes for biocatalysis is currently very limited.[89,91]



dopamine
Levenberg *et al.*, 1960

Scheme 14: Hydroxylation of dopamine by dopamine β-monooxygenase

Cytochrome P450

In 1958, Klingenberg and Garfinkel independently reported the discovery of a CO-binding pigment (with the CO-complex of the reduced form exhibiting a characteristic absorption at 450 nm) in microsomes isolated from rat and pig livers respectively.[92,93] Later, Omura and Sato identified this pigment as a hemoprotein (specifically a haem b containing protein) and named it P450 (pigment 450 - based on the characteristic absorption frequency mentioned above) (Figure 1).[94,95]

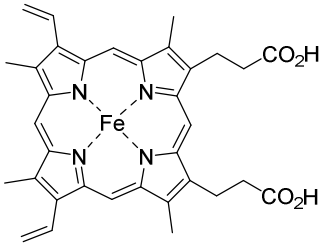


Figure 1: Structure of haem b

Since the early identification in mammalian livers, cytochrome P450 (CYP) enzymes have been discovered in all kingdoms of life: animals, plants, bacteria, protists, fungi, and even archea and viruses. *Escherichia coli* has no cytochrome P450 enzymes of its own: this and the fast growth speeds make *E. coli* very useful for expressing cytochrome P450.[96–98] The presence of cytochrome P450 in archea has led to speculation that this superfamily is of ancient origin, and may have evolved in response to the toxicity arising from increased levels of atmospheric oxygen.[99,100] The large number of cytochrome P450s are usefully divided into smaller groups. By considering the method of transfer of electrons to the active site (required for catalytic turnover), it is possible to classify cytochrome P450s into four groups:

1. Mitochondrial systems employ two proteins, an NADPH specific adrenodoxin reductase with FAD cofactor and adrenodoxin (a [2Fe-2S] ferredoxin-type iron-sulfur protein).

2. Microsomal systems require an FAD and FMN cofactor containing, NADPH specific reductase such as cytochrome P450 reductase.
3. Bacterial $P450_{CAM}$-type systems utilise a ferredoxin reductase and a ferredoxin (Fe-S protein).
4. Bacterial $P450_{MEG}$-type systems are composed of one large protein consisting of a P450 reductase domain fused to a cytochrome P450 domain.[101]

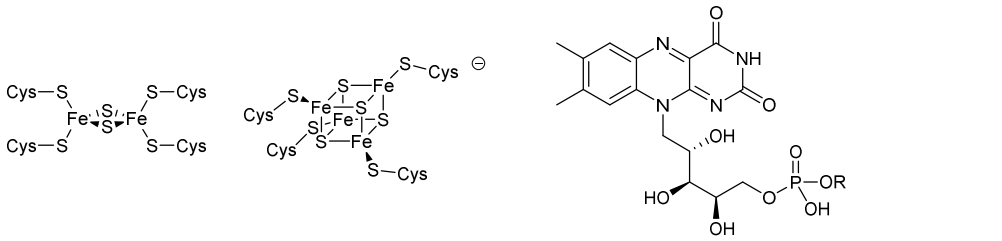


Figure 2: Structures of electron transfer partners

The genes which encode for a certain P450 enzyme, and the enzyme, are identified with CYP for the superfamily, a number for the relevant gene family, a capital letter for the subfamily, and a number again for the individual gene; for example, cytochrome $P450_{BM3}$ is identified as CYP102A1 with 102 identifying the family (family of bacterial P450s), A identifying the subfamily within the 102 gene family (from the genus *Bacillus*), and 1 identifying this specific gene within that family.[102]

The reactions mediated by the P450s rely on generation of an axial-thiolate ligated Fe(IV) oxo (ferryl) species (known as Compound I, **1**). Study of this species is complicated as it does not accumulate during turnover, and this has bought into question its existence and identity as the active species.[103] In 2010, Green put an end to the controversy surrounding the identity of the active species by its generation with *m*-CPBA, which facilitated characterisation by EPR, UV/VIS, and Mössbauer spectroscopy.[103] Other previously mooted active species were Fe(III) (ferric) hydroperoxo complex **2** (Compound 0) or Fe(V) oxo (perferryl) complex **3**; Mössbauer and EPR spectroscopy dismissed these suggestions and identified Compound 1 (**1**) as an Fe(IV) unit coupled to a ligand based radical (delocalised between the porphyrin system and the thiolate ligand) (Figure 3).

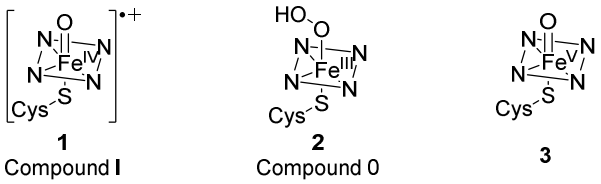
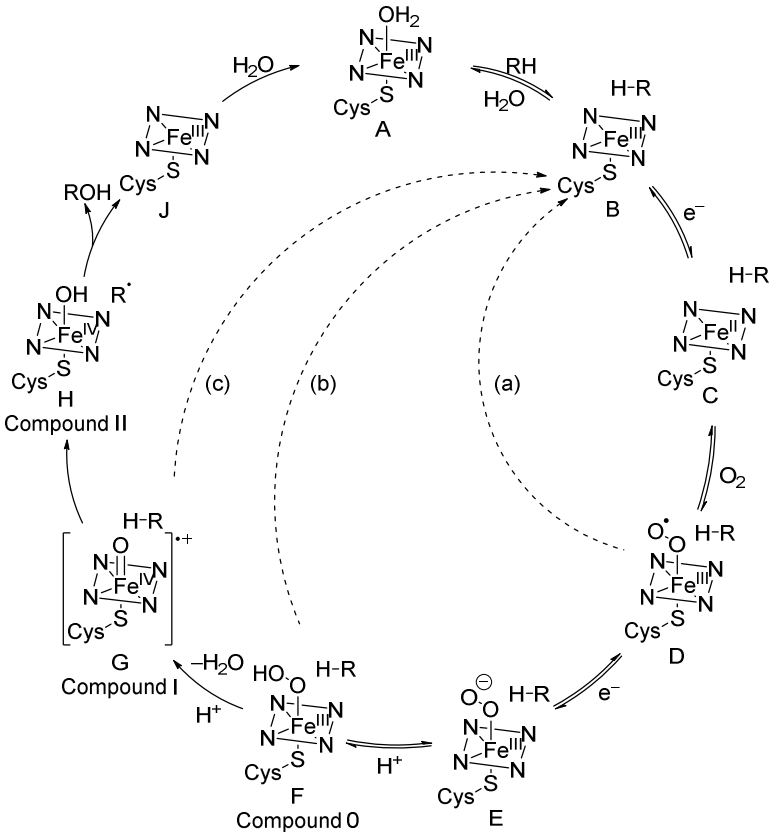


Figure 3: Proposed structures of catalytically active species

The axial thiolate ligand is important for the activity of the P450s because it enables redox-gating of dioxygen activation and also prejudices the systems towards heterolysis of dioxygen over homolysis without requiring a hydrogen-bonding network for this induction.[104] Furthermore, the presence of an axial thiolate ligand effects an increase in the pK_a of Compound II (the conjugate acid of compound I, Scheme 15 (H)) to 12 (compared to ~3.5) and a decrease in the reduction potential compared to histidine ligated Fe(IV) oxo species.[105] This decrease in reduction potential, coupled with an increase in basicity, biases the enzyme towards C–H oxidation and away from non-productive single-electron oxidation of proximal amino acids.[105] For haem species with other ligands (e.g. histidine), far too much uncoupling (*via* non-productive protein oxidation) is liable to occur which is unsustainable in a P450-like system for use of histidine-ligation in Nature.[106] Furthering this point, Arnold has shown that substitution of the axial thiolate with any other ligand (by mutagenesis) abolishes all monooxygenase activity.[107]

Catalytic Cycle

With the identity of the active species known, it is possible to complete the catalytic cycle, beginning with the enzyme in its resting state (A), a hexa-coordinate iron(III) haem b complex, axially ligated by water and a cysteine thiolate (Scheme 15).[102]



Scheme 15: The catalytic cycle for the cytochrome P450s

Arrival of the substrate in the active site leads to displacement of water from the axial position.[102] This is not necessarily due to direct displacement by the substrate: for example, in cytochrome $P450_{BM3}$, displacement of water occurs as a result of rotation of the benzyl group of a phenylalanine-87 (F87) through 90°, moving it close enough to the ferric centre to impede the haem-water interaction.[108] The displacement of water and formation of a pentacoordinate Fe(III) species (**B**) is accompanied by a switch in the spin state from low-spin (S = 1/2) to high-spin (S = 5/2) and the reduction potential becoming more oxidising (an increase in the positive direction); this can be monitored spectrophotometrically and is seen as a blue shift in the Soret band (shift in absorbance from 420 nm to 390 nm, corresponding to the π-π* transition in the porphyrin π-system).[109–112]

Now that the system is more oxidising, a single electron is supplied to the haem iron (*via* electron transfer partners, see page 12) from a reduced pyridine nucleotide (NADH or NADPH), reducing the metal to the Fe(II) (ferrous) species; dioxygen binds to this ferrous species (**C**) and another electron is supplied to form ferric peroxy complex **E**. From this compound, protonation forms Fe(III) hydroperoxy complex **F** (Compound 0; once postulated to be the active species, see

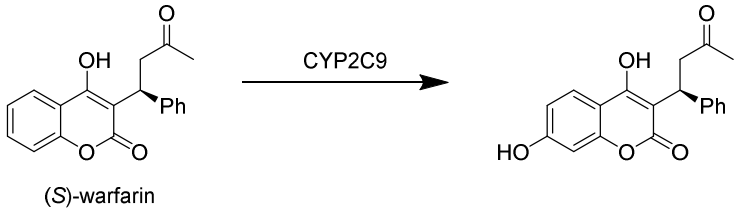
above), another protonation followed by heterolytic O-O bond breaking (with loss of water) generates the active Fe(IV) oxo species, Compound I (**G**). At this point, Compound I can abstract a hydrogen atom from the substrate to form an alkyl radical and an Fe(IV) hydroxide complex (**H**, Compound II); radical rebound with the Fe(IV)-bound OH group produces an alcohol. Following removal of product from the active site, re-binding of the axial water ligand to return the enzyme to its resting state (**A**) or arrival of another substrate molecule is possible.

In the radical rebound mechanism, an oxygen atom from atmospheric dioxygen is formally inserted into a C–H bond. Whilst the radical rebound mechanism is generally accepted, there is still some controversy surrounding the mechanism; much evidence was gathered in support of the radical rebound mechanism, with large kinetic isotope effects and stereochemical scrambling supporting a two-step mechanism featuring a discrete intermediate, formed by full breakage of the C–H bond, which has a limited lifetime.[113] However, other experimental evidence disagrees with the purported radical rebound mechanism; radical clocks have been employed and inconsistencies with these radical clocks and the expected results have been observed, consistent with radical lifetimes that are too short for a species to be considered an intermediate.[114] These inconsistencies have led to the development of a two-state reactivity theory where it is acknowledged that P450s can act *via* the radical rebound mechanism but another mechanism whereby, rather than a formal insertion of an atom of oxygen, there is a direct insertion into the C–H bond by an oxene species. This has been attributed to P450s being able to react as a high-spin species (a diradical with spin of the electrons on Fe and O unpaired), and follow the radical rebound mechanism, or as a low-spin species (spins on Fe and O paired with a formal Fe=O double bond); *cf*. the structures of triplet and singlet oxygen.[114,115] Good evidence exists for both mechanisms and it is possible that the mechanism followed in a particular oxidation is structure-dependent.[114]

Conversion of substrate is not always completely coupled to consumption of NADH (or NADPH) (e.g. 75% substrate conversion but 100% NAD(P)H consumption). There are three mechanisms by which the catalytic cycle can become uncoupled (shown by the dashed arrows in

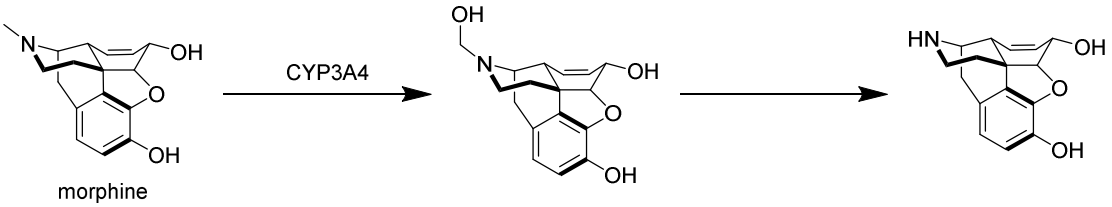
Scheme 17). In superoxide uncoupling (a), retardation of the second electron transfer due to impeded binding of dioxygen can result in loss of superoxide (O_2^-) from complex D. If a substrate is loosely bound in the active site, peroxide uncoupling (b) can occur when water is allowed too close to the ferric centre and the iron-bound oxygen atom can be protonated with loss of hydrogen peroxide. This is detrimental to the enzyme as hydrogen peroxide can oxidise amino acid residues (particularly methionine and cysteine) in the active site. Finally, oxidase uncoupling (c) can occur if no hydrogen atom on the substrate is available for abstraction and the Fe(IV) oxo species (G) is reduced to Fe(III) and water.[102] Reversal of the peroxide uncoupling pathway (at high peroxide concentrations) is known as the peroxide shunt and allows the P450s to function without a cofactor to supply electrons; the CYP152 family can efficiently use H_2O_2 as a feedstock for oxidation and some genetically-engineered mutants demonstrate the ability to use the peroxide shunt pathway and act as peroxygenases.[116–118]

Cytochrome P450s are very powerful enzymes for the catalysis of C–H hydroxylation; however, many other modes of action are possible, including: heteroatom oxidation and dealkylation, olefin epoxidation, arene oxidation, dehydrogenation, and C–C bond cleavage.[113] By these modes of action, cytochrome P450s are vital in a large number of biosynthetic and metabolic processes; they play a particularly important role in phase 1 metabolism (for ≥80% of drugs metabolised by enzymes involved in phase 1 metabolism, the enzyme responsible is a cytochrome P450).[119] Phase 1 metabolism invokes oxidations, reductions, or hydrolases to activate the substrate for conjugation in phase 2.[120] In phase 1 metabolism with cytochrome P450, typically either hydroxylation or heteroatom dealkylation prepares the substrate for conjugation to a charged species to prevent diffusion across lipid membranes so the substrate can now be removed from the cell by active transport.[119,121] Hydroxylation of an aromatic ring is seen, for example, in the metabolism of (*S*)-warfarin by mammalian liver P450 CYP2C9 (C7-hydroxylation) (Scheme 16).[122]



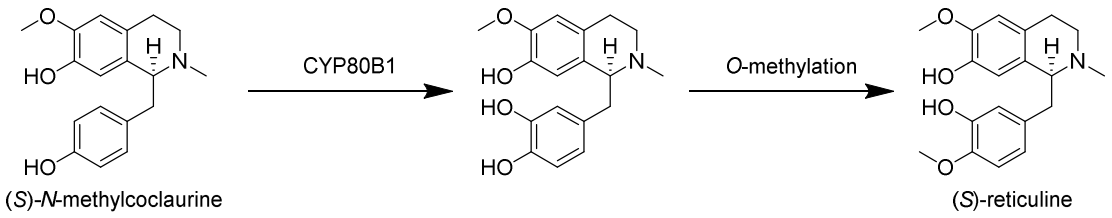
Scheme 16: Arene hydroxylation in metabolism of (*S*)-warfarin

Metabolism of morphine occurs by *N*-demethylation catalysed by mammalian P450s CYP3A4 and, to a lesser degree, CYP2C8. Hydroxylation of the *N*-methyl group and collapse of the intermediate hemi-aminal affords normorphine and formaldehyde as a by-product (Scheme 17).[123]



Scheme 17: *N*-demethylation during metabolism of morphine

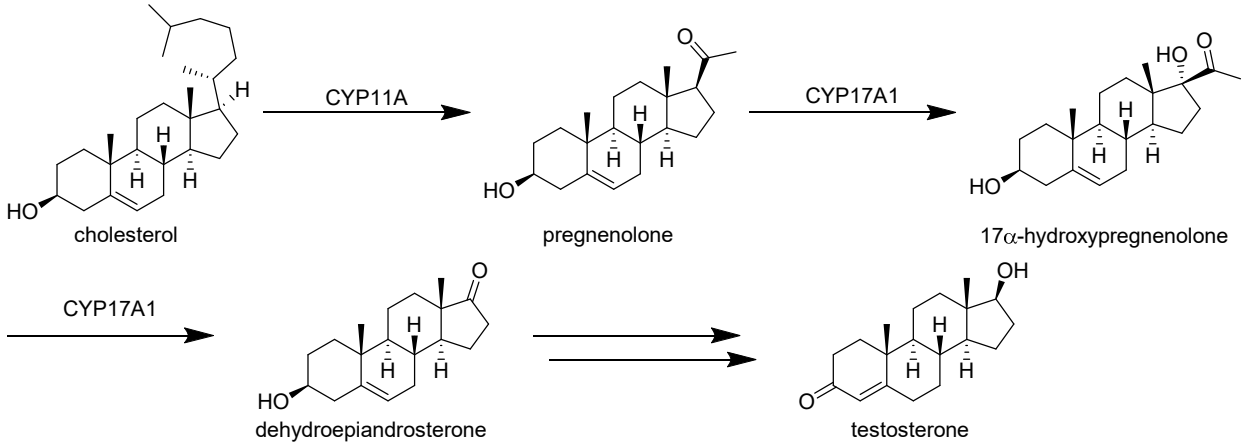
The cytochrome P450s are important in biosynthetic pathways as well as in metabolism. Aromatic hydroxylation of (*S*)-*N*-methylcoclaurine is catalysed by plant cytochrome P450 CYP80B1 (*Eschscholzia californica,* California poppy) in a key step of the biosynthesis of (*S*)-reticuline, a precursor to many alkaloids including morphine (opioid pain medication) and berberine (clothing dye and histology stain) (Scheme 18).[124,125]



Scheme 18: Biosynthesis of (*S*)-reticuline

The utility of the P450s extends to steroid biosynthesis, with the first step in the biosynthesis of many of the steroid hormones being oxidative side-chain cleavage of cholesterol catalysed by mammalian P450 CYP11A to form pregnenolone. This is hydroxylated to 17α-hydroxypregnenolone by CYP17A1, which then catalyses C-C bond cleavage, to form dehydroepiandrosterone (Scheme 19).[126] Two more, non-P450 catalysed, steps are required (alcohol oxidation and ketone reduction) to form testosterone, the male sex hormone. These compounds are precursors to a range of other

important steroid hormones including estrone, cortisol, and progesterone, amongst others, with many steps requiring cytochrome P450 enzymes.[126,127]



Scheme 19: Biosynthesis of steroid hormones

The P450s are also important for the biosynthesis of terpene-derived natural products such as anti-cancer drugs (e.g. taxol) and fragrance compounds (e.g. nootkatone) (Figure 4).[128–132]

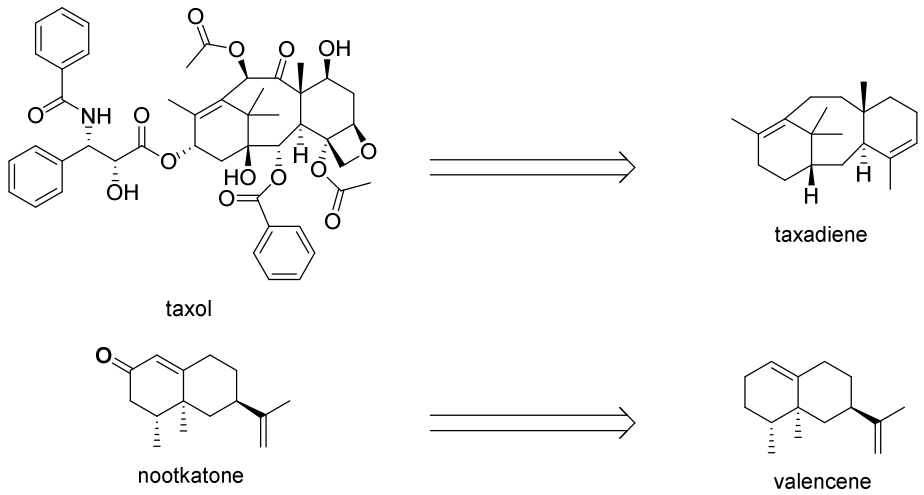
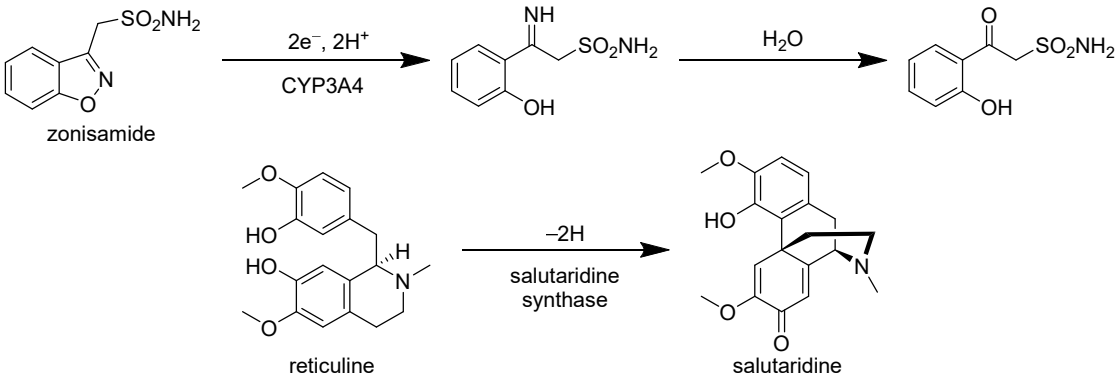


Figure 4: Structures of taxol and nootkatone

With the exception of the CYP11A1 and CYP17A1-catalysed C–C bond cleavages in the formation of pregnenolone and dehydroepiandrosterone (Scheme 19), the majority of the oxidations outlined above demonstrate the more common modes of action of cytochrome P450 enzymes: arene-hydroxylation, alkyl C–H hydroxylation, and heteroatom dealkylation.[119] Whilst they may be the typical modes of action for the P450s, this superfamily is very adaptable to catalysing different reactions when required (as reviewed by Guengerich).[121] A striking example is the ability to act as a reductase, catalysing, for example, reductions of alkyl hydroperoxides, nitro-compounds, *N*-oxides,

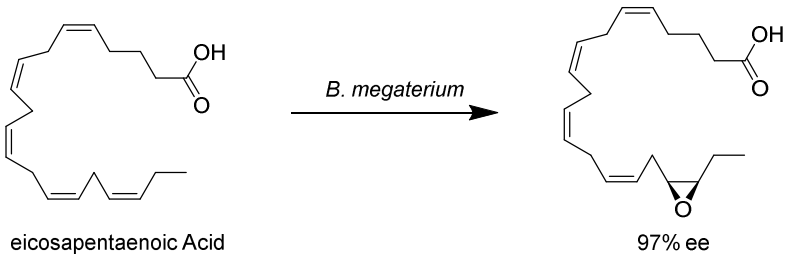
and N-N bonds (in hydrazines and diazo-compounds).[121] In the metabolism of zonisamide, an anticonvulsant agent, the 1,2-benzisoxalole ring undergoes ring-opening (*via* reduction of an N–O bond) then non-enzymatic hydrolysis of the so-formed imine (Scheme 20).[133] The ability to catalyse bond formation is seen in the conversion of reticuline to salutaridine with a P450-mediated (from *Papaver somniferum*, opium poppy) oxidative C–C bond coupling between two phenols for ring formation (Scheme 20).[134,135]



Scheme 20: Unusual reactivity of cytochrome P450s

Cytochrome $P450_{BM3}$ (CYP102A1)

Cytochrome $P450_{BM3}$ (CYP102A1) is so named because it is the third P450 enzyme isolated from *Bacillus megaterium*, a bacterium found in a diverse range of habitats; products formed by this bacterium have found use in industrial processes such as toxic waste clean-up and the construction of synthetic penicillins.[136] It was discovered in the mid-1970s and identified as a solely NADPH and O_2-dependent medium- to long-chain fatty acid hydroxylase, hydroxylating selectively at sub-terminal positions (ω–1, ω–2, ω–3).[137–139] For saturated fatty acids, $P450_{BM3}$ hydroxylates these sub-terminal positions with fairly poor selectivity between the three.[137] However, when unsaturated fatty acids are oxidised by the bacterium, an improvement in selectivity is generally seen for ω–2 hydroxylation, whereas, for some unsaturated fatty acids, epoxidation is the favoured product; for example, when eicosapentaenoic acid is incubated with the bacterium, selectivity for epoxidation of one of the double bonds is >99% with good enantioselectivity (Scheme 21).[140]



Scheme 21: Epoxidation of eicosapentaenoic acid

One of the key features that distinguishes $P450_{BM3}$ from most other P450s is its self-sufficiency; whilst the majority of cytochrome P450 enzymes require an external reductase cofactor to supply electrons to the active site (needed for catalytic turnover), $P450_{BM3}$ is composed of two domains: a haem domain (BMP) and a reductase domain (BMR).[141,142] $P450_{BM3}$ was first considered to be a single polypeptide of mass ~119 kDa; however, proteolysis confirmed that it was actually two fused polypeptides of 55 and 66 kDa respectively.[142] The haem domain contains the active site and is of similar size to other, non-self-sufficient, P450s (sizes typically 50 ± 10 kDa).[141] On the other hand, the rarer reductase domain consists of an equimolar ratio of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) and is no doubt responsible for the supply of electrons to the active site from a cofactor such as NADH or NADPH.[142] Both domains can, when separated, display their original activity (as a reductase and a CO-binding pigment, respectively), but they show no mutual affinity for each other once separated and monooxygenase activity cannot be returned; monooxygenase activity is also not recovered when the domains are expressed and grown separately then mixed.[142,143] The existence of these fused domains negates the need for the haem domain to encounter a reductase cofactor in order to effect turnover, and far higher activity is possible in comparison to other fatty acid monooxygenases.[144]

$P450_{BM3}$ may have been the first, but is not the only P450 featuring a haem domain fused to its reductase partner; other examples include: $P450_{foxy}$, isolated from *Fusarium oxysporum* (a fungal pathogen) that has a similar fusion-redox protein to $P450_{BM3}$ and demonstrates sub-terminal fatty acid hydroxylase activity; in PFOR (phthalate family oxygenase reductase), the fused-reductase protein partner consists of mixed FMN and Fe–S centres for transport of electrons to the phthalate

dioxygenase domain; a haem domain fused to a flavodoxin domain (solely FMN) was found in *Rhodococcus rhodochrous* bacteria grown in soil rich in RDX (Research Department Preparation X; hexahydro-1,3,5-trinito-1,3,5-triazine) and is capable of degrading RDX; P450-ferredoxin fusion proteins are also known, one having been isolated from *Methylococcus capsulatus* which catalyses the three-step oxidation of sterols (e.g. lanosterol) for the cleavage of the 14α-methyl group.[145]

Structure

The crystal structure of the haem (BMP) dimer of CYP102A1 was first recorded in the early 1990s, revealing a long hydrophobic access channel consisting mostly of non-aromatic amino acid residues, and a region displaying a large degree of flexibility (F/G loop and F- and G-helices; residues 190–196, 172–189, and 197–226, respectively) (Figure 5).[146,147] The haem centre is bound to cysteine-400 (Cys400), between the K- and L-helices, and the axial water ligand (Wat23) is hydrogen bonded to alanine-264 (Ala264) in the I-helix (250–282).

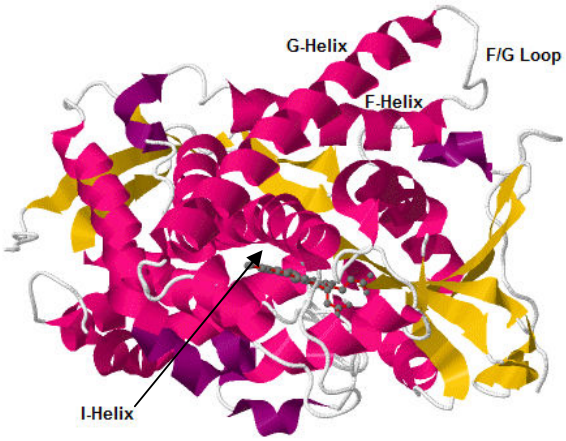


Figure 5: Crystal structure of substrate-free wild-type CYP102A1 (PDB code: 2HPD)[147]

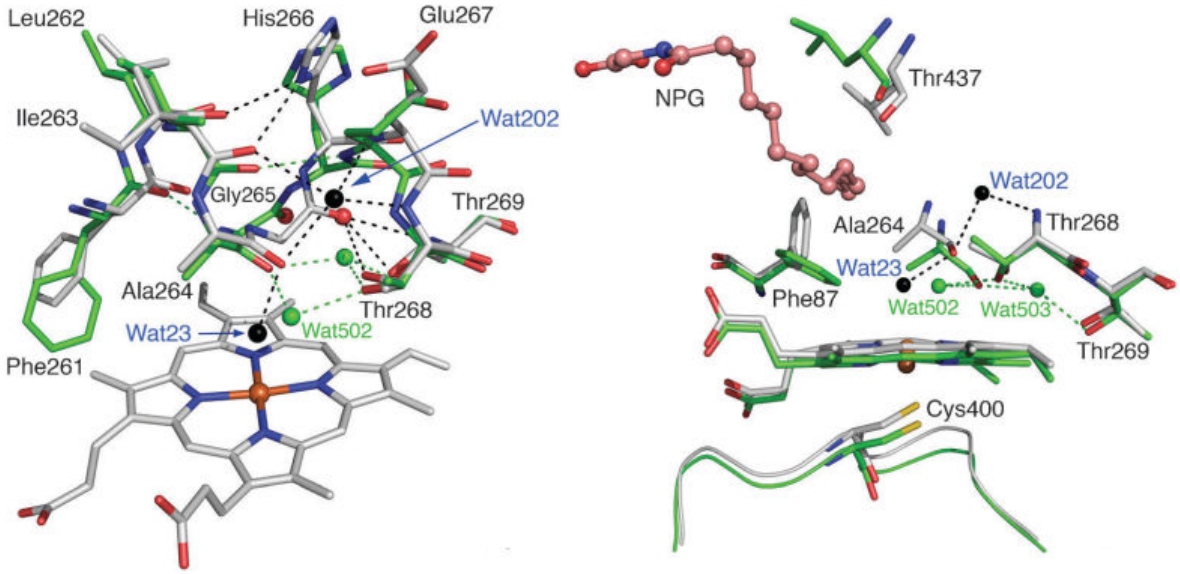


Figure 6: Close-up of the substrate-free (grey, PDB code 1BU7) and *N*-palmitoyl glycine-bound (green, PDB code 1JP7) active site focusing on the kink region of the I-helix (left) and the active site (right)[102]

As discussed earlier, the axial thiolate ligand is the most important residue for retention of monooxygenase activity.[107] Other important residues for binding and oxidation of native substrates in the wild type enzyme include phenylalanine-87 (Phe87), which may be responsible for the displacement of the axial water ligand upon binding of substrate in the active site. It has also been argued that Phe87 is responsible for the selectivity towards sub-terminal hydroxylation; however, it has since become accepted that the innate reactivity of C–H bonds (2° > 1°) is largely responsible.[102,108,148]

Arginine-47 (Arg47) and tyrosine-51 (Tyr51) are the key residues (close to the entrance to the substrate access channel, between the A and B helices), for activity towards the native substrates (medium- to long-chain fatty acids), in the active site. The crystal structure of palmitoleic acid-bound $P450_{BM3}$ (and of *N*-palmitoyl glycine-bound $P450_{BM3}$) displayed an interaction between the carboxylate group of the acid and the phenol group of Tyr51; Arg47 is not involved directly in binding to the acid *via* hydrogen bonding but stabilises the binding by ion pairing.[108,149,150]

A key acid-alcohol pair, highly conserved in the P450 superfamily, is located in the I-helix; for $P450_{BM3}$ the pair is Glu267 and Thr268.[102] It has been suggested that the acid partner, Glu267, plays an important role in the mechanism of activation of dioxygen, potentially by playing a role in the proton transfer network.[151] The alcohol residue, Thr268, is believed to play a more complicated role,

with particular importance in proton transfer, dioxygen activation, substrate recognition, electron transfer, and providing an alternative binding site for the axial haem-water ligand.[152]

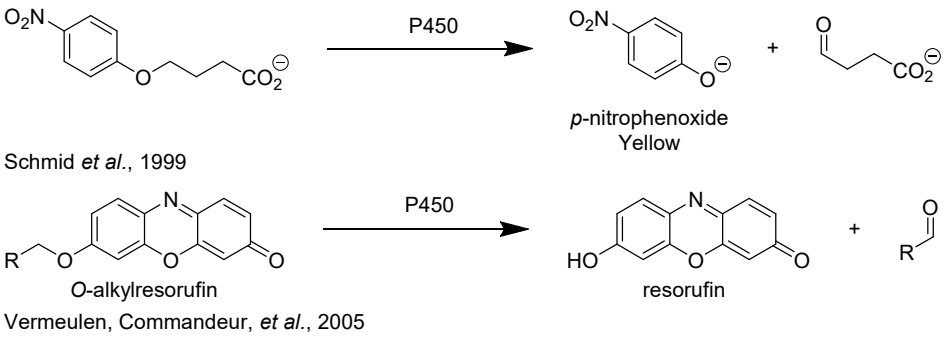
Engineering

The excellent ability of the P450 superfamily to catalyse hydroxylations with high levels of selectivity, whilst also demonstrating a large degree of variability in monooxygenase activity (e.g. heteroatom oxidation, C–C bond formation, *etc.*), makes it a desirable target for biocatalysis.[153] Bacterial P450s have many advantages for this purpose over eukaryotic P450s as they are typically more soluble, tractable, and easier to isolate.[102] A particularly attractive member of the superfamily is $P450_{BM3}$ owing to its self-sufficiency; the existence of the fused reductase domain makes the required biocatalytic system simpler by negating the need for an external redox cofactor.[141,142] Whilst some of the oxidation products of the natural substrates are potentially useful for industrial purposes (e.g. fragrances and pharmaceuticals), a much wider variety of substrates for oxidation is desired; however, due to the narrow substrate specificity typical of enzymes, other substrates will not necessarily fit the active site well and turnover of substrate may be slow or, in cases where binding is much poorer, non-existent.[154,155]

Mutagenesis is a useful tool for probing the catalytic cycle and the mechanistic importance of certain residues for P450s; it can also be used to improve the activity and selectivity of the enzymes towards both natural and unnatural substrates compared to the wild-type enzyme.[153] Typical methods for generating mutant enzymes include directed evolution, where error-prone polymerase chain reaction (PCR) is utilised to generate mutations at random positions; whilst this method does not allow for thought-out design of mutants, it is possible to effect mutations at residues that may not be expected to exert a great influence on reactivity (and are therefore unlikely to be selected for a designed mutation), and this can lead to unexpected and potentially desirable activity.[156] A complementary method, which provides greater control, is rational design (or site-selective mutagenesis). Here, certain residues are targeted due to their perceived importance for reactivity or

selectivity (based on mechanistic and structural knowledge); a list of mutations reported for $P450_{BM3}$ (accurate as of 2012) is available.[102] These two methods can be combined as "rational evolution" where certain regions, believed to be important to activity, are concentrated on for random mutagenesis; the resulting more focused library requires less high-throughput screening for identification of useful mutants.[157]

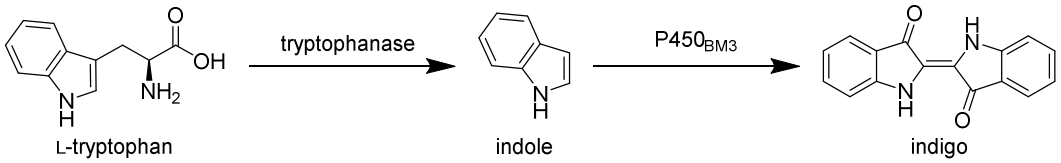
Screening can be improved if a probe is used which resembles the targeted substrates; good examples of this are the commonly used spectrophotometric assay developed by Schmid in which medium-chain fatty acid hydroxylase activity is easily observed by release of the yellow chromophore, *p*-nitrophenoxide, *via* *O*-dealkylation of *p*-nitrophenoxycarboxylic acids (*p*NCAs) (Scheme 22).[158] Another example, which focuses on oxidation of drug-like compounds, was developed by Vermeulen and Commandeur; employing *O*-alkylresorufins as probes for detection of activity by fluorescence spectroscopy (*O*-dealkylation releases the dye, resorufin) (Scheme 22).[159]



Scheme 22: Probes for screening assays

Unexpected formation of the blue, water-insoluble dye indigo during the growth of new mutants proved to be an effective method for identification of mutants capable of oxidising indole during high-throughput screening.[160] Indigo is formed by oxidation and dimerisation of indole which, in turn, is formed in the growth medium by degradation of tryptophan (Scheme 23).[161] Easy, visual identification of mutants capable of oxidising substrates which have a large degree of structural difference to the native substrates is possible by formation of this dye (which does not occur with the wild-type enzyme). Other substrates, including polycyclic aromatic hydrocarbons, showed good

activity with mutants identified as being more active than wild-type by this method.[162] Other known methods of analysis for high-throughput screening have been reviewed.[163,164]



Scheme 23: Formation of indigo from L-tryptophan

The engineering of $P450_{BM3}$ and the increase in the range of substrates accepted by the enzymes has been well reviewed.[102,165,166] In the first attempt to employ random mutagenesis of $P450_{BM3}$ a single-site mutation, in a region thought to be important for substrate binding (proline-25 to glutamine, P25Q), resulted in a decrease in affinity of the natural substrate and an alteration in the regioselectivity of hydroxylation.[167]

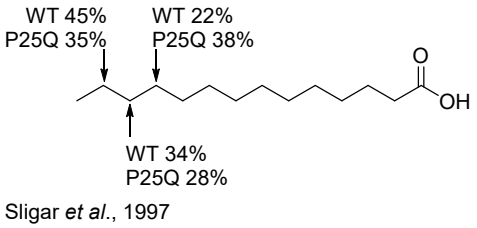
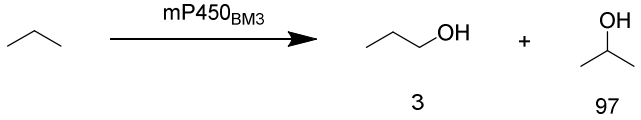


Figure 7: Sites of hydroxylation of myristic acid with wild-type (WT) $P450_{BM3}$ and variant P25Q

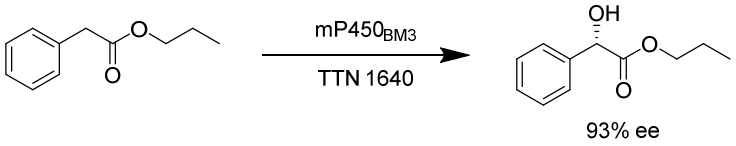
Directed evolution has also been employed to generate far more complex mutants; for example, Arnold demonstrated the use of a colourimetric screen (using *p*-nitrophenoxy octane, similar to above with *p*NCAs) to identify a mutant, with 11 amino acid residue substitutions, capable of oxidising octane at a much greater rate than wild-type, although the hydroxylation was not particularly regioselective.[168] This mutant was also shown to oxidise shorter-chain alkanes (heptane, hexane, butane, and propane), cycloalkanes (cyclohexane), and longer chain alkanes (nonane and decane), with good selectivity only seen for butane, propane, and cyclohexane (in which only one mono-hydroxylation product, cyclohexanol, was formed) (Scheme 24).[169]



Arnold *et al.*, 2003

Scheme 24: Hydroxylation of propane by mutant $P450_{BM3}$ enzyme

By combining the mutation of alanine-328 (directly above the haem: Figure 8, page 28) to the larger valine (A328V) with this alkane-hydroxylating mutant, it was possible to improve the selectivity for 2-hydroxylation of heptanes and the longer chain alkanes.[169] Some of the mutants, which had demonstrated ability to hydroxylate alkanes, also hydroxylated structurally diverse compounds, such as in the enantioselective hydroxylation of phenylacetic acid esters by mutants which have 14 or 15 amino acid residues different from the wild type (Scheme 25).[170]



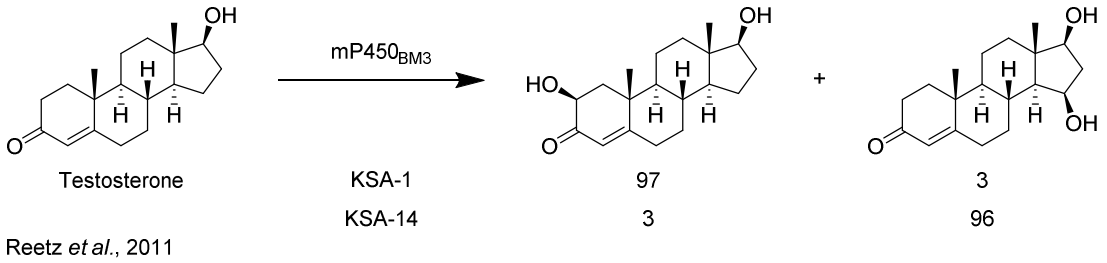
Arnold *et al.*, 2006

Scheme 25: Enantioselective hydroxylation of phenylacetic acid esters

Smaller-chain alkanes have proven more difficult to oxidise, partially due to the large size of the active site in which it is statistically unlikely that the small substrates will be in the appropriate orientation to be oxidised by the active species; also, the substrate is required to be present for initiation of the catalytic cycle, and the small size of these substrates limits, for example, their ability to carry out this role. This requirement can be avoided by use of a decoy substrate; perfluorocarboxylic acids can occupy the active site, initiating the catalytic cycle and limiting the available volume, allowing oxidation of small-chain alkanes including ethane, propane, and butane.[171,172] These decoy substrates have been improved in the second generation of their development; here, *N*-perfluoroacyl amino acid decoys increase the product formation rate and the coupling efficiency for oxidation of ethane, propane, and cyclohexane, believed to be due to improved binding of the decoy substrates allowing more efficient initiation of the catalytic cycle.[173]

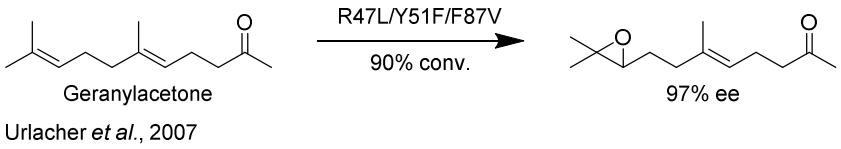
An effective method for the improvement of $P450_{BM3}$ variants for activity and selectivity, and generation of a binding pocket focused library, is a saturation mutation based directed evolution

known as iterative combinatorial active-site saturation test (CAST), which involves subjecting residues in groups to saturation mutagenesis or making substitutions covering polar or non-polar, charged or uncharged, and aromatic or non-aromatic amino acids, and screening for activity and selectivity.[174] Once a promising mutant has been identified, it can be used as the basis for further rounds of mutagenesis at other residues until the desired selectivity is achieved. This was applied successfully to the highly selective hydroxylation of steroids such as testosterone (KSA-1: A330W/F87A; KSA-14: R47Y/T49F/V78L/A82M/F87A) (Scheme 26).[175]



Scheme 26: Selective hydroxylation of testosterone

Early efforts to use rational design for generating mutants with altered reactivities focused on mutations close to the haem (Phe87) or close to the mouth of the substrate access channel (Pro25, Arg47, Tyr51), both for investigation of the role they play in the activity of the enzyme and for the oxidation of unnatural substrates.[176–179] It was discovered by Wong in 2001 that a double mutation, which increases the hydrophobicity of the substrate access channel (substituting Arg47 and Tyr51 for non-polar residues: R47L, Y51F), raised the activity towards the non-polar polycyclic aromatic hydrocarbons (PAHs) phenanthrene, fluoranthene, and pyrene by up to 40 times that of the wild-type enzyme.[180] Combination with other, active site, mutations resulted in much larger increases in activity. These two mutations have proven to improve activity for oxidation of a variety of non-polar substrates.[181–185] Urlacher applied these mutations (combined with another: R47L/Y51F/F87V) for the oxidation of acyclic monoterpenes, such as geranylacetone, which was highly selective for 9,10-epoxidation (Scheme 27).[184]

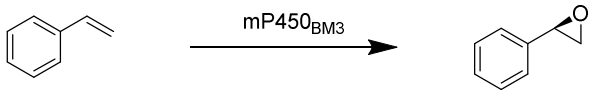


Urlacher *et al.*, 2007

Scheme 27: Epoxidation of geranylacetone

Selective mutation was used to design mutants that target oxidation of fatty acids of shorter chain lengths than the native substrates; by substituting two hydrophobic residues for polar ones (Leu181 to Lys and Leu75 to Thr) to create a carboxylate binding site closer to the haem than the Arg47/Tyr51 pair, it was possible to drastically increase both the activity of the enzyme towards shorter-chain fatty acids (C_4, C_6, and C_8) and their binding strength.[186] For hexanoic, octanoic, and decanoic acid, k_{cat} was decreased relative to wild-type for the single-residue mutation L181K; however, combination with L75T resulted in a dramatic increase in k_{cat} for hexanoic and octanoic acid, and a decrease for decanoic acid. For butyric acid, an increase in k_{cat} relative to wild-type was found for both the single- and double-residue mutation. Combination of L181K with substitutions of Arg47 and Tyr51 with non-polar residues resulted in severe loss of activity for substrates $<C_{10}$ suggesting the two residues play a role in substrate recognition.

A hydrophobic pocket between Phe81 and Phe87 was considered to bind small hydrophobic molecules unproductively. Roberts found that incorporation of a large group inside this pocket (at Ala82: A82F or A82W) could raise in the binding affinity of laurate and palmitate.[187] Following on from this work, a new mutant was designed for oxidation of styrene; it was discovered that, in combination with A82F, incorporation of larger groups at Thr438 (Val/Ile/Leu/Phe) constricted the active site, limiting freedom of movement for the substrate, and altering the activity and enantioselectivity of styrene epoxidation (Scheme 28).[188]



Roberts *et al.*, 2011

Scheme 28: Enantioselective epoxidation of styrene

Efforts to generate a library of $P450_{BM3}$ mutants can lead to surprising results. For example, during directed evolution, using indigo formation to identify mutants with promising activity, a new, single mutation variant was discovered (Ala330 to Pro; A330P).[181] This mutation, either on its own or in combination with other mutants (e.g. in R47L/Y51F/A330P), led to an increase in activity from wild type and often unique selectivity in the oxidation of small molecules such as octane or alkylbenzenes.[181,189] Examples include increasing selectivity for phenol formation in the oxidation of propylbenzene with R47L/Y51F/A330P, whereas R47L/Y51F favoured benzylic hydroxylation (Scheme 29).[181] Solving the crystal structure of A330P allowed for elucidation of a reason for the observed selectivity differences: the substitution of Ala300 for Pro forced the neighbouring residue, Pro329 into the substrate access channel, constricting the active site and altering the substrate binding conformation (Figure 8).[190]

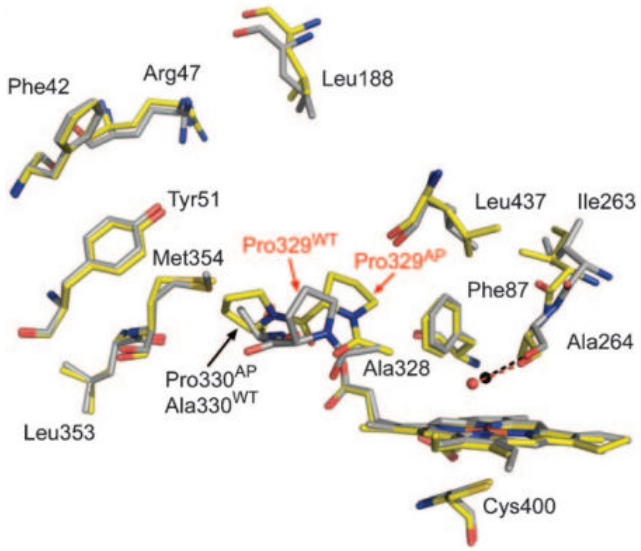
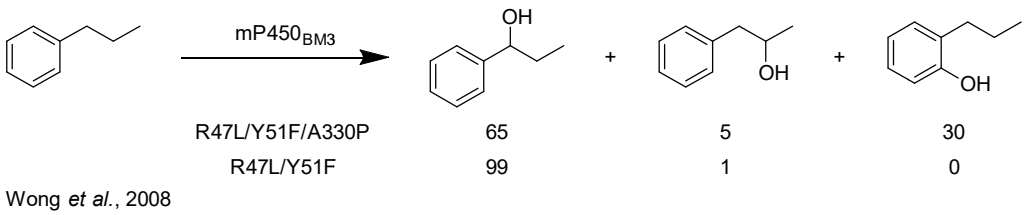


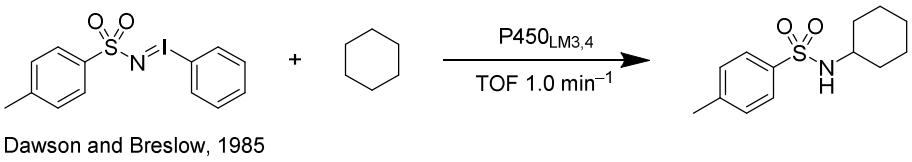
Figure 8: Crystal structure of active site for mutant A330P (yellow, PDB code 3M4V) overlaid with wild-type $P450_{BM3}$ (grey, PDB code 1BU7)



Scheme 29: Differing selectivity in propylbenzene hydroxylation

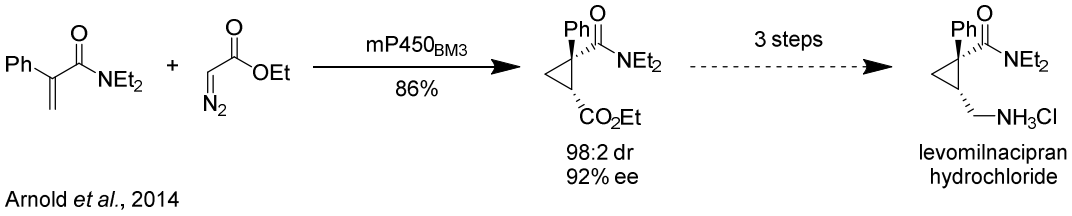
Engineering the enzyme alters selectivity or activity towards certain substrates, and can also alter the nature of the enzyme. Dawson and Breslow demonstrated that a rabbit liver microsomal

P450 could effect C–H amination of cyclohexane by nitrene transfer from an iminoiodane (Scheme 30).[191] Turnovers were too low to be synthetically useful (TOF 1.0 min^{-1}) and work on using P450 to transfer atoms other than oxygen was not reported for a further 28 years.



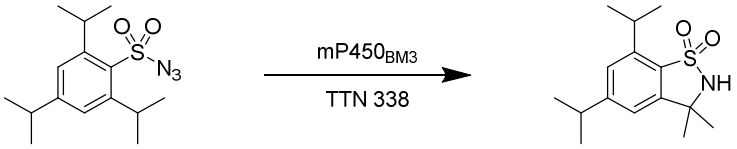
Scheme 30: $P450_{LM3,4}$-catalysed nitrene transfer from an iminoiodane

Arnold applied a mutant $P450_{BM3}$ library to identify carbene and nitrene transfer activity (for alkene cyclopropanation and intramolecular C–H amination, respectively) and saw improved turnover and differing stereoselectivities compared to wild type; this was greatly improved upon by substituting the haem-ligating Cys400 for Ser, losing monooxygenase activity, but increasing turnovers to up to 67,800 for carbene transfer and 680 for nitrene transfer.[192–194] Axial histidine ligation of the haem (Cys400 to His) also facilitates cyclopropanation and a variant containing this mutation was employed in the formal synthesis of the clinical anti-depressant, levomilnacipran hydrochloride (Fetzima), an inhibitor of serotonin and norepinephrine reuptake (Scheme 31).[195]



Scheme 31: Formal synthesis of levomilnacipran hydrochloride

Fasan has also demonstrated the use of $P450_{BM3}$ for intramolecular nitrene transfer, although, rather than altering the haem-ligation to improve activity, a different tactic was employed; by increasing the size of the active site and carrying out reactions under anaerobic conditions (as monooxygenase activity is still present), intramolecular C–H amination with sulfonyl azides or carbonazidates could be achieved (Scheme 32).[196,197]

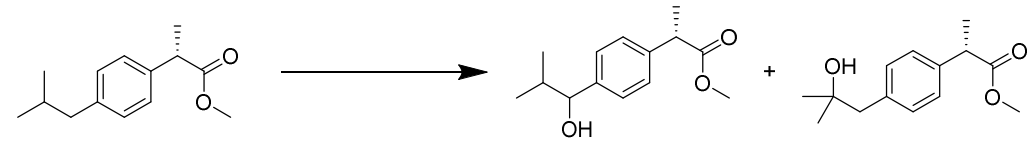


Fasan *et al.*, 2014

Scheme 32: $P450_{BM3}$-catalysed intramolecular C–H amination

In addition to natural amino acid mutations in $P450_{BM3}$, the installation of unnatural amino acids has also been demonstrated. This has been achieved by two different strategies: global substitution of a particular amino acid with a particular unnatural amino acid, or site-selective substitution with unnatural amino acids. Arnold demonstrated that a two-fold increase in peroxygenase activity (relying on the peroxide shunt pathway for generation of Compound I) could be achieved by the global substitution of methionine residues (13 out of 463 in the haem domain) for the isosteric norleucine which, unlike methionine, is resistant to oxidation by hydrogen peroxide.[198]

Site-selective installation of unnatural amino acids was employed by Fasan, modifying a mutant $P450_{BM3}$ with the unnatural amino acids *para*-aminophenylalanine (pAmF), *para*-acetylphenylalanine (pAcF), 3-(2-naphthyl) alanine (NapA), and *O*-benzyl tyrosine (OBnY), and screening them for activity and selectivity towards (*S*)-ibuprofen methyl ester and (+)-nootkatone (Scheme 33).[199] These unnatural mutants were able to alter the product selectivity and increase the activity, with mutants incorporating *para*-aminophenylalanine exerting a particularly strong enhancement of turnover numbers.



Fasan *et al.*, 2014

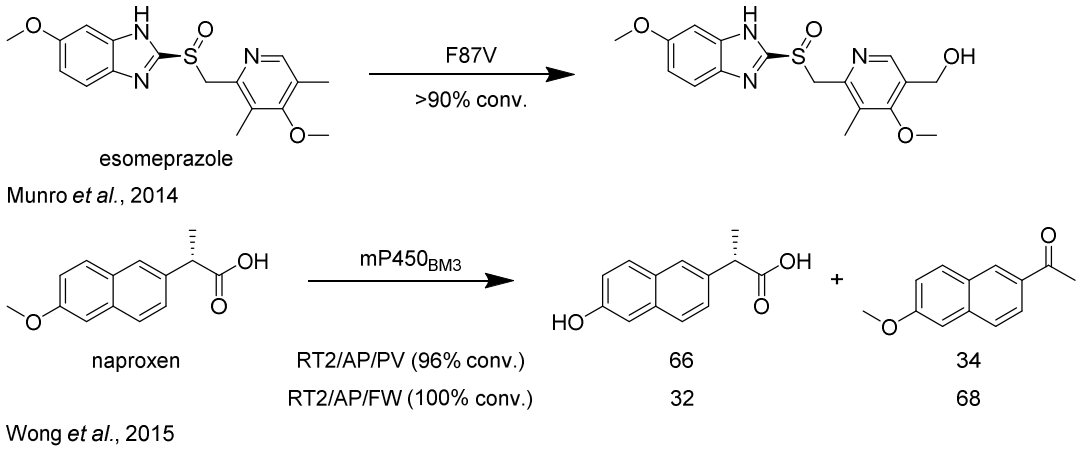
Scheme 33: Oxidation with mutant $P450_{BM3}$ containing an unnatural amino acid

A more extreme unnatural modification applied to $P450_{BM3}$ is the installation of a non-native haem unit (replacing the vinyl groups of the natural haem b with protons), and the mutation of amino acid residues in the haem-binding cavity to selectively bind the non-native haem (even in the

presence of the native haem b).[200] Ultimately, this level of modification disrupts the enzyme sufficiently that monooxygenase capability is lost; however, this engineered enzyme can act as a catalyst for carbenoid transfer, see above.

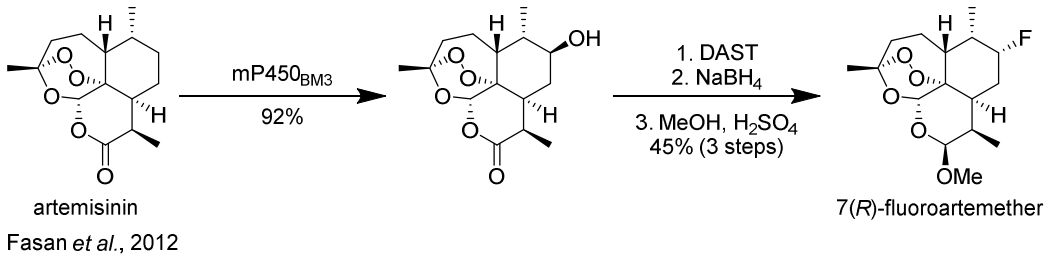
Applications of mutant $P450_{BM3}$

Whether developed by directed evolution, rational design, or a combination of the two (rational evolution), libraries have been created that exhibit widely differing activities and selectivities. These mutant libraries have been applied for a range of purposes, which have been well reviewed.[82,102,165,201–203] Of particular relevance is the synthesis of drug metabolites on preparative scale to aid the analysis of metabolite activity and toxicity, a vital aspect of drug development.[204] Synthesis of these metabolites by chemical means in the quantities necessary for activity and toxicity analysis can be difficult and an alternative is to generate the metabolites by P450 oxidation, given that P450 enzymes are responsible for metabolism of ≥80% of drugs processed by enzymes associated with phase 1 metabolism.[119] Whilst it could be expected that human liver microsomes could be used to this effect as they are responsible for the *in vivo* metabolism of the drugs, in practice, their limited availability and the highly unpredictable levels of expression of P450 enzymes within them make this option unfeasible.[205] The libraries of self-sufficient, readily expressible $P450_{BM3}$ mutants; however, have proven to be valuable substitutes, providing metabolites of a range of drugs in sufficient quantities for analysis.[206–214] A successful example is the synthesis of the major human metabolite of esomeprazole, a proton-pump inhibitor, which was achieved by Munro *via* a mutant $P450_{BM3}$-catalysed benzylic hydroxylation with good conversion (Scheme 34).[213] Another example is the synthesis of the major human metabolite of naproxen, a non-steroidal anti-inflammatory drug (NSAID), as well as a non-natural metabolite, by *O*-demethylation and oxidative decarboxylation, respectively (Scheme 34).[206]



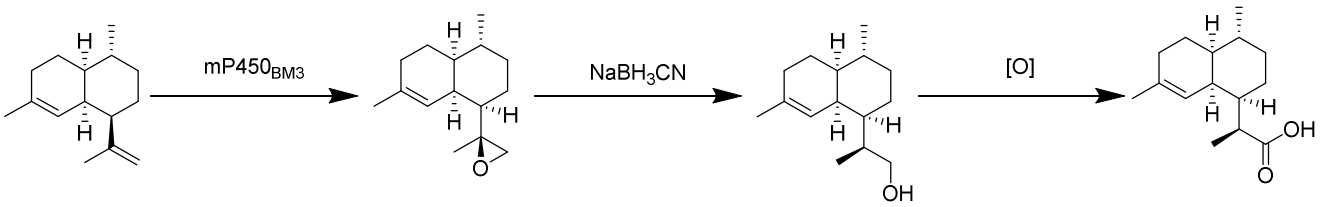
Scheme 34: Oxidation of drugs by $mP450_{BM3}$

This approach can be also used to develop analogues of drugs in which the normal sites of metabolism are blocked by transformation of the enzymatically synthesised metabolite. Fasan applied this approach to the synthesis of 7(*R*)-fluoroartemether, a fluorinated derivative of the antimalarial drug artemether, in which a metabolic site is blocked by a fluorine atom.[215,216] This is achieved by a stereoselective hydroxylation of artemisinin, catalysed by a mutant $P450_{BM3}$, followed by a series of non-enzymatic steps (Scheme 35). This strategy for the synthesis of fluorinated drug derivatives *via* late-stage functionalisation has been applied to a range of drug-like molecules.[217]



Scheme 35: Chemoenzymatic synthesis of 7(*R*)-fluoroartemether

In addition to the synthesis of drug metabolites and analogues as analytical standards, mutant $P450_{BM3}$ enzymes have been applied in the synthesis of the drugs themselves. $P450_{BM3}$-mediated enantioselective epoxidation of amorpha-4,11-diene, is a key step in a semi-biosynthetic route towards dihydroartemisinic acid, a direct precursor to artemisinin (Scheme 36).[218,219]

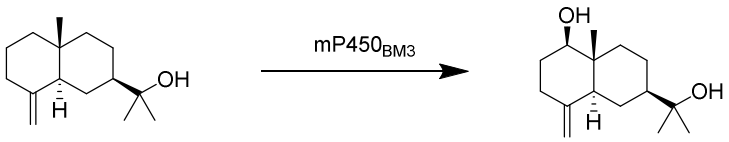


amorpha-4,11 -diene | artmeisinic-11*S*,12-epoxide | (*R*)-dihydroartemisinic alcohol | dihydroartemisinic acid

Keasling *et al.*, 2009

Scheme 36: Semi-biosynthetic route to dihydroartemisinic acid

Numerous sesquiterpenes and their oxidised derivatives have, or are precursors to metabolites that have, desirable biological or medicinal properties or have found use as flavourings or fragrances. The sites of oxidation are often chemically inaccessible, which has made these compounds interesting targets for P450-mediated oxidation.[102,220,221] Epoxidation of the acyclic sesquiterpene, methyl farnesoate, with a $P450_{BM3}$ variant achieved the synthesis of juvenile hormone III, methyl-10,11-epoxyfarnesoate, which is found in most insect species for regulating larval growth.[222] In another example, β-eudesmol, a bicyclic sesquiterpene which is used as an antidote for organophosphate intoxication, was hydroxylated at the 5-position selectively (Scheme 37).[223,224]



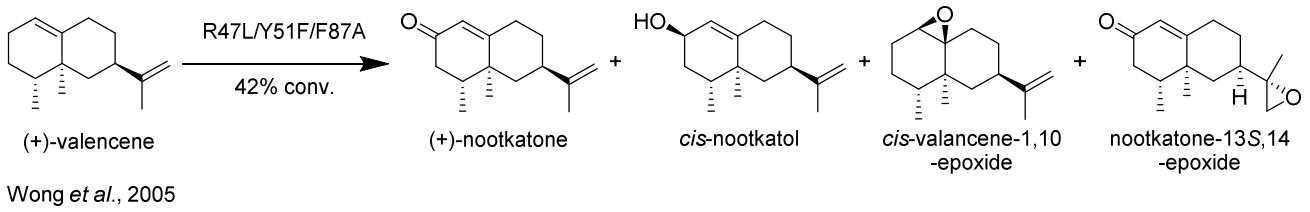
β-eudesmol

Misawa *et al.*, 2011

Scheme 37: Hydroxylation of β-eudesmol by mutant $P450_{BM3}$

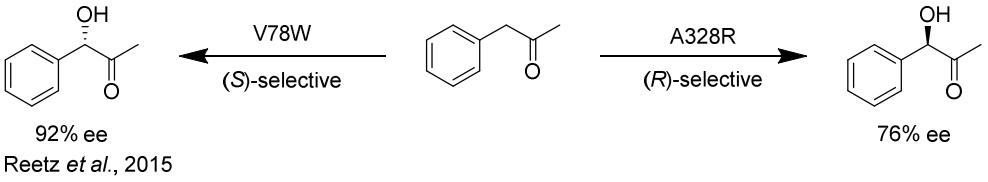
(+)-Nootkatone, a sesquiterpenoid isolated from grapefruit, has a unique odour and is highly desirable as a flavouring and fragrance component; its insect-repellent ability has led to interest in its pharmaceutical qualities.[225] Extraction of (+)-nootkatone from natural sources fails to supply adequate quantities due, in part, to variable harvests and low concentrations in biomass, whereas chemical synthesis, which can satisfy the high demand, typically requires harsh, environmentally damaging methods; these factors have resulted in (+)-nootkatone being a long sought-after, challenging target for enzymatic synthesis.[225] An attractive solution is offered by biocatalytic oxidation of (+)-valencene, a fragrance compound isolated from Valencia oranges, which is much cheaper than (+)-nootkatone and more reliably produced.[225,226] Wong investigated the application of

$P450_{BM3}$ mutants (and $P450_{cam}$ mutants) for this biotransformation and initial results showed that $mP450_{cam}$ were more selective but less active than $mP450_{BM3}$. The most successful mutant for (+)-nootkatone production gave a 42% conversion with (+)-nootkatone comprising ~10% of the product along with nootkatol, epoxidation products, and products of unknown structure (Scheme 38).[227] Since this initial study, the conversion and selectivity have been greatly improved.[228]



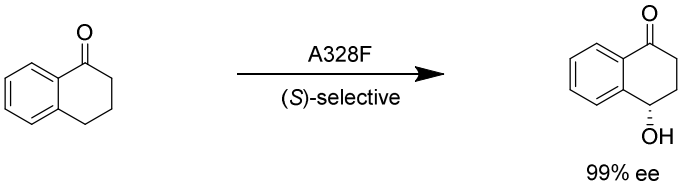
Scheme 38: Biotransformation of (+)-valencene with $mP450_{BM3}$

$P450_{BM3}$ has begun to be more widely applied in asymmetric catalysis.[165] Examples mentioned earlier include the enantioselective α-hydroxylation of 2-arylacetic acid derivatives (Scheme 25), hydroxylation of steroidal compounds (Scheme 26), and, in a less classical application, asymmetric cyclopropanation of alkenes (Scheme 31).[170,175,195] Reetz has been particularly active in this area. His group has employed a library of $P450_{BM3}$ mutants to achieve enantioselective (and regio- and diastereoselective) hydroxylations for a variety of structurally differing compounds.[229–235] Enantioselective benzylic hydroxylations were achieved with both acyclic and cyclic substrates; the enantioselective synthesis of acyloins was achieved by ketone α-hydroxylation and, for a number of substrates, either enantiomer was accessible with good enantioselectivity (Scheme 39).[234]



Scheme 39: Enantioselective hydroxylation of 1-aryl-propan-2-ones

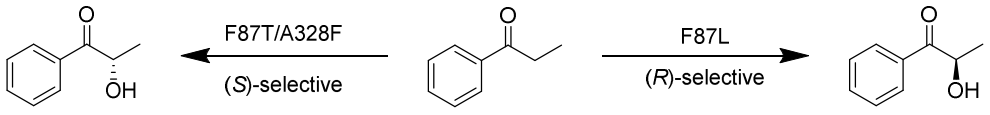
Cyclic substrates could also be utilised as demonstrated by the hydroxylation of 1-tetralones with high levels of enantioselectivity; this method was also applicable to 1-indanones, indane, and tetralin, with lower levels of enantioselectivity found for tetralin hydroxylation (Scheme 40).[232]



Reetz *et al.*, 2014

Scheme 40: Enantioselective benzylic hydroxylations

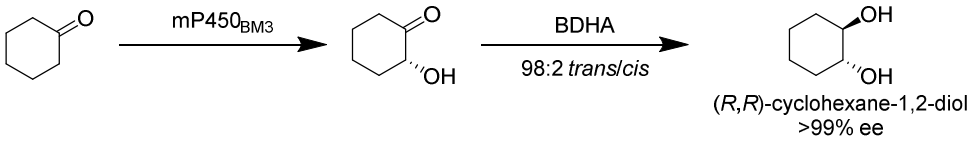
Enantioselective α-hydroxylation of ketones in non-benzylic positions was also achieved with substituted propiophenones; again, selectivity for either enantiomer of product was possible with generally higher enantioselectivities than for 1-aryl-propan-2-ones (Scheme 41).[234]



Reetz *et al.*, 2015

Scheme 41: Enantioselective α-hydroxylation of propiophenone

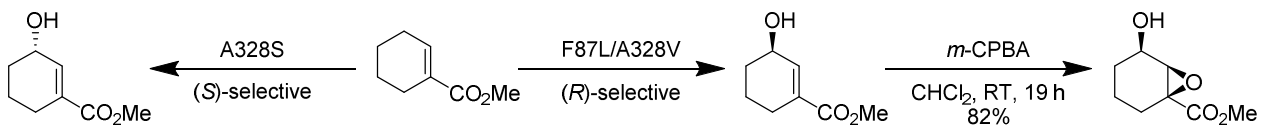
A similar enantioselective acyloin synthesis was employed, using *E. coli* co-expressing a mutant $P450_{BM3}$ and an alcohol dehydrogenase [e.g. butane-1,2-diol dehydrogenase (BDHA)], for the conversion of cyclohexanone into enantiopure 1,2-cyclohexanediol (Scheme 42).[235] Selectivity was possible for (*R*,*R*)-, *meso*, or (*S*,*S*)-cyclohexane-1,2-diol.



Reetz *et al.*, 2016

Scheme 42: Enantioselective synthesis of (*R*,*R*)-cyclohexane-1,2-diol

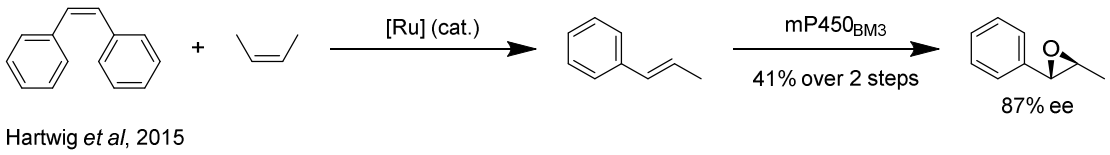
Enantioselective $P450_{BM3}$-mediated allylic hydroxylation of cyclohexenes gives products that can serve as intermediates in the synthesis of more complex structures; for example, reaction with *m*-CPBA can be directed by the alcohol to effect diastereoselective epoxidation (Scheme 43).[229]



Reetz *et al.*, 2012

Scheme 43: Enantioselective allylic hydroxylation and directed epoxidation

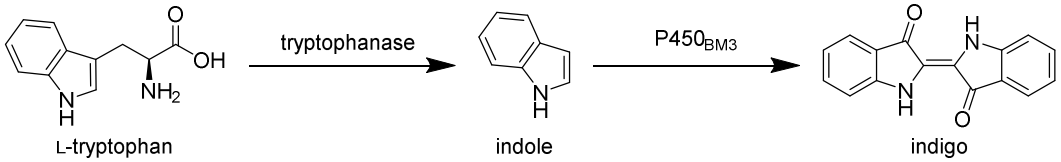
In addition to enantioselective hydroxylation, the application of mutant $P450_{BM3}$ in enantioselective epoxidations is well established, as illustrated by the enantioselective epoxidation of geranylacetone and styrene (Schemes 27 and 28, respectively).[184,188] Enantioselective epoxidation was applied by Hartwig in a tandem reaction comprising cross-metathesis of two alkenes, which are not turned over by the enzyme employed for the transformation, and enantioselective epoxidation catalysed by a mutant $P450_{BM3}$ in a one-pot procedure (Scheme 44).[236]



Scheme 44: Tandem alkene metathesis/ asymmetric epoxidation

Research in the Wong Group

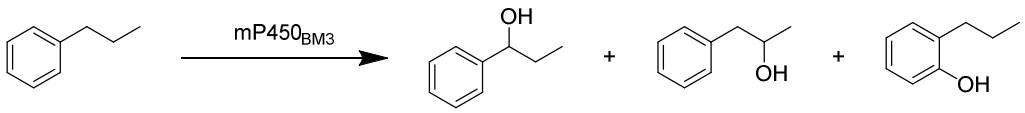
Within the Wong group, a library of mutant $P450_{BM3}$ has been developed that contains >200 different mutants, each member typically containing one or more point substitutions although, in certain cases amino acid insertions or deletions are employed. A large number of these are based on mutants discovered by error-prone PCR. By monitoring indigo formation during colony growth, blue colonies were selected to indentify mutants which displayed increased activity, which were then subjected to further screening.[181]



Scheme 45: Formation of indigo from L-tryptophan metabolism

Purification and analysis was carried out on four exciting variants and these were identified as A330P (single mutation variant), KT2 (5 mutations, A191T/N239H/I259V/A276T/L353I), KSK19 (4 mutations, F87A/H171L/Q307H/N319Y), and KT5 (4 mutations, F87A/A330P/E377A/D425N); some of these mutants were also combined with variants already established to increase activity (RLYF, R47L/Y51F or F87A) and the range of mutants was screened for activity and selectivity with propylbenzene and naphthalene.

Compared to wild type, product formation rates were generally increased across the board. Product profiles for KT2 and RLYF (and the combination thereof) were markedly similar to those of the wild-type enzyme (selecting for benzylic hydroxylation of propylbenzene, 1-naphthol was the only detected product for naphthalene oxidation). A330P displayed a different product profile, increasing the selectivity for *ortho*-hydroxylation of propylbenzene (see Scheme 24); this selectivity was not affected when it was combined with RLYF. KT5 (which contains F87A and A330P) displayed a product profile more similar to that of F87A, but with a greater degree of selectivity for 1-phenyl-2-propanol than for F87A alone (Scheme 46).



WT	99	0	1
F87A	46	54	0
KT5	20	80	0
A330P	2	30	68

Wong *et al.*, 2008

Scheme 46: Regioselectivity in $P450_{BM3}$-catalysed hydroxylation of propylbenzene

Another mutation which has been widely used is I401P where the isoleucine adjacent to Cys400 (which ligates the haem unit) has been substituted for a proline; this mutation sees an increase in the NADPH turnover rate and the product formation rate for natural substrates (lauric acid) and for non-natural substrates [(+)-α-pinene, fluorene, 3-methylpentane, and propylbenzene], although little change in product profiles is observed (Scheme 47).[237]



(+)-α-pinene

(+)-verbenol
74% (57:43 dr)

2,3-epoxypinane
19% (19:1 dr)

Wong *et al.*, 2009

Scheme 47: Oxidation of (+)-α-pinene by I401P (minor products not shown)

For some of these mutants, the structural basis for the change in reactivity or selectivity has been investigated by X-ray crystallography; this was discussed for A330P, which forces Pro329 into the substrate access channel, constricting the active site.[190] The crystal structure has also been solved for the other single-site proline mutant, I401P, which (in the substrate-free form) displays a

much greater degree of similarity to the wild-type substrate-bound form than the substrate-free form, showing that the enzyme is in a catalytically ready conformation prior to substrate binding.[190,237]

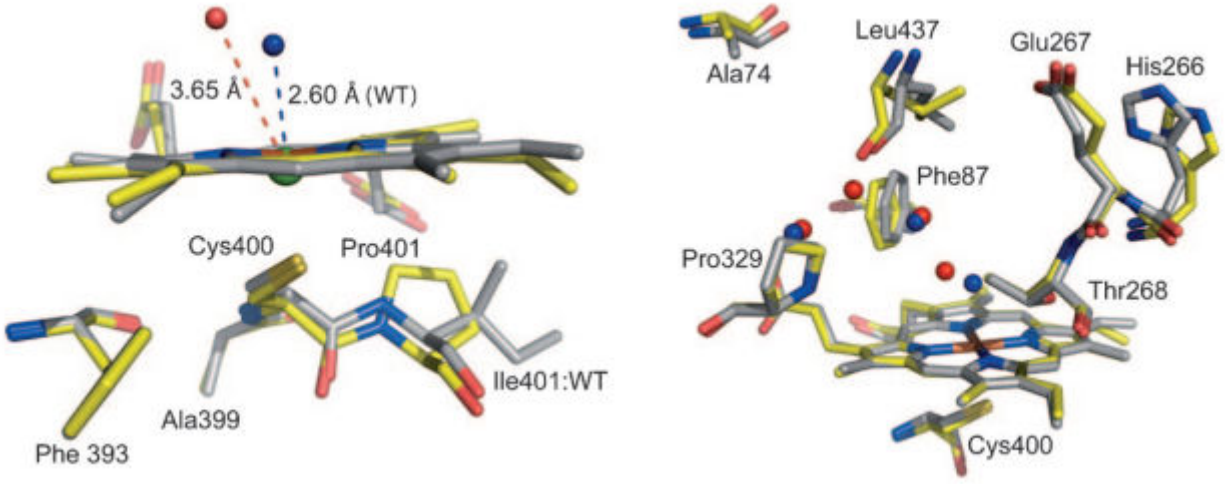


Figure 9: Close-ups of an overlay of the wild-type substrate-free form (grey, water molecules in blue, PDB code 1BU7) and I401P (yellow, water molecules in red, PDB code 3HF2)[237]

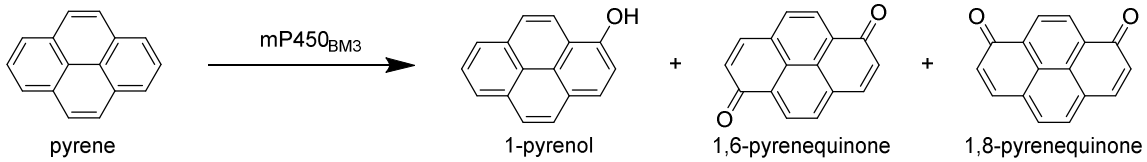
The differences from the substrate-free wild type include an increase in distance of the Ala399-Glu402 loop and the haem unit placing the iron atom out of the plane of the porphyrin ring. The axial water ligand, which is still hydrogen-bonded to Ala264, is further away from the iron atom and off axis (with an S-Fe-O angle of 161.5°) meaning it is no longer considered a ligand for the iron centre (*cf*. displacement of the axial water ligand on substrate binding, Scheme 15). Another change in conformation, more distant from the mutation, occurs in the I-helix (250-282) where the reorientation of Gly265 and His266 results in a reduction of the I-helix kink angle which is believed to assist in the binding and/or activation of dioxygen.[190]

For the variant KT2 (A191T/N239H/I259V/A276T/L353I) the crystal structure again displays more similarity to the wild-type substrate-bound structure rather than the substrate-free, and again, it is the adoption of this catalytically ready conformation that is thought to be responsible for the increased activity.[238] There are certain structural similarities between KT2 and I401P, in that the iron atom is out of the plane of the porphyrin and the kink angle in the I helix is reduced (due to the disruption of two salt bridges seen in substrate-free wild type: Lys224-Asp251 and Asp217-Arg255),

altering the region in which dioxygen binds. In KT2 the axial water ligand is still present, although it is no longer hydrogen bonded to Ala264, which has formed an interaction with an ordered active-site water molecule that is not found in the wild-type enzyme. For both KT2 and I401P the rate of the first electron transfer, in the absence of substrate, is orders of magnitude greater than that of substrate-free wild-type enzyme, and is comparable to that of palmitate-bound wild-type, suggesting that substrate binding is less important for initiation of the catalytic cycle for these mutants than they are for the wild-type enzyme.[190,238]

Previously, research in the Wong group has focused largely on developing variants of $P450_{BM3}$ which demonstrate improved activity and altered product selectivity compared to wild-type $P450_{BM3}$, and applying the libraries of mutant enzymes for the production or oxidation of specific targets. Examples discussed include the regioselective hydroxylation of propylbenzene, the oxidation of polycyclic aromatic hydrocarbons and alkylbenzenes (other than propylbenzene), production of (+)-nootkatone from (+)-valencene, and the synthesis of metabolites of common pharmaceutical compounds.[180–182,189,206,227]

The polycyclic aromatic hydrocarbons, phenanthrene, fluoranthene, and pyrene were submitted for screening against a small library of mutants (mostly based on R47L/Y51F); all three compounds formed varying amounts of mono-hydroxylated products, and quinones formed by further oxidation of those products.[180] The oxidations generally displayed only mild selectivity between the products; however, for pyrene, complete selectivity for 1-pyrenol was observed with R47L/Y51F/M354A. Use of mutants containing the mutation A264G altered the selectivity to favour quinone formation (Scheme 48).



pyrene		1-pyrenol	1,6-pyrenequinone	1,8-pyrenequinone
	R47L/Y51F/M354A	100	0	0
	R47L/Y51F/A264G	13	11	76
	R47L/Y51F/F87A/A264G	64	6	29

Wong *et al.*, 2001

Scheme 48: Different product profiles in oxidation of pyrene

A larger library (92 variants) was used to probe the oxidation of common, commercially available, drugs (Figure 10). To this end, the library was screened against lidocaine, naproxen (Scheme 34), chlorzoxazone, testosterone, diclofenac, and amitriptyline; conversion of >90% was possible with all six drugs.[206]

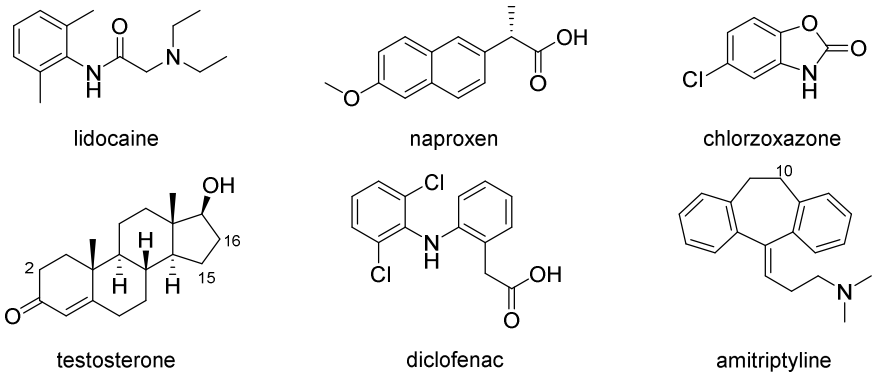
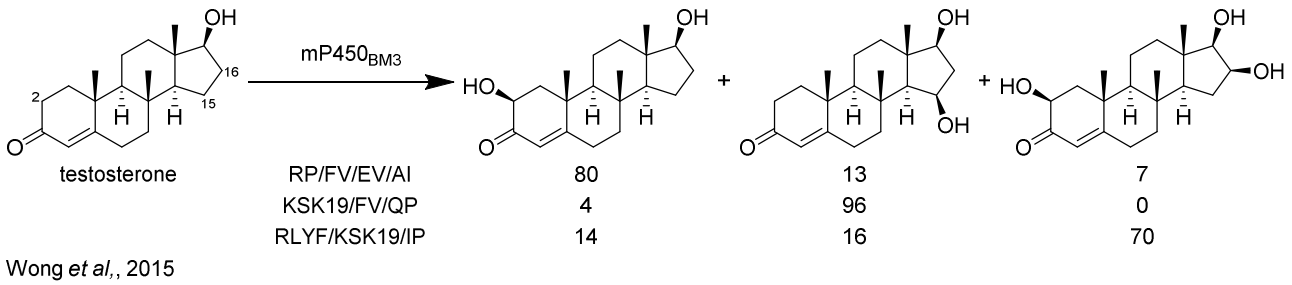


Figure 10: Common drugs screened against the library of mutant $P450_{BM3}$ variants

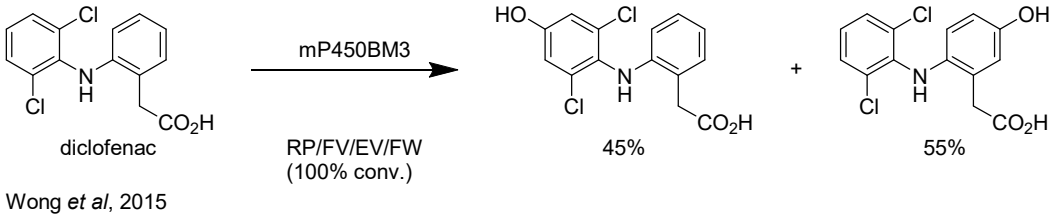
Lidocaine formed two products, with very good selectivity for either one possible. The two products were formed by *N*-dealkylation and oxidative cyclisation, respectively; see below. Testosterone was either mono-hydroxylated or di-hydroxylated to form three major products: 2β- or 15β-mono-hydroxylated, and 2β,16β-dihydroxylated. By selecting the appropriate mutant, any of these products could be chosen as the major product; the mono-hydroxylated products are found as natural metabolites formed in the human liver, whereas, the di-hydroxylated product is not a known metabolite of this drug (Scheme 49).



Scheme 49: Regio- and stereoselective hydroxylation of testosterone

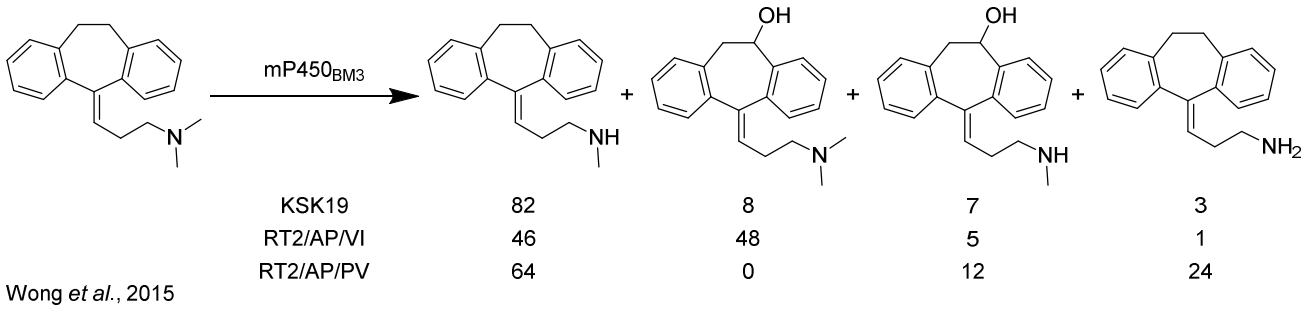
Diclofenac (NSAID) and chlorzoxazone (muscle relaxant) were both oxidised by hydroxylation of the aromatic ring *para*- to the nitrogen atom present in both cases. For diclofenac, *para*-

hydroxylation was possible on both aromatic rings, with just a slight preference being observed for hydroxylation of the non-chlorinated ring (Scheme 50).



Scheme 50: Oxidation of diclofenac by RP/FV/EV/FW

Finally, amitriptyline (treatment of mental illnesses) was oxidised to four products; mutants were mostly selective for mono-*N*-demethylation, but with certain mutants, mild selectivity for C-10$_{cis}$ hydroxylation was achieved (Scheme 51).



Scheme 51: Mutant P450$_{BM3}$-mediated oxidation of amitriptyline

Project Aims

Previous work in the Wong group focused on generating a more active library of mutants in order to impart different selectivities for a range of natural and non-natural substrates and then applying this library to the synthesis and oxidation of specific targets. In contrast, the aim of this project was not to develop new mutants and expand the library but to examine the utility of the library for application as a set of catalysts for oxidations more generally. The library of mutant P450$_{BM3}$ enzymes has demonstrated great potential for this purpose; this work aimed to build upon the previous work within the group, and the wider P450$_{BM3}$ community, to develop these enzymes into valuable catalysts capable of effecting transformations that are not readily achievable by traditional chemical synthesis. Another aim was to gain a better understanding of the library by presenting it with diverse, challenging substrates from which activity trends could be drawn to

ultimately aid the synthesis of complex molecules. To achieve these goals, the intention was initially to carry out screens on both a wide range of compounds with varied structural features and functional groups, and focused groups of structurally similar compounds; both would be followed up by elaborating the results of these initial screens. This would be assisted by collaboration with Syngenta who would supply a range of commercially available herbicides and small molecules, the oxidation products of which are of interest for analysis of toxicity and activity. A few examples of compounds to be screened in this project are shown in Figure 11 and a list of most commonly used mutants and the mutations contained within them is shown in Table 1.

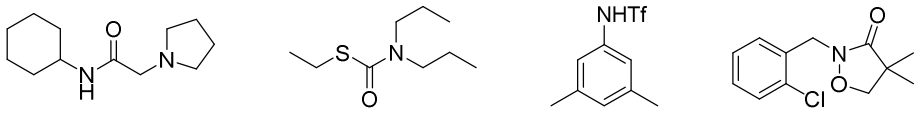


Figure 11: Examples of substrates to be screened in this project

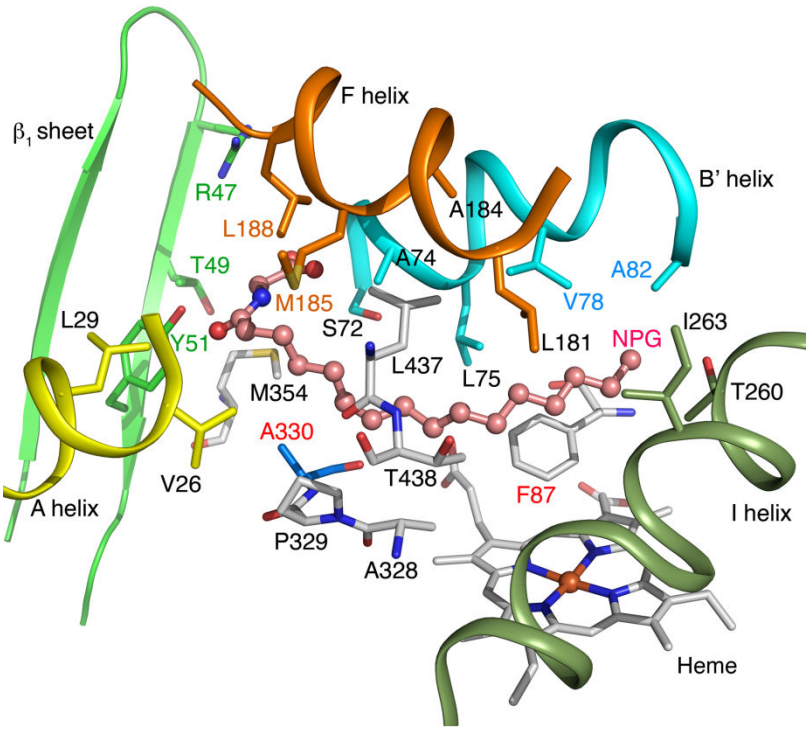


Figure 12: Close-up of the *N*-palmitoyl glycine-bound active site for wild-type $P450_{BM3}$ (PDB code 1JP7)

Mutant	Mutations
Library A	
RLYF/KSK19/AI	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
KSK19/AM	A82M/F87A/H171L/Q307H/N319Y
KSK19/AI/YL	F87A/ H171L/I263A/Q307H/N319Y/A328I
KSK19/AI/AI	F87A/H171L/A184I/Q307H/N319Y/A328I
RP/FV/EV/L437VL	R47L/Y51F/F87V/E267V/I401P/L437VL
KSK19/IA	F87A/H171L/I263A/Q307H/N319Y
RP	R47L/Y51F/I401P
RP/EV	R47L/Y51F/E267V/I401P

RT2/FW	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/L353I
RP/FV/EV	R47L/Y51F/F87V/E267V/I401P
KSK19/AI/FV	F87V/H171L/Q307H/N319Y/A328I
RP/FV/EV/L437LV	R47L/Y51F/F87V/E267V/I401P/L437LV
KSK19/AI/VI	V78I/F87A/H171L/Q307H/N319Y/A328I
RT2/AP/IM	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I/I401M
RT2/AP/AM	R47L/Y51F/A82M/A191T/N239H/I259V/A276T/A330P/L353I
RT2/IP	R47L/Y51F/A191T/N239H/I259V/A276T/L353I/I401P
RT2/AP	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/AI	R47L/Y51F/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/VI	R47L/Y51F/V78I/A191T/N239H/I259V/A276T/A330P/L353I
RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
RP/IA/EV	R47L/Y51F/I263A/E267F/I401P
WT	–
RP/AP	R47L/Y51F/A330P/I401P
RP/FV	R47L/Y51F/F87V/I401P
Library B	
A330P	A330P
RP/FW	R47L/Y51F/I401P/ F81W
RT2/AP/AI/SA	R47L/Y51F/S72A/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RP/HL/IG	R47L/Y51F/I263G/H171L/I401P
RT2/AP/PV	R47L/Y51F/A191T/N239H/I259V/A276T/P329V/A330P/L353I
GVQ	A74G/F87V/L188Q
RT2/AW	R47L/Y51F/A191T/N239H/I259V/A276T/A330W/L353I
F87A	F87A
RLYF/KSK19	R47L/Y51F/F87A/H171L/Q307H/N319Y
RP/AM/IA	R47L/Y51F/A82M/I263A/I401P
RT2/VF	R47L/Y51F/V78F/A191T/N239H/I259V/A276T/L353I
RLYF	R47L/Y51F
KSK19/AM/EF	A82M/F87A/H171L/E267F/Q307H/N319Y
RP/HL	R47L/Y51F/H171L/I401P
RP/EG	R47L/Y51F/E267G/I401P
KSK48	R179H/L233V/S270I/Q307H/N319Y
KSK19/QP/FV	F87V/H171L/Q307H/N319Y/Q403P
RT2/AP/VI/AI	R47L/Y51F/V78I/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RP/FV/EV/LQ	R47L/Y51F/F87V/L188Q/E267V/I401P
RP/FV/EV/VF	R47L/Y51F/F87V/L188Q/E267V/I401P
RT2/FW/AI	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/A328I/L353I
RP/EM	R47L/Y51F/E267M/I401P
KSK19/AI/IA	F87A/ H171L/I263A/Q307H/N319Y/A328I
RT2/SW/AW	R47L/Y51F/S72W/A191T/N239H/I259V/A276T/A330W/L353I

Table 1: List of mutants contained in most commonly used libraries

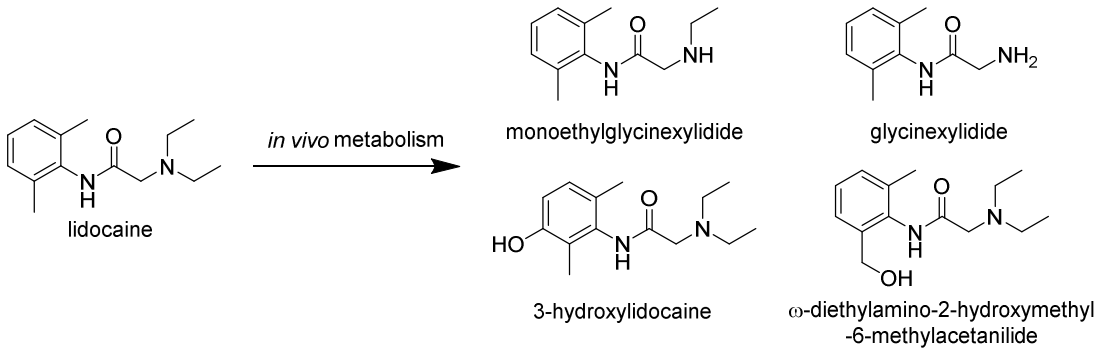
Chapter 1: Synthesis of Imidazolidin-4-ones

Aims

The library of $P450_{BM3}$ mutants has the potential to catalyse reactions or impart selectivity which may be difficult to achieve by traditional chemical methods due to the less reactive nature of the desired position of oxidation or the presence of other, more reactive functional groups. These reactions can be targeted by screening of carefully designed substrates or discovered by the (possibly unexpected) formation of certain products when carrying out a broader screen of compounds. Once discovered, the scope of the reaction can be expanded upon to include a wider range of substrates to make the reaction more generally applicable.

Background

Lidocaine is a pharmaceutical compound most commonly employed as a local anaesthetic or as an antiarrhythmic medication; it is considered one of the medicines required for a basic healthcare systems as demonstrated by its place on the World Health Organisation (WHO) Model List of Essential Medicines.[239] The metabolism of lidocaine in humans and in animals has been extensively studied, and the major metabolites have been identified as arising from mono- and di-*N*-de-ethylation to form monoethylglycinexylidide (MEGX) and glycinexylidide, respectively, 3-hydroxylation of the aromatic ring, and benzylic hydroxylation (Scheme 52).[240–242]

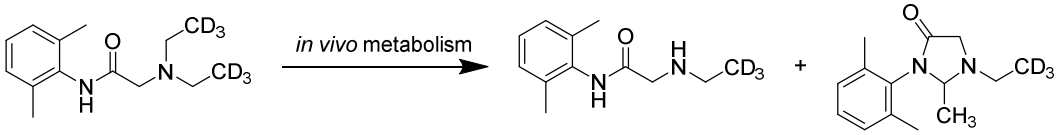


Scheme 52: Major *in vivo* metabolites of lidocaine

In separate studies concerning the metabolism of lidocaine by humans and Rhesus monkeys, a metabolite was discovered which appeared to have been formed by an oxidative cyclisation.[243,244]

1. Synthesis of Imidazolidin-4-ones

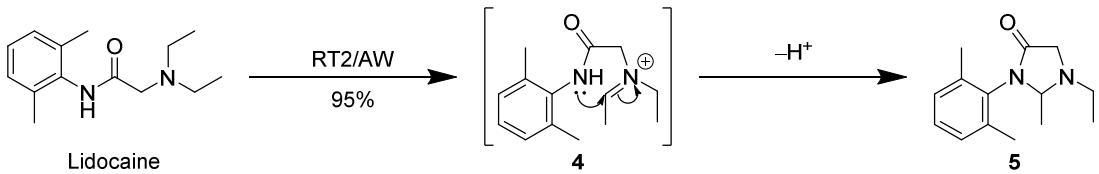
The imidazolidin-4-one product was shown, by isotopic labelling studies, to have been formed by mono-de-ethylation with subsequent condensation with acetaldehyde (produced by metabolism of ethanol), rather than being formed directly from lidocaine itself. In this study, the methyl group in both ethyl side chains was fully deuterium labelled and the deuterated drug was administered to the monkeys; MS analysis determined that the cyclised product contained only one deuterated methyl group (Scheme 53).



Trager *et al.*, 1973

Scheme 53: Deuterium-labelled lidocaine metabolic study

As mentioned in the introduction, lidocaine was one of six drugs screened by the Wong group against the library of $P450_{BM3}$ mutants.[206] From this screen two major products were detected, with 24 mutants giving greater than 50% conversion. Known human metabolite MEGX, formed by mono-*N*-de-ethylation, was typically the minor product where formed (produced by 12 out of 45 mutants, and was the minor product for 9 of these mutants). The highest selectivity for this product was achieved with RP/FV/EV/FW which gave 93% conversion with 96% selectivity for mono-*N*-de-ethylation and 4% of the doubly de-ethylated product. Other mutants were fully selective for imidazolidin-4-one **5**, accessible by oxidative cyclisation (*via* an intermediate iminium ion). The most successful mutant, RT2/AW, gave 100% conversion with full selectivity for this product, and scale-up of the oxidation with this mutant facilitated synthesis of **5** in 95% yield (Scheme 54).



Lidocaine

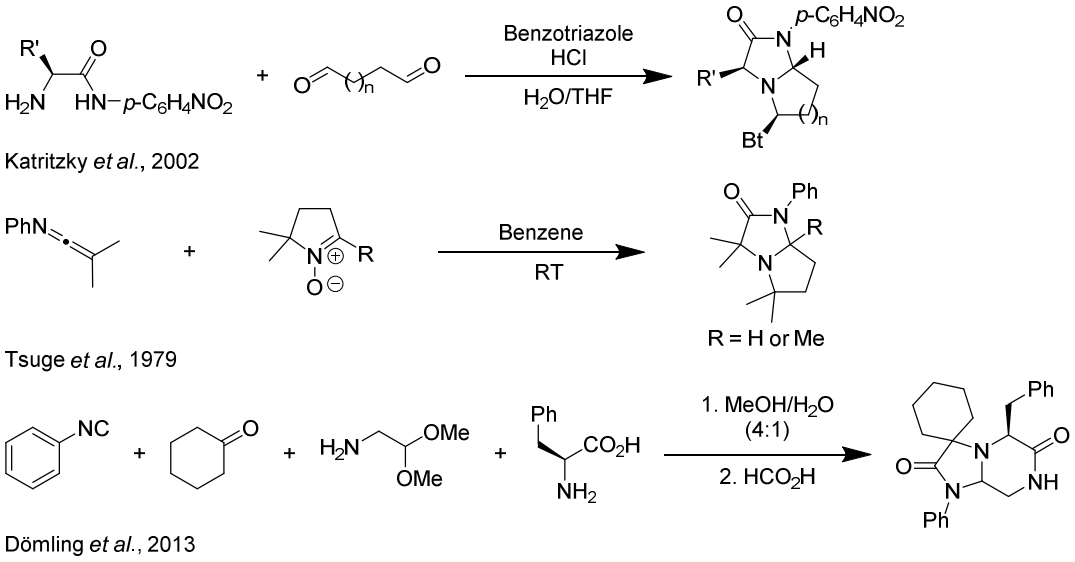
Wong *et al.*, 2015

4 **5**

Scheme 54: Synthesis of imidazolidin-4-one **5** mediated by $P450_{BM3}$ mutant RT2/AW

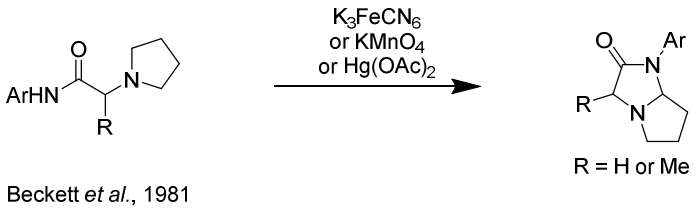
Synthesis of monocyclic imidazolidin-4-ones is easily achievable by simple condensation chemistry; on the other hand, bicyclic imidazolidin-4-ones are more difficult to access, with only a

few methods available of limited scope and utility. Katritzky prepared 5,5- and 5,6- fused bicyclic imidazolidin-4-ones by condensing α-amino amides with succindialdehyde and glutaraldehyde, respectively, trapping the iminium formed (following the second condensation) with benzotriazole (Bt) which can then be removed by reduction (Scheme 55).[245] Other intermolecular approaches include the reaction of keteneimines with nitrones, which occurs *via O*-attack of the nitrone onto the imine then rearrangement to an imidazolidin-4-one; construction of the bicyclic system *via* a two-step 4 component Ugi reaction/cyclisation was also successful (Scheme 55).[246,247]



Scheme 55: Synthesis of imidazolidin-4-ones *via* intermolecular reaction

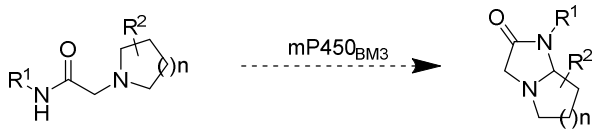
Alternatively, intramolecular oxidative cyclisation of an α-amino amide bearing a cyclic amine substituent was achieved under harsh oxidising conditions (K_3FeCN_6 in 2.0 M KOH (aq.); $KMnO_4$; or $Hg(OAc)_2$ in acetic acid) applied to a limited range of substrates (Scheme 56).[248]



Scheme 56: Oxidative cyclisation for synthesis of imidazolidin-4-ones

The formation of imidazolidin-4-one **5** by $P450_{BM3}$ variants in the oxidation of lidocaine, and the precedent of the oxidative cyclisation in Scheme 56, suggested that a library of $P450_{BM3}$ variants could provide a less toxic, more environmentally friendly, and more selective method to access

bicyclic imidazolidin-4-ones. This prospect was investigated, in collaboration with Xinkun Ren of the Wong group, by the screening of various α-cycloamino acetamides with the mutant $P450_{BM3}$ library (Scheme 57).



Scheme 57: Proposed biocatalytic synthesis of bicyclic imidazolidin-4-ones

The compounds formed by the proposed oxidative cyclisations could offer interesting biological activity and bear structural resemblance to the nootropic drug dimiracetam and mannosidase I enzyme inhibitor kifunensine (Figure 12).[249,250]

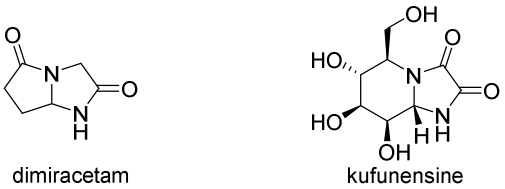
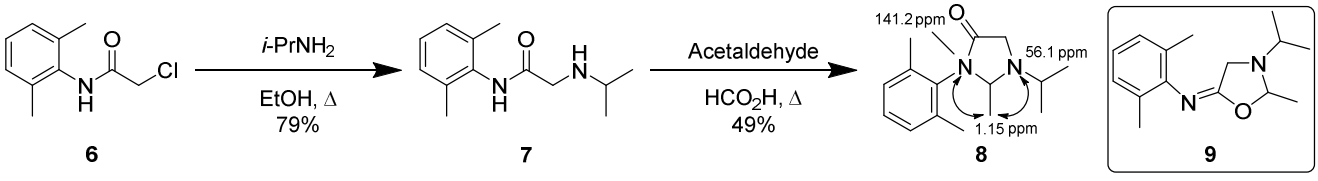


Figure 12: Structure of dimiracetam and kifunensine

Structure elucidation

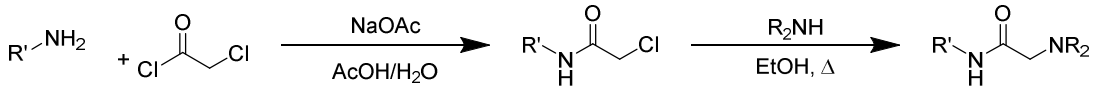
When the oxidative cyclisation of lidocaine was discovered, there was uncertainty about the structure of the product. Iminium intermediate **4** can cyclise through nitrogen to form an imidazolidin-4-one or through oxygen to form an oxazolidin-5-imine. Given that neutral amides are typically more reactive towards electrophiles through the oxygen rather than the nitrogen, it was initially thought that an oxazolidin-5-imine was the product formed in the $P450_{BM3}$-mediated oxidative cyclisation. Synthesis of a structurally similar compound by a traditional chemical method facilitated a $^{1}H–^{15}N$ HMBC experiment which confirmed the structure as imidazolidin-4-one **8** arising from cyclisation through nitrogen (Scheme 58).[251]



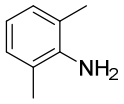
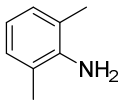
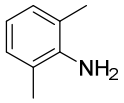
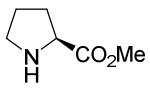
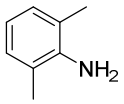
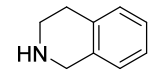
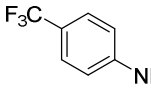
Scheme 59: Synthesis and key $^{1}H–^{15}N$ HMBC correlations of imidazolidin-4-one **8**

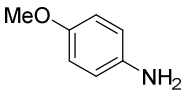
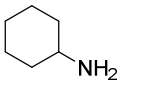
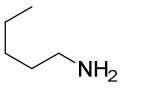
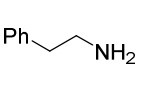
Substrate Synthesis

The majority of the substrates were prepared by simple S_N2 substitution of the relevant α-chloro acetamide by the desired cyclic amine. The α-chloro acetamides are, in turn, available by condensation of the appropriate amine with chloroacetyl chloride (Table 2).[252]



Synthesis of α-amino acetamides

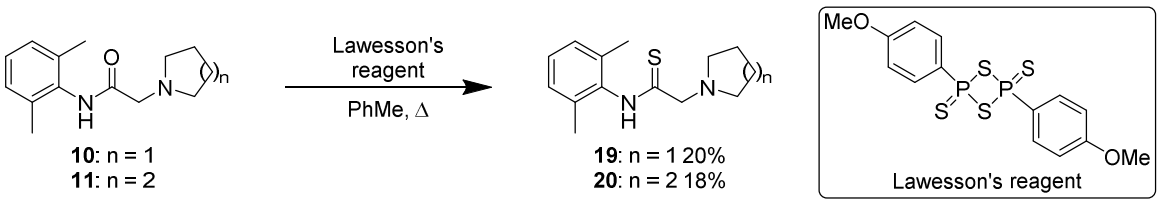
Compound	R'	R	Yield (over 2 steps)
10			42%
11			65%
12[a]			52%
13			41%
14			65%

Compound	R'	R	Yield (over 2 steps)
15			73%
16			86%
17			85%
18			73%

a. Methanol was used as the solvent

Table 2: Synthesis of α-amino acetamides

Two α-amino thioacetamides were also synthesised by the action of Lawesson's reagent on the respective α-amino acetamide (Scheme 60).[253] Thionation reactions were typically low yielding with numerous species visible in the NMR spectra of the crude products.

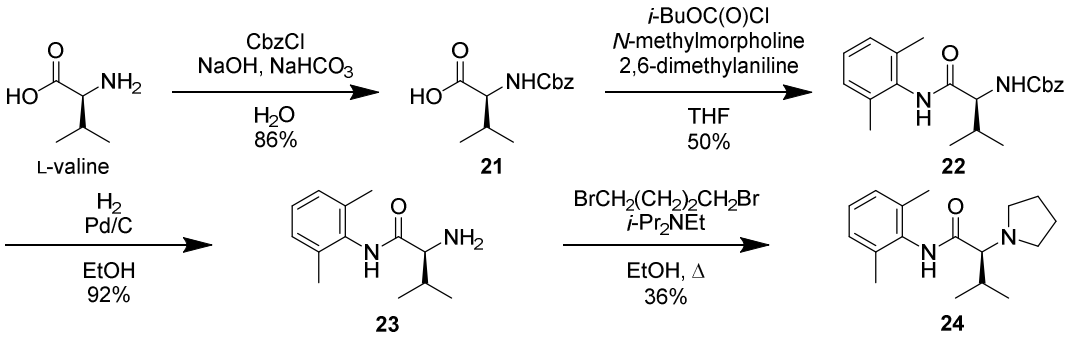


Scheme 60: Synthesis of α-amino thioacetamides

Finally, a valine derived substrate was synthesised in order to investigate the stereochemical induction of a potential cyclisation (complementary to proline-derived substrate **12** in Table 1); the substrate was prepared in four steps from L-valine (Scheme 61). The yield for the synthesis of amide

1. Synthesis of Imidazolidin-4-ones

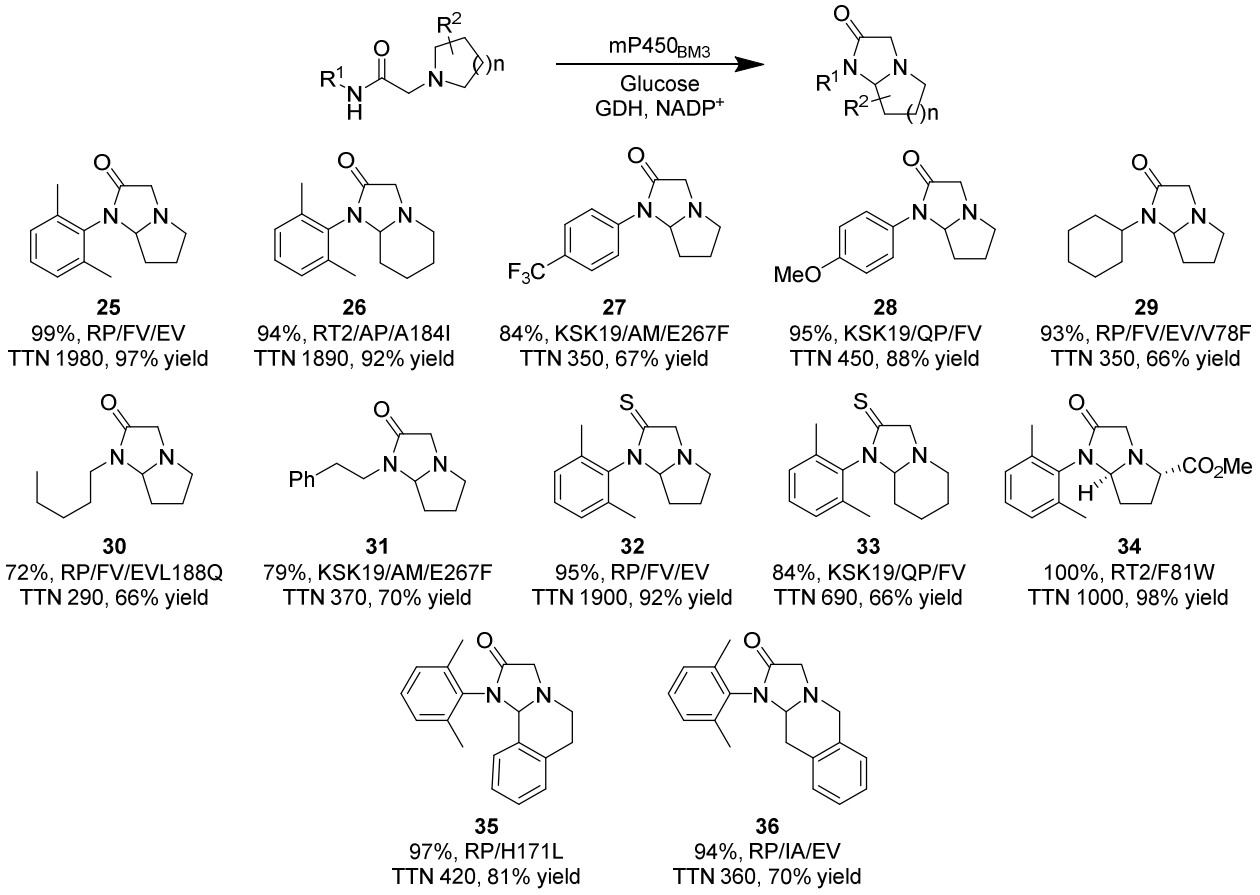
22 was lowered due to competing carbamoylation of 2,6-dimethylaniline with excess isobutyl chloroformate.



Scheme 61: Synthesis of valine-derived α-amino acetamide (the ee of these compounds was not measured)

Substrate Screening

Screening of substrates was carried out with an NADPH regeneration system using GC for analysis. Mutants that displayed promising turnover were utilised in scale-up reactions for isolation of the oxidation products, depicted below (Scheme 62). Conversions are estimated by GC and TTN refers to the total turnover number (defined in this case as the concentration of cyclisation product formed per unit enzyme concentration).



Scheme 62: Substrate scope for biocatalytic synthesis of bicyclic imidazolidin-4-(thi)ones

Initial experiments explored substrates bearing a 2,6-dimethylphenyl group on the amide as they were most similar to lidocaine, the lead compound for this investigation. Two simple substrates were screened to assess the feasibility of effecting this transformation biocatalytically; pyrrolidinyl-based substrate **10** and piperidinyl-based substrate **11**. These were screened against a library of $P450_{BM3}$ mutants and were converted successfully to the respective 5,5- and 5,6-bicyclic compounds **25** and **26** in good conversion and good yield upon scale-up. For both substrates, the transformation was selective for a small number of mutants, with <35% conversion observed with almost all mutants and <10% conversion with >75% of mutants. In the screening of pyrrolidinyl substrate **10,** only one mutant had >35% conversion, with 99% conversion (TTN 1980) possible when incubated with RP/FV/EV (Figure 13). Conversion of >35% towards piperidinyl substrate **11** occurred with a slightly wider range of mutants, with RP/FV/EV affording 77% conversion (TTN 1550) and RT2/AP/A184I affording 94% conversion (TTN 1890) (Figure 13). The location of key active-site amino acid residues that will be discussed are shown below in Figure 12.

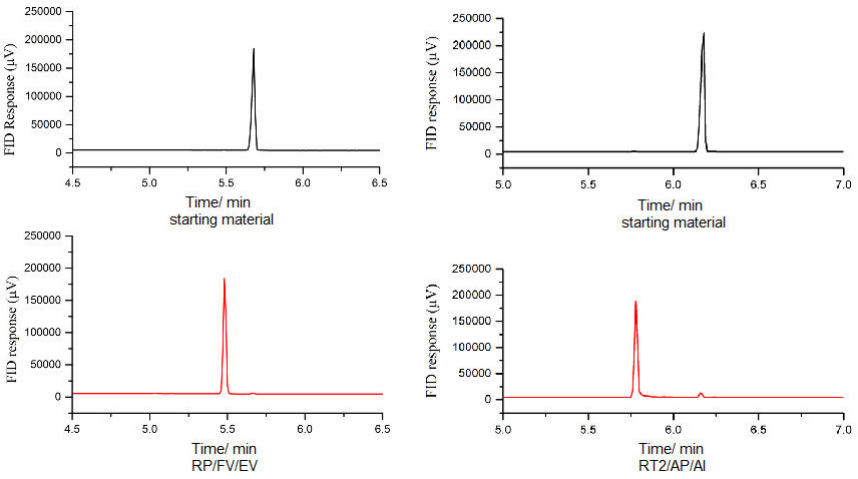


Figure 13: GC traces for pyrrolidinyl substrate **10** (left) and piperidinyl substrate **11** (right)

Following on from the proof of concept screens, the tolerance of functionality surrounding the amide and the amine was investigated. Variation of the amide, with the amine remaining as pyrrolidinyl, was possible with both electron poor and electron rich *N*-aryl rings being tolerated; *para*-trifluoromethyl-phenyl derived substrate **14** displayed good conversion with a wide range of mutants; however, selectivity was an issue. It is believed that the reduced nucleophilicity of the amide (resulting from the attached electron-deficient aromatic ring) retards cyclisation sufficiently that it is possible for other, non-cyclisation pathways to compete although products of these pathways were not isolated. As a result, care had to be taken when selecting a mutant for scale-up: whilst RP/FV/EV/V78F gave the best conversion (99%), its selectivity for cyclisation over other product formation was 44:56; mutant KSK19/A82M/E267F gave a lower conversion (84%) but was far more selective for cyclisation (86:14 cyclisation to other) and was used to synthesise 5,5-bicyclic imidazolidin-4-one **27** in 67% yield (Figure 14). *para*-Anisidine-derived substrate **15** was employed to assess the tolerance of the reaction towards electron-rich amides. This substrate also displayed good conversion across a wide range of mutants but, in this case, selectivity was not as much of an issue, with complete selectivity for imidazolidin-4-one **28** possible in some cases. A mutant which gave both appreciable conversion and selectivity (KSK19/QP/F87V: 95% conversion, 97:3 selectivity) was chosen for the scale-up reaction which afforded **28** in 88% yield (Figure 14).

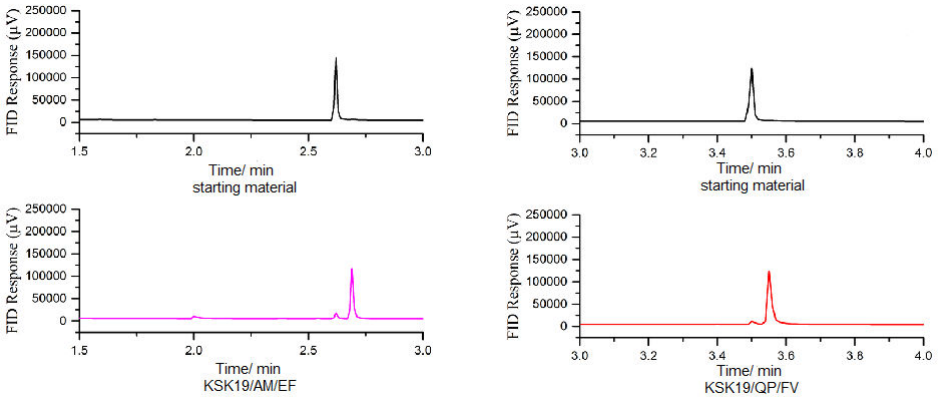


Figure 14: GC traces for 4-CF_3-Ph derivative **14** (left) and *p*-anisidine derivative **15** (right)

In addition to anilides, 1°- and 2°-alkyl-based amides, as exemplified by substrates **16**, **17**, and **18**, were synthesised from cyclohexylamine, *n*-pentylamine, and phenethylamine, respectively. The oxidation of these substrates proved that an *N*-aryl group was not required for substrate binding or appropriate orientation of the substrate in the active site in order for oxidative cyclisation to occur. Although turnover was possible with these alkyl side chains, for all three substrates a mutant was not found which gave as high a TTN as for 2,6-dimethylaniline derived substrate **10**; the same is true for the electron poor and electron rich amide substrates discussed above, suggesting that the 2,6-substitution pattern assists in binding/orientation of the substrate.

Cyclohexyl-2-pyrrolidin-1-ylacetamide **16** was turned over to form cyclisation product **29** in high conversion; its reactions were generally reasonably selective although the highest selectivity was seen for mutants in which conversion was below 16%; for example, KSK19/A82M/E267F gave 15% conversion (TTN 76) with 100% selectivity for cyclisation product **29**. Whilst selectivity with KSK19/A82M/E267F was good, conversion was poor and, as a result, a mutant which effected higher turnover was selected for scale-up; RP/FV/EV/V78F gave good conversion (93%, TTN 350) and catalysed the oxidative cyclisation with reasonable selectivity (79:21 **29**/other products) to afford **29** in 66% yield (Figure 15). For this substrate, the majority of mutants which afforded >50% conversion contained were based on the mutant RP.

A similar pattern was seen with *n*-pentyl analogue **17** where the best selectivity was found for mutants showing lower conversions. In this case, all mutants with conversion <41% were completely

selective for imidazolidin-4-one **30**; whereas, the best selectivity for the mutants with >41% conversion was found with mutant RP/H171L, which afforded 61% conversion to desired product **30** and another, unidentified product, in a ratio of 86:14. Use of RP/FV/EV/L188Q effected a 72% conversion (82:18 **30**/other products) of starting material to form desired product **30** in 55% yield (Figure 15). For this substrate, and cyclohexylamine-derived substrate **16**, variants containing the mutations R47L/Y51L generally gave better conversions, although combination with other mutations was required for good conversion. The mutant R47L/Y51L/I401P (RP) gave only 19% conversion for *n*-pentyl derivative **17** and 12% conversion for cyclohexyl derivative **16**.

The final alkylamino acetamide derivative, phenethyl-2-pyrrolidin-1-ylacetamide **18**, did not demonstrate the same preference for mutants containing the mutations R47L/Y51F. In this case, the most successful four mutants (in terms of conversion) were all based on the KSK19 mutant, with KSK19/A82M/E267F affording 79% conversion (selectivity 93:7) for the preparation of imidazolidin-4-one **31** in 70% yield (Figure 15).

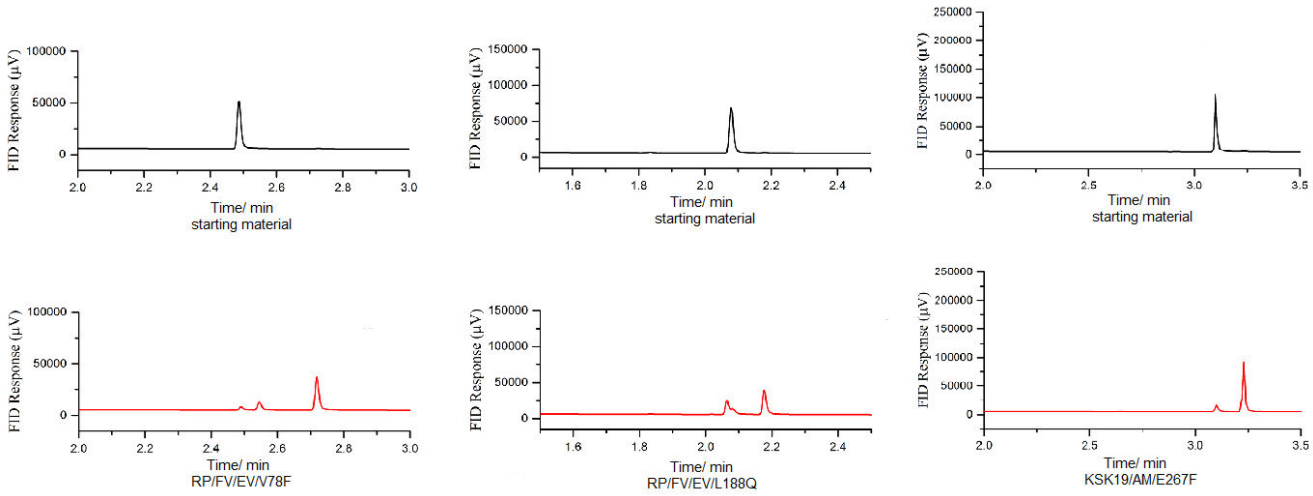


Figure 15: GC traces for cyclohexyl **16** (left), *n*-pentyl **17** (centre), and phenethyl **18** (right) derivatives

Replacement of the amide oxygen with sulfur (i.e. conversion to a thioamide) can lead to greatly modified reactivity with changes including an increase in rotational barrier about the C–N bond, and a decrease in stability towards oxidation.[254,255] Given that thioamides are readily oxidisable, they present an interesting challenge to the selectivity of the $P450_{BM3}$ mutants.[256,257] 2,6-Dimethylaniline was present as the amide *N*-substituent for both thioamide substrates and the

chosen amine groups were pyrrolidinyl and piperidinyl, for substrates **19** and **20**, respectively. The presence of the thioamide group proved largely unproblematic in both cases, with pyrrolidinyl substrate **19** forming cyclised product **32** with 95% conversion (TTN 1900) and 92% yield with RP/FV/EV, and piperidinyl substrate forming cyclised product **33** with 84% conversion (TTN 690, 82:18 selectivity) and 66% yield with KSK19/QP/F87V (Figure 16). Selectivity was generally higher for pyrrolidinyl substrate **19**, for which imidazolidine-4-thione **32** was often the sole product formed, than for piperidinyl substrate **20**, which formed multiple products.

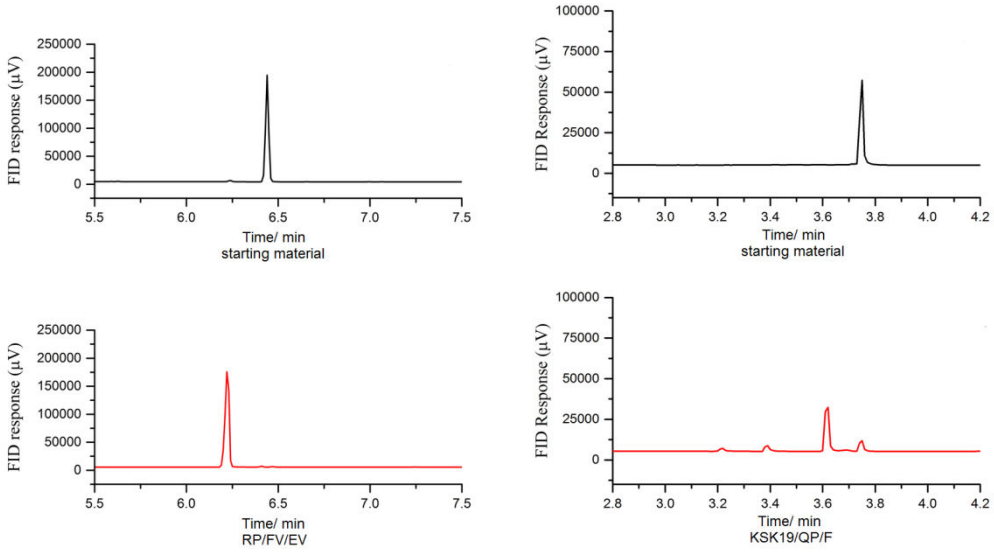


Figure 16: GC traces for thioamide substrates bearing pyrrolidinyl **19** (left) and piperidinyl **20** (right) side chains

The transformation was also compatible with further substitution on the amine side chain, which raised interesting aspects of regio- and diastereo-control. In tetrahydroisoquinoline-derived α-amino amide **13**, there are two potential activation sites flanking the ring nitrogen: the more chemically reactive benzylic (1) position or the less reactive non-benzylic (3) position. In unbiased examples, α-functionalisation of tetrahydroisoquinolines typically occurs at the benzylic position; however, examples of non-selective functionalisation are known, as are examples of 3-selective functionalisation which require thermal equilibration or bulky transition metal catalysts.[258–263]

When subjected to oxidation by the library of $P450_{BM3}$ variants, oxidation at both positions on the tetrahydroisoquinoline ring occurred, forming the regioisomeric tricyclic imidazolidin-4-ones **35** and **36**. Good conversion of the starting material was seen with reasonable selectivity; for example

RP/H171L gave 97% conversion with 9:1 selectivity for benzylic/non-benzylic with an 81% isolated yield for benzylic oxidation product **35**, whereas RP/IA/EV gave 94% conversion with 15:85 selectivity for benzylic/non-benzylic for a 70% isolated yield for non-benzylic oxidation product **36**. Improved levels of selectivity were possible with other mutants; selectivity was achieved for benzylic functionalisation with IP/QP (22% conversion, 98:2 selectivity) and for non-benzylic functionalisation with RP/F81W (25% conversion, 0:100 selectivity) (Figure 17).

Although these mutants were highly selective, conversion was poor; this was expected to be improved by incubating the substrate with *E. coli* expressing the relevant mutant, rather than using the purified enzymes (i.e. *in vivo* reaction instead of *in vitro*). With whole-cell reactions, the conversions were increased to >95% for both mutants, enabling the preparation of either product in >90% isolated yield. *In vivo* reactions have the potential to achieve higher conversions because product inhibition can be avoided as the product diffuses out of the cell, and the enzyme is not vulnerable to harmful organics (e.g. co-solvent). A downside to whole cell reactions is that if the substrate cannot cross the cell wall, it cannot reach the enzyme and turnover is not possible; this substrate is clearly able to cross the cell wall.

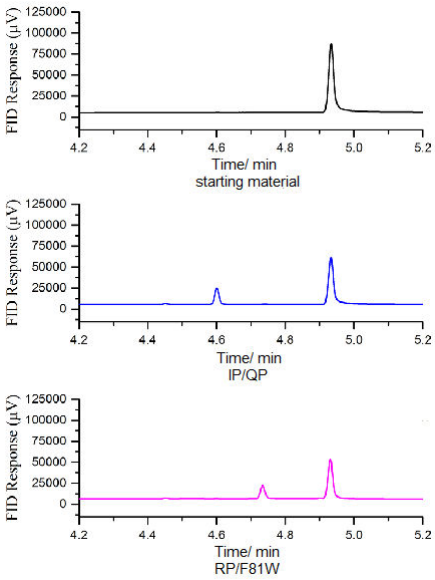


Figure 17: GC traces for formation of benzylic oxidation product **35** (centre) and non-benzylic oxidation product **36** (bottom)

To investigate the cyclisation diastereoselectivity, substrates derived from proline and valine were screened. Proline derivative **12** proved to be an excellent substrate for the enzyme, with

$P450_{BM3}$ variant RT2/F81W achieving 100% conversion (TTN 1000) to a single product. Mutants which turned over with the highest conversion typically contained the R47L/Y51F mutation, although it was, once again, required to be in combination with other mutations [R47/Y51F/I401P (RP) gave conversion of 6%] (Figure 18). Mutation at E267 seemed to have a particularly productive effect on conversion (six of the nine mutants with >50% conversion contained a mutation at E267); this effect is demonstrated strongly in the conversions for the mutants RP (6%), RP/FV (23%), RP/FV/EV (85%), and RP/EV (86%). Computational work to investigate the ability of this mutation to increase activity has suggested that the mutation is responsible for closing off the solvent channel, thus limiting the number of water molecules in the active site, and may be important for protection against uncoupling pathways.[108,264]

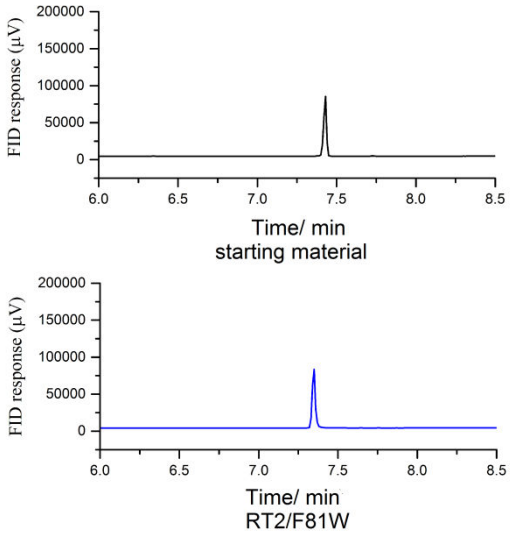
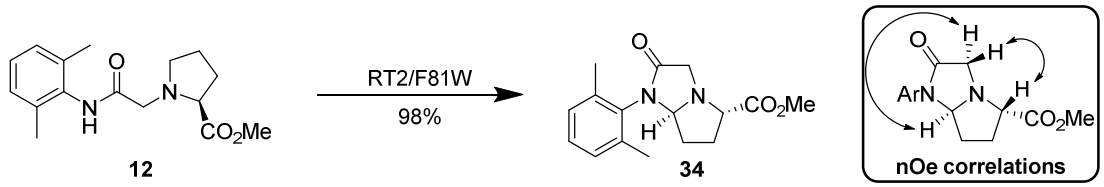


Figure 18: GC traces for oxidation of proline derived substrate **12**

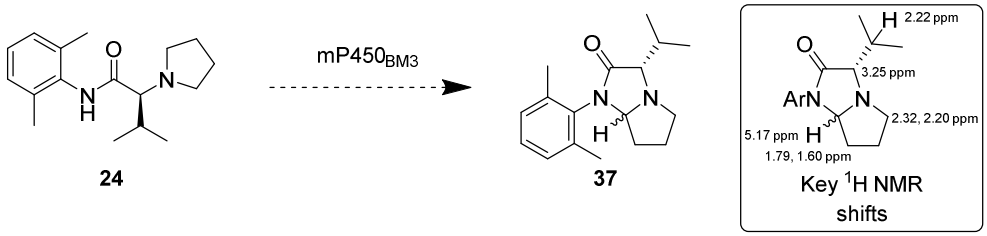
In the oxidative cyclisation of proline derived substrate **12**, only one isomer was observed and isolated in the scale-up reaction, that in which the methyl ester is *cis* to the aminal proton, on the *exo*-face of bicyclic imidazolidin-4-one **34** (no cyclisation was observed α- to the ester of the proline unit). This was confirmed by nOe spectroscopy following the isolation of the product in 98% yield from scale-up reaction with RT2/F81W (Scheme 63). It is likely that the enzyme is not responsible for this diastereoselectivity; rather the diastereomers interconvert in solution by reversible opening of the imidazoldin-4-one ring at the aminal centre and an equilibrium mixture of diastereomers would

then be obtained. It has been calculated computationally that the *exo*- isomer is 2.46 kcalmol^{-1} more stable than the *endo*- isomer, which corresponds to a ~63:1 *exo*/*endo* ratio at equilibrium.[a,265] In solution, equilibration at the aminal position is consistent with the lack of enantioselectivity found for other substrates without a pre-installed stereogenic centre (demonstrated by chiral-phase GC spectroscopy).



Scheme 63: Oxidative cyclisation of proline derived substrate **12**

In the case of the valine-derived substrate, oxidative cyclisation would form a product in which the imidazolidin-4-one ring is functionalised at every position. Screening of this compound afforded two products in up to 72% conversion with mixed selectivity for either product; at lower conversions (<10%), up to 100% selectivity for the later retention time product was achievable. In a scale-up reaction, the NMR spectrum of the crude mixture suggested that the earlier retention time product was likely to have been formed by the oxidative cyclisation; however, both this product and the other, unidentified, product decomposed during purification so characterisation and determination of diastereoselectivity was not achieved (Scheme 64).



Scheme 64: Potential structure formed by oxidative cyclisation of L-valine derived substrate **24**

This study showed that monocyclic imidazolidin-4-ones are accessible *via* P450$_{BM3}$ mutant catalysed transformation; trisubstituted and disubstituted amine side chains are viable substrates for synthesis of imidazolidin-4-ones either by the aforementioned oxidative cyclisation or by

[a] For each diastereomer of 3, conformers were obtained in Spartan 14 following a Monte Carlo search (MMFF). Each conformer was then submitted for an equilibrium geometry calculation (B3LYP/6-31G**) with a water solvation model, and a Boltzmann-weighted G^{o} was obtained from a thermodynamics calculation.

incorporation of formaldehyde or acetaldehyde formed by oxidation of the co-solvent (methanol or ethanol respectively) (Appendix A).

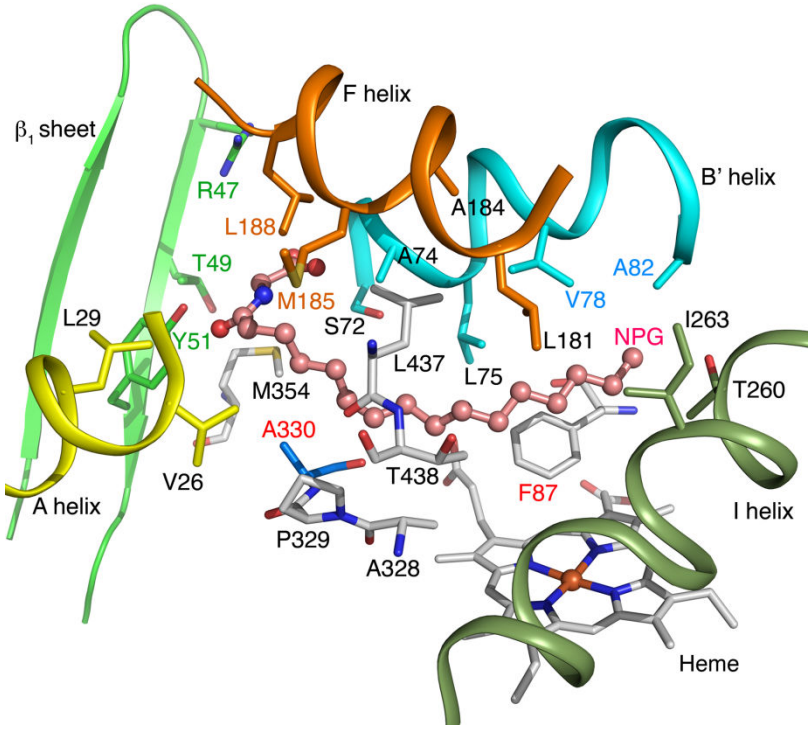
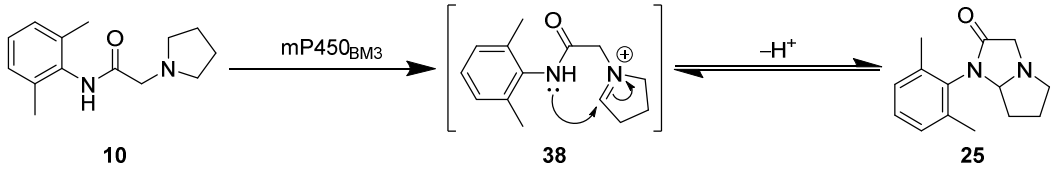


Figure 12: Close-up of the *N*-palmitoyl glycine-bound active site for wild-type $P450_{BM3}$ (PDB code 1JP7)

Mechanism

As mentioned earlier (Scheme 53) the oxidative cyclisation proceeds *via* formation of an iminium intermediate then ring closure by the amide nitrogen, believed to be reversible under the reaction/purification conditions (Scheme 65).



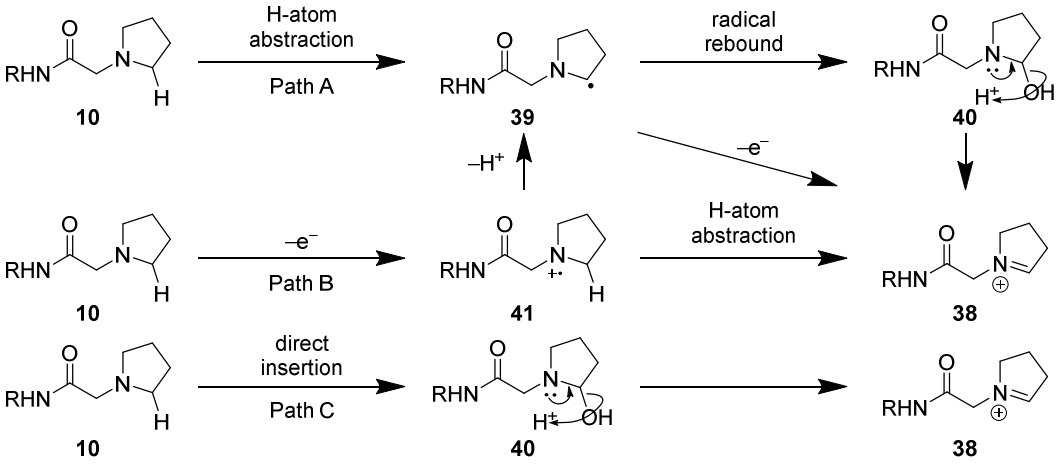
Scheme 65: Proposed mechanism for oxidative cyclisation

This mechanism is a simplification. Iminium species **38** is a necessary and obvious intermediate in the transformation; however, there are at least three potential mechanistic pathways for its formation:

1. hydrogen atom abstraction followed by radical rebound to provide a hemiaminal which can lose water to form iminium **38** (path A);

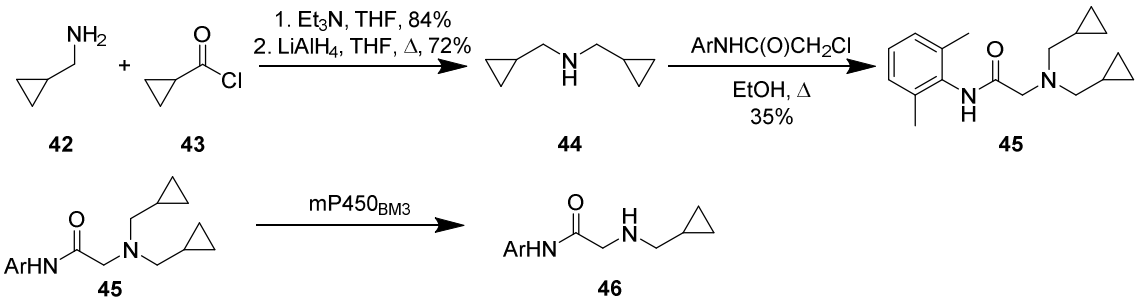
2. single electron transfer from the nitrogen of the amine can precede hydrogen atom abstraction to avoid hemiaminal intermediate **40** (path B);

3. hemiaminal intermediate **40** can be formed by direct insertion of the oxygen atom into the C–H bond (path C) (Scheme 66).



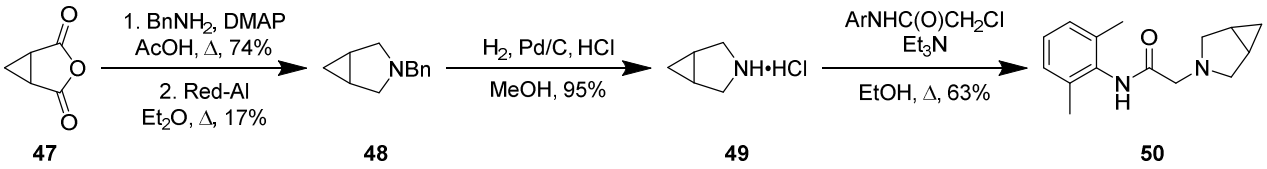
Scheme 66: Mechanistic pathways for formation of iminium **38**

To gain insight into which pathway may be followed during the oxidative cyclisation, two mechanistic probes were prepared. Both probes contain a cyclopropane ring β- to the nitrogen atom of the amine side chain which could be expected to undergo ring-opening rearrangement on formation of a radical α- to the nitrogen atom. The first, based on bis(cyclopropylmethyl)amine, was obtained in three steps from cyclopropylmethylamine and cyclopropanecarbonyl chloride (Scheme 67). Treatment of this substrate with the library of $P450_{BM3}$ variants resulted primarily in mono-*N*-dealkylation; it is unknown if this occurs by collapse of a hemiaminal or rearrangement of the radical formed by abstraction of the α-hydrogen atom then hydrolysis (Scheme 67).



Scheme 67: Synthesis and screening of bis(cyclopropylmethyl)amine derived mechanistic probe **45**

α-Amino amides derived from acyclic amines are more prone to dealkylation than those based on cyclic amines as the aldehyde formed in the acyclic amine case (such as the example above) can diffuse away from the substrate whereas, when cyclic amines are employed, the aldehyde remains tethered to the amine and is unable to diffuse away. As a result, a cyclic amine would be expected to be a more robust mechanistic probe and, to this end, probe **50** was prepared from 3-azabicyclo[3.1.0]hexane, where any rearrangement products should be easier to identify (Scheme 68).



Scheme 68: Synthesis of 3-azabicyclo[3.1.0]hexane derived mechanistic probe **50**

This compound proved to be a good substrate for the $P450_{BM3}$ mutants, with higher conversions typically seen for mutants based on the mutant RP than for KSK19-based mutants or those containing the mutation A330P. For the mutant RP/EV, 100% conversion (TTN 1000) was achieved for the formation of a single product.

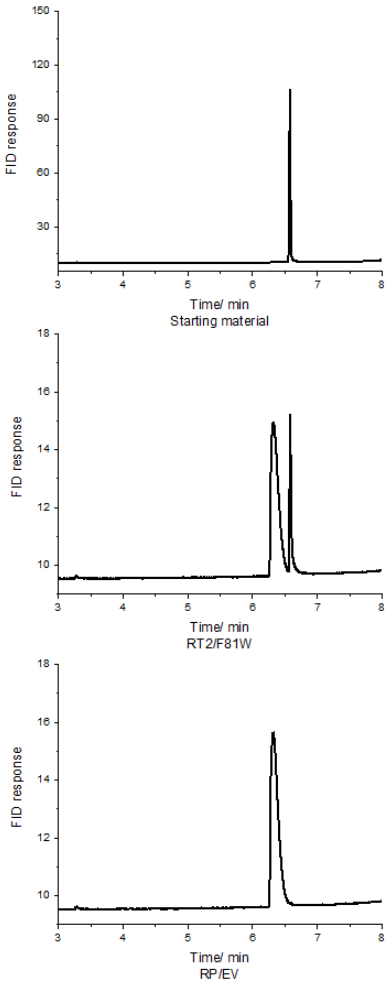
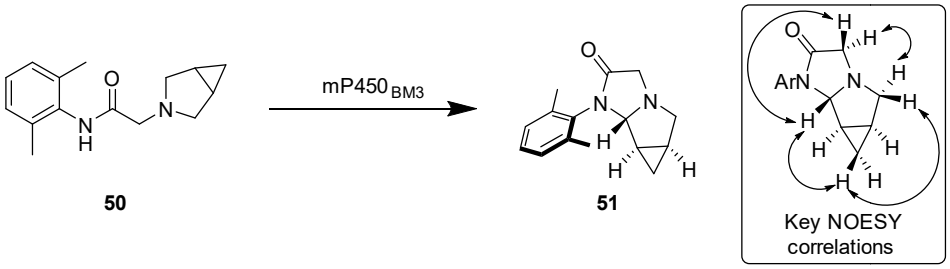


Figure 19: GC traces for oxidation of **50** by library of $P450_{BM3}$ mutants

In fact, all mutants were highly selective for this product; combination of the products of the screening reactions which gave appreciable conversion, followed by extraction with ethyl acetate and purification by column chromatography, facilitated identification of this product as imidazolidin-4-one **51**, arising from oxidative cyclisation without any rearrangement of the fused ring system (Scheme 69). As in the case of proline-derived substrate **12**, this cyclisation proved to be highly diastereoselective, with the cyclopropane ring being *cis* to the aminal proton (again, likely to be a result of equilibration in solution rather than diastereoselectivity controlled by the enzyme).

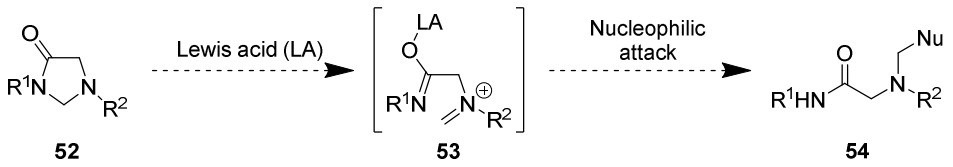


Scheme 69: Oxidation of 3-azabicyclo[3.1.0]hexane derived mechanistic probe **50**

In bicyclo[3.1.0]hexane, the rate of rearrangement of the 2-bicyclohexyl radical has been shown to be 2.9×10^7 s^{-1}; given that radicals α- to amines are stabilised (due to conjugation with the adjacent nitrogen lone pair), it can be expected that 3-aza-bicyclo[3.1.0]hexane will have a slower rate of rearrangement of the 2°-radical.[266,267] Oxygen rebound rates have been seen to reach 10^{10}–10^{11} s^{-1} for P450 systems,[b] which means that the lack of rearrangement for this does not necessarily rule out the intermediacy of a radical (isotopic labelling experiments could prove useful for this purpose).[268–270] It is also possible for the corresponding iminium ion intermediate to rearrange, as it is adjacent to a cyclopropyl ring, and the lack of rearrangement in the formation of the product suggests that any iminium species must be relatively short lived and that cyclisation occurs quickly (and reversibly as discussed previously).[271]

Imidazolidin-4-one Ring-opening Functionalisation

Synthetically, imidazolidin-4-ones have largely been applied in the temporary protection of α-amino amides to enable reactions at other reactive centres under conditions to which the amide/amine would be sensitive. Imidazolidin-4-ones in which the aminal centre is stereogenic, can react with asymmetric induction at other positions; subsequent aminal hydrolysis can then lead to enantiomerically enriched α-amino amides.[272–275] As the aminal position is labile, it was envisaged that these imidazolidin-4-ones could be considered as masked iminiums, revealed by exposure to Lewis acids and leading to α-functionalisation of the amine (Scheme 70).

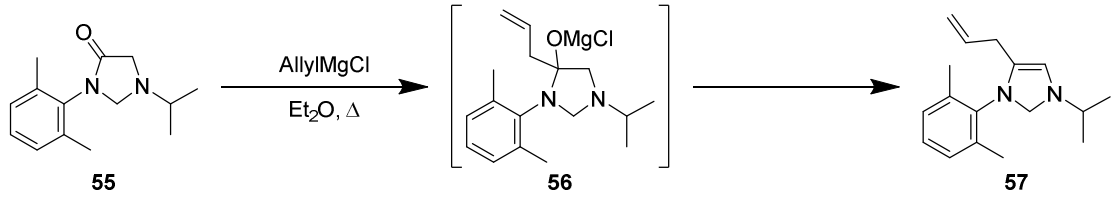


Scheme 70: Proposed functionalisation of imidazolidin-4-ones

Efforts focused initially on attempting to functionalise monocyclic imidazolidin-4-ones, as they were readily available in large quantities by classical condensation chemistry as demonstrated

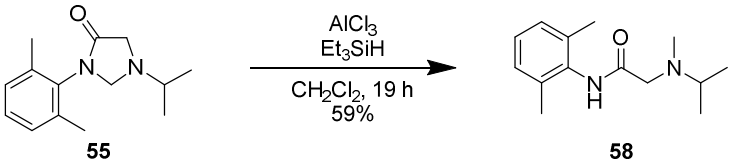
[b] Apparent rebound rates of ~10^{13} s^{-1} have been found but this corresponds to radical lifetimes of <200 fs, considered too short for a true intermediate so the reactions are believed to proceed *via* a different mechanism.

earlier (Scheme 58). The first efforts employed allylmagnesium chloride with the hope that the Grignard reagent could act both as a Lewis acid and a nucleophile due to the Schlenk equilibrium ($2RMgX \rightleftharpoons R_2Mg + MgX_2$).[276] In practice, this did not occur; allylmagnesium chloride preferentially added into the carbonyl group then elimination of water from that intermediate led to formation of dihydroimidazole **57** (Scheme 71). This reaction was poorly reproducible and the instability of the product to purification meant that its isolation as a pure compound was not possible.



Scheme 71: Reaction of imidazolidin-4-one **55** with allylmagnesium chloride

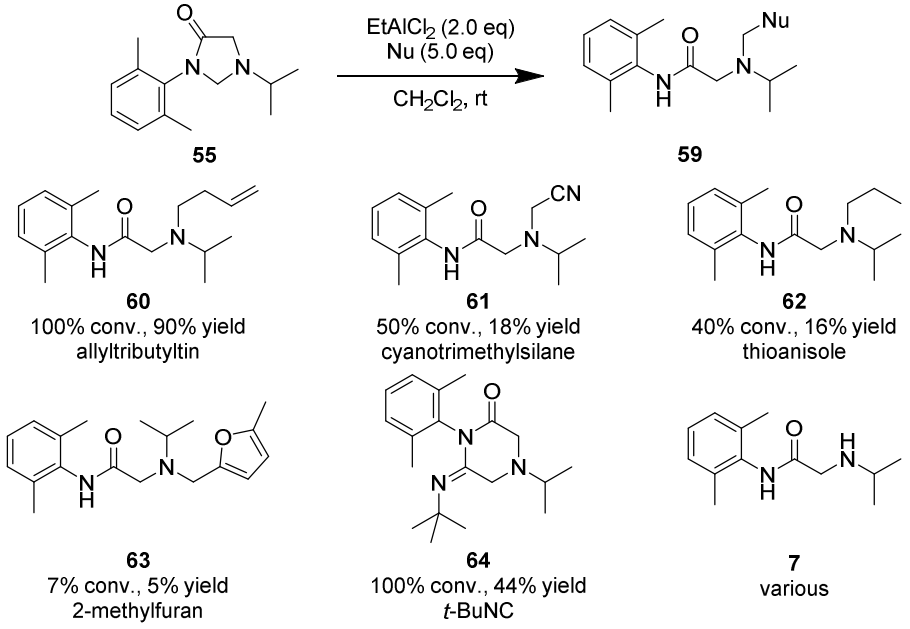
Efforts shifted to the use of a separate Lewis acid and nucleophiles. The use of $TiCl_4$ as the Lewis acid led to low conversion with a variety of nucleophiles, potentially related to an observed precipitation of an insoluble species following addition of $TiCl_4$ to the reaction mixture. Success was first achieved with $AlCl_3$ as the Lewis acid and triethylsilane as the nucleophile, in a ring-opening reduction of the aminal (Scheme 72).[277]



Scheme 72: Reaction of imidazolidin-4-one **55** with $AlCl_3$ and Et_3SiH

Aiming to build on this result, and generate more complex structures, the reaction was attempted with allyltrimethylsilane in place of triethylsilane which successfully effected ring-opening allylation of imidazolidin-4-one **55**; however, the reaction was much slower, reaching 40% conversion after two days as opposed to 100% conversion after 19 hours in the example above. Considering that the poor solubility of $AlCl_3$ in dichloromethane may have been problematic, ethylaluminium dichloride was employed instead, resulting in a slight improvement to 50% conversion after two days. Replacement of allyltrimethylsilane with the more reactive allyltributyltin improved the

reaction to achieve full conversion after two days and isolation of the product in 90% yield.[278] Expanding the scope proved troublesome as many nucleophiles were either insufficiently reactive or led to further reactions of the product; however, some nucleophiles proved successful in the ring opening/nucleophilic attack (Scheme 73).

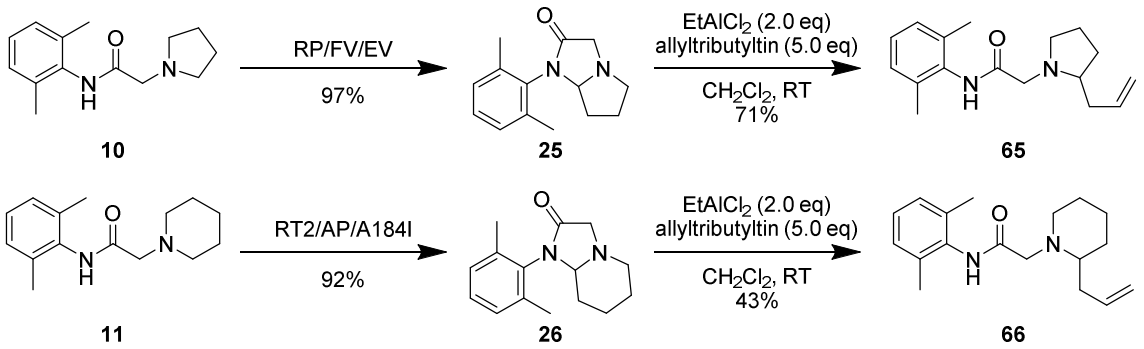


Scheme 73: Scope of $EtAlCl_2$-mediated ring opening nucleophilic functionalisation

As mentioned, allyltributyltin was very successful with high conversion and good yield, although five equivalents of the reagent were required (attempts to reduce this resulted in much poorer performance of the reaction). 2-Methylfuran and cyanotrimethylsilane were poorer nucleophiles; however, both successfully functionalised the substrate with cyanotrimethylsilane performing significantly better than 2-methyl furan (50% conversion and 18% isolated yield compared to 7% conversion and 5% isolated yield). When thioanisole was chosen as the nucleophile, rather than attacking the iminium intermediate, activation of ethylaluminium dichloride occurred, resulting in transfer of the ethyl group. It is presumed that activation of ethylaluminium dichloride by thioanisole occurs *via* formation of an aluminate complex, the formation of such complexes between thioethers and $AlCl_3$ being known.[279] Some nucleophiles were (presumably) able to successfully attack the iminium; however, the Lewis acid could still mediate further reaction, which resulted in the dealkylation of the product and formation of **7**. This occurred with a ketene silyl acetal, an

enamine, (furan-2-yloxy)trimethylsilane, pyrrole, and *N*-methylpyrrole. No reactivity was seen for anisole, 2-methylthiophene, and *N*-Boc-pyrrole.

The potential application of the $P450_{BM3}$ mutant catalysed oxidative cyclisation for α-functionalisation of amines was further demonstrated for two of the bicyclic imidazolidin-4-one products. Pyrrolidine-derived imidazolidin-4-one **25** and piperidine-derived imidazolidin-4-one **26** were treated with ethylaluminium dichloride and allyltributyltin; the reaction occurred more rapidly than for monocyclic substrate **55** (full conversion after 22 hours rather than two days) with α-allyl amines **65** and **66** being accessible in reasonable yield (Scheme 74). Proline-derived imidazolidin-4-one **34** was not as successful a substrate for this reaction, with addition of the allyl group preferentially occurring at the ester. These products could potentially be subjected to $P450_{BM3}$-mediated oxidation for further α-functionalisation or oxidation at other positions.



Scheme 74: Allylation of pyrrolidine and piperidine-derived imidazolidin-4-ones

Whilst the ring opening functionalisation has scope for improvement, the biocatalytic oxidative cyclisation of α-amino amides has proven to be applicable to differentially substituted aryl and alkyl amides, with variation of the amine moiety tolerated as well. In cases where the amine ring is substituted, high levels of regioselectivity are possible. Diastereoselectivity is also possible *via* thermodynamic control. The cyclisation mechanism is believed to require an iminium intermediate; however, the details of the generation of this intermediate are unclear. Some probing of the mechanism has been carried out, but these have not shed light on the pathway followed to the iminium species; isotopic labelling studies may provide useful insight.

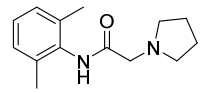
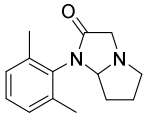
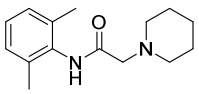
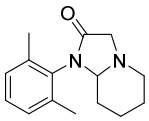
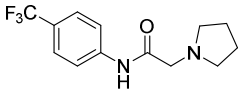
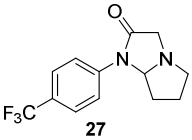
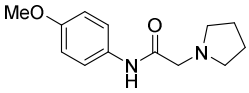
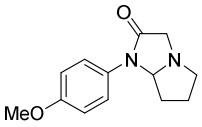
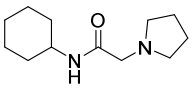
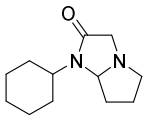
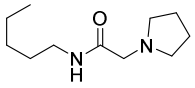
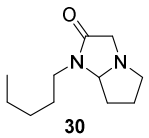
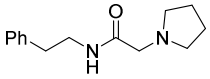
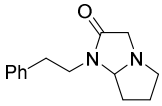
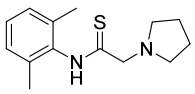
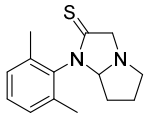
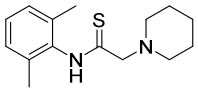
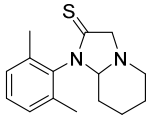
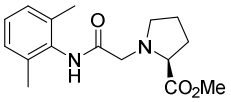
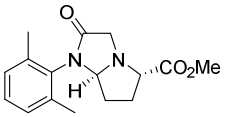
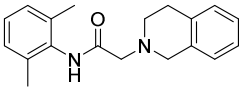
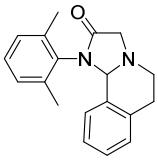
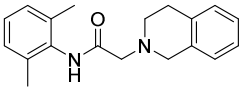
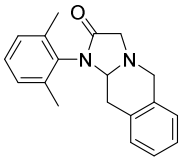
Substrate	Mutant (yield)	Product	Substrate	Mutant (yield)	Product
 10	RP/FV/EV (97%)	 25	 11	RT2/AP/AI (92%)	 26
 14	KSK19/AM/EF (67%)	 27	 15	KSK19/QP/FV (88%)	 28
 16	RP/FV/EV/EF (66%)	 29	 17	RP/FV/EV/LQ (66%)	 30
 18	KSK19/AM/EF (70%)	 31	 19	RP/FV/EV (92%)	 32
 20	KSK19/QP/FV (66%)	 33	 12	RT2/F81W (98%)	 34
 13	RP/H171L (81%)	 35	 33	RP/IA/EV (70%)	 36

Table 3: Compounds screened in this chapter and the products identified

Chapter 2: Oxidation of Anilides

Background/ Aims

4-Aminophenols are a class of compounds with demonstrated widespread importance in organic chemistry, both for medicinal and material uses. They are found in natural products with antitumour activity, act as precursors to a range of pharmaceutical compounds, and have been applied as synthetic precursors to heterocycles and as useful intermediates for natural product total synthesis.[280–289] In materials chemistry, they have application in liquid crystals and dyes.[290–293] Examples of such compounds which have found applications include sulfonamide **67**, an inhibitor of a D-glutamic acid ligase, and urea derivative **68**, a fluorescent probe for intracellular detection of tyrosinase activity (Figure 20).[285,293]

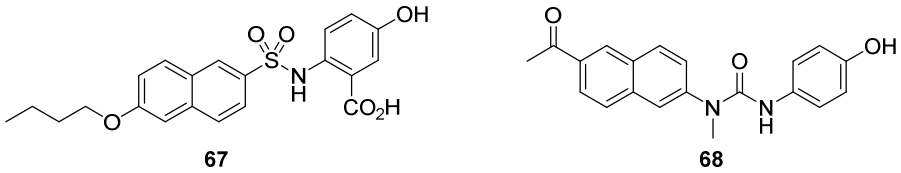
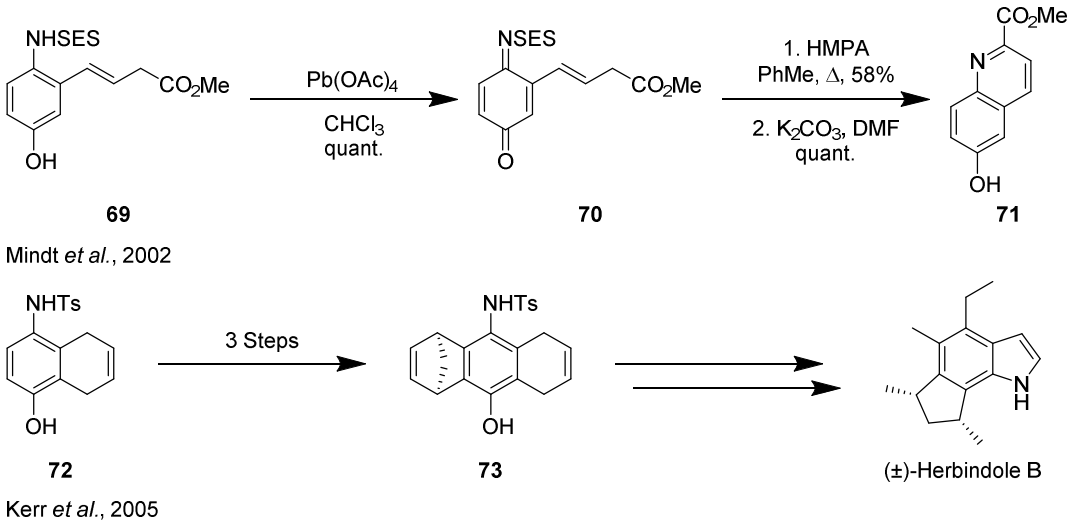


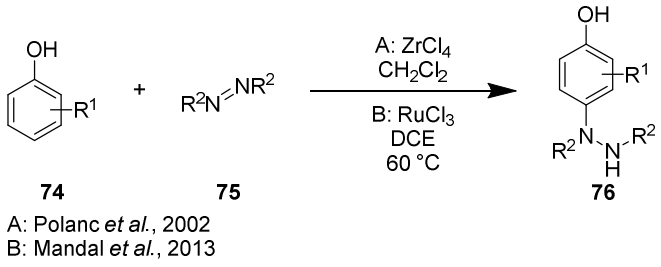
Figure 20: Examples of pharmaceutical compounds containing a 4-aminophenol scaffold

The use of 4-aminophenols as precursors to heterocycles was demonstrated by Mindt for the preparation of 6-hydroxyquinolines *via* a 6π-electrocyclisation of a quinone imine (generated from a 4-aminophenol) (Scheme 75; SES = 2-trimethylsilylethanesulfonyl).[289] The intermediacy of a quinone imine was also used in the total synthesis of (±)-herbindole B, a cyclopenta[*g*]indole isolated from a Western Australian sponge (Scheme 75).[287]



Scheme 75: Application of 4-aminophenols in heterocycle and natural product synthesis

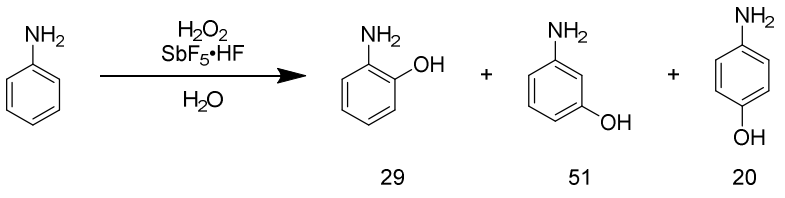
Traditionally, widely applicable methods for the synthesis of 4-aminophenols involve reduction of nitro groups and/or deprotection of *O*-protected aminophenols.[294–300] Although many syntheses have been made possible only by the use of protecting groups, the necessity of the addition of protection and deprotection adds steps to any synthesis, leading to a decrease in the total yield and a negative impact on atom economy.[301,302] Thus, methods which limit the use of protecting groups are favourable. In the synthesis of 4-aminophenols, limiting the use of protecting groups can be achieved by introduction of the alcohol or amine functionality at a later stage in the synthesis, as exemplified in the Lewis acid-mediated *para*-amination of phenols with azodicarboxylates (Scheme 76).[303,304]



Scheme 76: *para*-Amination of phenols with azodicarboxylates

Alternatively, the strategy can be reversed so as to effect the hydroxylation of anilines; this has been achieved with H_2O_2 in a superacidic medium for the hydroxylation of aniline and *N*-alkylanilines. This reaction occurs *via* electrophilic aromatic substitution, preferentially hydroxylating at the *meta*-position, but with little regioselectivity (Scheme 77).[305]

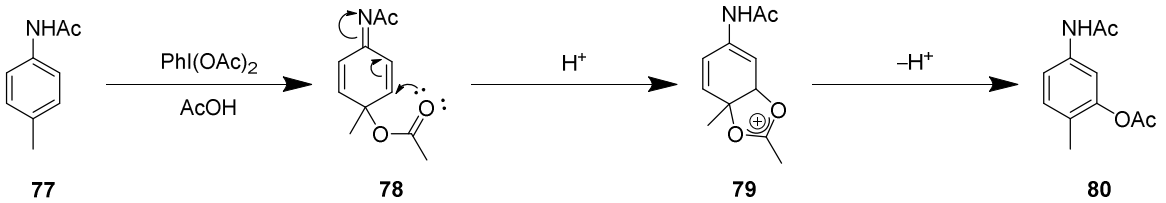
2. Oxidation of Anilides



Vidal *et al.*, 1984

Scheme 77: Hydroxylation of aniline in superacidic media

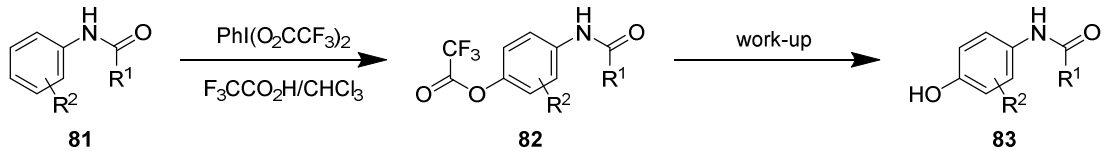
A different method has been developed in which, rather than installing the oxygen functionality by electrophilic aromatic substitution, the aniline is activated towards attack by an oxygen-based nucleophile by the use of hypervalent iodine.[306–310] This was first demonstrated in the acetoxylation of *para*-substituted acetanilides, selectively affording *meta*-acetoxylated products *via* *para*-acetoxylation and subsequent rearrangement (Scheme 78).[306] Hydrolysis of the ester affords the 3-aminophenol.



Barlin and Riggs., 1954

Scheme 78: Acetoxylation of 4-subsituted acetanilides

Almost 50 years after the publication of this paper, a further example of hypervalent iodine-mediated oxidation of anilides was reported, this time for *para*-hydroxylation.[307] The reaction proceeded by trifluoroacetoxylation, with ester hydrolysis taking place during work-up (Scheme 79).

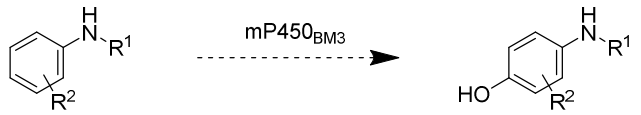


Kikugawa *et al*,, 2002

Scheme 79: I(III)-mediated *para*-hydroxylation of anilides

Building upon these discoveries, various groups have developed the reaction for the selective *para*-carboxylation, etherification, and sulfonyloxylation of anilides. Selectivity for *meta*-functionalised products is possible for 4-substituted anilides; however, as in the example above, the initial attack occurs at the 4-position then rearrangement to the 3-position takes place.[308–310]

Hydroxylation of aromatic rings is a known mode of action for cytochrome P450s, as observed in the metabolism of aniline and anilides, benzene and alkyl benzenes, phenols, and halogenated arenes, amongst others.[119,311] It has been demonstrated that wild type $P450_{BM3}$ can catalyse the *para*-hydroxylation of aniline, although the reaction is fairly inefficient with turnover largely uncoupled from NADPH consumption (72% uncoupled).[312] In previous work within the Wong group, aniline has been screened against the library of $P450_{BM3}$ mutants, resulting in low conversion with a small number of mutants, possibly due to substrate inhibition of the enzyme.[313] It was thought anilides may be more successful substrates since the nitrogen is less nucleophilic. Given the ability of cytochrome P450s to metabolise aniline and potentially anilides in this fashion, it was expected that the library of $P450_{BM3}$ variants would be able to catalyse this reaction for a range of diverse anilides, including more densely functionalised herbicidal compounds.



84 **85**

Proposed hydroxylation of anilines mediated by $P450_{BM3}$ mutants

Oxidation of Trifluoromethanesulfonanilide Containing Herbicides

It is important to identify the metabolic products of herbicides to confirm that they are not hazardous to the environment or to humans and to investigate whether they are viable herbicides in their own right. Synthesis of the metabolites in quantities necessary for analysis is not always possible from the parent herbicide by chemical synthetic methods; on the other hand, biocatalytic oxidation employing the library of mutant $P450_{BM3}$ variants is expected to be applicable to the formation of natural and non-natural metabolites in analogy to the direct synthesis of drug metabolites from the parent drug.[205–209,211–214,312,314] Four herbicidal compounds, supplied by Syngenta, were selected to be screened for synthesis of their metabolites. These compounds, originally patented by Nissan Chemical Industries, had demonstrated strong herbicidal activity in

paddy fields, crop plants, and orchards.[315] The compounds share the common functional motif of a lactam connected *via* an *ortho*-methylene group to a trifluoromethanesulfonanilide (Figure 21).

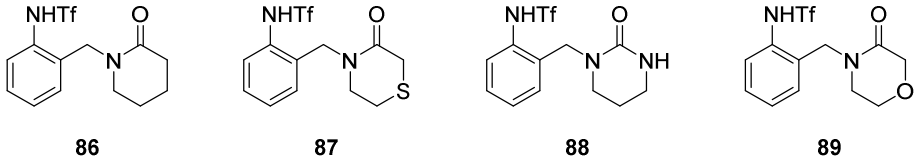
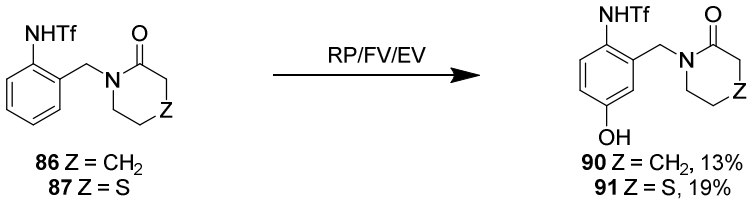


Figure 21: Herbicidal compounds selected for screening against the $P450_{BM3}$ variant library

Initial screening of these substrates was carried out with 2 mM substrate and 1 µM enzyme and no turnover of substrate was found for any mutant. An attempt to improve upon this, by altering the concentrations to 1 mM substrate vs 2 µM enzyme, proved successful for three of the substrates, with morpholinone-containing substrate **89** still showing no conversion with any of the 21 mutants screened. Substrates **86** and **87,** which contain a piperidinone ring and a thiomorpholinone ring, respectively, gave reasonable to good conversion (37% and 83%) with $P450_{BM3}$ variant RP/FV/EV. Conversion was not observed with any of the other mutants used in the screening. Both substrates were amenable to scale up to facilitate identification of the products as those arising from *para*-hydroxylation of the trifluoromethanesulfonanilide ring (Scheme 80). For substrate **87**, the oxidation was 75% selective for *para*-hydroxylation over *S*-oxidation of the thiomorpholinone ring. *S*-oxidation is not necessarily enzyme-mediated; it is possible that H_2O_2 formed by the peroxide uncoupling pathway was responsible.



Scheme 80: *para*-hydroxylation of **86** and **87**

Urea derivative **88** was turned over with good conversion on analytical scale (RP/FV/EV, 85%, TTN 275); however, this result was difficult to repeat with the same levels of conversion when carried out on larger scale. Conversions for the scale-up reactions were typically <20% and, whilst the NMR spectrum of the crude product [400 MHz, $CDCl_3$: δ_H 7.43 (1H, d, *J* = 9.0 Hz), 6.80 (1H, dd, *J* = 9.0,

3.0 Hz), 6.76 (1H, d, *J* = 3.0 Hz)] suggested that *para*-hydroxylation of the anilide had occurred, the product was never isolated. For this substrate, the pH of the scale-up reactions led to rapid acidification of the mixture and denaturing of the enzyme. This rapid lowering of pH is likely due to the turnover being largely decoupled from NADPH consumption causing formation of a large amount of gluconic acid by GDH-catalysed oxidation of glucose and lactone hydrolysis. The fourth herbicide, **89**, was a very poor substrate, with conversion only observed for one mutant out of 50 screened; conversion in this case was <20% and a mixture of two products resulted, both seemingly from mono-oxygenation (demonstrated by the presence of a compound with mass +16 in MS of screening products).

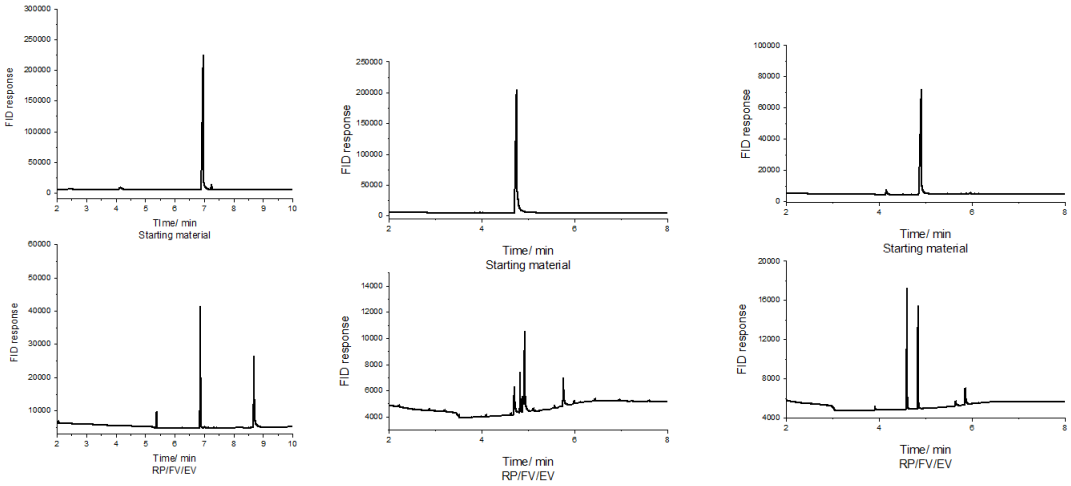
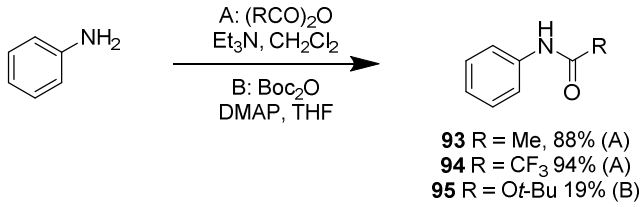


Figure 22: GC traces for oxidation of lactam **86** (left), thiomorpholinone **87** (centre), and urea **88** (right) by the library of $P450_{BM3}$ mutants

Substrate Synthesis

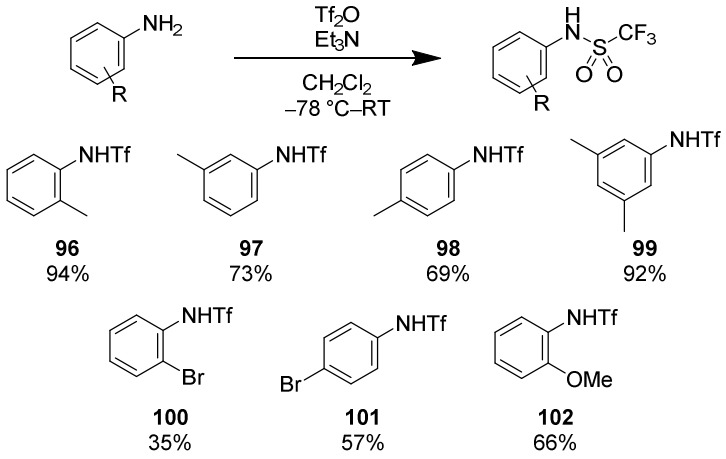
para-Hydroxylation of anilides as the major process in three of the four screened herbicides suggested that the library of $P450_{BM3}$ variants could effect this transformation more generally. Whilst aniline itself had proven to be a poor substrate, the sulfonanilides shown above were readily hydroxylated with good conversions. Whilst trifluoromethanesulfonyl was a successful *N*-substituent, it was not necessarily the best option; therefore, four differently *N*-substituted aniline derivatives were tested for activity and selectivity against the library of $P450_{BM3}$ variants (see below for discussion of the screening). Trifluoromethanesulfonanilide (PhNHTf) **92** is commercially available; acetanilide **93**, trifluoroacetanilide **94**, and *tert*-butyloxycarboxanilide **95** were synthesised by

reaction of aniline with acetic anhydride, trifluoroacetic anhydride, and di-*tert*-butyl dicarbonate (Boc_2O), respectively (Scheme 81).



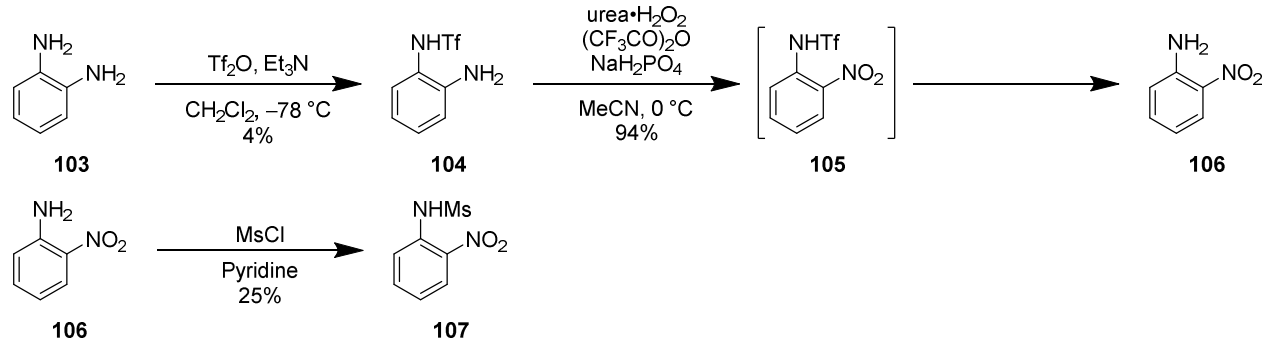
Scheme 81: Synthesis of anilides bearing different *N*-substituents

Based on the results of the initial screen (see below), it was decided that trifluoromethanesulfonanilide substrates would be used to explore the scope of the transformation. Substrates bearing this functionality are readily synthesised in generally acceptable yields by the reaction of diversely substituted anilines with trifluoromethanesulfonic anhydride (Scheme 82).[316]



Scheme 82: Synthesis of trifluoromethanesulfonanilide substrates

It was not possible to synthesise all of the intended substrates by this method; for example, derivatisation of 2-nitroaniline performed extremely poorly, with isolated yields <1%. This was likely due to the instability of the product towards hydrolysis of the triflamide group, demonstrated by the oxidation of **104**, the mono-triflyl derivative of *o*-phenylenediamine, which afforded only 2-nitroaniline as the product (Scheme 83).[317] Because of this instability, the less electrophilic methanesulfonyl derivative was synthesised for screening instead (Scheme 83).



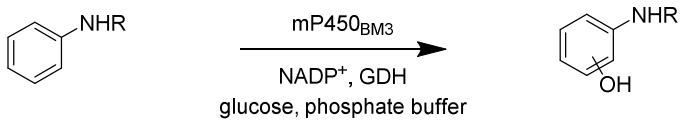
Scheme 83: Synthesis of derivatives of 2-nitroaniline

Substrate Screening

Following synthesis of the substrates, screening was carried out against a library of 24 or 48 $P450_{BM3}$ variants. Initial investigations were focused on determining whether *N*-acetyl-, *N*-trifluoroacetyl-, *N*-triflyl-, or *N*-Boc-anilines were more readily converted than aniline itself and whether one proved to be superior in terms of activity and selectivity. The anilide which offered the greatest conversion and selectivity in the screening would be chosen as the basis for the rest of the substrates.

The compounds were screened using an NADPH regeneration system and analysed by GC. The screening results for the mutants which afforded the highest conversion for the four aniline derivatives are displayed in Table 4. Conversion was estimated by GC with residual starting material as an internal standard and TTN (total turnover number) refers to the concentration of substrate turned over per unit concentration of enzyme.

2. Oxidation of Anilides



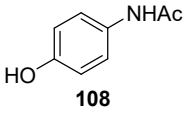
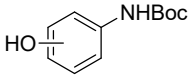
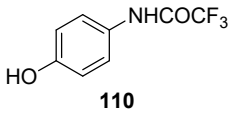
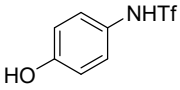
R	Mutant	Conversion (TTN)	Product
Ac **93**	KSK19/AI/AI	17% (170)	 **108**
Boc **95**	RP/FV/EV/L437LV	66% (660)	 **109**
$C(O)CF_3$ **94**	KSK19/AI/AI	100% (1000)	 **110**
Tf **92**	RP/FV/EV/L437LV	100% (1000)	 **111**

Table 4: Initial screening of anilides bearing different *N*-functionalities

Acetanilide **93** was a poor substrate, with turnover only found with six mutants, five of them effecting turnover of ≤2%. Mutant KSK19/AI/AI, however, afforded a 17% conversion with *para*-hydroxylation as the minor product (18:82 selectivity for *para*-hydroxylation/unidentified product). The *para*-hydroxylated product was identified by GC comparison with an authentic standard of 4-hydroxyphenylacetamide **108**, extracted from commercially available paracetamol tablets.

Slightly more success was had with *N*-Boc aniline **95** which gave conversion with the majority of mutants. Conversion up to 93% was observed; however, selectivity was typically poor and reactions were difficult to reproduce on scale which impeded the isolation and identification of the products. MS analysis of a crude extract showed a product with mass SM+16 suggesting mono-oxidation is occurring. Analysis of the screens was troubled by what appeared to be loss of the *tert*-butyloxycarbonyl group, either in solution or thermally, during GC analysis.

Improved conversion was found with trifluoroacetanilide **94** which gave up to 100% conversion to the *para*-hydroxylated product **110**. This product was accessible in 5% isolated yield by scale-up with mutant KSK19/AI/AI. The low yield is likely a result of formation of an emulsion during

work-up of the reaction, impeding extraction of the product. High conversion was possible for a range of different mutants; however, selectivity was variable with by-products likely including amide hydrolysis and a quinone-like product arising from further oxidation. The increased conversion for trifluoroacetanilide compared to acetanilide suggests that either the increase in polarity or lipophilicity aids the binding or orientating of the substrate within the active site.

High levels of conversion were also possible with *N*-triflyl derivative **92**, which bears the most similarity to the lead compounds, herbicides **86**, **87**, and **88**. The highest levels of conversion were found with mutants containing the mutations R47L/Y51F/I401P (RP) and E267V (EV) with RP/EV, RP/FV/EV/L437LV, and RP/IA/EV all affording ≥97% conversion. 4-Aminophenol **111** was isolated in 56% yield by incubation with RP/FV/EV/L437LV. Whilst trifluoroacetanilide **94** and *N*-triflyl derivative **92** both gave high conversions, reactions with **92** were generally more selective for the *para*-hydroxylation product with little signs of further oxidation or sulfonanilide cleavage; as a result, it was decided that trifluoromethanesulfonanilides would be employed to investigate the widening of the reaction scope.

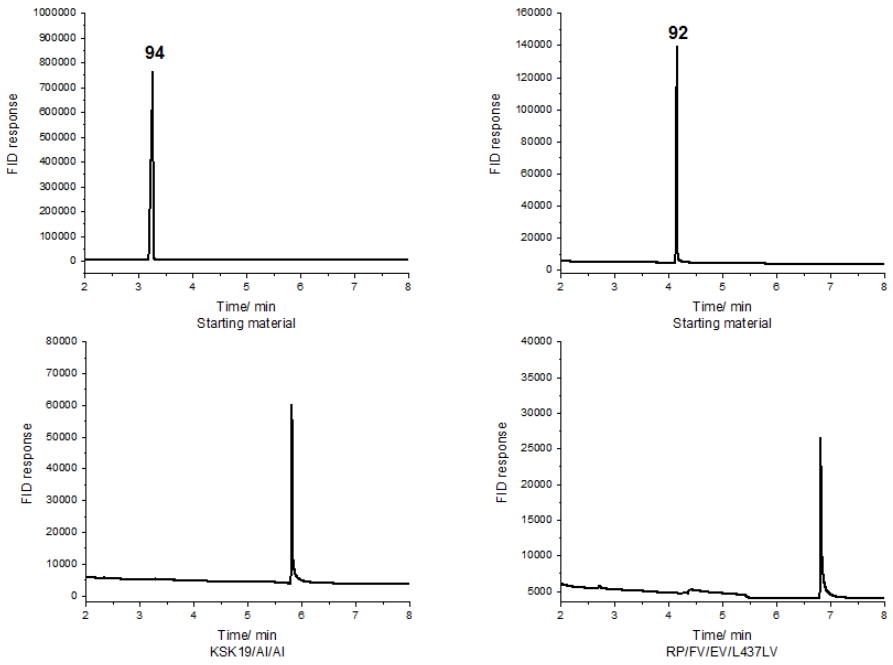
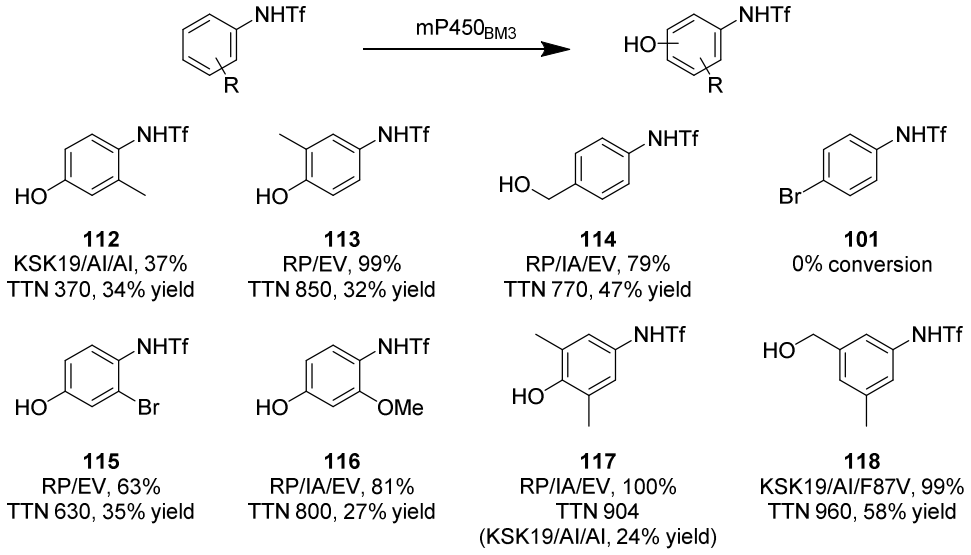


Figure 23: GC traces for oxidation of trifluoroacetamide **94** (left) and triflamide **92** (right) by the mutant $P450_{BM3}$ library

2. Oxidation of Anilides

Eight sulfonanilides were screened against the library of $P450_{BM3}$ variants; the results of the screens are discussed below (Scheme 84). Conversions were estimated by GC for all products apart from **115,** where NMR spectroscopy proved more accurate. In this case, TTN refers to the concentration of the pictured product formed per unit concentration of enzyme. The locations of key active-site amino acid residues are pictured below in Figure 12.



Scheme 84: Substrate scope for anilide hydroxylation

Simple toluidine derivatives **96**, **97**, and **98** were investigated first: *o*-toluidine-derivative **96** gave high levels of conversion; however, with certain mutants, analysis was made difficult by irregular and uncontrolled (possibly non-enzyme-mediated) detriflation of the 4-hydroxylated product. For mutant RP/FV/EV, an analytical-scale reaction gave a 78:22 mixture of detriflated product and **112** but scale-up reactions proved inconsistent, with a 50:50 mixture seen for one scale-up with mutant RP/FV/EV and a 9:91 mixture for another scale-up with the same mutant. Complete selectivity was seen in screening for the detriflated product but, on scale-up, very little was found with **112** being the major product (Figure 24). Selectivity up to 100% for *para*-hydroxylation product **112** was possible for the majority of mutants that effected turnover, one of which (KSK19/AI/AI) was employed for scale-up for synthesis of 4-aminophenol **112** in 34% yield. The highest conversions for this substrate were found with variants containing the RP mutations which were present in all mutants that gave >90% conversion (RP/EV, RP/FV/EV, RP/H171L/I263G, RP/IA/EV, and

RP/FV/EV/L188Q). Mutant RLYF (R47L/Y51F) gave 0% conversion suggesting that the I401P mutation may be responsible for the high conversions, likely due to it occupying a catalytically ready conformation in the substrate-free form (see above).[237]

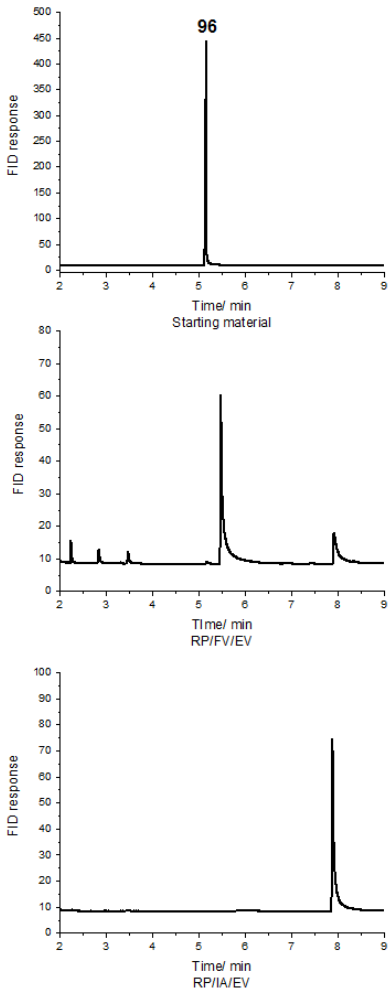


Figure 24: GC traces for oxidation of 2-methyl substrate **96** with the mutant $P450_{BM3}$ library

3-Methyl derivative **97** was also a good substrate, with conversions >85% observed for seven mutants. The major product in the screens, and the only product observed and isolated in scale-up reactions, was *para*-hydroxylated product **113** which was accessible in 32% yield (TTN 1000) by reaction with mutant RP/EV. Again, conversion was typically better for variants containing the RP mutations, although KSK19/AI/AI also gave >90% conversion; selectivity was variable on analytical scale with four products formed but, as mentioned above, only 4-aminophenol **113** was observed in the scale-up reactions.

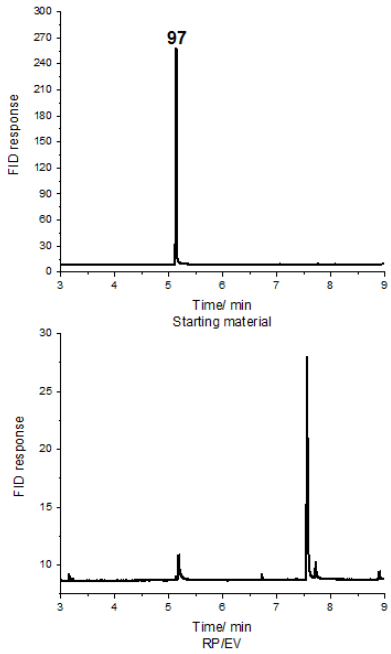


Figure 25: GC traces for oxidation of 3-methyl derivative **97** by mutant $P450_{BM3}$ library

4-Methyl derivative **98** was not accepted as a substrate by many mutants, with turnover seen for only 8 out of 24 variants. For this substrate, oxidation of the anilide ring was not observed and benzylic hydroxylation was favoured instead. Conversion of >50% was seen only for variants containing the RP mutations, with the best conversion found for RP/IA/EV (79%, TTN 770), which facilitated synthesis of alcohol **114** in 47% yield. The position of hydroxylation suggests the substrate binds in a similar orientation to the other derivatives; in this case, that orients the methyl group closest to the catalytic centre to favour benzylic hydroxylation, but, in the other two examples, ring oxidation is preferred.

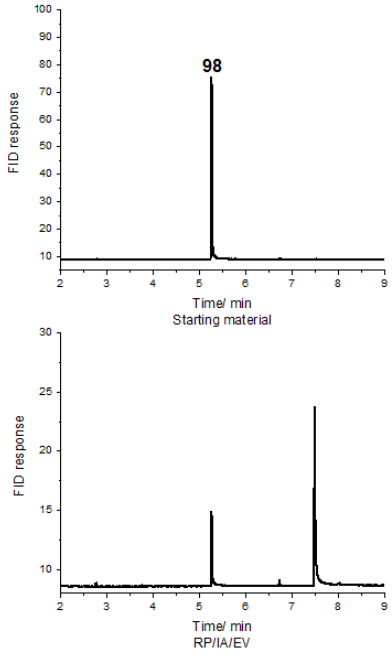
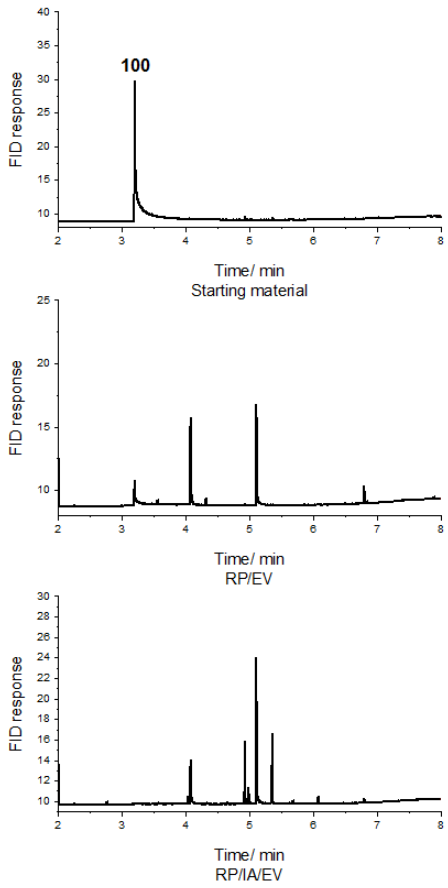


Figure 26: GC traces for oxidation of 4-methyl derivative **98** by the mutant $P450_{BM3}$ library

Screening of substrate **100**, synthesised from 4-bromoaniline, revealed no oxidation activity, with no substrate turnover seen across the whole library. Substrate binding in a similar orientation to the previously discussed substrates positions the bromine atom proximal to the haem centre, preventing oxidation at this or other positions. 2-Bromoaniline-derived substrate **100** fared much better, with conversion of over 75% achieved for four variants, all of which contain the RP and EV mutations, with a maximum of 100% conversion for RP/IA/EV. Whilst the conversion was highest for RP/IA/EV, RP/EV gave better selectivity, and was employed in a scale-up reaction for synthesis of 4-aminophenol **115** in 35% yield. GC traces for the analytical scale reactions appeared to contain multiple products; however, the NMR spectrum of the crude product obtained from the scale-up reaction suggested that only one product (4-aminophenol **115**) was being formed.



Scheme 27: GC traces for oxidation of 2-bromo derivative **100** by the mutant $P450_{BM3}$ library

The more electron rich sulfonanilide **102**, synthesised from *o*-anisidine, was also accepted as a substrate by the $P450_{BM3}$ variants, with half of the 24 variants screened effecting substrate turnover. Only three of these mutants gave >45% turnover, all of which contained the mutations RP and EV (RP/EV - 49%, 98:2 selectivity for 4-aminophenol/other products; RP/FV/EV - 97%, 6:94 selectivity; RP/IA/EV - 81%, 99:1 selectivity) (Figure 28). RP/IA/EV, as the most selective mutant, was used in a scale-up reaction, from which 4-aminophenol **106** was isolated in 27% yield. Other products formed in this reaction were not isolated (their formation in scale-up reactions was not consistent with GC analysis of analytical scale reactions), but NMR spectroscopic analysis of the crude extracts suggested that at least one of them was a result of further oxidation and quinone formation.

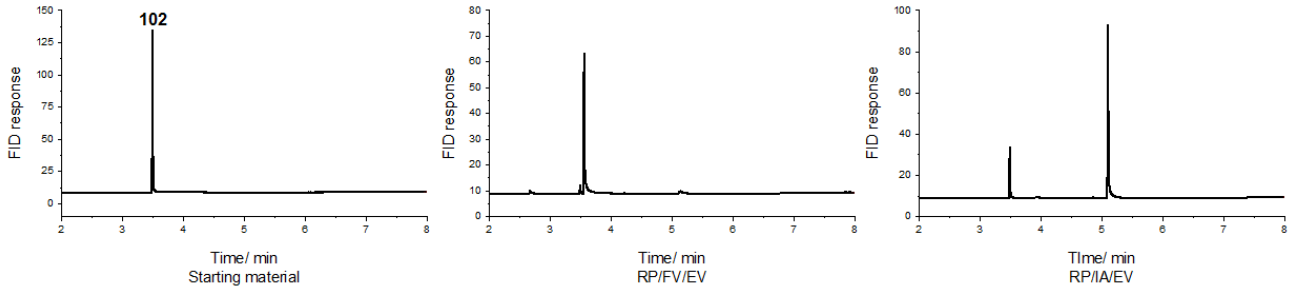


Figure 28: GC traces for oxidation of 2-methoxy derivative **102** by the library of $P450_{BM3}$ mutants

Electron poor sulfonanilide **107**, bearing an *ortho*-nitro group (and methanesulfonyl rather than trifluoromethanesulfonyl), was screened with the majority of mutants affording no conversion, although 35% conversion was possible with RT2/AP/I401M. Attempts to scale-up reactions with this substrate proved unfruitful with mostly starting material returned; very minor amounts of other compounds were observed, potentially arising from sulfonamide hydrolysis or reduction of the nitro group (a known mode of action for cytochrome P450s).[318]

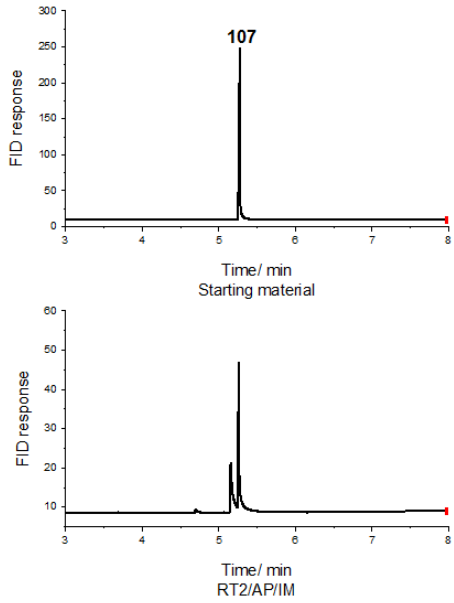


Figure 29: GC traces for oxidation of 2-nitro derivative **107** by the library of $P450_{BM3}$ mutants

Interesting selectivity was found for compound **99**, synthesised from 3,5-dimethylaniline, where products arising from benzylic hydroxylation and from *para*-hydroxylation of the aromatic ring were accessible. Of the 22 mutants which afforded turnover of the substrate, 12 were selective for *para*-hydroxylation and 10 were selective for benzylic hydroxylation. For mutants which gave >10% conversion, selectivity was generally low; however, high levels of selectivity (>90% selectivity) for

either product were possible. Mutants RP/IA/EV (100% conversion, 90% selectivity) and RT2/AP/A184I (26% conversion, 100% selectivity) preferentially reacted *via para*-hydroxylation of the aromatic ring, with RP/IA/EV enabling the synthesis of 4-aminophenol **117** in 24% yield (Figure 27). The A330P mutation, which constricts the space above the haem, and the I263A/E267V pair of mutations, which creates space in the active site further away from the haem, do not alter the active site in the same manner; however, both promote *para*-hydroxylation. Excellent selectivity for benzylic hydroxylation was found for mutant RT2 (80% conversion, 96% selectivity) and KSK19/AI/F87V (99% conversion, 97% selectivity) with the latter affording 58% yield of benzylic alcohol **118** from scale up of the reaction (Figure 30). The F87V mutation generates space above the haem, potentially allowing for closer approach of the methyl group, favouring benzylic hydroxylation.

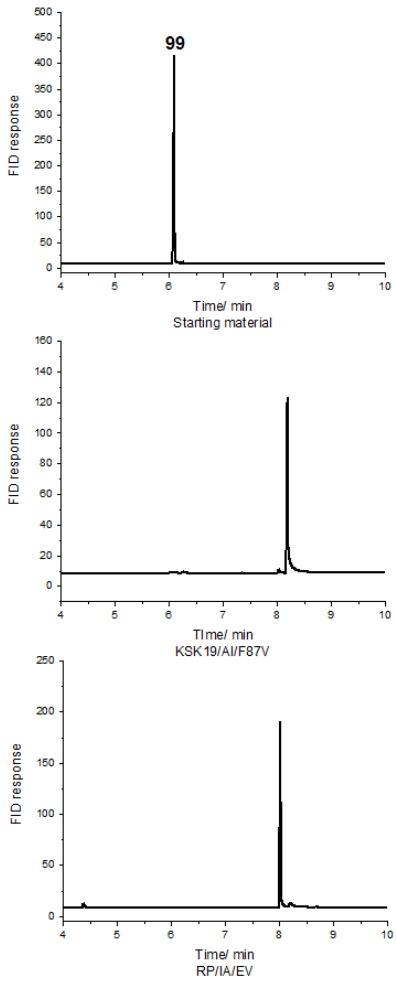


Figure 30: GC traces for oxidation of 3,5-dimethylaniline derived substrate **99**

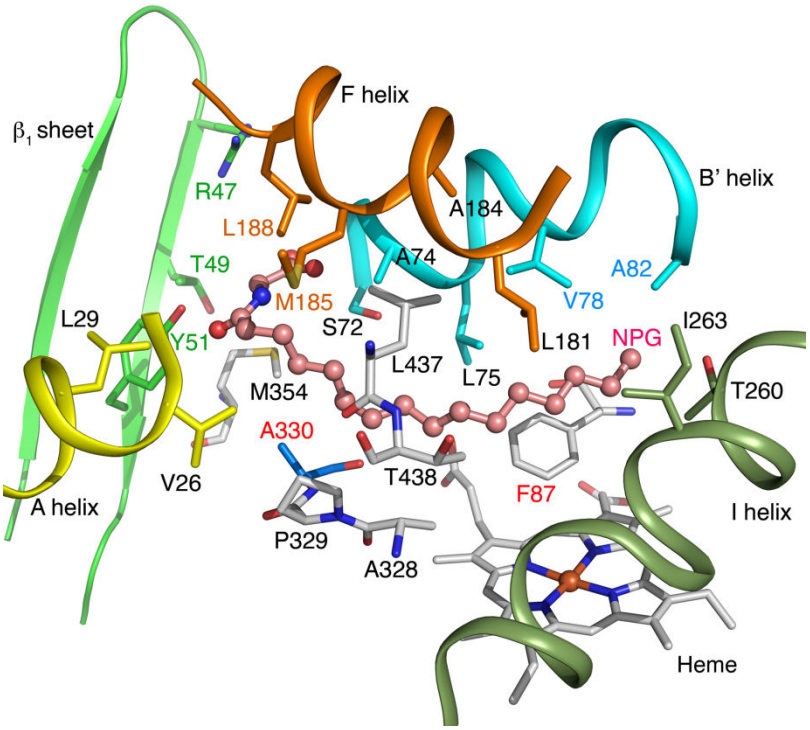
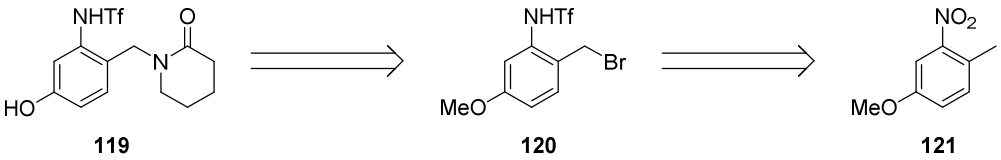


Figure 12: Close-up of the *N*-palmitoyl glycine-bound active site for wild-type $P450_{BM3}$ (PDB code 1JP7)

Investigation into *meta*-Hydroxylation of Herbicides

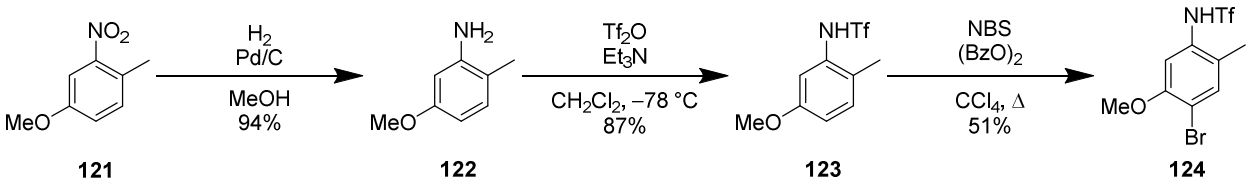
To ascertain whether *meta*-hydroxylation of the herbicidal compounds discussed above was occurring to some extent in the screens, to provide a product that could have herbicidal activity or be a previously unidentified natural metabolite, the 3-hydroxy derivative of one of them was synthesised chemically as an analytical standard for GC. The initial plan was to begin with 4-methyl-3-nitroanisole and proceed *via* benzylic halogenation and *N*-alkylation (Scheme 85).



Scheme 85: Synthetic plan for synthesis of 3-hydroxy derivative **119**

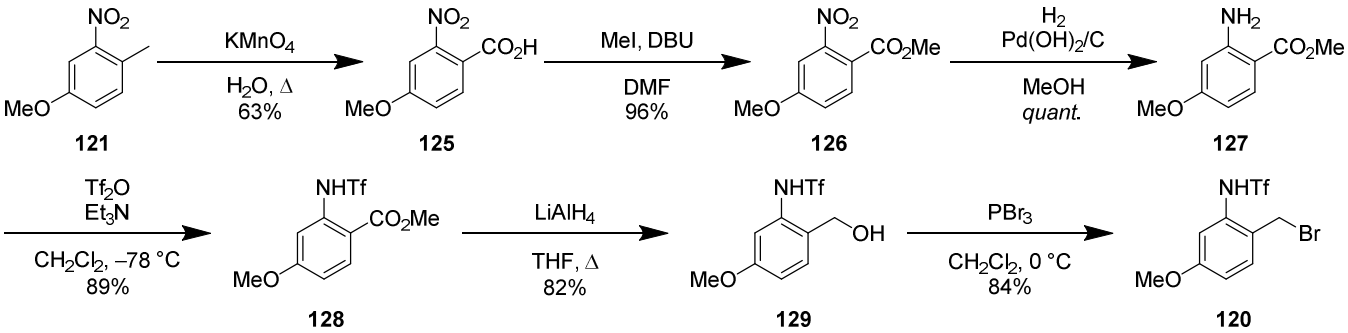
Hydrogenation of 4-methyl-3-nitroanisole was high yielding, as was the subsequent *N*-triflation of aniline **122**; however, efforts to brominate the benzylic position led only to bromination of the aromatic ring (Scheme 86). Subjecting aryl bromide **124** to the same conditions also effected ring bromination, the position of which was not determined. Ring bromination, *ortho*- to the sulfonamide and ether groups, occurred when directed lithiation and trapping with Br_2 was attempted on triflamide **123**.

2. Oxidation of Anilides



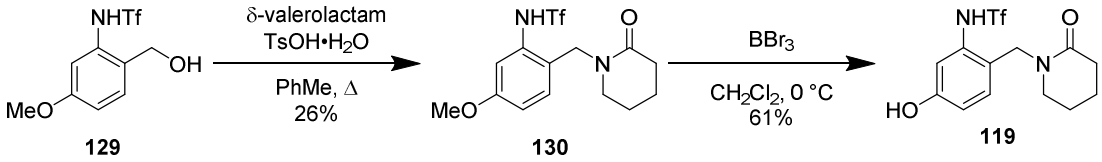
Scheme 86: Failed synthesis of benzyl bromide **120**

An alternative route was planned, in which the bromide was installed *via* Appel reaction on benzyl alcohol **129**. Again, 4-methyl-3-nitroanisole was chosen as the starting point for this synthesis (Scheme 87). Synthesis of benzyl bromide **120** was possible by this route, although substitution with δ-valerolactam proved unsuccessful, with the reaction returning starting material.



Scheme 87: Synthesis of benzyl bromide **120**

Synthesis of 3-hydroxy derivative **119** was finally achieved by displacement of benzyl alcohol **129** with δ-valerolactam, followed by BBr_3 mediated demethylation (Scheme 88). By this route, synthesis of 3-hydroxyl derivative **119** was possible in seven steps with a total yield of 7%, highlighting the desirability of direct hydroxylation. By using this compound as a GC standard it was shown that it was not formed by any of the mutants screened; use of an expanded library may enable discovery of a mutant capable of catalysing the necessary *meta*-hydroxylation.

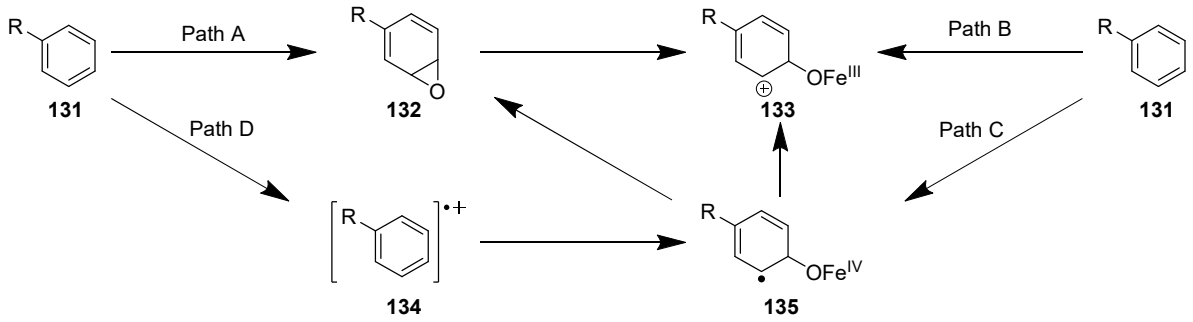


Scheme 88: Successful synthesis of 3-hydroxyl derivative **119**

Mechanism

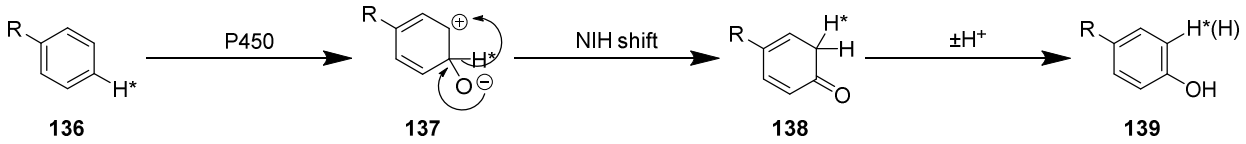
The mechanism of the cytochrome P450-catalysed hydroxylation of aromatic rings has long been a source of disagreement, with at least four options considered and claimed as the correct

mechanism; however, it is generally accepted that the oxidation proceeds through cationic σ-complex **133** (Scheme 89).[119,319,320]



Scheme 89: Potential pathways for the formation of cationic σ-complex **133**

This complex is generally accepted to be converted to a ketone *via* a 1,2-shift of the *ipso*-group; this shift is known as the NIH shift (demonstrated by isotopic labelling), and is followed by tautomerisation to the phenol (Scheme 90).[119,321–325] When a deuterium or tritium atom is positioned *para*- to the functional group on an aromatic ring, it is seen to migrate to the neighbouring carbon, and is partially retained in the final product (Scheme 90). It is only partially retained because some is lost in the final tautomerisation. This shift provides evidence for oxidation of the π-system rather than direct hydroxylation of the C–H bond.



Scheme 90: Evidence for the NIH shift mechanism

Whilst the fate of cationic σ-complex **133** is well established, its mechanism of formation is not as secure. Path A, originally held as the accepted mechanism, involves direct arene oxide formation, then a non-enzymatic rearrangement of the epoxide. The evidence for this mechanism was the aforementioned NIH shift; however, this shift is possible for all the pathways in Scheme 89. Path D involves initial charge transfer then formation of a radical σ-complex **135** which can undergo electron transfer to form cationic σ-complex **133**; the formation of a stable radical cation from 1,2,4,5-tetramethoxybenzene, a very electron rich aromatic ring, and Marcus plot analysis, which

concluded that electron transfer was reversible and occurring in a solvent cage, have been used as evidence for path D.[320,326]

The direct epoxidation mechanism (path A) and the charge-transfer mechanism (path D) have been strongly disputed by investigations of kinetic isotope effects for P450-catalysed arene hydroxylations.[119,327–329] A small inverse kinetic isotope effect (0.83–0.94) was seen for hydroxylation of *ortho*- and *para*-xylene which disqualified path D (initial electron transfer) as the mechanism for these substrates, as there would be no kinetic isotope effect expected to be associated with electron transfer from the ring.[329] Strong evidence against the direct formation of arene oxide **132** was displayed in an investigation of the kinetic isotope effects associated with hydroxylation of differently deuterated chlorobenzenes.[327] For *meta*-hydroxylation of *meta*-deuterated chlorobenzene, a small normal kinetic isotope effect (~1.1) was observed. However, for *ortho*- or *para*-hydroxylation of chlorobenzenes where the position of oxidation is deuterated, a small inverse kinetic isotope effect (~0.95) was found. Concerted formation of the two C–O bonds of an epoxide would be expected to exhibit a small inverse isotope effect with the deuteration of either site (due to rehybridisation at those centres); this result (differing isotope effects) suggests that the rate determining step is not the concerted formation of an arene oxide. This result is supported by the computational work of Shaik which suggests that the dominant pathway proceeds *via* cationic σ-complex **133**, with intermediacy of radical σ-complex **135** representing a minor pathway; arene oxide formation is believed to occur *via* this radical σ-complex.[319]

Conclusions

The library of $P450_{BM3}$ variants has been successfully applied to the *para*-hydroxylation of a range of diversely substituted sulfonanilides with high levels of conversion and selectivity. Benzylic hydroxylation has also been observed with switchable selectivity between this pathway and *para*-hydroxylation possible for one of the substrates. This reaction was also successful for anilides bearing a variety of *N*-substituents and for more structurally complex herbicidal compounds, with selectivity

found for arene hydroxylation over *S*-oxidation (which may be difficult to achieve with chemical oxidants). Screening against an expanded library could facilitate the identification of mutants that catalyse the more difficult *meta*-hydroxylation.

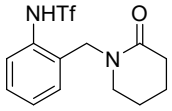
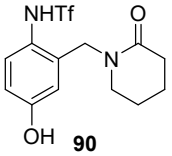
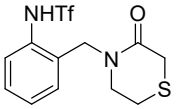
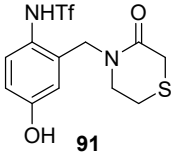
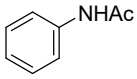
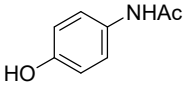
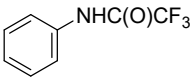
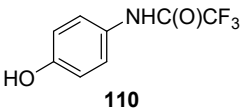
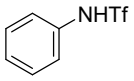
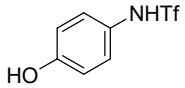
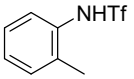
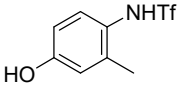
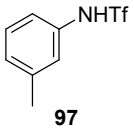
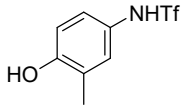
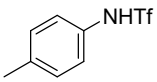
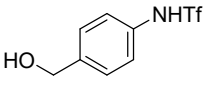
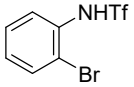
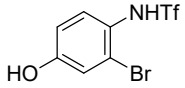
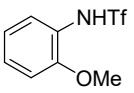
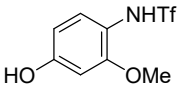
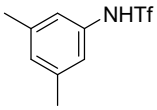
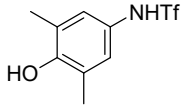
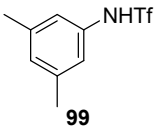
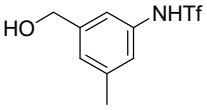
Substrate	Mutant (yield)	Product	Substrate	Mutant (yield)	Product
 **86**	RP/FV/EV (13%)	 **90**	 **87**	RP/FV/EV (19%)	 **91**
 **93**	KSK19/AI/AI (17%)	 **108**	 **94**	KSK19/AI/AI (5%)	 **110**
 **92**	RP/FV/EV/L437LV (56%)	 **111**	 **96**	KSK19/AI/AI (34%)	 **112**
 **97**	RP/EV (32%)	 **113**	 **98**	RP/IA/EV (47%)	 **114**
 **100**	RP/EV (35%)	 **115**	 **102**	RP/IA/EV (27%)	 **116**
 **99**	KSK19/AI/AI (24%)	 **117**	 **99**	KSK19/AI/FV (58%)	 **118**

Table 5: Hydroxylation of anilides by the library of $P450_{BM3}$ mutants

Chapter 3: Efforts Toward the Synthesis of Trigoxyphin K and L

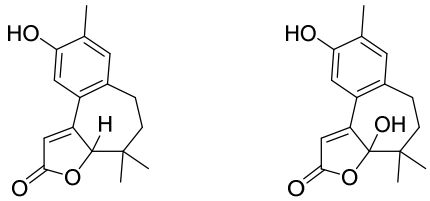
Aims

The synthetic strategy of late-stage functionalisation considers the C–H bonds of a molecule to be functional groups which can be used for diversification, avoiding the need for *de novo* synthesis.[330] An obvious difficulty had with this approach is achieving selectivity between different C–H bonds with typically similar reactivities. Wild-type $P450_{BM3}$ and its mutants are capable of catalysing C–H hydroxylation at unactivated positions with high levels of selectivity, making them highly applicable for use in late-stage functionalisation.[102,165,166,207] To demonstrate this, the total syntheses of trigoxyphin K and L were targeted, with construction of the core framework followed by late-stage $P450_{BM3}$-mediated C–H hydroxylation being the key strategy of the route.

Isolation

The isolation of trigoxyphin K and trigoxyphin L from *Trigonostemon xyphophylloides,* an evergreen shrub found in the Hainan province of China, was reported by Wu and Han in 2013; this was achieved by percolation of the air-dried, powdered twigs with ethanol, extraction with ethyl acetate, and column chromatography.[331] Plants of the genus *Trigonostemon* grow predominantly in tropical and sub-tropical Asia and comprise roughly 50 species.[331,332] A wide range of diterpenoid and degraded diterpenoid natural products (including trigoxyphin A–T) have been isolated from plants belonging to this genus and some have demonstrated interesting biological activities including cytotoxic activities, flea insecticidal activity, and MET tyrosine kinase inhibitory activity.[331,333] The structures of trigoxyphin K and trigoxyphin L were elucidated by high-resolution mass spectrometry, infra-red spectroscopy, and a variety of ^{1}H and ^{13}C NMR spectroscopy experiments (COSY, HSQC, HMBC, and ROESY). Trigoxyphin K and L both contain a 6,7,5-fused tricyclic ring system, differing only in that trigoxyphin K contains a butenolide where trigoxyphin L contains a γ-hydroxy-butenolide (Figure 28). Discrepancies in the labelling of these compounds in the isolation paper have created some confusion about the structures with trigoxyphin K drawn as a γ-hydroxy-butenolide but with

NMR, MS, and IR data identifying a butenolide, and trigoxyphin L drawn as a butenolide but with NMR, MS, and IR data identifying a γ-hydroxy-butenolide; for the purposes of this study structures will be identified based on the data, not the drawn structures.



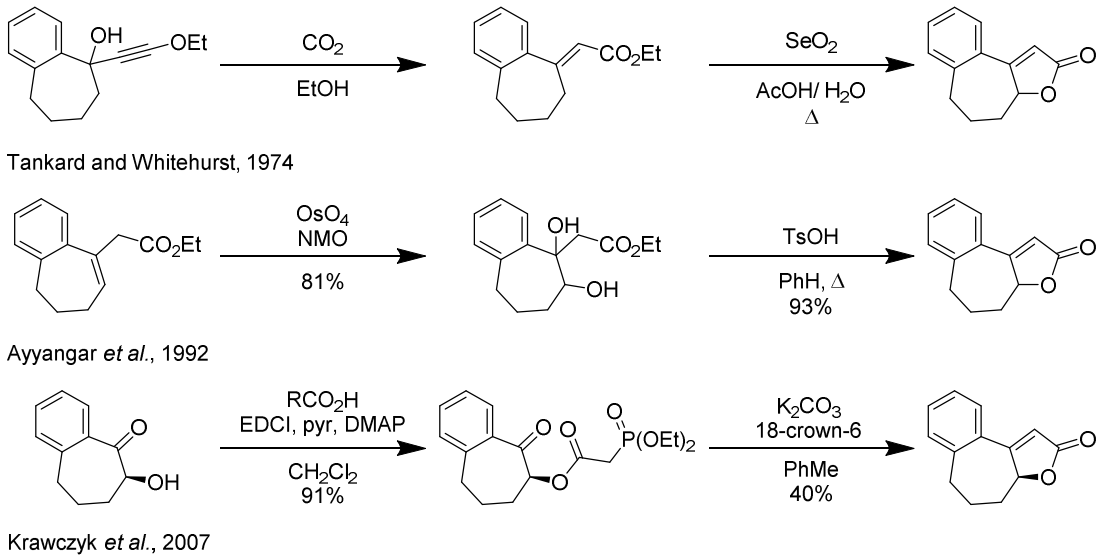
141 Triogxyphin K **142** Trigoxyphin L

Figure 31: Proposed structures of trigoxyphin K and trigoxyphin L

Synthesis of Similar Structures

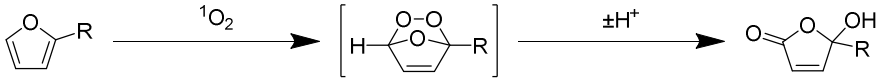
No total, or partial, synthesis of trigoxyphin K or trigoxyphin L has been published; however, similar 6,7,5-fused ring systems containing a butenolide group have been synthesised.[334–336] In all cases, the 6,7-ring system (6,7,8,9-tetrahydro-5*H*-benzo[7]annulene: fused benzene/cycloheptane rings) was constructed first, with a ketone α- to the aromatic ring; further chemistry on these ketones (conversion into an ethoxyethynylcarbinol and hydrolysis with carbonic acid; Reformatsky reaction, elimination, and alkene dihydroxylation; or α-hydroxylation and esterification) affords precursors for the installation of the butenolide moiety.[334–336] Formation of the butenolide ring was achieved by SeO_2 mediated hydroxylation with subsequent lactonisation, diol lactonisation and concomitant dehydration, and Horner–Wadsworth–Emmons olefination (Scheme 91). [334–336]

3. Efforts Toward the Synthesis of Trigoxyphin K and L



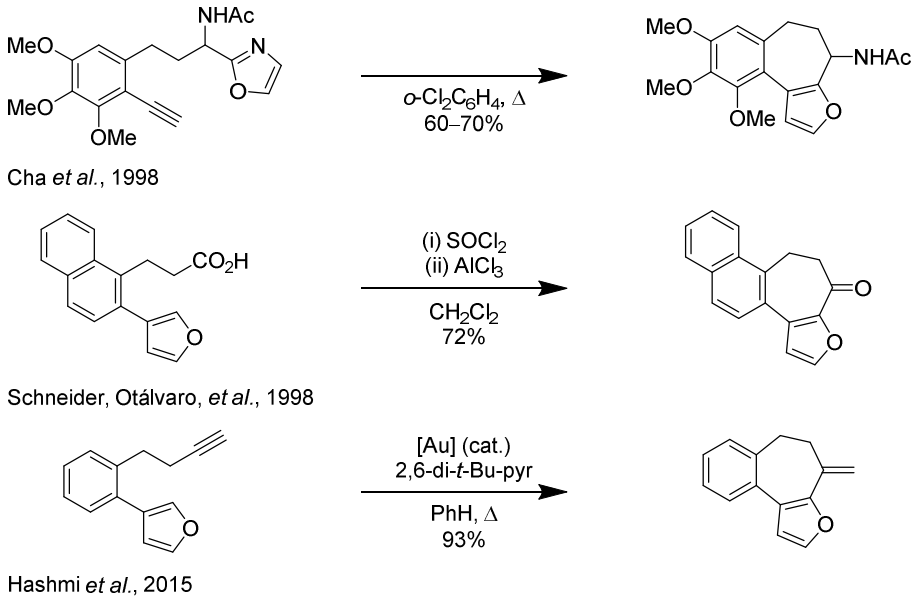
Scheme 91: Synthesis of 6,7,5-fused ring systems

Furans are commonly employed precursors to γ-hydroxy-butenolides, with singlet oxygen oxidation being a particularly popular method for effecting the transformation.[337,338] This is typically achieved by photosensitised excitation of triplet oxygen to form singlet oxygen which undergoes [4+2] cycloaddition with a furan to form the (isolable) endoperoxide; base-induced fragmentation of the intermediate peroxide affords a γ-hydroxy-butenolide (Scheme 92).[339,340]



Scheme 92: Singlet oxygen oxidation of a furan

Given that furans are such reliable butenolide precursors, a method for synthesising a furan-containing 6,7,5-fused tricyclic ring system could be applied to the core of trigoxyphin K and L. Access to such structures has been achieved by intramolecular [4+2]/retro-[4+2] cycloaddition, intramolecular Friedel–Crafts acylation, and Au-catalysed intramolecular cyclisation (Scheme 93).[341–344]

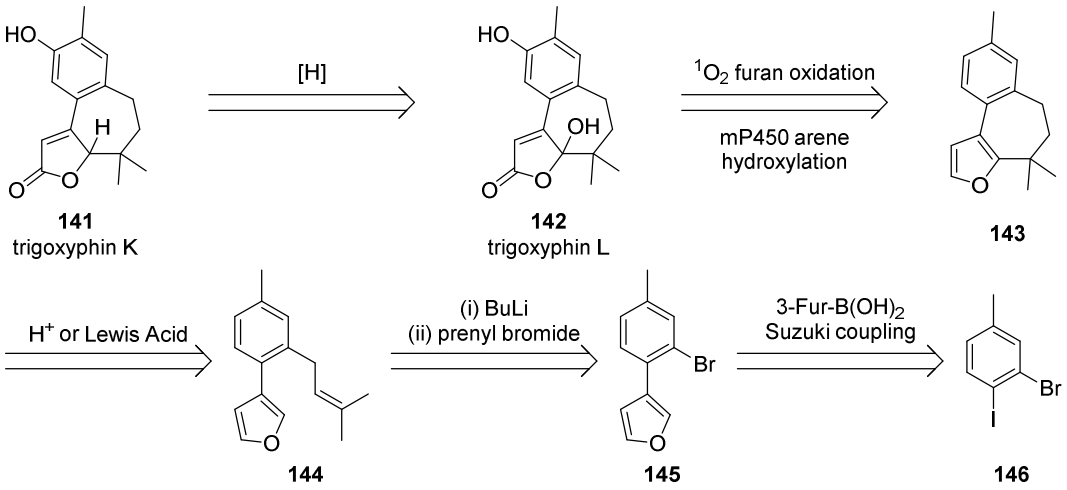


Scheme 93: Synthesis of furan containing 6,7,5-fused ring systems

Retrosynthetic Plan

As discussed previously, cytochrome P450s are capable of hydroxylating aromatic rings in metabolism and biosynthesis; also, within the Wong group, the ring-hydroxylation of alkylbenzenes by the library of $P450_{BM3}$ variants has been developed with high levels of selectivity possible.[102,119,181,189,206,345] It was intended that this ability to enact hydroxylations of aromatic rings could be employed as a key step of the synthesis of trigoxyphin K and L, for the late-stage installation of the phenolic hydroxyl group (Scheme 94). Trigoxyphin K was expected to be accessible *via* reduction of the γ-hydroxy-butenolide of trigoxyphin L which would be formed by singlet oxygen oxidation of furan **143**. The 6,7,5-fused ring system could be created by a Brønsted or Lewis acid-mediated cyclisation of furan onto an alkene. The requisite alkene was proposed to be synthesised *via* Suzuki coupling of aryl iodide **146** with 3-furanylboronic acid followed by lithium-halogen exchange and trapping with prenyl bromide.

3. Efforts Toward the Synthesis of Trigoxyphin K and L

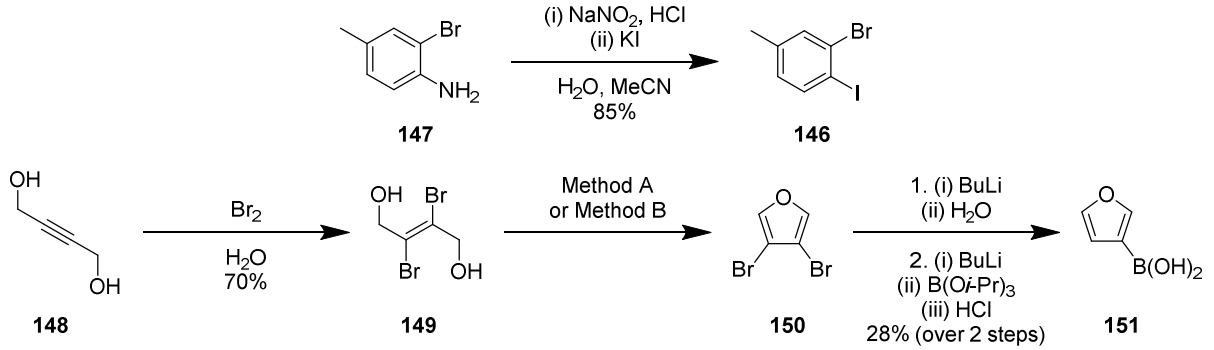


Scheme 94: Retrosynthetic plan for synthesis of trigoxyphin K and trigoxyphin L

Synthesis of 3-(2-bromo-4-methylphenyl)furan

Plan A: Suzuki coupling

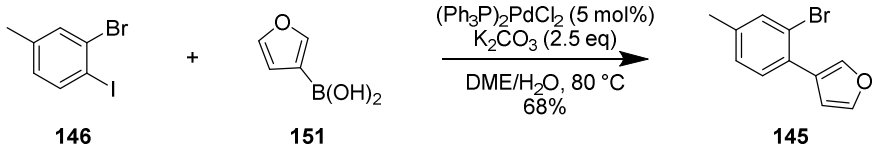
Aryl iodide **146** was accessible in good yield by Sandmeyer iodination of 2-bromo-4-methylaniline (Scheme 95). 3-Furanyl-boronic acid was prepared in four steps from 2-butyn-1,4-diol starting with dibromination of the alkyne followed by double-bond isomerisation and oxidative furan formation to provide 3,4-dibromofuran. Two methods, both employing Jones oxidations, were attempted for the oxidative furan formation: method A used a biphasic solvent system of water and hexane, and method B used distillation to remove the product from the reaction mixture (in which it is unstable) (Scheme 95).[346,347] Method B was the more successful, affording 3,4-dibromofuran in more than double the yield of method A. With 3,4-dibromofuran in hand, lithium-halogen exchange and trapping furnished the boronic acid (Scheme 95).[348,349]



Method A: $K_2Cr_2O_7$, H_2SO_4, hexane/H_2O, 85 °C, 17%
Method B: $K_2Cr_2O_7$, H_2SO_4, H_2O, 130 °C, distillation, 36%

Scheme 95: Synthesis of aryl iodide **146** and 3-furanylboronic acid **151**

With both coupling partners in hand the desired Suzuki coupling was attempted. Initial efforts resulted in poor conversions (60–70% conversion); however, increasing the volume of 1,2-dimethoxyethane (DME) in the reaction (from 3.7 mL/mmol to 6.7 mL/mmol substrate) gave an increase to 80% conversion with isolation of **145** in 68% yield (Scheme 96).



146 151 145

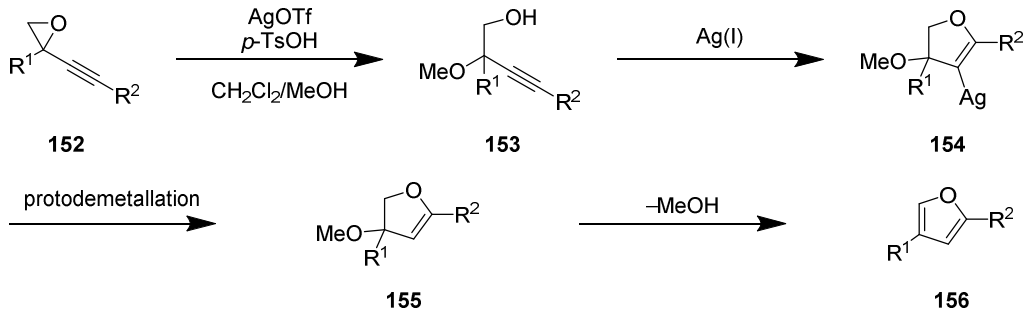
Scheme 96: Synthesis of 3-(2-bromo-4-methylphenyl)furan **145** by Suzuki coupling

Whilst the Suzuki coupling proved to be a productive method for the synthesis of 3-arylfuran **145**, the synthesis of 3-furanylboronic acid **151** was both low yielding and atom uneconomic. It was decided that it was more viable to use commercially available 3-furanylboronic acid (Fluorochem, 5 g, £86.00) so that the synthesis could be progressed on a useful scale. For the initial attempts at gram-scale reactions, catalyst loading was lowered to 1 mol% to avoid using large amounts of the expensive catalyst; however, this negatively affected conversion, resulting in a reduced yield (<40%). Addition of bidentate phosphine ligands did not improve matters and carrying out the reaction at higher temperature with 1,4-dioxane as co-solvent proved particularly poor, with the yield dropping to 7% (possibly due to decomposition at elevated temperature). Eventually, increase of the catalyst loading to 2 mol% improved the gram-scale reaction conversions and yields to be comparable to those of the smaller-scale reactions, making 1.1 g (64% yield) of 3-arylfuran **145** readily available.

Plan B: Alkynyl epoxide rearrangement

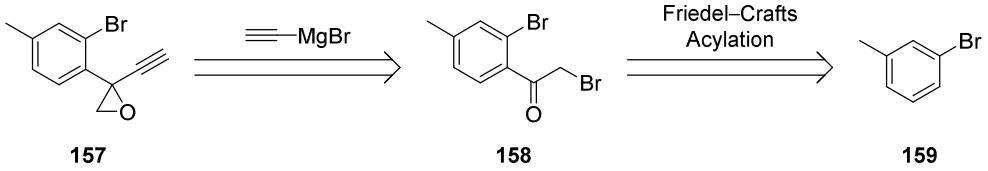
While the synthesis of 3-furanylboronic acid was proving troublesome, an alternative route to 3-arylfuran **145** was sought. It is known that Ag(I) (and other metals) can catalyse the rearrangement of an alkynyl epoxide to a 3-substituted furan.[350] Under these conditions the epoxide is opened by methanol and the hydroxyl group cyclises onto the alkyne, catalysed by Ag(I); protodemetallation and methanol elimination then afford the desired furan (Scheme 97).

3. Efforts Toward the Synthesis of Trigoxyphin K and L



Scheme 97: Ag(I) catalysed synthesis of 3-substituted furans

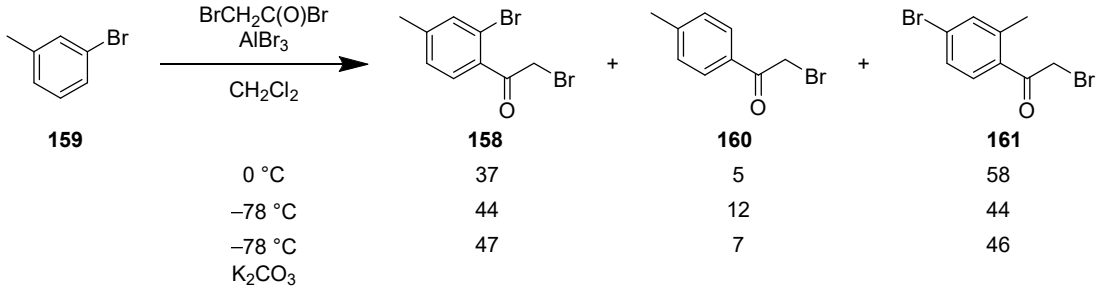
For this reaction to be applicable to the planned total synthesis, a route to alkynyl epoxide **157** was required and a route was planned beginning with 3-bromotoluene (Scheme 98).



Scheme 98: Retrosynthesis plan for alkynyl epoxide **157**

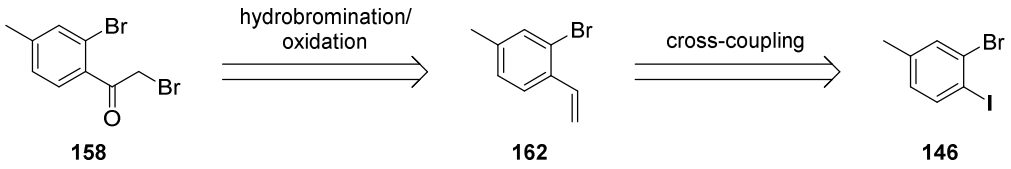
Attempts to effect the Friedel–Crafts acylation of **159** with $AlBr_3$ and bromoacetyl bromide were complicated by the production of regioisomers; when the addition of $AlBr_3$ was carried out at 0 °C, a 40:60 mix of desired bromoacetophenone **158**, from acylation *para*- to the methyl group, and undesired bromoacetophenone **161**, from acylation *ortho*- to the methyl group, resulted (Scheme 99). Adding $AlBr_3$ at a lower temperature (–78 °C) improved the regioselectivity to 50:50 *para*/*ortho* acylation. This result, whilst not perfect, appeared to be good enough to examine the feasibility of the further steps as the regioisomers were separable by column chromatography; however, desired product **158** was inseparable from 2-bromo-4'-methylacetophenone **160**, formed by Friedel–Crafts acylation of toluene (from debromination of starting material) (Scheme 87). The products of the subsequent reactions carried out on this mixture also proved to be inseparable. It was believed that the debromination was mediated by small amounts of HBr so a small selection of bases was screened for the ability to suppress this. 2,4,6-Collidine and Hünig's base both completely suppressed acylation, 2,6-di-*tert*-butylpyridine had no effect on the extent of debromination, and K_2CO_3 had just a minor effect, but increasing the amounts of base from one to three equivalents did not further improve the reaction (Scheme 99). Debromination was seen to a lesser extent when addition of $AlBr_3$

was carried out at 0 °C, addition of K_2CO_3 to this reaction could have potentially resulted in an improvement on this. Use of a weaker Lewis acid ($AlCl_3$) made the reaction much slower and saw an increase in selectivity for undesired product **161** (20:80 *para*/*ortho*-acylation), and with a weaker acylating agent (bromoacetic anhydride with $BF_3{\cdot}OEt_2$) there was no reactivity.



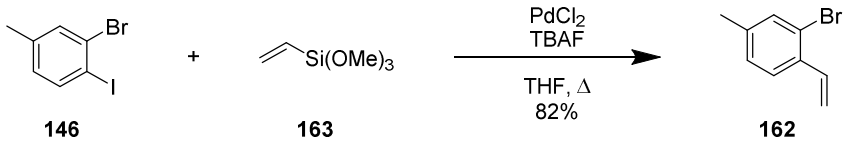
Scheme 99: Attempts to optimise synthesis of bromoacetophenone **158** by Friedel–Crafts acylation

An alternative synthesis of bromoacetophenone **158** was required and, in turn, it was planned for this to be achieved by hydrobromination/oxidation of styrene **162** which, in turn, could be accessed *via* a cross-coupling reaction with 3-bromo-4-iodotoluene **146** (Scheme 100).



Scheme 100: Alternative proposal for the synthesis of bromoacetophenone **158**

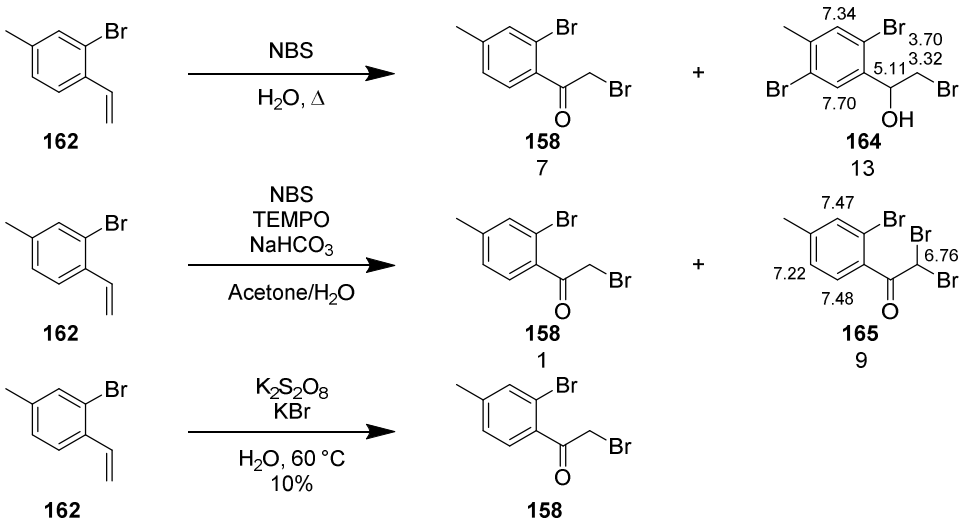
Hiyama coupling with vinyltrimethoxysilane was selected for cross-coupling to form styrene **162**.[351] This proved to be a successful choice as, by this method, styrene **162** was obtained in good yield (Scheme 101). Early attempts suffered from formation of a gelatinous substance which made separation and filtration difficult; decreasing the concentration of the reaction and evaporation of the reaction solvent prior to work up helped to alleviate this.



Scheme 101: Synthesis of styrene **162** by Hiyama cross-coupling

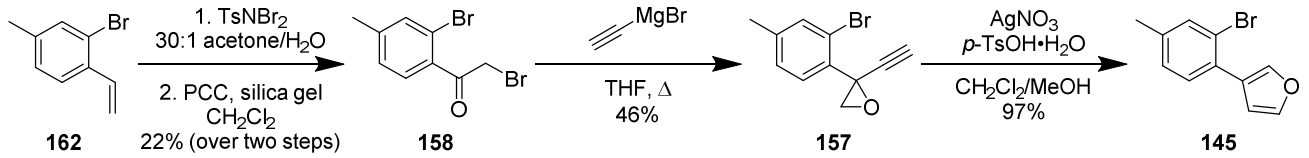
With styrene **162** readily available, it was intended that a one-step hydrobromination/oxidation procedure would be employed to generate bromoacetophenone **158**.

Bromoacetophenone **158** was made by this method, but it was generally only formed as a minor product with the reaction conditions proving to be either too vigorous or not vigorous enough. *N*-bromosuccinimide (NBS) has been used as both electrophilic source of bromine and as oxidant for the synthesis of α-bromoketones from alkenes; however, when applied to this system, the major product formed (**164**) was that arising from ring-bromination of the intermediate bromohydrin (Scheme 102).[352] Use of less harsh conditions, where NBS is used as a co-oxidant for a TEMPO-mediated oxidation following bromohydrin formation, suffered from over-bromination α- to the ketone formed, with dibromoketone **165** being obtained as the major product (Scheme 102). A third method, which employed KBr and $K_2S_2O_8$ for tandem hydrobromination/oxidation, did not appear to form any over-brominated products; however, bromoacetophenone **158** was isolated in only 10% yield from this reaction (Scheme 102).[353]



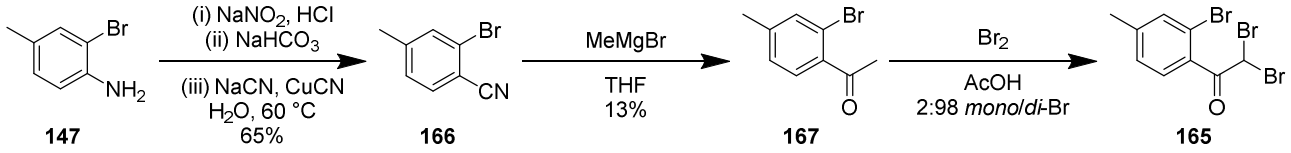
Scheme 102: Attempts to achieve a one-step hydrobromination/oxidation of bromostyrene **162** (product ratios are estimated from NMR spectra of the crude product)

Although the one-step process was proving troublesome, bromoacetophenone **158** was prepared in pure form in a two-step sequence. From this point, the next two steps caused little trouble, with formation of alkynyl epoxide **157** and Ag(I) catalysed rearrangement to 3-arylfuran **145** occurring in reasonable to good yield (Scheme 103).



Scheme 103: Successful synthesis of 3-arylfuran **145** *via* Ag(I) catalysed rearrangement

Bromoacetophenone **158** was also targeted *via* a third route. Sandmeyer reaction for the cyanation of 2-bromo-4-methyl-aniline was straightforward; however, the steps that followed were less successful. Addition of methylmagnesium bromide suffered due to issues of poor mass recovery and debromination (the product of which was inseparable from acetophenone **160**), and the following ketone α-bromination formed dibromoketone **165** predominantly (Scheme 104).



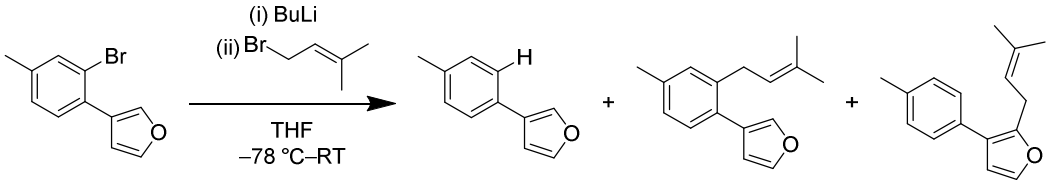
Scheme 104: Attempted synthesis of bromoacetophenone **158** *via* nitrile **166**

Although the alkynyl epoxide rearrangement route is a viable pathway to 3-arylfuran **145**, it did not compete with the Suzuki coupling route from commercially-available 3-furanylboronic acid. As a result, the Suzuki coupling pathway was chosen for continuation of the synthesis.

Prenylation of 3-Arylfuran 145

Next, it was necessary to install the prenyl side chain, required for the planned formation of the 7-membered ring. It was intended for this to be achieved by lithium-halogen exchange and trapping with prenyl bromide.[354] In practice, lithium-halogen exchange with BuLi occurred readily, indicated by the lack of returned starting material, but the fate of the anion proved to be an issue. Lithium-halogen exchange was carried out at –78 °C and prenyl bromide was added at the same temperature; the reaction was allowed to warm to room temperature overnight following the addition. In the first experiment, a very small amount of desired product **144** was formed, with the major products being **168** and **169** arising, respectively, from protonation of the aryllithium and an unexpected selective proton transfer from the 2-position of the furan followed by trapping with prenyl bromide (Scheme 105). By decreasing the concentration of the reaction from 0.75 M to

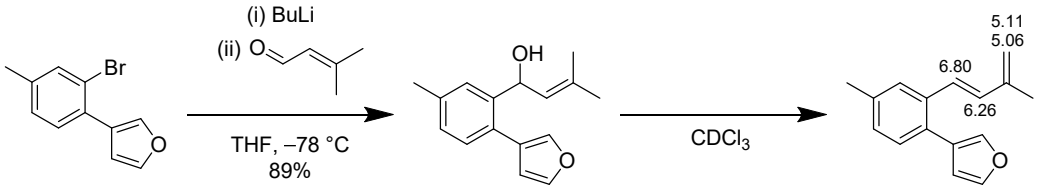
0.15 M it was possible to partially suppress this proton transfer (suggesting a predominantly intermolecular proton transfer); however, the major product was still arising from protonation rather than prenylation (Scheme 105). Desired prenylated product **144** and lithiation/protonation product **168** were separable from disubstituted furan **169** by column chromatography, but they were not separable from each other and neither could be isolated in pure form. Attempts to form the desired product by Negishi coupling failed using two methods for forming the prenylzinc species: (i) insertion of Zn into prenyl bromide; and (ii) lithium-halogen exchange of prenyl bromide followed by transmetallation with $ZnCl_2$. Both methods returned only starting material and unreacted prenyl bromide.[355]



145		168	144	169
	0.75M	54	8	38
	0.15 M	48	47	5

Scheme 105: Attempted prenylation of 3-arylfuran **145** (product ratios are estimated from NMR spectra of the crude product)

The proton transfer from the furan to the lithiated benzene ring was believed to occur as the reaction warmed to room temperature. Because the trapping with prenyl bromide occurred slowly, it appeared that a more reactive electrophile was required. To this end, the reaction was attempted with 3-methylcrotonaldehyde as the electrophile and the reaction reached completion after one hour at –78 °C affording alcohol **170** in high yield (Scheme 106).



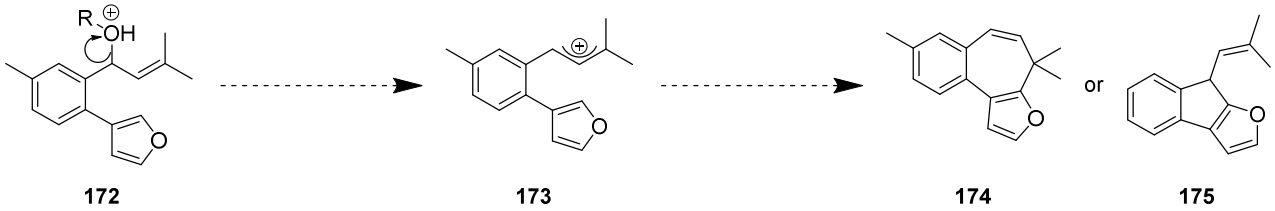
145 **170** **171**

Scheme 106: Conversion of 3-arylfuran **145** into alcohol **170** and formation of diene **171** [key ^{1}H-shifts for diene **171** are shown (400 MHz, $CDCl_3$)]

As well as being more easily accessible than prenylated compound **144**, this alcohol was also expected to be more reactive, potentially offering a more secure route to the desired 7-membered ring. This reactivity was observed when elimination of the alcohol occurred during the preparation of the NMR sample in $CDCl_3$, showing that the alcohol is labile even in mildly acidic conditions.

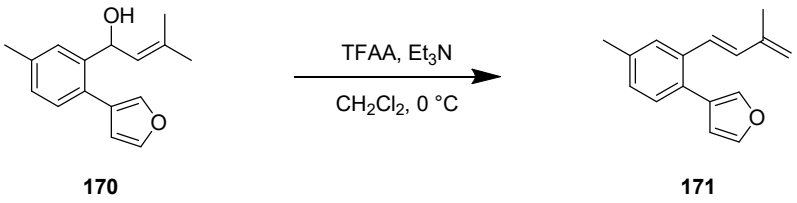
Formation of the Seven-Membered Ring

In view of the lability of the alcohol, as demonstrated by its ready elimination, it was expected that activation of the alcohol by acylation or treatment with a Lewis or Brønsted acid could enable formation of an allyl cation onto which the furan would cyclise to form either a 5- or 7-membered ring (Scheme 107).



Scheme 107: Potential cyclisation pathways for allyl cation **173**

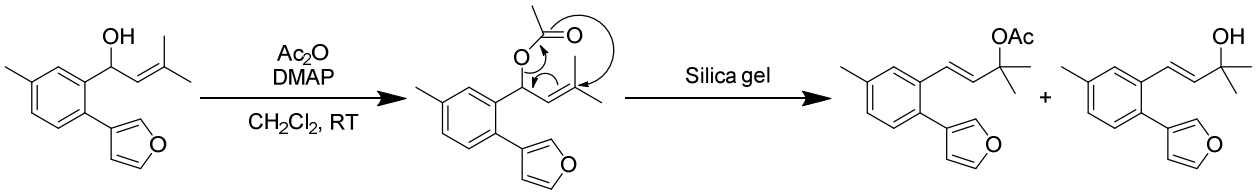
Treatment of the alcohol with trifluoroacetic anhydride (TFAA) or $BF_3{\cdot}OEt_2$ at 0 °C or –78 °C did not appear to effect cyclisation, and the products (which were not identified) were unstable on silica, with NMR spectra of the collected fractions appearing much more complex than those of the crude mixtures. In an effort to negate the potentially detrimental effect of the strongly acidic trifluoroacetic acid [pK_a 0.23 (25 °C, H_2O)] on the reaction, triethylamine was added to the mixture prior to the addition of trifluoroacetic anhydride; this reaction was much cleaner but diene formation was preferred at the expense of cyclisation (Scheme 108).[356] Diene formation also dominated in reactions with ethyl chloroformate or *p*-nitrobenzoyl chloride (which also formed some of the *p*-nitrobenzoate ester; roughly 2:1 diene/ester). When a Brønsted acid, formic acid, was used, a complex mixture of products was obtained; clearly these conditions were too harsh.[357]



170 **171**

Scheme 108: Formation of diene **171** during attempted cyclisation

The only cyclisation attempt that did not result primarily in decomposition or diene formation was when the alcohol was treated with acetic anhydride. In this case, acetylation was successful, but cyclisation did not occur during the reaction. Acetate **176** was observed in the NMR spectrum of the crude product, but it could not be isolated by column chromatography. On silica, the benzylic acetate underwent a [3,3]-sigmatropic rearrangement to afford tertiary acetate **177** and tertiary alcohol **178** by hydrolysis of the acetate (Scheme 109). This rearrangement is well known both thermally and on silica and is believed to be driven by the stability gained from the alkene moving into conjugation with the aromatic ring.[358–361]

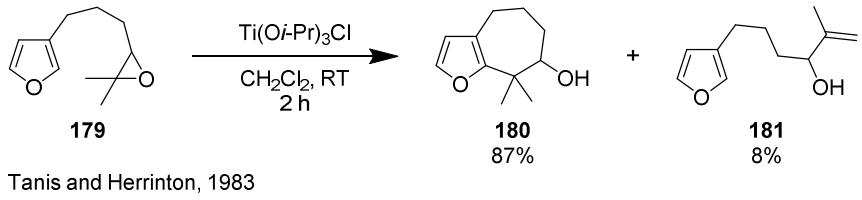


170 **176** **177** 60% **178** 9%

Scheme 109: [3,3]-Sigmatropic rearrangement of benzylic acetate **176**

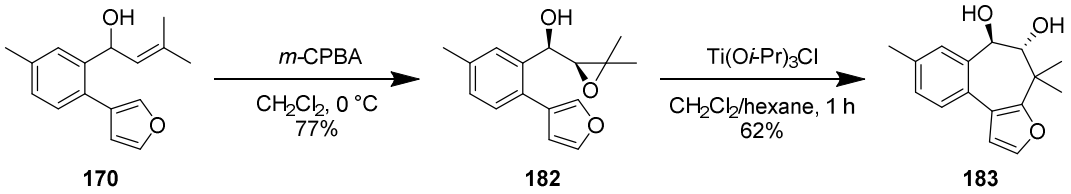
Whilst not the desired product, tertiary acetate **177** was thought to be potentially amenable to cyclisation *via* a Tsuji–Trost reaction mediated by Pd(0) catalysis.[362] Heating the substrate in dichloromethane with a small amount of $PdCl_2$ (20 mol%) afforded only diene **171**, whilst stirring with $PdCl_2$ at room temperature in DMF did not appear to effect any reaction whatsoever.

An alternative approach was sought and attention turned to the precedent of furan cyclisation onto an epoxide, mediated by $Ti(O\textit{i}\text{-}Pr)_3Cl$ (Scheme 110).[363]



Scheme 110: Synthesis of 7-membered ring *via* Ti(O*i*-Pr)$_3$Cl mediated epoxide opening

Application of this method to the formation of the 6,7,5-fused ring system began with synthesis of epoxide **182** with *m*-CPBA, which was highly chemoselective for epoxidation over furan oxidation and diastereoselective (dr>95:5). After column chromatography, epoxide **182** was isolated as a single diastereomer (stereocentre established later in the route) in good yield (Scheme 111). Subjecting this epoxide to the cyclisation conditions provided *anti*-diol **183** in 62% yield; again, the relative stereochemistry was established later in the route (see opposite) (Scheme 112). The stereochemistry of the directed epoxidation is believed to arise from minimisation of $A^{1,3}$-strain in the transition state.

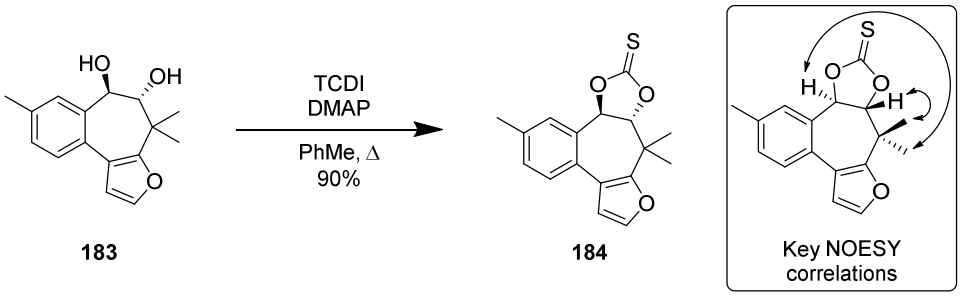


Scheme 111: Synthesis of *anti*-diol **183** *via* Ti(O*i*-Pr)$_3$ mediated intramolecular epoxide opening

The successful cyclisation gave access to the desired tricyclic ring system and deoxygenation of the diol was projected to provide the core framework for submission to biocatalytic oxidation by the library of P450$_{BM3}$ variants.

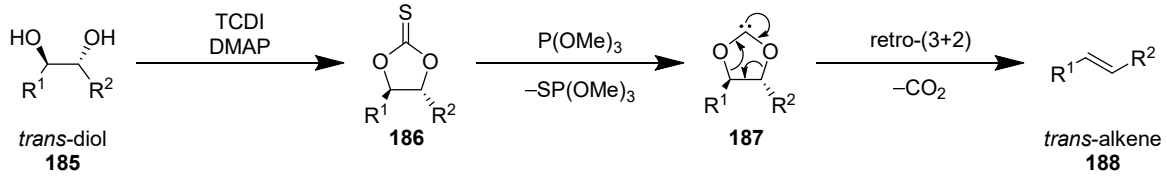
Synthesis of the Core Structure of Trigoxyphin K and L

Before the relative stereochemistry of the diol was known, a Corey–Winter reaction was planned for converting diol **183** into alkene **174**.[364] Reaction of the diol with thiophosgene was low-yielding (11%), but use of 1,1'-thiocarbonyldiimidazole (TCDI) was much more successful with a yield of 90% being obtained (Scheme 112).[365,366] Probing the relative stereochemistry by NOESY (nuclear Overhauser effect spectroscopy) revealed that the two C–O bonds were *anti*- to each other (which informed the stereochemistry of epoxide **182** and diol **183**, see above) (Scheme 112).



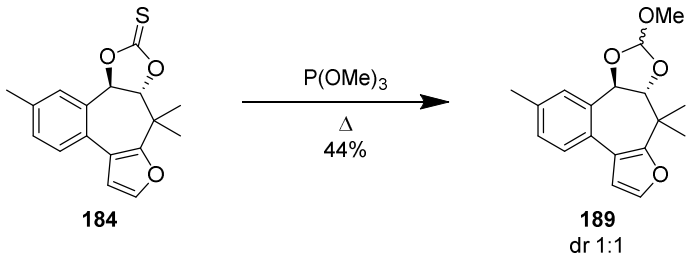
Scheme 112: Synthesis of thiocarbonate **184**

The Corey–Winter reaction is most commonly formulated as the formation of a carbene then concerted extrusion of CO_2 [*via* retro-(3+2)] to provide the alkene (Scheme 113).[364,367] Because of the concerted nature of the mechanism, this reaction is stereospecific and *trans*-diols form *trans*-alkenes, and *cis*-diols form *cis*-alkenes.[364]



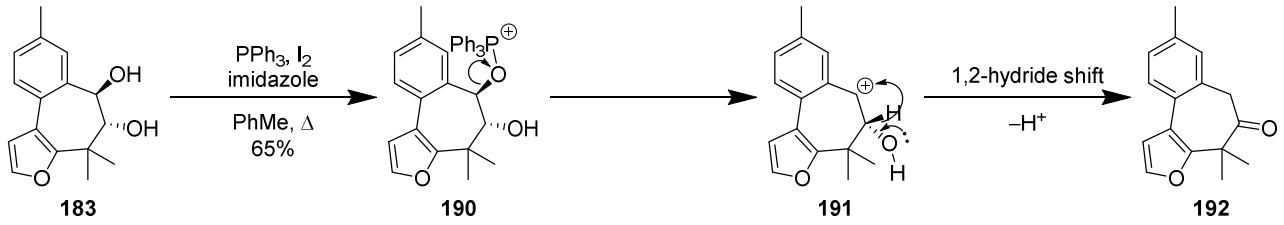
Scheme 113: Mechanism of the Corey-Winter reaction

This suggested that thiocarbonate **184** would form a *trans*-cycloheptene. *trans*-Cycloheptene itself is unstable at room temperature (though it can be formed as an intermediate and trapped), and this system would form a particularly strained cycloheptene with six out of seven atoms in the ring being sp^2-hybridised.[368,369] However, it was thought that, being adjacent to an electron-rich aromatic system, the benzylic oxygen might be sufficiently labile to access a *cis*-cycloheptene by a stepwise mechanism. In practice, however, this did not occur; formation of the carbene was possible upon treatment of thiocarbonate **184** with trimethylphosphite, but the retro-(3+2) cycloaddition did not proceed. Instead of extruding CO_2, the carbene inserted into the O–H bond of methanol (presumably present as an impurity in trimethylphosphite) to afford orthoester **189** as a mixture of diastereomers (Scheme 114).



Scheme 114: Attempted Corey–Winter olefination of thiocarbonate **184**

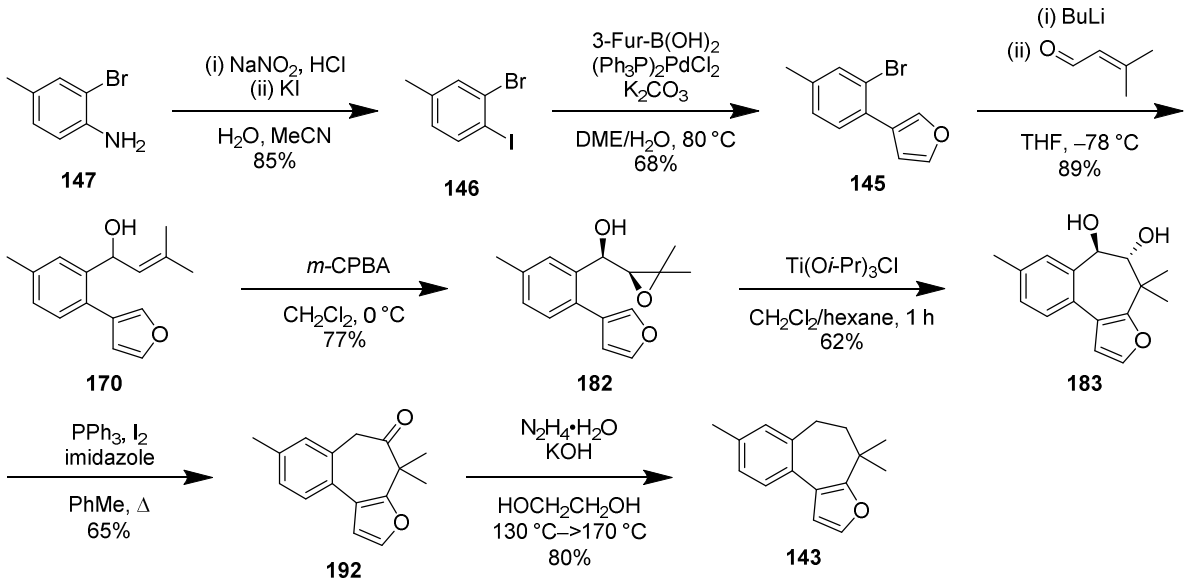
An alternative to the Corey–Winter reaction is the Garegg–Samuelsson olefination, which employs triphenylphosphine, imidazole, and I_2 to effect substitution of both alcohols by iodide (with loss of triphenylphosphine oxide) and elimination to form the alkene.[370] When diol **183** was submitted to these conditions ketone **192** was obtained *via* [1,2]-hydride shift to quench benzylic cation **191** (Scheme 115). Formation of ketones (presumably by this mechanism) has been reported previously under these conditions.[371]



Scheme 115: Synthesis of ketone **192** under Garegg–Samuelsson conditions

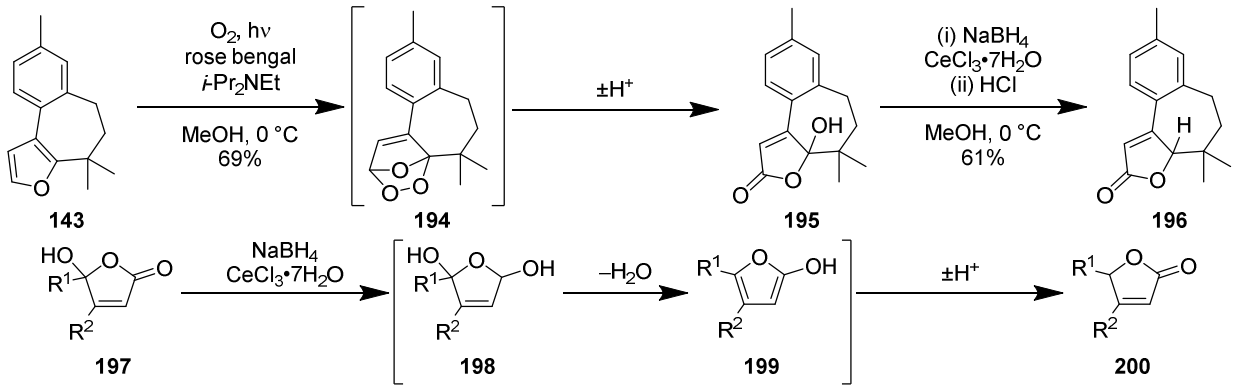
Complete reduction of the carbonyl group in ketone **192** would provide the core structure of trigoxyphin K and L, with oxidations being the only steps required to reach the natural products. The obvious choice for deoxygenation, Wolff–Kishner reduction, was successful, with hydrazone formation occurring smoothly and the only hindrance being consistently maintaining the requisite temperature (>170 °C) for a sufficiently long enough period for base-induced loss of N_2 to proceed. This approach made synthesis of unfunctionalised core structure **143** possible in good yield without requirement of column chromatography (Scheme 116).[372,373]

3. Efforts Toward the Synthesis of Trigoxyphin K and L



Scheme 116: Synthesis of tricycle **143** (seven steps, 13% total yield)

It was decided that tricycle **143** would be screened against the library of $P450_{BM3}$ variants (as would ketone **192**); however, it also provided a good test substrate for the necessary furan oxidation (and subsequent γ-hydroxy-butenolide reduction), the products of which would be des-oxy variants of trigoxyphin L and K, respectively, and could also be subjected to screening. To achieve this, tricycle **143** was subjected to singlet-oxygen oxidation with rose Bengal as sensitiser, supplying γ-hydroxy-butenolide (des-oxy-trigoxyphin K) **195** in good yield (Scheme 117). Luche reduction of this compound and acidic hydrolysis afforded butenolide **196** (des-oxy-trigoxyphin L) in fair yield, following the pathway outlined in Scheme 117.



Scheme 117: Singlet oxygen oxidation of furan **143** and formation of butenolide **196**

With the synthesis of the core structures of trigoxyphin L and K successfully completed in 8 steps, 9% overall yield and 9 steps, 6% overall yield, respectively, all that remained was the

biocatalytic installation of the phenolic hydroxyl group, which was intended to be performed by a member of the library of $P450_{BM3}$ variants.

Screening against library of $P450_{BM3}$ variants

Two compounds were selected for screening against the mutant library, ketone **192** and tricycle **143**; this was primarily due to the quantity of compound available, and that the product of their oxidation could be a precursor to both natural products. Both substrates, though highly insoluble in the reaction medium, were good substrates for the enzymes, with conversions of up to 95% and 89% achievable, respectively.

For ketone **192**, the variants which gave the highest conversion all contained the R47L/Y51F pair of mutations with RP/F81W, RP/H171L/I263G, and RLYF/KSK19 all giving greater than 90% conversion, with up to four products being formed. Subjecting ketone **192** to a scale-up reaction with RP/H171L/I263G effected turnover of starting material; however, it was not possible to isolate the products formed by column chromatography. The NMR spectrum of the crude product suggested that the furan ring was still intact and that, for at least one of the products, the protons on the aromatic ring were still present, implying that oxidation was taking place in another part of the molecule. Ultimately, the complexity of the NMR spectrum, and the loss of products during column chromatography (potentially due to decomposition) made identification of the oxidation products impossible.

For tricycle **143**, the highest conversion was found generally (but not exclusively) with variants based on the KSK19 mutant (F87A/H171L/Q307H/N319Y) with KSK19/AI/Y51L, KSK19/AI/AI, KSK19/AI/F87V, and the non-KSK19 containing RP/FV/EV/L437VL all giving between 80–90% conversion. Turnover was generally selective for one product (retention time 7.90) and, in an effort to identify the product, samples for all mutants which were selective for this product and gave reasonable conversion were combined and extracted with ethyl acetate. The NMR spectrum of this crude product suggested that the furan had been oxidised (as evidenced by the disappearance of the

furan peaks). Not all mutants were selective for this product; for example, RP/IA/EV was selective for products with retention times 4.99 and 6.73, although overall conversion was just 30% (which could probably be improved upon by increasing the relative amount of the enzyme). The earlier product (retention time 4.99) has a very similar retention time to ketone **192** suggesting that it is being formed by this mutant.

A scale-up reaction was attempted with this substrate and $P450_{BM3}$ mutant RT2/IP (which gave 55% conversion to a 73:27 mixture of this product and a product with retention time 6.73); however, no information could be gained from the NMR spectrum due to the presence of large amounts of dimethylsulfoxide (used as a co-solvent for the substrate), and no products were retrieved from column chromatography. GC traces for selected mutants from the screening of both compounds are displayed in Figure 32.

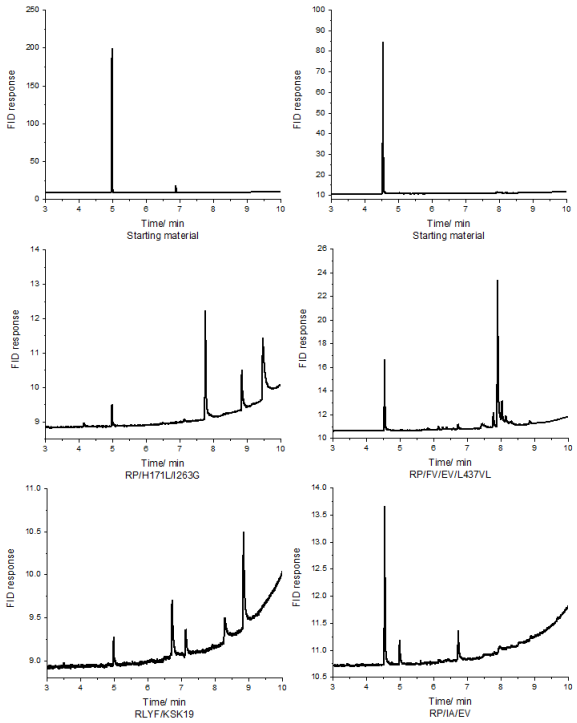


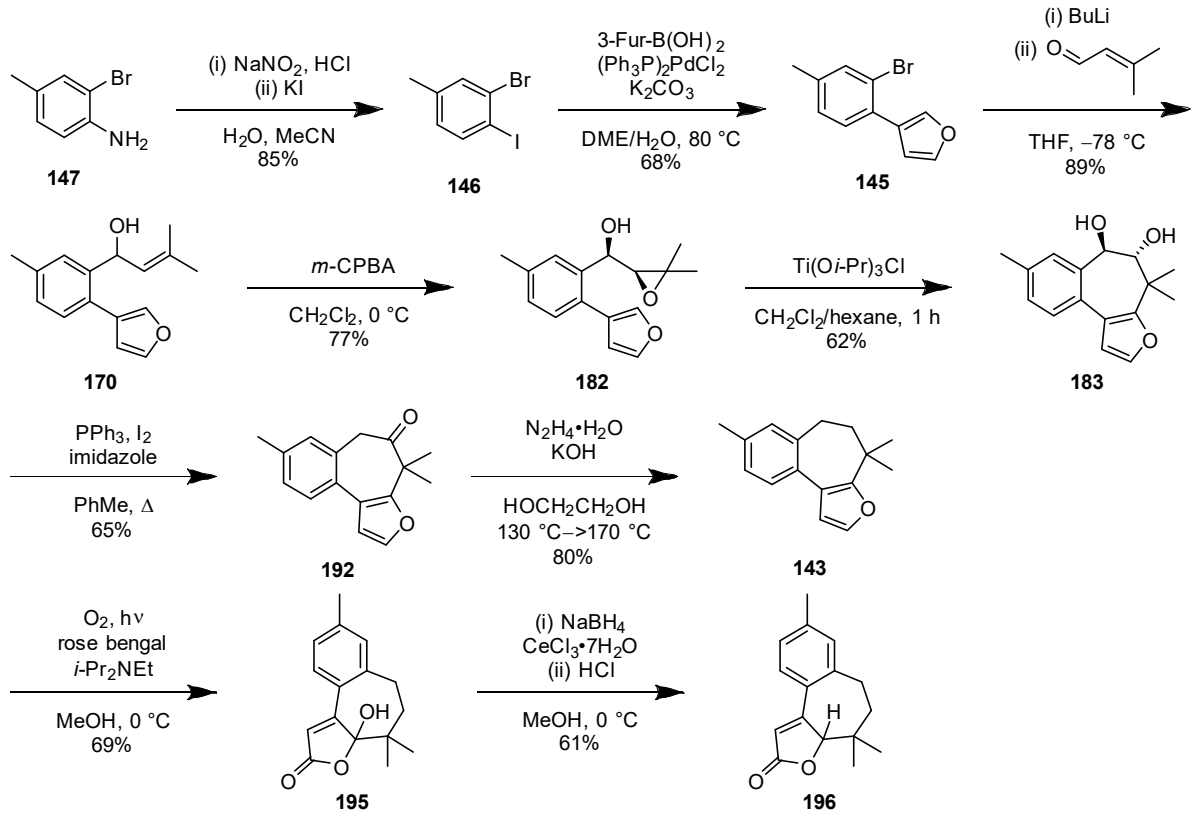
Figure 32: GC traces from screening of ketone **192** (left) and tricycle **143** (right)

Whilst a mutant has not been found for the installation of the phenolic hydroxyl group, there are numerous products formed in the screens of ketone **192** and tricycle **143** that have not been identified and could possibly be arising from the desired hydroxylation. Production of more material would have enabled a more thorough investigation of the identity of these products and may have

made completion of the total synthesis possible (if a mutant was found that catalysed the desired hydroxylation). Directed evolution or the use of a larger library of mutants (the Wong group has >200) could identify greater selectivity for these products. If a mutant cannot be found to catalyse the necessary hydroxylation, other oxidases could be screened to effect this transformation (see introduction). Hydroxylation at other positions may not be desired for the synthesis of trigoxyphin K and L but could facilitate the synthesis of isomers or analogues which may offer different and interesting biological activity.

Conclusions

In summary, a short route has been developed for the synthesis of the core-framework of trigoxyphin K and L and the late stage oxidation for completion of the synthesis has been investigated for two possible precursors. γ-Hydroxy-butenolide **195** or butenolide **196** could also be screened with the library of $P450_{BM3}$ variants to make the natural products available with the synthesis being completed with installation of the phenolic hydroxyl group. Success in this endeavour, currently being actively pursued in the group, would demonstrate the power of these enzymes for application to late-stage functionalisation in total synthesis.



Scheme 118: Summary of synthesis of trigoxyphin K and L core structures

Chapter 4: Screening of Miscellaneous Compounds

Background and Aims

When formulating a biocidal product (herbicide, fungicide, pesticide), intended for use in areas where humans could be exposed to it, it is crucial that its effects on human health are known. As these products are expected to be metabolised by the organism they are applied to and targeted against, it is also important that the major metabolites are identified and their effects on human health are known. The synthesis of these metabolites is required in the necessary amounts to examine their toxicity. Their synthesis is not always readily achievable by traditional chemical means and their isolation from the organism metabolising the biocide is also not always a practical method for their isolation in quantity.

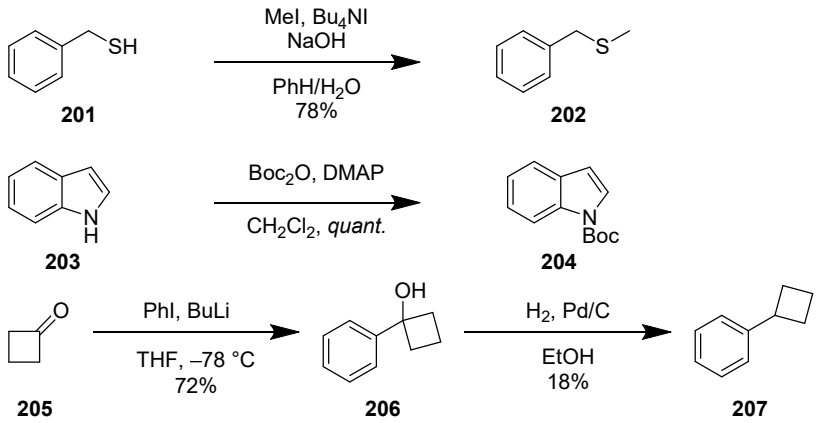
Cytochrome P450 enzymes are important for the phase 1 metabolism of a wide range of herbicides based on a variety of different structural motifs and bearing various functional groups.[374] It was expected that the library of $P450_{BM3}$ mutants could demonstrate use in the synthesis of useful amounts of herbicide metabolites for investigation of their toxicity. Screening of herbicides with the mutant $P450_{BM3}$ library was also expected to provide access to non-natural metabolites which may demonstrate herbicidal activity of their own, possibly greater than that of the parent herbicide.

Finally, screening the mutant library against a structurally diverse set of herbicides and other small molecules was expected to provide information about how the enzymes tolerate or behave towards certain functional groups for optimisation of the library for use with certain motif. Such a study was also expected to lead to the discovery of interesting reactivity, upon which more general procedures could be built (*cf*. the development of the oxidative cyclisation discussed in Chapter 1).

Substrate Synthesis

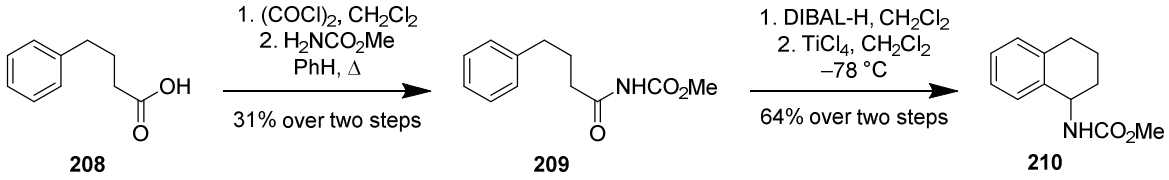
The herbicides screened were commercially available (or provided by Syngenta); however, a number of small molecules for screening required synthesis. In some cases the synthesis was

straightforward; this was the case for benzyl methyl sulfide **202**, *N*-Boc-indole **204**, and cyclobutylbenzene **207** (Scheme 119).



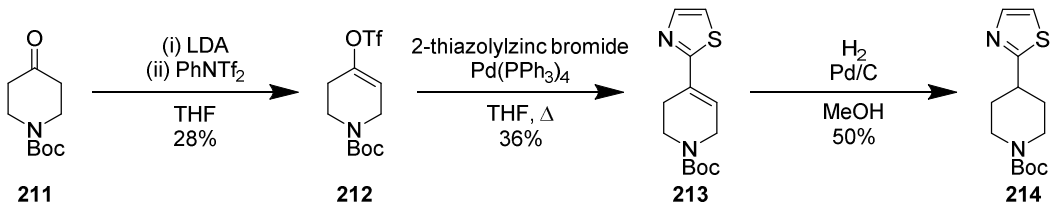
Scheme 119: Synthesis of benzyl methyl sulfide **202**, *N*-Boc-indole **204**, and cyclobutylbenzene **207**

The other small molecules under investigation required slightly more complex syntheses; for example, *N*-tetrahydronaphthalen-1-yl carbamate **210** was prepared in four steps, with the synthesis finished by a Lewis acid mediated Vilsmeier reaction (Scheme 120).



Scheme 120: Synthesis of *N*-tetrahydronaphthalen-1-yl carbamate **210**

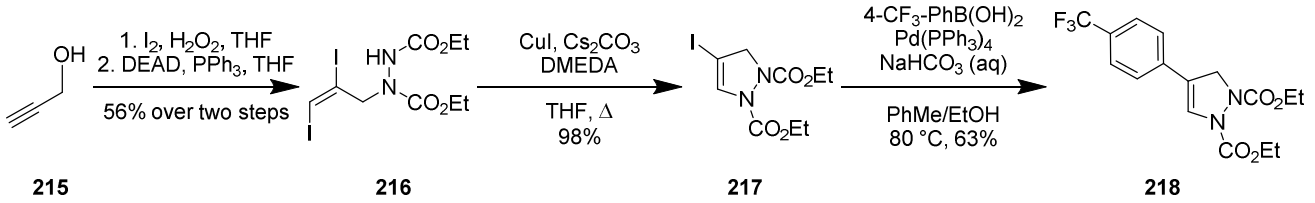
Another substrate, *N*-Boc-4-(thiazol-2-yl)piperidine, was synthesised in three steps from *N*-Boc-piperidin-4-one **211** with a Negishi coupling featuring as the key step; addition/elimination with 2-thiazolyllithium was also possible but gave much poorer yields (Scheme 121).



Scheme 121: Synthesis of 2-substituted thiazole **214**

Finally, dihydropyrazole **218** was accessible in four steps with key steps including Cu-catalysed intramolecular coupling and a Suzuki coupling (Scheme 122).

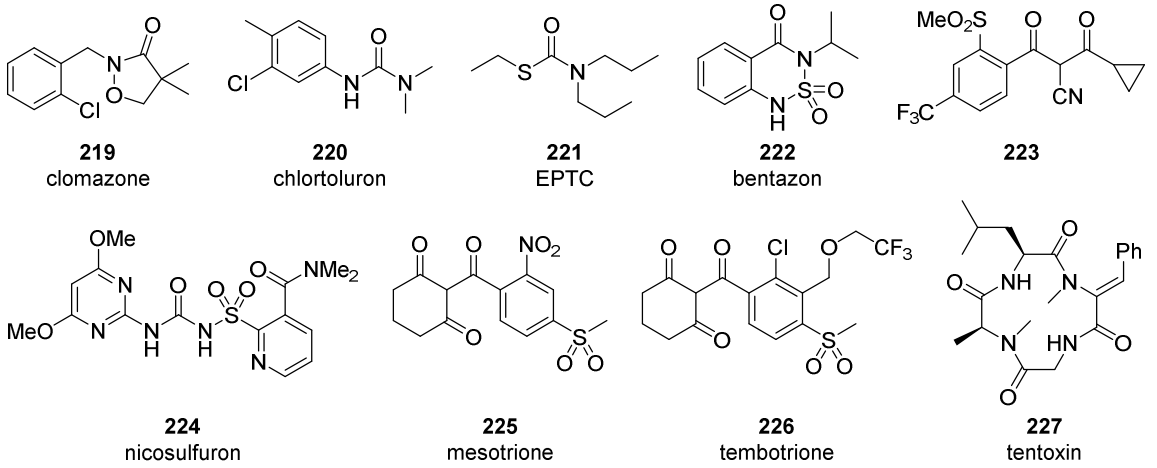
4. Screening of Miscellaneous Compounds



215 **216** **217** **218**

Scheme 122: Synthesis of pyrazole **218** *via* intramolecular C-N coupling

The synthetic accessibility of the aforementioned small molecules and the availability of the herbicides shown in Figure 33 meant that a diverse array of compounds bearing a range of different functional groups were now at hand for screening against the library of $P450_{BM3}$ variants.



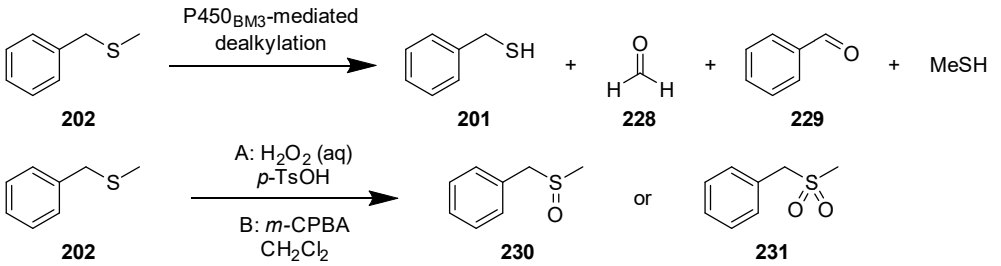
219 clomazone
220 chlortoluron
221 EPTC
222 bentazon
223
224 nicosulfuron
225 mesotrione
226 tembotrione
227 tentoxin

Figure 33: Herbicides to be screened against the library of mutant $P450_{BM3}$ variants

Substrate Screening

Small Molecules

Benzyl methyl sulfide **202** was an excellent substrate for $P450_{BM3}$-catalysed oxidation with seemingly high levels of conversion found for the majority of mutants, though accurate calculation of conversion was complicated by overlap with solvent peaks. It was suspected that dealkylation of either the benzyl or methyl group was a likely oxidation pathway (Scheme 123). Commercially available benzyl mercaptan and benzaldehyde were used as standards for GC analysis to ascertain if either dealkylation pathway was being followed. Oxidation of sulfur was another expected outcome of the oxidation so sulfoxide **230** and sulfone **231** were synthesised for use as GC standards (Scheme 123).



Scheme 123: Potential products of dealkylation or sulfoxidation of benzyl methyl sulfide **202**

Comparison of the commercially available and synthetic standards with the GC traces of the screens showed that these products were not formed in the screen, suggesting oxidation at other positions (Figure 34).

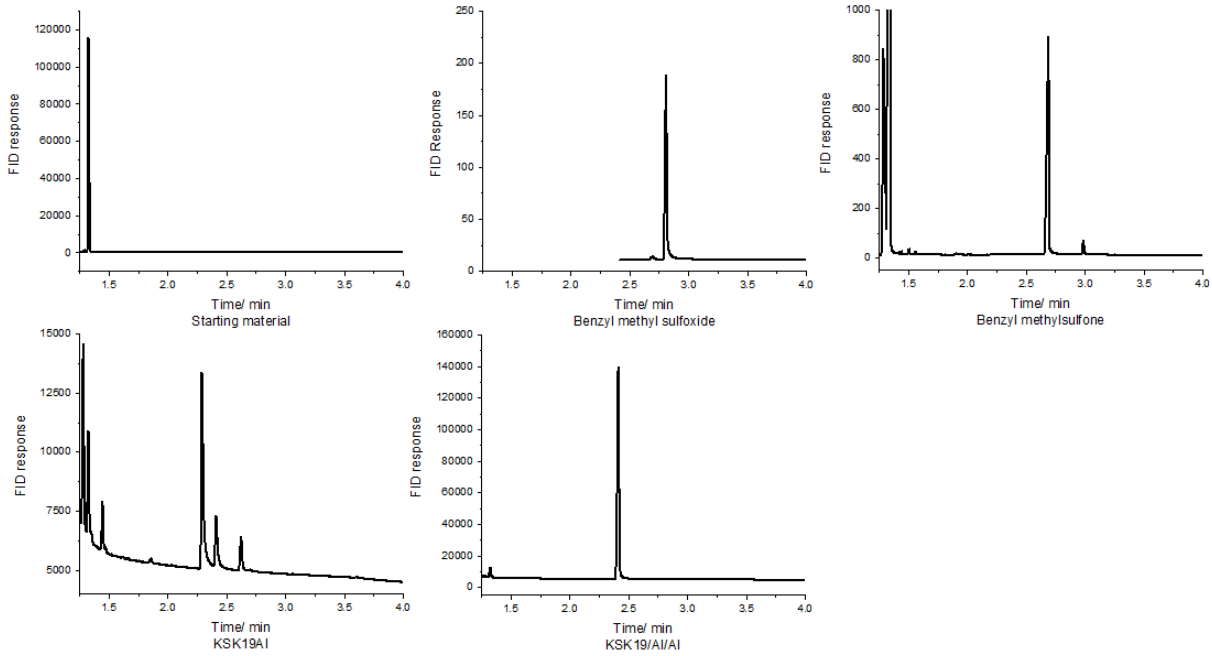


Figure 34: GC traces for oxidation of benzyl methyl sulfide **202** with the mutant $P450_{BM3}$ library

Cyclobutylbenzene also proved to be a good substrate with every mutant screened affording complete conversion of starting material. The major product was the synthetic precursor to the screening substrate, 1-phenylcyclobutan-1-ol **206**; high levels of selectivity were possible for this product whereas, for the formation of other products, very little selectivity was seen with GC traces typically being quite complex (Figure 35). The poorest selectivity (both for benzylic alcohol **206** and in general for other products) was typically seen with variants containing the mutations R47L/Y51F, a pair which has been shown to increase activity of $P450_{BM3}$ towards non-polar substrates.[180–184,218]

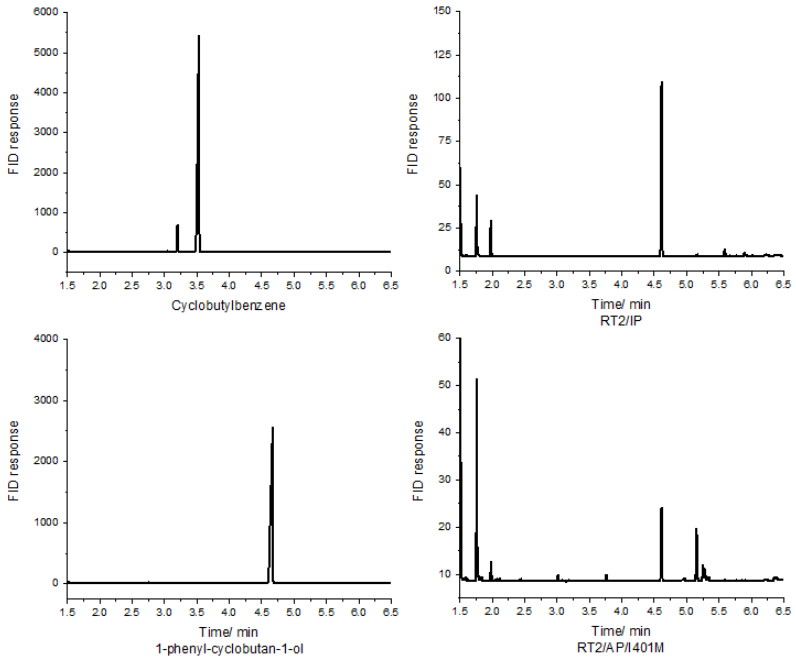


Figure 35: GC traces for oxidation of cyclobutylbenzene **207** with the mutant $P450_{BM3}$ library

Thiazole **214** and pyrazole **218** were both poor substrates, with no conversion observed for the majority of mutants. In the screening of thiazole **214**, only four mutants gave any conversion whatsoever, with two of those mutants affording <10% conversion. Two mutants (KSK19 and RP/FV/EV) gave 61% and 33% conversion, respectively, to mixtures of products; MS analysis of the screening samples suggested products of mass SM–2 ($–H_2$), SM+14 (+O, $–H_2$), and SM+16 (+O) were being formed (with little selectivity for any product in particular); none of these products was isolated (Figure 36).

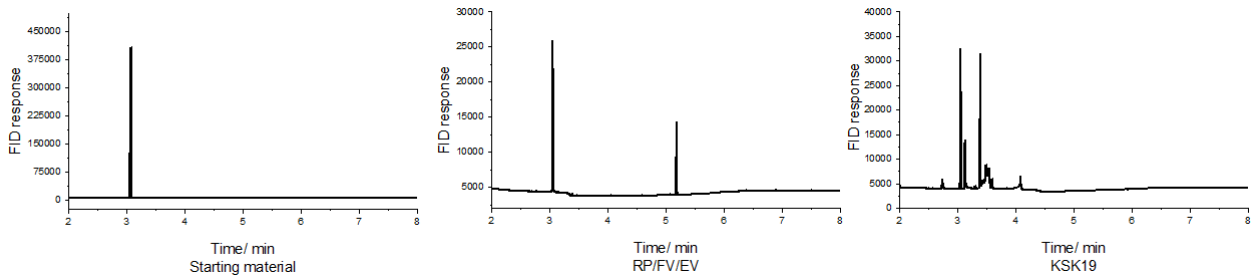
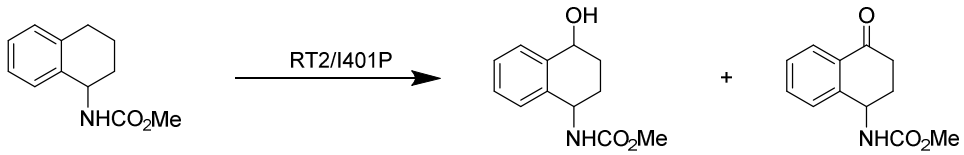


Figure 36: GC traces for oxidation of thiazole **214** with the mutant $P450_{BM3}$ library

Analysis of the screening reactions of pyrazole **218** proved difficult, with no products visible by GC, just a reduction (partial or complete) in the intensity of the starting material peak. It is possible that this arises due to formation of an acidic product, likely by dealkylation or hydrolysis of

the enamine carbamate which remains in the basic aqueous layer rather than being extracted into the organic solvent.

Oxidation of *N*-tetrahydronaphthalen-1-yl carbamate **210** proceeded with up to 100% conversion; variants based on KSK19 (F87A/H171L/Q307H/N319Y), RP (R47L/Y51F/I401P), and KT2 (A191T/N239H/I259V/A276T/L353I) gave >90% conversion, with no specific mutation or combination of mutations proving to be particularly effective for boosting activity towards this substrate. Two products were isolated from scale-up of the oxidation of this substrate with RT2/IP (100% conversion, TTN 2000). Benzylic hydroxylation product **232**, as a mixture of diastereomers, and benzylic ketone **233**, formed by oxidation of alcohol **232**, were isolated from the reaction in 59% and 24% yields, respectively (Scheme 124).



210 **232** 59% **233** 24%

Scheme 124: Oxidation of carbamate **210**

Ketone **233** and alcohol **232** were usually obtained as a mixture with selectivity favouring alcohol **232** (ranging from 13:87 to 50:50 ketone **233**/alcohol **232**); however, with mutant RP/FV/EV/L437LV, 100% selectivity was seen for alcohol **232** (96:4 dr) albeit in conversion of only 26%. One diastereomer was formed as the major component for all mutants with 14 out of 21 giving >95:1 selectivity (major:minor) with dr of >99:1 accessible for six mutants; it is not known if this is the *cis*- or *trans*-isomer. Another, typically minor, product was observed, which appeared to be a product of hydroxylation at another position on the ring (again forming a mixture of diastereomers); however, its separation from benzylic alcohol **232** was not possible, and no mutants screened proved to be solely selective for its formation, and isolation in pure form was not possible. Mutant KSK19/AI was the only variant which favoured formation of this product over ketone **233** and alcohol **232**, with a product ratio of 60:25:15 (unidentified product/alcohol **232**/ketone **233**).

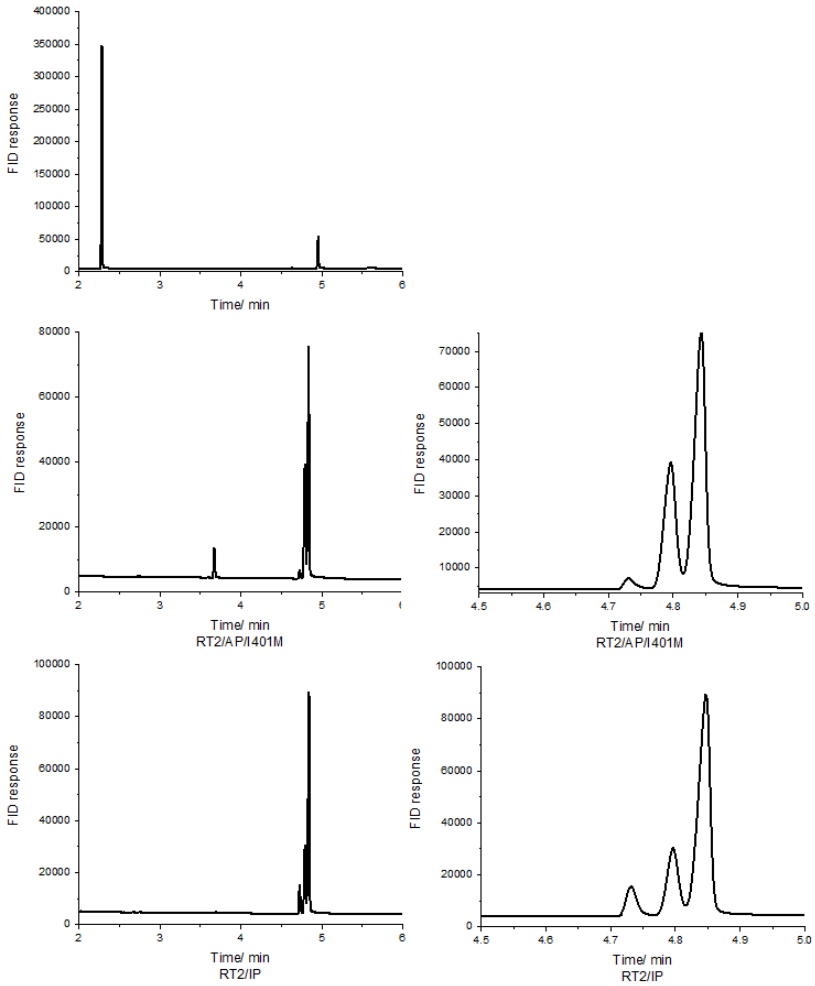


Figure 37: GC traces for oxidation of carbamate **210** with the mutant $P450_{BM3}$ library

The products are formed first by hydroxylation of substrate **234** in the typical fashion (likely hydrogen atom abstraction and radical rebound) to form benzylic alcohol **235**; repetition of this process forms geminal diol (hydrate) **236**, which collapses to produce ketone **237** (Scheme 125). Use of ^{18}O-labelled alcohols has provided evidence for this mechanism.[375]



234 **235** **236** **237**

Scheme 125: Mechanism for formation of ketone **237**

The last of the synthesised small molecules to be screened was *N*-Boc-indole **204**, for which the screen analysis was carried out by liquid chromatography-mass spectrometry (LCMS). This substrate was readily accepted for turnover by a variety of mutants, with complete consumption of

starting material found for four mutants. The best mutants were typically those which contained the mutation pair R47L/Y51F although 100% conversion was also seen for mutant KSK19. Mutant RP/FV appeared to be more selective than the other variants that gave high conversion, and was therefore employed in a scale-up reaction.

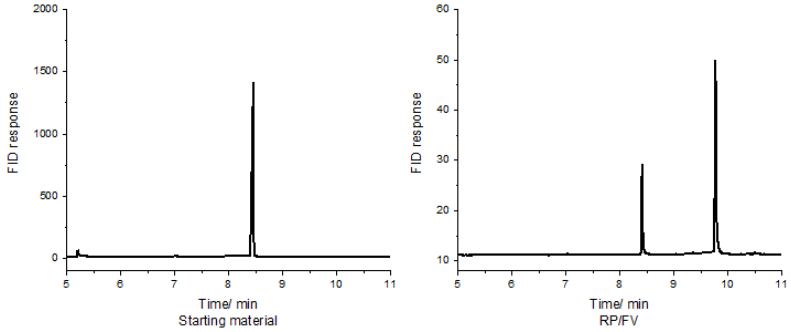
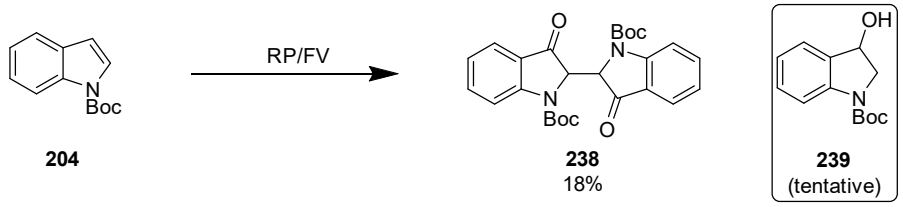


Figure 38: GC traces for oxidation of *N*-Boc-indole **204** with the mutant $P450_{BM3}$ library

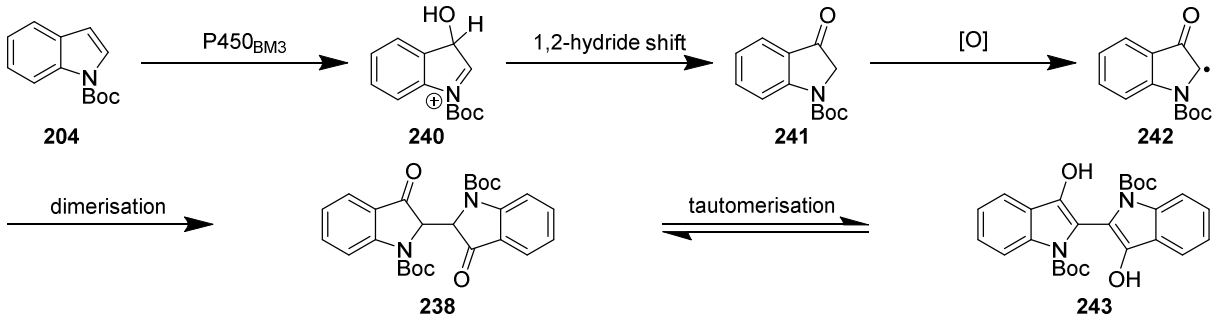
Two products were observed from scale-up, a monomeric and a dimeric oxidation product. The monomeric product, which was suggested to be indolin-3-ol **239** by MS and NMR analysis, could not be isolated in pure form, and its formation suffered from irreproducibility. Dimeric product **238** was formed as the major product on scale-up, isolated in 18% yield (Scheme 126).



Scheme 126: Mutant $P450_{BM3}$-mediated oxidation of *N*-Boc-indole **204**

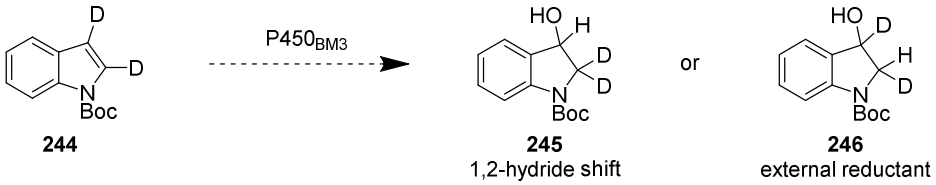
Both products are thought to be formed from a common intermediate, *N*-Boc-indolin-3-one **241**. This product could be formed by oxidation of the indole ring followed by subsequent 1,2-hydride shift in iminium intermediate **240** (Scheme 127). From this point, ketone **241** can be reduced (either by $P450_{BM3}$ or GDH) to form alcohol **239** or undergo an oxidative dimerisation (potentially non-enzymatic) to form dimer **238** which likely exists in equilibrium with its tautomer, biindole **243** (Scheme 127).[376]

4. Screening of Miscellaneous Compounds



Scheme 127: Mechanism for formation of dimer **238**

Whilst it is possible that alcohol **239** is formed from ketone **241** by $P450_{BM3}$ or GDH mediated reduction, it is also plausible that for proposed intermediate, iminium **240**, to be reduced by an external reductant. By use of NADPH itself rather than the regeneration system, it should be possible to determine whether $P450_{BM3}$ or GDH is responsible for the reduction (if it proceeds *via* ketone **241**). This was attempted on an analytical scale, but very little conversion (<5%) was seen, suggesting that turnover is significantly decoupled from NADPH consumption. It is also possible that the partially purified $P450_{BM3}$ mutant contained an alcohol dehydrogenase or ketoreductase contaminant which catalysed the reduction. Which of ketone **241** or iminium **240** becomes reduced to alcohol **239** may be determined by deuterium labelling at C-2 and C-3; the location of the deuterium atoms in the product would confirm whether a 1,2-hydride shift had taken place or if an external reductant was involved (Scheme 128).

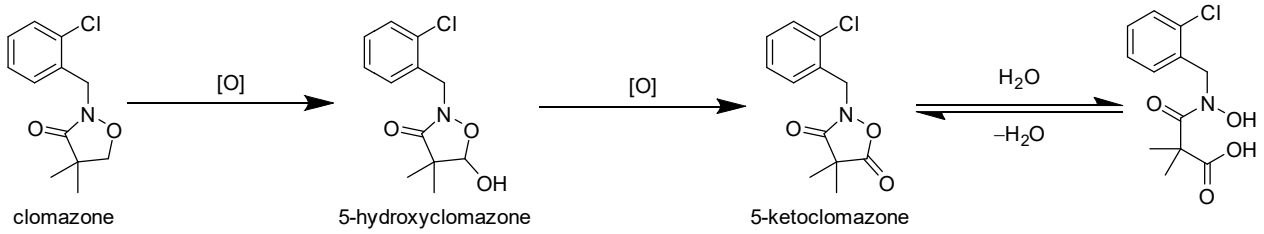


Scheme 128: Potential outcomes of deuterium-labelling experiment

Commercially-Available Herbicides

Clomazone is a proherbicide used for protection against a variety of species of annual broad-leafed and grassy weeds.[377] As a proherbicide, it is inactive in its standard form and requires metabolism to 5-ketoclomazone, which exists in an open- and closed-ring form, with 5-hydroxyclomazone as an intermediate (Scheme 129).[378] Other, non-activating, metabolic products

include arene hydroxylation, benzylic hydroxylation (and dealkylation), and methyl hydroxylation, believed to be mediated by P450s.[374,377–380]



Scheme 129: Formation of active metabolite 5-ketoclomazone

When screened against the library of $P450_{BM3}$ variants, conversion up to 100% was possible, with very good selectivity for a single product found for the majority of mutants. Mutants which afforded the highest conversions (>95%) were based on the KSK19 (F87A/H171L/Q307H/N319Y) set of mutations, or mutant GVQ (A74G/F87V/L188Q).[381]

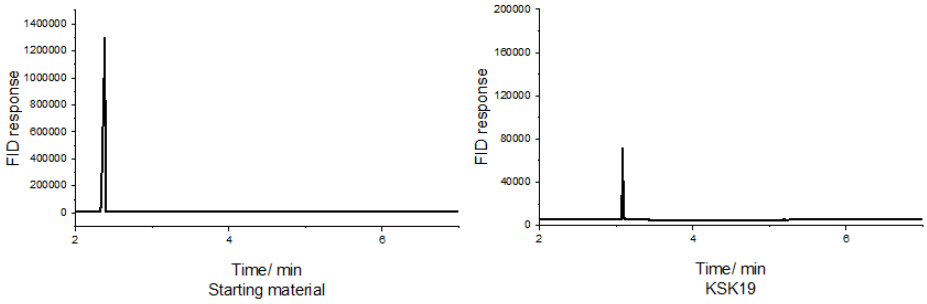
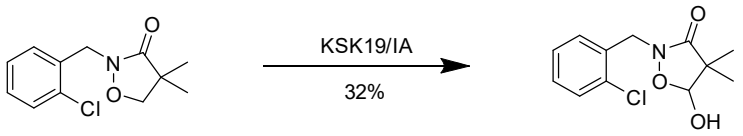


Figure 39: GC traces for oxidation of clomazone with the mutant $P450_{BM3}$ library

The mutation A328I was particularly detrimental to conversion of this substrate, exemplified by the conversions observed for KSK19 (100%) and KSK19/A328I (8%); this dramatic loss of conversion is likely due to the increase in bulk at a residue directly above the haem unit, preventing the substrate getting close enough to the iron centre, or inhibiting binding.[382] By scale-up reaction with KSK19/IA the product was identified as 5-hydroxyclomazone, which was isolated in 32% yield (Scheme 130). This product was used as a substrate for further screening against the library of $P450_{BM3}$ mutants, with turnover effected by seven (out of 24) mutants, giving conversion up to 44% with five different products formed. MS analyses of the crude extracts suggest that 5-ketoclomazone was one of the products formed (with the mass of the open-ring form visible) as well as mono-hydroxylated products (shown by the presence of peaks with mass SM+16).

4. Screening of Miscellaneous Compounds



219
clomazone

247
5-hydroxyclomazone

Scheme 130: Synthesis of 5-hydroxyclomazone **219** with KSK19/IA

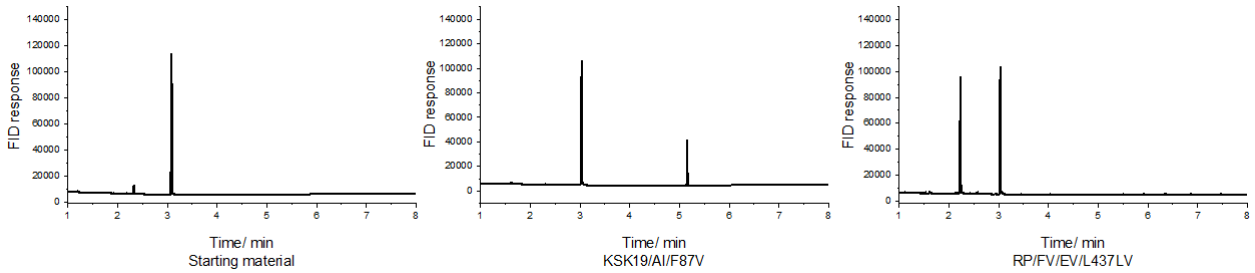
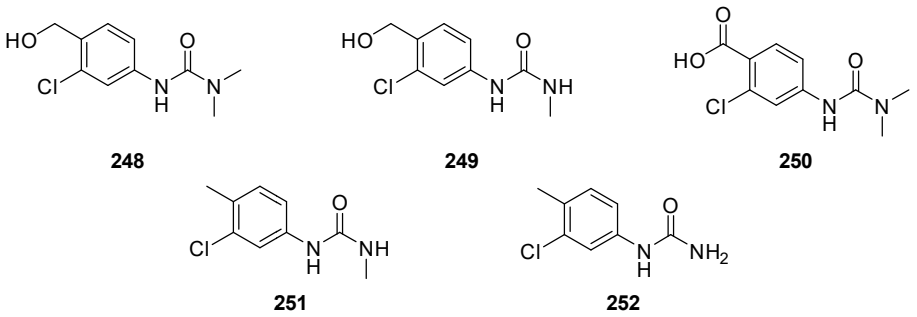


Figure 40: GC traces for the oxidation of 5-hydroxyclomazone **247** with the library of $P450_{BM3}$ mutants

Chlortoluron is a herbicide used for selective weed control in cereals crops; it acts by inhibiting photosynthetic electron transfer in photosystem II.[383,384] The major metabolites of chlortoluron in mammals and plants arise from benzylic hydroxylation, *N*-mono-demethylation, and *N*-di-demethylation, with the minor metabolites being formed by further oxidations or combinations thereof (Figure 51).[374,383–386] Benzylic hydroxylation is believed to confer resistance to plants.[386]

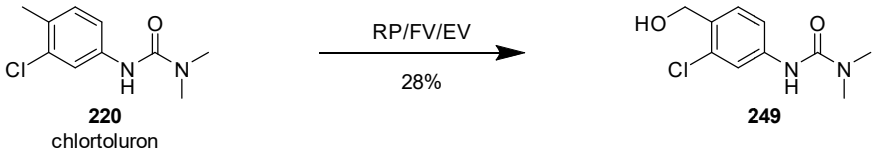


248 **249** **250**

251 **252**

Figure 41: Natural metabolites of chlortoluron

In the first screens against the library of $P450_{BM3}$ mutants, with substrate concentration of 2 mM and enzyme concentration of 1 µM, chlortoluron performed well with conversions up to 86%; increasing the enzyme concentration to 2 µM facilitated complete conversion of substrate. The highest conversion was found for mutants containing the mutations R47L/Y51F with very few of the other mutants effecting any turnover. Mutants appeared to give mixtures of products by GC analysis although, on scale-up, only one product was isolated. Oxidation with mutant RP/FV/EV facilitated synthesis of benzylic alcohol **249** in 28% yield (Scheme 131).



Scheme 131: Oxidation of chlortoluron **220** with RP/FV/EV

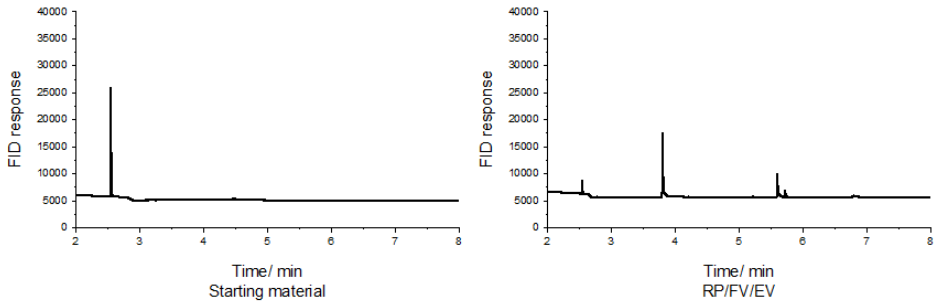


Figure 42: GC traces for the oxidation of chlortoluron by mutant RP/FV/EV

S-Ethyl dipropylcarbamothioate (EPTC) is a pre-emergence herbicide for control of monocot weeds in corn which requires combination with chemical safeners such as 1,2-naphthalic anhydride or biosafeners (bacterial strains) to protect the crop against the phytotoxicity of EPTC.[387] Metabolism of EPTC occurs by hydroxylation at every position, and *S*-oxidation.[374,387–389] *S*-oxidation (forming sulfoxide **253** and sulfone **254**) is the most important pathway, followed by hydroxylation α- to the heteroatoms with subsequent dealkylation; minor pathways derive from hydroxylation at other positions (Figure 43).[388]

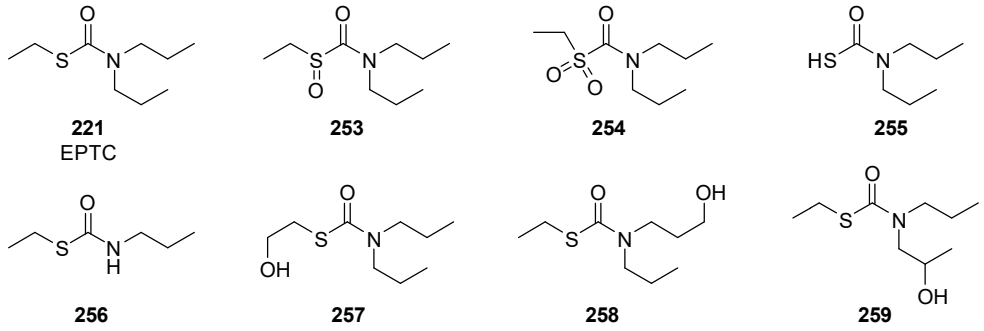


Figure 43: EPTC and the natural metabolites of EPTC

EPTC was a very good substrate for the library of $P450_{BM3}$ mutants, with 100% conversion achieved for 10 of the 21 mutants screened. For this substrate, mutants based on the mutations KSK19 performed very well with six (out of seven) mutants based on KSK19 affording conversion of 100%.

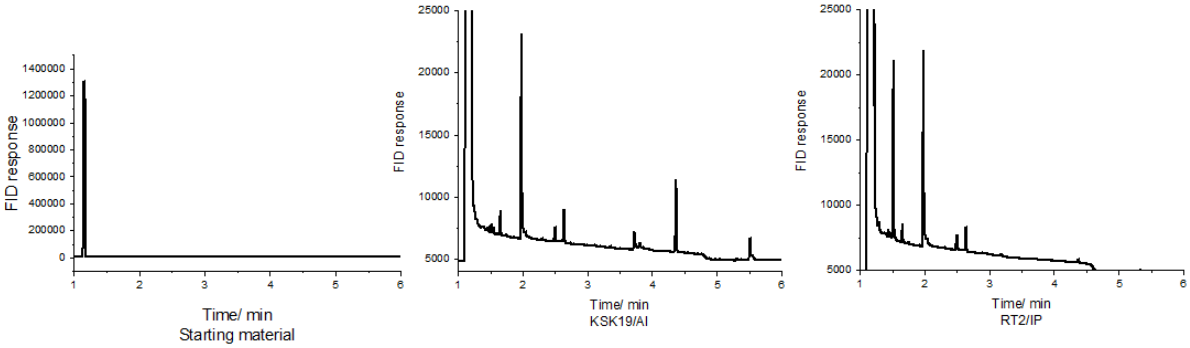
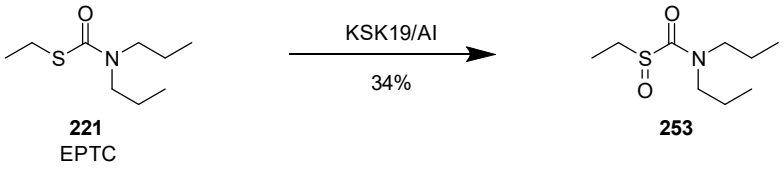


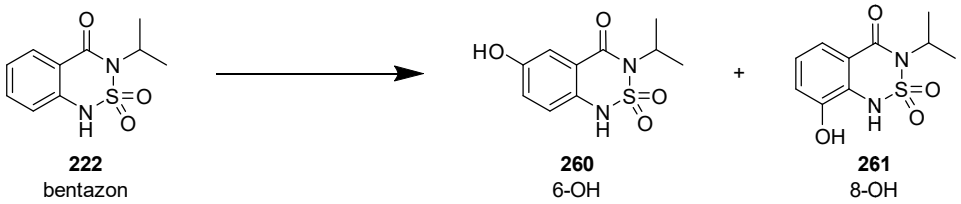
Figure 44: GC traces for oxidation of EPTC by the library of $P450_{BM3}$ mutants

The seventh KSK19-based mutant, KSK19/I263A performed very poorly with conversion of only 9%; the only difference between this mutant and its parent (KSK19), which gave full conversion, is the mutation I263A which increases the space around the haem centre, potentially resulting in loose binding of EPTC.[227] The major product (retention time 1.98) formed by most mutants, the only isolated product, was sulfoxide **253**, which was prepared in 34% yield by scale-up with KSK19/A328I (100% conversion, TTN 2000) (Scheme 132).



Scheme 132: Oxidation of EPTC by KSK19/AI

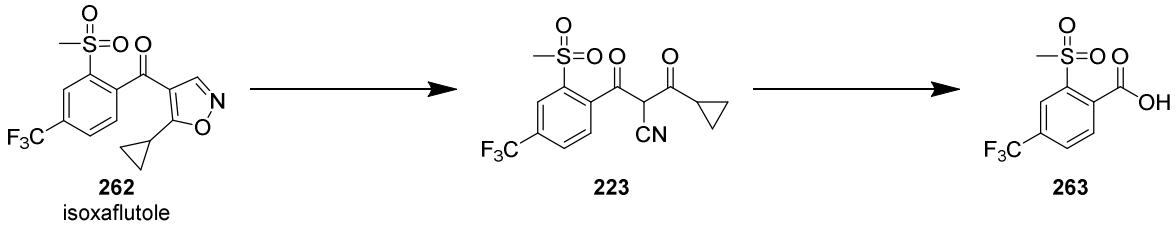
Bentazon is a post-emergence herbicide which has been employed for selective weed control in soybeans, cereals, and rice; its herbicidal activity is believed to arise from inhibition of photosynthesis.[390] The major metabolites of bentazon in soybean and rice arise from hydroxylation of the aromatic ring at the 6- and 8- positions; these phenols are then *O*-glucosylated in phase II metabolism (Scheme 133).[390–392]



Scheme 133: Metabolism of bentazon

Whilst bentazon appeared to be a good substrate on analytical scale, with conversion up to 87% achieved (with KSK19/A328I, TTN 870), this result was not reproducible on larger scale. The first scale-up reactions with bentazon used ethanol as co-solvent (where DMSO was used in the screens) for ease of purification which led to complete loss of turnover (0% conversion). Re-screening of the substrate with ethanol as co-solvent (and repeating the screen with DMSO as co-solvent) showed that ethanol hinders the reaction. However, even with DMSO as co-solvent, the results obtained on analytical scale could not be replicated on larger scale; MS data for the crude extract of the analytical scale reactions showed the presence of a peak with mass SM+16 (+O).

Isoxaflutole is a pre-emergence pro-herbicide for use on corn and sugarcane crops, which is metabolised to the active species, diketonitrile **223** (Scheme 134).[393–395] In non-susceptible plants, diketonitrile is rapidly metabolised to non-phytotoxic benzoic acid **263** whereas in the susceptible weed targets this does not occur and diketonitrile **223** inhibits pigment biosynthesis and prevents chloroplast development (Scheme 134).[393–395]



262
isoxaflutole

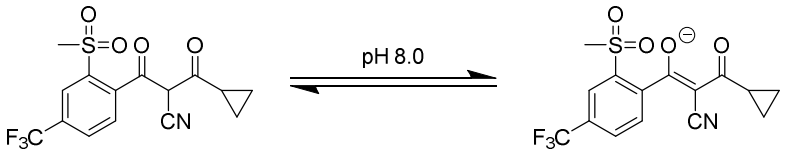
223

263

Scheme 134: Formation and metabolism of diketonitrile **223**

Diketonitrile **223** was initially thought to be an excellent substrate for oxidation by the library of $P450_{BM3}$ mutants, with every mutant screened affording complete turnover of starting material. When scale-up proved unsuccessful, it was suspected that the results of the first screen were misleading. A control reaction, in which diketonitrile was stirred in a solution of pH 8.0 buffer, gave the same result as the screen showing that the apparent consumption of starting material (observed by GC analysis) was caused by tautomerisation of the α-cyano-β-diketone to its enol form (Scheme 135).

4. Screening of Miscellaneous Compounds



223 **264**

Scheme 135: Tautomerisation of diketonitrile

Re-screening against an expanded library in less basic buffer (pH 7.5) afforded 0% conversion for all but two mutants. Mutant RP/EV gave 100% conversion, selectively forming a product with a MS peak of SM+16, and mutant R19 (R47L/Y51F/H171L/Q307H/N319Y) gave 87% conversion, selectively to a different product with a MS peak of SM+32. Neither of these products were isolated as these conversions could not be reproduced on larger scale; it is possible that oxidation of this substrate is pH sensitive.

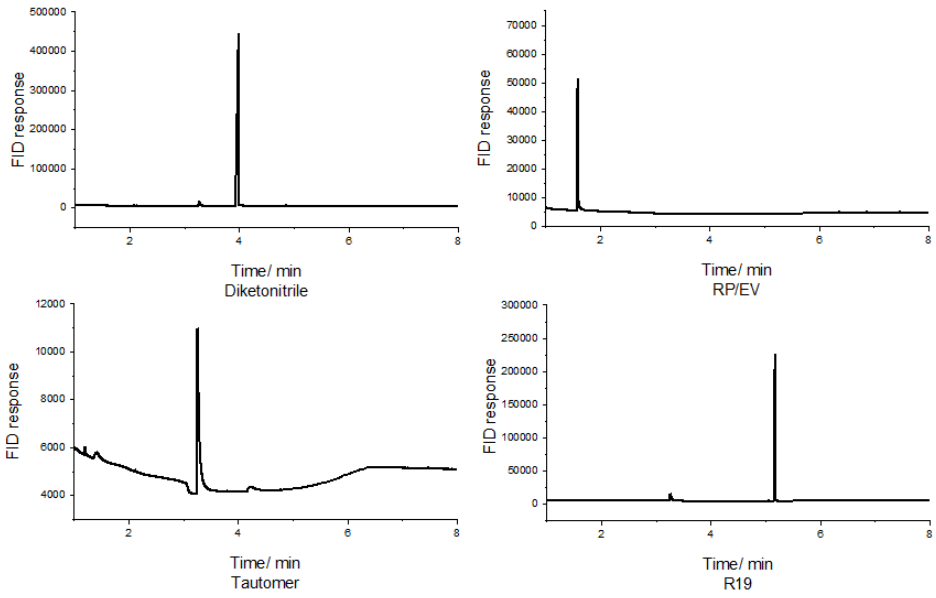


Figure 45: GC traces for oxidation of diketonitrile by the library of $P450_{BM3}$ mutants

Nicosulfuron is employed in corn fields for control of broad-leaved weeds and grasses.[396,397] The metabolic pathways are similar in soil, animals, and humans; urea hydrolysis dominates, and minor pathways include pyrimidine hydroxylation, amide *N*-demethylation, Smiles rearrangement of the sulfonylurea, and combinations thereof (Figure 46).[397]

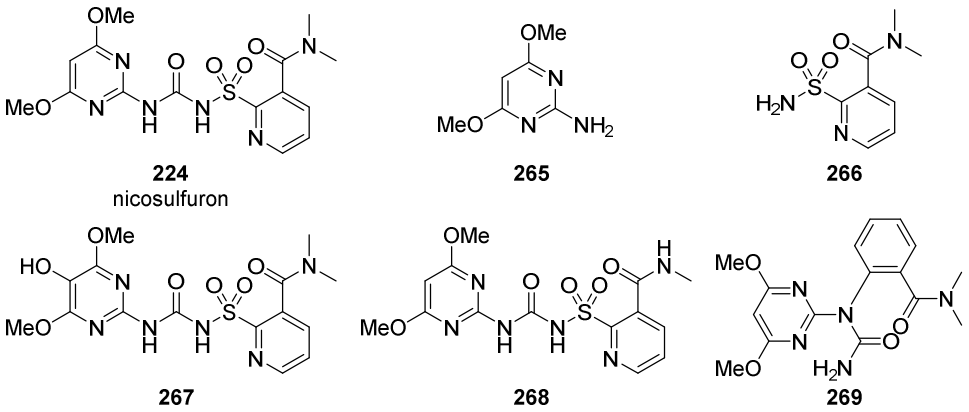
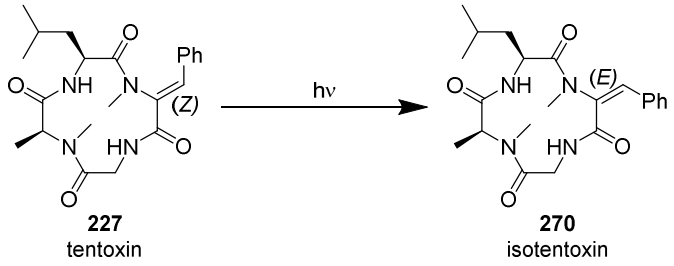


Figure 46: Nicosulfuron and the natural metabolites of nicosulfuron

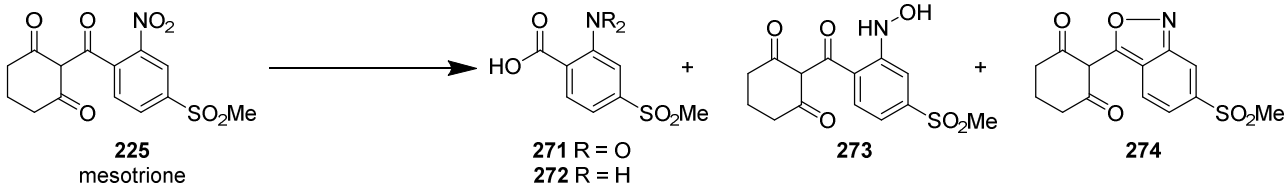
When nicosulfuron was screened against the library of $P450_{BM3}$ mutants no starting material was observed in the GC traces for almost all mutants; however, no products were observed either. It is likely that the metabolites were not extracted into an organic solvent; LCMS analysis of the aqueous layer may have provided insight into the identity of the oxidation products or if any were being formed at all.

Tentoxin is a cyclic tetrapeptide, formed by the pathogenic fungus *Alternaria alternata*, which induces chlorosis in the seedlings of various plants and can inhibit polyphenol oxidase, an enzyme important for the synthesis of hydroxylated flavonoids.[398–400] The major metabolites isolated from incubation with rat liver microsomes or yeast expressing human P450s (from the CYP3A family) were formed by *N*-demethylation of the *N*-Me-alanine peptide unit, hydroxylation of the methyl group of the same unit, or di-*N*-demethylation.[400] Screening against the library of $P450_{BM3}$ mutants produced low conversions (<14%) to a single product with very similar retention time and identical mass; it is believed that this product is simply an isomer of tentoxin, potentially arising from conversion of the alkene configuration from *Z*- to *E*-, known to occur upon exposure to UV light (Scheme 136).[401] Given the size of tentoxin it is possible that a library of mutants in which the active site volume is increased could show improved turnovers.



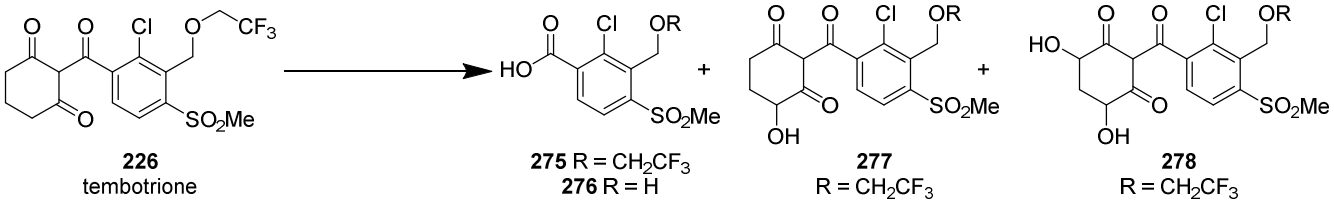
Scheme 136: Isomerisation of tentoxin

Mesotrione and tembotrione are members of the triketone class of herbicides which act by inhibition of 4-hydroxyphenolpyruvate dioxygenase (HPPD), thus hindering the synthesis of carotenoids which are vital for chloroplast stability.[402,403] Metabolism of mesotrione occurs simultaneously by two pathways: reduction of the nitro- group, to form hydroxylamine **273** which can undergo further transformations, and hydrolytic loss of the cyclohexanedione group which can also undergo further transformations (Scheme 137).[404,405]



Scheme 137: Metabolism of mesotrione

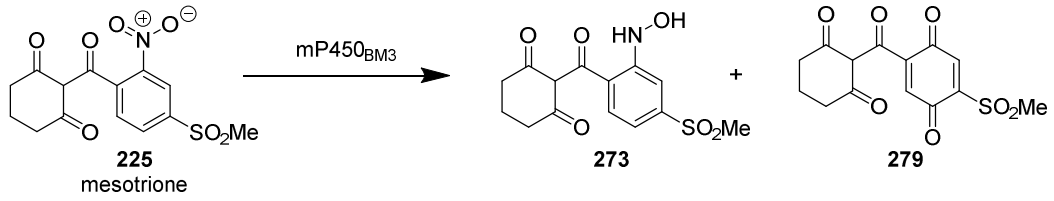
In the metabolism of tembotrione in plants, two pathways are also followed: hydrolytic loss of the cyclohexanedione moiety and hydroxylation of the cyclohexanedione ring (Scheme 138).[406]



Scheme 138: Metabolism of tembotrione in plants

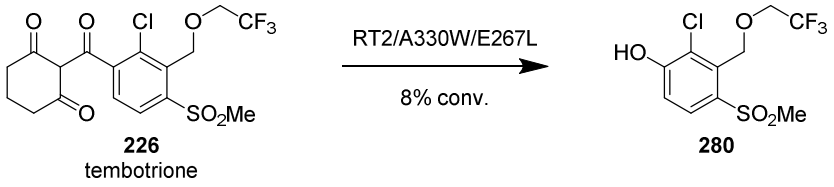
LCMS analysis was used for the mutant $P450_{BM3}$ screens of mesotrione and tembotrione with the products being identified based on MS data and comparison with known metabolic pathways. Mesotrione was more readily metabolised by the mutant library, with conversion of up to 47% observed. A mixture of two products was formed in the majority of cases, with MS data suggesting they were hydroxylamine **273** and quinone **279** formed by reduction of the nitro group, and further

oxidation of **273**, respectively (Scheme 139).[407] Reduction of the nitro group is likely to occur by a series of electron and proton transfers.



Scheme 139: Nitro-reduction and subsequent oxidation of mesotrione **225** by $P450_{BM3}$ mutants

The library showed poor activity towards tembotrione, with product formation only observed with four mutants with conversion <10% in each case. Two products were formed in these screens, one of which appeared to be phenol **280**, formed by oxidative cleavage, and another product which could not be identified, although MS data showed that no Cl was present in the molecule (Scheme 140).



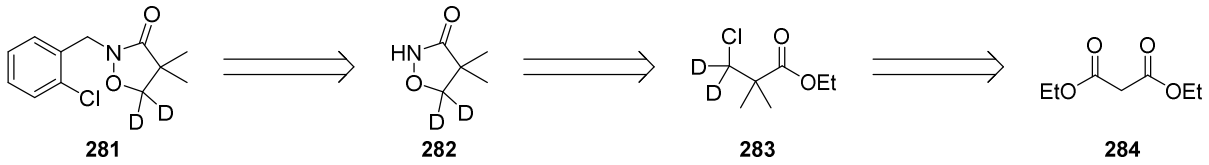
Scheme 140: Oxidation of tembotrione by mutant RT2/A330W/E267L

Studies on Deuterated Clomazone

Deuterated analogues of drugs or herbicides have been used to aid the identification of metabolites and uncover information about the mechanism of metabolism; however, efforts are increasingly turning towards the use of deuterium-containing compounds as drugs and herbicides themselves.[408–413] Mechanistic information can be gained by use of MS to assess if a deuterated part of a molecule is still present or by the measurement of kinetic isotope effects at reactive centres. Metabolism by C–H oxidation is prevalent for drugs and herbicides and can potentially lead to formation of toxic metabolites; a common method for preventing metabolism at these sites is the substitution of hydrogen for fluorine which may be a successful strategy for preventing oxidation, but the properties of the compound will be altered due to the greater size and electronegativity of fluorine, and its ability to form hydrogen bonds.[412] An alternative strategy for blocking metabolic

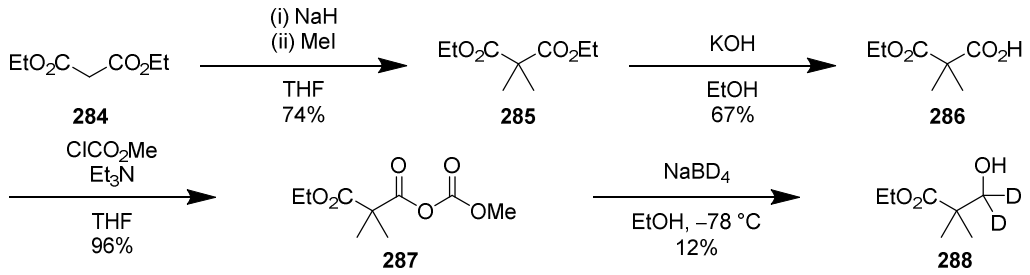
sites is to replace hydrogen with deuterium, relying on the kinetic isotope effect to prevent oxidation whilst having a negligible effect on other chemical properties.[412]

The oxidation of clomazone was discussed above, with the major product arising from hydroxylation of the isoxazolidinone ring (Scheme 130). To investigate the potential for altering the site of metabolism by deuteration at this position, a synthesis of clomazone-d_2 was required. It was intended that it would be accessible from diethylmalonate with deuterium being introduced by reduction with $NaBD_4$ (Scheme 141).



Scheme 141: Retrosynthesis plan for clomazone-d_2

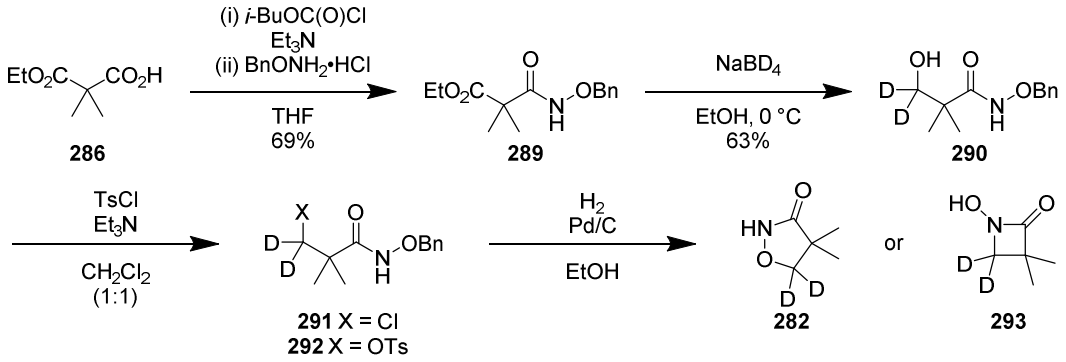
The route proceeded with good yields, and few steps required purification by chromatography; however, reduction of **287** was extremely susceptible to additional, undesired, reduction of the ester group for formation of a diol, with desired alcohol **288** only isolated in yields ≤12% (Scheme 142). This reduction occurred before complete consumption of starting material; cooling the reaction to –78 °C did not prevent its preferential formation.



Scheme 142: Synthesis of alcohol **288** *via* $NaBD_4$ reduction of **287**

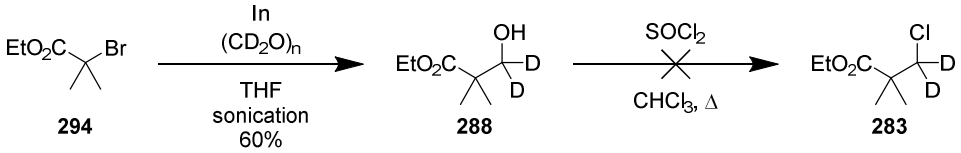
To avoid this undesired reduction, it was decided that the hydroxamate would be installed at an earlier point in the synthesis. Reduction with $NaBD_4$ was much more successful for substrate **289**; attempts to convert alcohol **290** to chloride **291**, however, were less successful. Use of $SOCl_2$ saw solely return of starting material, whereas, use of tosyl chloride gave an inseparable mixture of chloride **291** and tosylate **292**. Hydrogenolysis of the *O*-benzyl group afforded what appeared to be a

cyclised species; however, it was uncertain whether cyclisation had occurred through nitrogen or oxygen, and attempts to reproduce the result were unsuccessful (Scheme 143).



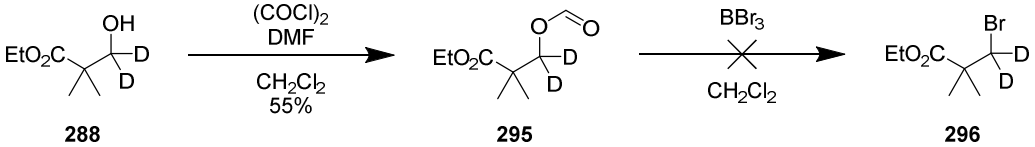
Scheme 143: Synthesis of alcohol **290** *via* $NaBD_4$ reduction of ester **289**

Attention was returned to synthesising alcohol **288**; rather than installing the deuterium by reduction of a carbonyl with $NaBD_4$, paraformaldehyde-d_2 was chosen as the source of the $–CD_2OH$ group. Attempted Zn-mediated Reformatsky reaction with paraformaldehyde-d_2 and ethyl 2-bromoisobutyrate gave only return of starting material; however, the synthesis of alcohol **288** was achieved by sonication of the reactants with In (Scheme 144). Conversion to chloride **283** by heating with $SOCl_2$ was unsuccessful and again, starting material was returned.



Scheme 144: Synthesis of alcohol **288** by In-mediated Reformatsky reaction

The conversion of alcohol **288** into chloride **283** with trichloroisocyanuric acid and DMF also proved unsuccessful. With oxalyl chloride and DMF, the product isolated was formate ester **295**; treatment of this compound with BBr_3 did not enable synthesis of bromide **296**, with decomposition favoured (Scheme 145).[414]



Scheme 145: Formation and attempted bromination of formate **295**

4. Screening of Miscellaneous Compounds

With the synthesis of clomazone-d_2 proving troublesome, focus was switched to other projects.

Conclusion

The library of $P450_{BM3}$ mutants has demonstrated ability to oxidise a variety of small molecules and commercially available herbicides bearing a diverse array of functional groups and structural motifs. Very high levels of conversion and selectivity have been achieved for some substrates whereas others require further screening to identify mutants capable of facilitating their oxidation. Screening of commercially available herbicides has enabled the preparation of natural and non-natural metabolites from the herbicide itself, a transformation that may not be possible by chemical synthesis.

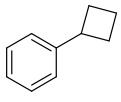
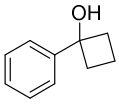
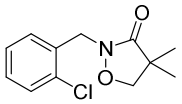
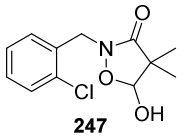
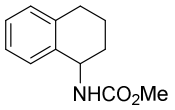
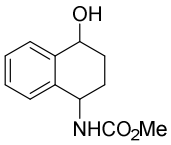
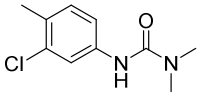
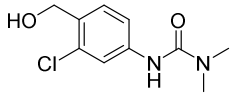
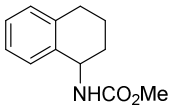
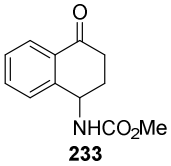
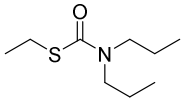
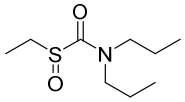
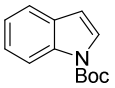
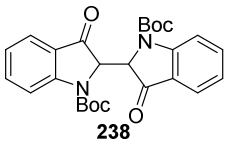
Substrate	Mutant (Yield)	Product	Substrate	Mutant (Yield)	Product
 **207**	RT2/IP (100% conv.)	 **206**	 **219**	KSK19/IA (32%)	 **247**
 **210**	RT2/IP (59%)	 **232**	 **220**	RP/FV/EV (28%)	 **249**
 **210**	RT2/IP (24%)	 **233**	 **221**	KSK19/AI 34%	 **253**
 **204**	RP/FV (18%)	 **238**	-	-	-

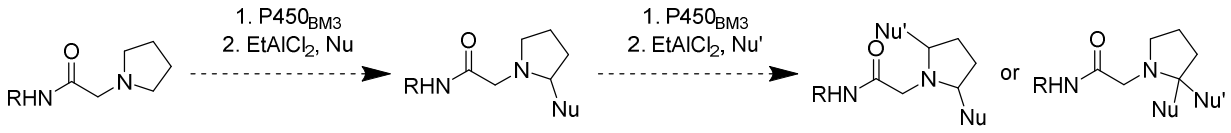
Table 6: Compounds screened in this chapter and the products identified

Chapter 5: Conclusions and Future Work

This work aimed to investigate the oxidation of a range of structurally diverse compounds by a library of $P450_{BM3}$ mutants to gain information about the functional group tolerance and selectivity and activity towards these compounds. It was intended that information gained from this screening could assist the use of the library for synthesis, potentially enabling the total synthesis of a natural product by implementation of a late-stage, $P450_{BM3}$-catalysed, oxidation strategy. Another aim was to build upon transformations discovered in the screening to develop the mutant library as a set of catalysts for a wider range of oxidations with investigation of the scope of these reactions.

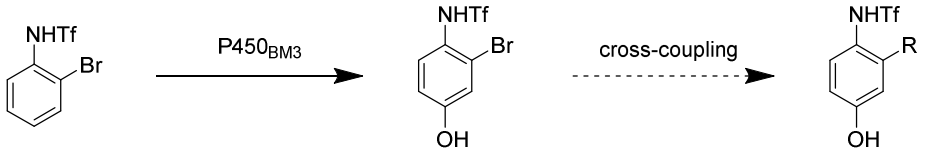
In Chapter 1, the synthesis of a variety of bicyclic imidazolidin-4-ones *via* $P450_{BM3}$-catalysed oxidative cyclisation of α-amino amides was discussed, expanding on the previously discovered oxidative cyclisation of lidocaine, discovered during the screening of commercially available drugs. The transformation was achieved with 12 substrates, including electron-rich and electron-poor aromatic and alkyl amides, and 5- and 6-membered cyclic amines. When substitution on the amine ring was present, high levels of regio- and diastereoselectivity were achieved, with control of regioselectivity possible by choice of $P450_{BM3}$ mutant. The mechanism of this reaction was investigated although its nature could not be identified conclusively; deuterium labelling of the sites α- to the nitrogen of the amine could enable a greater understanding of the mechanism.

A method was discovered for employing these imidazolidinones as masked iminium ions using a Lewis-acid mediated ring-opening with subsequent trapping by a nucleophile; the overall transformation achieves α-functionalisation of the amines. Screening of the imidazolidin-4-one products against the library of $P450_{BM3}$ mutants could enable the preparation of densely functionalised imidazolidinones which may offer useful biological activity. Submission of the α-functionalised products to the library of $P450_{BM3}$ mutants could enable the preparation of 2,2- or 2,4-disubstituted pyrrolidine rings from unsubstituted pyrrolidine rings.



Scheme 146: Proposed synthesis of disubstituted α-cycloamino amides

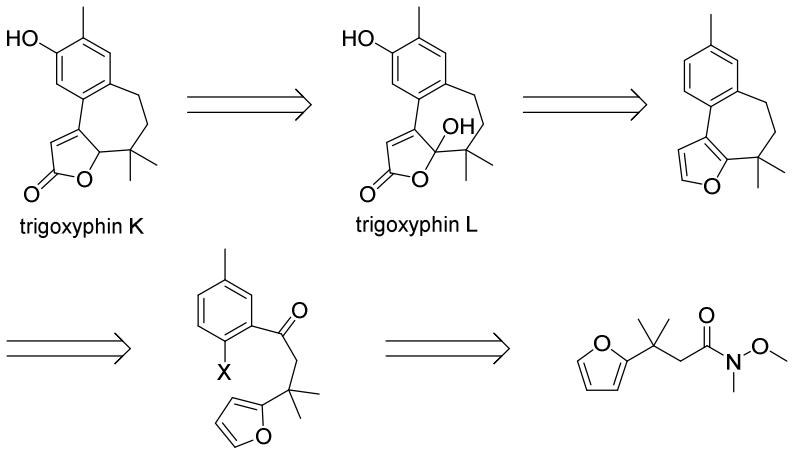
Another oxidation demonstrated for a range of substrates was the *para*-hydroxylation of anilides, which was discovered during the screening of herbicidal compounds. This transformation was achieved with amide and sulfonamide derivatives of a range of mono- and disubstituted anilines, with one substrate exhibiting selectivity between *para*- and benzylic hydroxylation. High levels of regioselectivity were achievable by selection of the appropriate $P450_{BM3}$ mutant. *para*-Hydroxylation was prevented in anilides bearing functionalisation at the *para*- position; benzylic hydroxylation was still possible for a *para*-methyl derivative but substrate turnover was not observed when a *para*-bromo derivative was screened. Further expansion of this work is currently being investigated within the group with the screening of a diverse range of *N*-phenyl heterocycles and *N*-aryl lactams. Further derivatisation of the oxidation products could enable the synthesis of a diverse range of substituted 4-aminophenols.



Scheme 147: Proposed synthesis of functionalised 4-aminophenols

Two syntheses of the core structures of trigoxyphin K and L have been achieved in 8 steps, 9% overall yield and 9 steps, 6% overall yield, respectively, from 2-bromo-4-methylaniline and 3-furanylboronic acid. The formation of the 6,7,5-fused ring system was achieved by Lewis acid-mediated intramolecular epoxide opening by a furan; singlet oxygen oxidation of the furan afforded the γ-hydroxybutenolide of trigoxyphin L and reduction of this gave the butenolide of trigoxyphin K. The oxidation of two potential precursors to the natural products by the library of $P450_{BM3}$ mutants for installation of the phenolic hydroxyl group was investigated; however, the required phenol product was not isolated, further screening is required to identify a mutant to

catalyse the appropriate hydroxylation. An alternative route to the core structure is under investigation, in which the 6,7,5-fused ring system is formed by C–H activation of a furan ring at the 3-position.



Scheme 148: Alternative synthesis of trigoxyphin K and L

Other natural products currently being targeted within the group for synthesis by late-stage oxidation with $P450_{BM3}$ mutants include the anti-cancer agents taxol, eleutherobin, and alvocidib, and the steroid antibiotic viridiol.

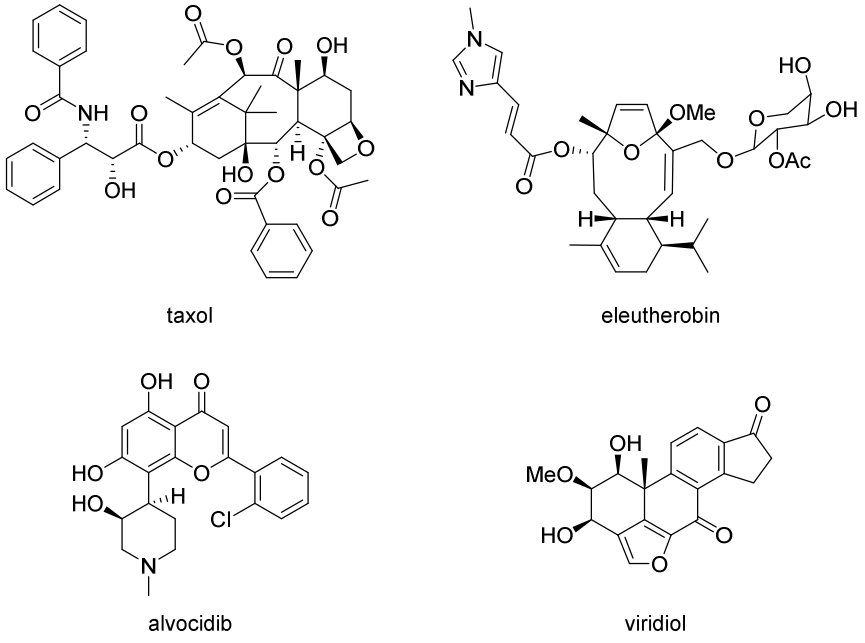


Figure 47: Natural products currently being targeted

Experimental Section

General Experimental

Novel compounds will be pictured and the proton (^{1}H) and carbon (^{13}C) NMR spectral data, and high resolution mass spectral data will be reported. Literature precedented compounds will have at least proton (^{1}H), carbon (^{13}C) NMR spectral data and low resolution mass spectral data. Boiling points and melting points will be reported where appropriate.

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AVIII600 (600/151 MHz), Bruker AVII500 (500/126 MHz), Bruker AVIII400 (400/101 MHz), or Bruker AV400 (400/101 MHz). Proton and carbon chemical shifts (δ_H, δ_C) are quoted in ppm with residual protonated solvent as the internal standard and referenced to tetramethylsilane unless otherwise stated. Peak assignments were made on the basis of chemical shift, coupling constants, COSY, NOESY, DEPT, HSQC, and HMBC data, and by comparison with spectra of related compounds. Coupling constants (*J*) are quoted as observed and rounded to the nearest 0.5 Hz. Atom numbering reflects NMR peak assignments and does not necessarily correspond to the IUPAC nomenclature.

Low resolution mass spectra were recorded on a Micromass LCT Premier spectrometer (ES) with values reported in Daltons. High resolution mass spectra were recorded by the Mass Spectrometry service of the Chemistry Research Laboratory, University of Oxford, using a Bruker microTOF Spectrometer. High resolution values are calculated to 4 decimal places from the chemical formula with all found values being within a tolerance of 5 ppm. Ultra performance liquid chromatography-mass spectrometry analysis was carried out on a Waters Acquity (SB-C18, 50 × 4.6 mm, 1.8 µm column, UV, mass spectroscopy, corona detection). Compounds were separated using a linear gradient system comprising of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a constant flow rate of 2.0 mL min^{-1}. Gas chromatographic analysis was performed on a Thermo Finnigan TRACE gas chromatograph equipped with a flame ionization detector (FID) and an AS3000 autosampler on a DB-1 capillary GC

column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injector temperature was 280 °C and the FID was at 250 °C.

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

Thin-layer chromatography was performed on Merck DC-Alufolien 60 F254 0.2 mm pre-coated plates with reaction process visualised using basic potassium permanganate dip, acidic anisaldehyde dip, or ultraviolet light. Retention factors (R_f) are quoted with the solvent system in parentheses. Column chromatography was performed on Merck Geduran® 60 SiO_2 (40–63 μm) and the solvent system used is recorded in parentheses. Solvents were either used as commercially supplied or as purified by standard techniques. Tetrahydrofuran was freshly distilled over Na and benzophenone prior to use. Petrol refers to the fraction of light petroleum ether boiling at 30–40 °C. Ether refers to diethyl ether. Brine refers to a saturated aqueous solution of sodium chloride. All reactions were carried out under an atmosphere of dry argon in oven–dried flasks unless otherwise stated.

Enzymes and Molecular Biology

The $P450_{BM3}$ enzymes were produced in *Escherichia coli* BL21 (DE3) and purified by anion exchange chromatography.[181] Alternatively, the enzymes were purified by addition of $(NH_4)_2SO_4$ to a concentration of 40% w/v and centrifugation at 37,500g for 10 min at 4 °C; the residue was discarded and $(NH_4)_2SO_4$ was added to a concentration of 60% w/v. The solution was submitted to centrifugation at 37,500g for 10 min at 4 °C, the supernatant was discarded and the residue was resuspended in phosphate buffer. The enzymes were stored as stock solutions in 1.0 mL aliquots at –20 °C and thawed at RT before use.

General Procedure for Screening of Compounds in Chapter 1

Screening of substrates was carried out with an NADPH regeneration system in 200 mM phosphate buffer (pH 7.5), at a total volume of 0.5 mL, contained in 14 mL glass vials. The substrate was added as a 100 mM stock solution in methanol or ethanol (final concentration 1 or 2 mM), and the mutant $P450_{BM3}$ enzyme was added as a 10 µM stock solution in phosphate buffer (pH 7.5, final concentration 1 or 2 µM). The NADPH regeneration system consisted of glucose dehydrogenase, added as a 2 U/µL stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 2 U/mL), glucose, added as a 1.0 M stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 100 mM), and $NADP^+$, added as a 4.0 mM stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 8 µM). The reactions were shaken at RT for 16 h and the pH was then adjusted to 11 with 2 M aqueous KOH. The solution was extracted with 300 µL of ethyl acetate was added and, following centrifugation at 14,300 g for 2 minutes, the organic phase was analysed by gas chromatography.

General Procedure for Screening of Compounds from Chapter 2, 3, and 4

Compounds were screened using an NADPH regeneration system in 100 mM phosphate buffer (pH 7.5), at a total volume of 0.5 mL, contained in 14 mL glass vials or 24 well plates. The substrate was added as a 100 mM stock solution in ethanol, methanol, isopropanol, or dimethylsulfoxide (final concentration 2 mM), and the mutant $P450_{BM3}$ enzyme was added as a 10 µM stock solution in phosphate buffer (pH 7.5, final concentration 2 µM). The NADPH regeneration system consisted of glucose dehydrogenase, added as a 2 U/µL stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 40 U/mL), glucose, added as a 1.0 M stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 100 mM), and $NADP^+$, added as a 4.0 mM stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 8 µM). The reactions were shaken at RT for 16 h, extracted with 300 µL of ethyl acetate and, following centrifugation at 14,300 g for 2

minutes, the organic phase was analysed by gas chromatography or liquid-chromatography mass spectrometry.

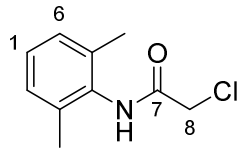
$P450_{BM3}$ Variants and the Mutations in Each

Enzymes	Mutations
A328V	A328V
AP	A330P
EV/VI/LI	E267V/V302I/L353I
F87A	F87A
F87A/I401P	F87A/I401P
F87A/T88A/I401P	F87A/T88A/I401P
FS/IP	F87S/I401P
GVQ	A74G/F87V/L188Q
IP/QP	I401P/Q403P
KSK19	F87A/H171L/Q307H/N319Y
KSK19/A82M	F87A/A82M/H171L/Q307H/N319Y
KSK19/A82M/E267F	F87A/A82M/H171L/E267F/Q307H/N319Y
KSK19/AI	F87A/H171L/Q307H/N319Y/A328I
KSK19/AI/IA	F87A/ H171L/I263A/Q307H/N319Y/A328I
KSK19/AI/Y51V	F87A/ H171L/I263A/Q307H/N319Y/A328I
KSK19/AI/Y51L	F87A/ H171L/I263A/Q307H/N319Y/A328I
KSK19/AI/F87V	F87V/H171L/Q307H/N319Y/A328I
KSK19/AI/AI	F87A/H171L/A184I/Q307H/N319Y/A328I
KSK19/AI/V78I	V78I/F87A/H171L/Q307H/N319Y/A328I
KSK19/I263A	V78I/F87A/H171L/Q307H/N319Y/A328I
KSK19/IA/QP	F87A/H171L/I263A/Q307H/N319Y/Q403P
KSK19/QP	F87A/H171L/Q307H/N319Y/Q403P
KSK19/QP/F87V	F87V/H171L/Q307H/N319Y/Q403P
KSK48	R179H/L233V/S270I/Q307H/N319Y
KT2	A191T/N239H/I259V/A276T/L353I
KT2/A82G	A82G/A191T/N239H/I259V/A276T/L353I
KT2/A328V	A191T/N239H/I259V/A276T/A328V/L353I
KT2/E267L	A191T/N239H/I259V/E267L/A276T/L353I
KT2/F87I	F87I/A191T/N239H/I259V/A276T/L353I
KSK19/T88A	T88A/A191T/N239H/I259V/A276T/L353I
QP	Q403P
QP/A82M	A82M/Q403P
RLYF	R47L/Y51F
RLYF/KSK19	R47L/Y51F/F87A/H171L/Q307H/N319Y
RLYF/KSK19/AI	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
RP	R47L/Y51F/I401P
RP/A82M	R47L/Y51F/A82M/I401P
RP/AM/A328I	R47L/Y51F/A82M/A328I/I401P
RP/AM/IA	R47L/Y51F/A82M/I263A/I401P
RP/AP	R47L/Y51F/A330P/I401P
RP/AP/AM	R47L/Y51F/A82M/A330P/I401P
RP/AP/PV	R47L/Y51F/P329V/A330P/I401P
RP/E267I	R47L/Y51F/E267I/I401P
RP/E267M	R47L/Y51F/E267M/I401P
RP/EV	R47L/Y51F/E267V/I401P

Enzymes	Mutations
RP/FA	R47L/Y51F//F87A/I401P
RP/F87A/A328I	R47L/Y51F//F87A/A328I/I401P
RP/F87A/A328P	R47L/Y51F//F87A/A328P/I401P
RP/FV	R47L/Y51F/F87V/I401P
RP/FV/EV	R47L/Y51F/F87V/E267V/I401P
RP/FV/EV/F81W	R47L/Y51F/F81W/F87V/E267V/I401P
RP/FV/EV/L188Q	R47L/Y51F/F87V/L188Q/E267V/I401P
RP/FV/EV/V78F	R47L/Y51F/F87V/L188Q/E267V/I401P
RP/F81W	R47L/Y51F/F81W/I401P
RP/H171L	R47L/Y51F/H171L/I401P
RP/H171L/IG	R47L/Y51F/I263G/H171L/I401P
RP/IA	R47L/Y51F/I263A/I401P
RP/IA/EV	R47L/Y51F/I263A/E267F/I401P
RQA	R47L/Y51F/A82M/I263A/Q403P
RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
RT2/A330W	R47L/Y51F/A191T/N239H/I259V/A276T/A330W/L353I
RT2/A330W/A82F	R47L/Y51F/A82F/A191T/N239H/I259V/A276T/A330W/L353I
RT2/A330W/A82M	R47L/Y51F/A82M/A191T/N239H/I259V/A276T/A330W/L353I
RT2/A330W/I263A	R47L/Y51F/I263A/A191T/N239H/I259V/A276T/A330W/L353I
RT2/AP	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/A184I	R47L/Y51F/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/A82F	R47L/Y51F/A82F/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/A82L	R47L/Y51F/A82L/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/AM	R47L/Y51F/A82M/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/AM/AI	R47L/Y51F/A82M/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/E267F	R47L/Y51F/A191T/N239H/I259V/E267F/A276T/A330P/L353I
RT2/AP/E267L	R47L/Y51F/A191T/N239H/I259V/E267L/A276T/A330P/L353I
RT2/AP/F81W	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/I401M	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I/I401M
RT2/AP/I401M/A82G	R47L/Y51F/A82G/A191T/N239H/I259V/A276T/A330P/L353I/I401M
RT2/AP/K440L	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I/K440L
RT2/AP/PV	R47L/Y51F/A191T/N239H/I259V/A276T/P329V/A330P/L353I
RT2/AP/V78I	R47L/Y51F/V78I/A191T/N239H/I259V/A276T/A330P/L353I
RT2/F81W	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/L353I
RT2/F81W/A82M	R47L/Y51F/F81W/A82M/A191T/N239H/I259V/A276T/L353I
RT2/F81W/A328I	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/A328I/L353I
RT2/IP	R47L/Y51F/A191T/N239H/I259V/A276T/L353I/I401P
RT2/IP/A184I	R47L/Y51F/A184I/A191T/N239H/I259V/A276T/L353I/I401P
RT2/IP/E267V	R47L/Y51F/A191T/N239H/I259V/E267V/A276T/L353I/I401P
RT2/IP/V78I	R47L/Y51F/V78I/A191T/N239H/I259V/A276T/L353I/I401P
RT2/V78F	R47L/Y51F/V78F/A191T/N239H/I259V/A276T/L353I
RT2/V78F/A330W	R47L/Y51F/V78F/A191T/N239H/I259V/A276T/A330W/L353I
T88M/IP	T88M/I401P
WT	WT

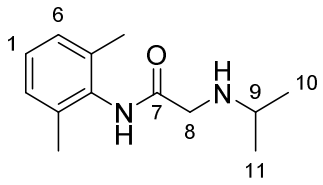
Experimental Procedures and Characterisation

2,6-Dimethylphenyl-2-chloroacetamide 6



Based on a literature procedure.[415] To a solution of 2,6-dimethylaniline (5.1 mL, 41.2 mmol, 1.0 eq) in acetic acid (41 mL) was added chloroacetyl chloride (3.7 mL, 46.2 mmol, 1.1 eq) slowly. The solution was stirred for 1 h. NaOAc (6.8 g, 82.4 mmol, 2.0 eq) in water (39 mL) was added slowly and the solution was stirred for 45 minutes. Water (48 mL) was added and the solution was stirred for 75 minutes during which time a white precipitate formed. The mixture was filtered and the residue was dried *in vacuo* to afford **6** as a white solid (6.1 g, 75%). $\mathbf{R_f}$ 0.10 (20% ethyl acetate / petrol); **mp** 139 °C (amorphous) (lit. mp 140 °C); **IR** (thin film) ν_{max} / cm^{-1} 3211 (w), 3036 (w), 1681 (w), 1642 (s), 1534 (m), 1476 (w), 1249 (w), 1147 (w), 761 (s), 708 (m), 664 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.87 (1H, br s, NH), 7.17–7.09 (3H, m, H1, H2, H6), 4.24 (2H, s, H8), 2.24 (6H, s, 2×Ar-CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 164.4 (C7), 135.4 (C3, C5), 132.7 (C4), 128.4 (C2, C6), 127.9 (C1), 42.8 (C8), 18.3 (2×Ar-CH_3); **LRMS** (ES^+) calc. for $C_{10}H_{13}{}^{35}ClNO$ $[M+H]^+$ 198.1, found 198.1. Data is consistent with literature values.[416]

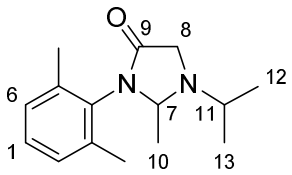
2,6-Dimethylphenyl-2-isopropylaminoacetamide 7



Based on a modified literature procedure.[415] To a solution of 2,6-dimethylphenyl-2-chloroacetamide **6** (1.0 g, 5.1 mmol, 1.0 eq) in ethanol (8.0 mL) was added isopropylamine (0.87 mL, 10.2 eq, 2.0 eq). The solution was heated at reflux for 18 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in water (5.0 mL) and aqueous HCl (3.0 M, 4.0 mL) and the mixture was extracted with ether. The pH of the aqueous layer was adjusted to 11 with

aqueous NaOH (50% w/v) and the mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a white solid (877 mg, 79%). **R_f** 0.10 (70% ethyl acetate / petrol); **mp** 68–70 °C (amorphous); **IR** (thin film) ν_{max}/ cm^{-1} 3265 (w), 2964 (m), 1662 (s), 1594 (w), 1503 (s), 1477 (m), 1441 (w), 768 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.92 (1H, br s, NH), 7.08 (3H, s, H1, H2, H6), 3.44 (2H, s, H8), 2.91 (1H, sept, *J* = 6.5 Hz, H9), 2.23 (6H, s, 2×Ar-CH_3), 1.52 (1H, br s, NH), 1.14 (6H, d, *J* = 6.5 Hz, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.7 (C7), 135.1 (C3, C5), 134.0 (C4), 128.2 (C2, C6), 127.1 (C1), 50.4 (C8), 49.8 (C9), 23.2 (C10, C11), 18.6 (2×Ar-CH_3); **HRMS** (ES^+) calc. for $C_{13}H_{21}N_2O$ $[M+H]^+$ 221.1648 found 221.1645.

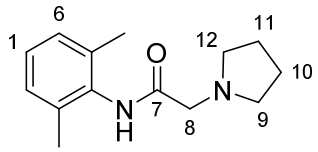
3-(2,6-Dimethylphenyl)-1-isopropyl-2-methylimidazolidin-4-one 8



Based on a modified literature procedure.[251] To a solution of 2,6-dimethylphenyl-2-isopropylaminoacetamide **7** (280 mg, 1.27 mmol, 1.0 eq) in formic acid (13 mL) was added acetaldehyde (1.4 mL, 25.4 mmol, 20 eq). The solution was heated at reflux for 75 minutes. The solution was cooled to RT and saturated aqueous $NaHCO_3$ was added carefully. The mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol + 1% Et_3N to 30% ethyl acetate /petrol + 1% Et_3N) to afford the *title compound* as a white solid (154 mg, 49%). **R_f** 0.35 (70% ethyl acetate / petrol); **mp** 58 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2970 (w), 1708 (s), 1475 (w), 1406 (w), 773 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.17–7.10 (2H, m, H2, H1), 7.08–7.06 (1H, m, H6), 4.72 (1H, qt, *J* = 5.5, 1.5 Hz, H7), 3.62 (1H, dd, *J* = 15.0, 2.0 Hz, H8), 3.52 (1H, dd, *J* = 15.0, 1.5 Hz, H8'), 3.06 (1H, sept, *J* = 6.5 Hz, H11), 2.26 (3H, s, Ar-CH_3), 2.22 (3H, s, Ar-CH_3), 1.18 (3H, d, *J* = 6.5 Hz, H13), 1.15 (3H, d, *J* = 5.5 Hz, H10), 1.09 (3H, d, *J* = 6.5 Hz, H12); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.3 (C9), 137.9 (C3), 135.7 (C5), 133.2 (C4), 128.9 (C2), 128.7

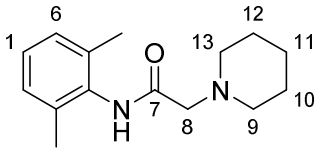
(C6), 128.4 (C1), 74.2 (C7), 49.9 (C8), 49.4 (C11), 21.9 (C13), 19.5 (C12), 18.8 (Ar-CH_3), 18.6 (Ar-CH_3), 17.0 (C12); **HRMS** (ES^+) calc. for $C_{15}H_{23}N_2O$ $[M+H]^+$ 247.1805, found 247.1800.

2,6-Dimethylphenyl-2-pyrrolidin-1-ylacetamide 10



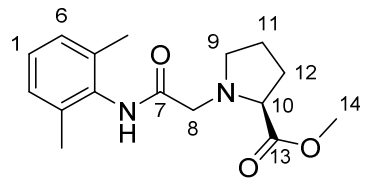
Based on a modified literature procedure.[415] To a solution of 2,6-dimethylaniline (3.0 g, 24.8 mmol, 1.0 eq) in acetic acid (25 mL) was added chloroacetyl chloride (2.2 mL, 27.7 mmol, 1.1 eq) dropwise. The solution was stirred for 1 h. NaOAc (4.1 g, 49.6 mmol, 2.0 eq) in water (24 mL) was added slowly and the solution was stirred for 45 minutes. Water (29 mL) was added and the solution was stirred for 75 minutes during which time a white solid precipitated out of solution. The mixture was filtered and the solid was washed with water. The solid was redissolved in ethanol (31 mL) and pyrrolidine (6.2 mL, 74.4 mmol, 3.0 eq) was added. The solution was heated at reflux for 14 h then cooled to RT and concentrated *in vacuo*. The residue was redissolved in water (23 mL) and aqueous HCl (3.0 M, 19 mL) and the solution was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v). The solution was extracted with DCM and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The mixture was recrystallised from hot hexane to afford **10** as a straw-coloured solid (2.4 g, 42%). $\mathbf{R_f}$ 0.30 (70% ethyl acetate / petrol); **mp** 78 °C (hexane) (lit. mp 82–84 °C); **IR** (thin film) ν_{max} / cm^{-1} 3214 (w), 2956 (w), 2925 (w), 2789 (m), 1656 (s), 1505 (m), 1476 (m), 1419 (w), 765 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.63 (1H, br s, NH), 7.12–7.05 (3H, m, H1, H2, H6), 3.34 (2H, s, H8), 2.76 (4H, t, *J* = 6.0 Hz, H9, H12), 2.24 (6H, s, 2×Ar-CH_3), 1.89–1.82 (4H, m, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.4 (C7), 135.2 (C4), 133.9 (C1), 128.2 (C2, C6), 127.1 (C3, C5), 59.3 (C8), 54.9 (C9, C12), 24.2 (C10, C11), 18.6 (2×Ar-CH_3); **LRMS** (ES^+) calc. for $C_{14}H_{21}N_2O$ $[M+H]^+$ 233.2, found 233.1. Data is consistent with literature values.[417]

2,6-Dimethylphenyl-2-piperidin-1-ylacetamide 11



Based on a modified literature procedure.[415] To a solution of 2,6-dimethylphenyl-2-chloroacetamide **6** (300 mg, 1.52 mmol, 1.0 eq) in ethanol (1.9 mL) was added piperidine (0.45 mL, 4.56 mmol, 3.0 eq). The solution was heated at reflux for 17 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in water (1.4 mL) and aqueous HCl (3.0 M, 1.2 mL). The mixture was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v). The mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **11** as an orange solid (355 mg, 87%). **R_f** 0.40 (70% ethyl acetate / petrol); **mp** 104 °C (amorphous) (lit. mp 114–116 °C); **IR** (thin film) ν_{max} / cm^{-1} 3229 (w), 2933 (m), 2855 (w), 2786 (w), 1661 (s), 1495 (s), 1422 (m), 1334 (w), 1165 (w), 766 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.81 (1H, br s, NH), 7.09 (3H, s, H1, H2, H6), 3.14 (2H, s, H8), 2.62 (4H, m, H9, H13), 2.24 (6H, s, 2×Ar-CH_3), 1.65 (4H, quin, *J* = 5.5 Hz, H10, H12), 1.52–1.46 (2H, m, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.1 (C7), 135.0 (C3, C5), 133.8 (C4), 128.3 (C2, C6), 127.1 (C1), 62.6 (C8), 55.3 (C9, C13), 26.4 (C10, C12), 18.6 (2×Ar-CH_3); **LRMS** (ES^+) calc. for $C_{15}H_{23}N_2O$ $[M+H]^+$ 247.2, found 247.2. Data consistent with literature values.[418]

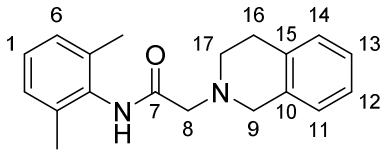
Methyl-{2-[(2,6-dimethylphenyl)amino]-2-oxoethyl}-L-prolinate 12



Based on a modified literature procedure.[415] To a solution of 2,6-dimethylphenyl-2-chloroacetamide **6** (400 mg, 2.02 mmol, 1.0 eq) in methanol (3.4 mL) was added methyl L-prolinate **297** (521 mg, 4.04 mmol, 2.0 eq). The solution was heated at reflux for 15 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in water (1.9 mL) and aqueous HCl (3.0 M, 1.6 mL). The mixture was extracted with ether. The pH of the aqueous layer was adjusted to

11 with aqueous NaOH (50% w/v) and the mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol + 1% Et_3N) to afford the *title compound* as a yellow oil (402 mg, 69%). **R_f** 0.35 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3290 (w), 2953 (w), 1736 (s), 1685 (s), 1499 (s), 1437 (w), 1204 (m), 770 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.99 (1H, br s, NH), 7.08 (3H, s, H1, H2, H6), 3.74 (3H, s, H14), 3.62 (1H, d, *J* = 16.5 Hz, H8), 3.55 (1H, dd, *J* = 9.0, 5.5 Hz, H10), 3.38–3.30 (2H, m, H13, H8'), 2.66 (1H, q, *J* = 8.0 Hz, H13'), 2.30–2.20 (7H, m, H12, 2×Ar-CH_3), 2.05–1.99 (1H, m, H11'), 1.97–1.90 (2H, m, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.7 (C13), 169.5 (C7), 135.3 (C3, C5), 128.2 (C2, C6), 127.1 (C1), 65.9 (C14), 58.6 (C10), 55.0 (C8), 52.1 (C9), 29.9 (C12), 24.4 (C11), 18.5 (2×Ar-CH_3); **HRMS** (ES^+) calc. for $C_{16}H_{23}N_2O_3$ $[M+H]^+$ 291.1703, found 291.1709.

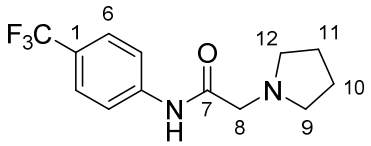
2,6-Dimethylphenyl-2-[3,4-dihydroisoquinolin-2(1*H*)-yl]acetamide 13



Based on a modified literature procedure.[415] To a solution of 2,6-dimethylphenyl-2-chloroacetamide **6** (400 mg, 2.02 mmol, 1.0 eq) in ethanol (3.4 mL) was added 1,2,3,4-tetrahydroisoquinoline (0.51 mL, 4.04 mmol, 2.0 eq). The solution was heated at reflux for 17 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in water (1.9 mL) and aqueous HCl (3.0 M, 1.6 mL). The mixture was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v). The mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow solid. The solid was recrystallised from hot hexane to afford the *title compound* as an orange solid (318 mg, 54%). **R_f** 0.35 (70% ethyl acetate / petrol); **mp** 140 °C (hexane); **IR** (thin film) ν_{max} / cm^{-1} 3302 (w), 2920 (w), 1679 (s), 1491 (s), 1100 (m), 934 (w), 739 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.72 (1H, br s, NH), 7.21–7.15 (3H, m, H12, H13, H14), 7.10–7.00 (4H, m, H1, H2, H6, H11), 3.89 (2H, s, H9),

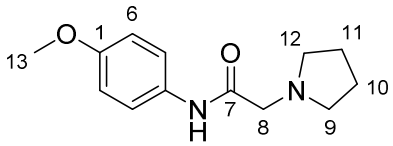
3.40 (2H, s, H8), 3.01 (4H, s, H16, H17), 2.25 (6H, s, 2×Ar-CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.6 (C7), 135.1 (C3, C5), 134.1 (C4), 133.6 (C15), 133.5 (C10), 128.9 (C14), 128.3 (C2, C6), 127.2 (C1), 126.7 (C11), 126.5 (C12), 126.0 (C13), 61.7 (C9), 56.5 (C9), 51.9 (C17), 29.4 (C16), 18.6 (2×Ar-CH_3); **HRMS** (ES^+) calc. for $C_{19}H_{23}N_2O$ $[M+H]^+$ 295.1805, found 295.1801.

4-Trifluoromethylphenyl-2-pyrrolidin-1-ylacetamide 14



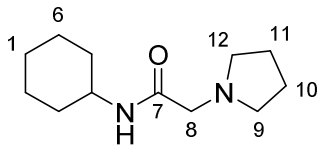
Based on a modified literature procedure.[415] A solution of 4-trifluoromethylphenyl-2-chloroacetamide **298** (300 mg, 1.26 mmol, 1.0 eq) and pyrrolidine (0.21 mL, 2.52 mmol, 2.0 eq) in ethanol (2.1 mL) was heated at reflux for 15 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (3.0 M) and the solution was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow solid (273 mg, 80%). **R_f** 0.45 (60% ethyl acetate / petrol); **mp** 52–54 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3283 (w), 2966 (w), 2816 (w), 1693 (s), 1613 (m), 1526 (s), 1410 (m), 1320 (s), 1112 (s), 1067 (s), 842 (m), 730 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 9.30 (1H, br s, NH), 7.70 (2H, d, *J* = 8.5 Hz, H3, H5), 7.57 (2H, d, *J* = 8.5 Hz, H2, H6), 3.29 (2H, s, H8), 2.71–2.67 (4H, m, H9, H12), 1.88–1.84 (4H, m, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.6 (C7), 140.8 (C4), 126.2 (q, *J* = 4.0 Hz, C2, C6), 125.8 (q, *J* = 33.0 Hz, C1), 124.2 (q, *J* = 271.0 Hz, CF_3), 119.1 (C3, C5), 59.7 (C8), 54.6 (C9, C12), 24.1 (C10, C11); **HRMS** (ES^+) calc. for $C_{13}H_{15}F_3N_2O$ $[M+H]^+$ 273.1209, found 273.1201.

4-Methoxyphenyl-2-pyrrolidin-1-ylacetamide 15



Based on a modified literature procedure.[415] A solution of 4-methoxyphenyl-2-chloroacetamide **299** (400 mg, 2.0 mmol, 1.0 eq), and pyrrolidine (0.33 mL, 4.0 mmol, 2.0 eq) in ethanol (3.3 mL) was heated at reflux for 14 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (3.0 M) and the solution was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown oil (435 mg, 93%). **R_f** 0.25 (60% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3276 (w), 2960 (w), 2812 (w), 1678 (s), 1607 (w), 1510 (s), 1242 (m), 1033 (m), 829 (m), 732 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.96 (1H, br s, NH), 7.47 (2H, d, *J* = 9.0 Hz, H3, H5), 6.86 (2H, d, *J* = 9.0 Hz, H2, H6), 3.78 (3H, s, H13), 3.25 (2H, s, H8), 2.69–2.66 (4H, m, H9, H12), 1.85–1.82 (4H, m, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.9 (C7), 156.2 (C1), 131.0 (C4), 121.2 (C3, C5), 114.1 (C2, C6), 59.7 (C8), 55.5 (C13), 54.6 (C9, C2), 24.1 (C10, C11); **HRMS** (ES^+) calc. for $C_{13}H_{19}N_2O_2$ $[M+H]^+$ 235.1441, found 235.1436.

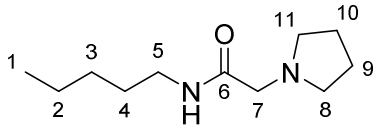
Cyclohexyl-2-pyrrolidin-1-ylacetamide 16



Based on modified literature procedures.[415,419] Chloroacetyl chloride (0.49 mL, 6.1 mmol, 1.0 eq) was added dropwise to a solution of cyclohexylamine (0.69 mL, 6.1 mmol, 1.0 eq) and triethylamine (0.93 mL, 6.7 mmol, 1.1 eq) in DCM (18 mL) at 0 °C. The solution was warmed to RT and stirred for 90 minutes. DCM was added and the solution was washed with water, saturated aqueous $NaHCO_3$, and saturated aqueous NH_4Cl, dried over $MgSO_4$, filtered, and concentrated *in*

vacuo. The residue was redissolved in ethanol (10 mL), pyrrolidine (1.0 mL, 12.2 mmol, 2.0 eq) was added, and the solution was heated at reflux for 15 h. The solution was cooled to RT and aqueous HCl (3.0 M) was added. The solution was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown oil (1.10 g, 86%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 6.94 (1H, br s, NH), 3.77–3.68 (1H, m, H4), 3.04 (2H, s, H8), 2.53–2.50 (4H, m, H9, H12), 1.84–1.81 (2H, m, H3, H5), 1.74–1.71 (4H, m, H10, H11), 1.67–1.61 (2H, m, H2', H6'), 1.58–1.51 (1H, m, H1'), 1.37–1.26 (2H, m, H2', H6'), 1.18–1.07 (3H, m, H1', H3', H5'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.7 (C7), 59.4 (C8), 54.4 (C9, C12), 47.3 (C4), 33.1 (C3, C5), 25.5 (C1), 24.8 (C2, C6), 23.9 (C10, C11); **HRMS** (ES^+) calc. for $C_{12}H_{23}N_2O$ $[M+H]^+$ 211.1805, found 211.1805.

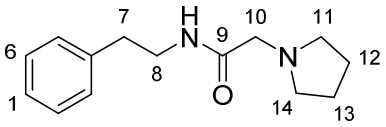
Pentyl-2-pyrrolidin-1-ylacetamide 17



Based on modified literature procedures.[415,419] Chloroacetyl chloride (0.55 mL, 6.9 mmol, 1.0 eq) was added dropwise to a solution of *n*-pentylamine (0.80 mL, 6.9 mmol, 1.0 eq) and triethylamine (1.1 mL, 7.6 mmol, 1.1 eq) in DCM (21 mL) at 0 °C. The solution was warmed to RT and stirred for 1 h. DCM was added and the solution was washed with water, saturated aqueous $NaHCO_3$, and saturated aqueous NH_4Cl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was redissolved in ethanol (12 mL), pyrrolidine (1.2 mL, 13.8 mmol, 2.0 eq) was added, and the solution was heated at reflux for 15 h. The solution was cooled to RT and aqueous HCl (3.0 M) was added. The solution was extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown oil (1.15 g, 84%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.10 (1H, br s, NH), 3.20 (2H,

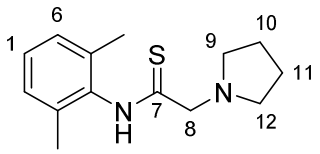
app q, *J* = 7.0 Hz, H5), 3.07 (2H, s, H7), 2.54–2.51 (4H, m, H8, H11), 1.75–1.71 (4H, m, H9, H10), 1.45 (2H, quin, *J* = 7.0 Hz, H4), 1.29–1.22 (4H, m, H2, H3), 0.83 (3H, t, *J* = 7.0 Hz, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.7 (C6), 59.3 (C7), 54.5 (C8, C11), 38.8 (C5), 29.3 (C4), 29.1 (C3), 23.9 (C9, C10), 22.3 (C2), 13.9 (C1); **HRMS** (ES^+) calc. for $C_{11}H_{23}N_2O$ $[M+H]^+$ 199.1805, found 199.1805.

Phenethyl-2-pyrrolidin-1-ylacetamide 18



Based on a modified literature procedure.[415] A solution of phenethyl-2-chloroacetamide **300** (200 mg, 1.0 mmol, 1.0 eq) and pyrrolidine (0.17 mL, 2.0 mmol, 2.0 eq) in ethanol (1.7 mL) was heated at reflux for 14 h then cooled to RT and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (3.0 M) and extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown oil (215 mg, 93%); **R_f** 0.30 (60% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3380 (m), 2802 (w), 1657 (s), 1520 (s), 1246 (w), 1148 (m), 748 (m), 699 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.31–7.27 (2H, m, H2, H6), 7.23–7.18 (3H, m, H1, H3, H5), 3.54 (2H, q, *J* = 7.0 Hz, H8), 3.09 (2H, s, H10), 2.83 (2H, t, *J* = 7.0 Hz, H7), 2.49–2.46 (4H, m, H11, H14), 1.72–1.68 (4H, m, H12, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 171.0 (C9), 139.0 (C4), 128.8 (C3, C5), 128.6 (C2, C6), 126.5 (C1), 59.1 (C10), 54.5 (C11, C14), 39.9 (C8), 35.7 (C7), 23.9 (C12, C13); **HRMS** (ES^+) calc. for $C_{14}H_{20}N_2NaO$ $[M+Na]^+$ 255.1468, found 255.1458.

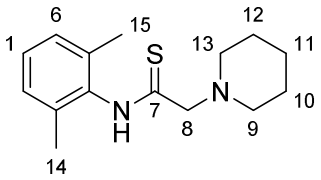
2,6-Dimethylphenyl-2-pyrrolidin-1-ylethanethioamide 19



Based on a modified literature procedure.[253] To a solution of 2,6-dimethylphenyl-2-pyrrolidin-1-ylacetamide **10** (500 mg, 2.2 mmol, 1.0 eq) in toluene (10.8 mL) was added Lawesson's reagent

(526 mg, 1.3 mmol, 0.6 eq). The solution was heated at 80 °C for 15 h then cooled to RT and concentrated *in vacuo*. The residue was redissolved in DCM and washed with aqueous HCl (3.0 M, 2×15 mL). The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow solid. The crude product was purified by flash chromatography (20% ethyl acetate / petrol) to afford the *title compound* as a yellow solid (103 mg, 20%). **R_f** 0.55 (50% ethyl acetate / petrol); **mp** 120 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3133 (w), 2971 (w), 2797 (w), 1486 (s), 1290 (m), 1095 (m), 772 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 10.50 (1H, br s, NH), 7.19 (1H, dd, *J* = 8.5, 6.0 Hz, H1), 7.13–7.11 (2H, m, H2, H6), 3.80 (2H, s, H8), 2.80–2.77 (4H, m, H9, H12), 2.23 (6H, s, 2×Ar-CH_3), 1.87 (4H, quin, *J* = 3.5 Hz, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 200.5 (C7), 135.8 (C4), 135.0 (C3, C5), 128.4 (C2, C6), 128.2 (C1), 67.3 (C8), 54.4 (C9, C12), 24.2 (C10, C11), 18.3 (2×Ar-CH_3); **HRMS** (ES^+) calc. for $C_{14}H_{21}N_2S$ $[M+H]^+$ 249.1420, found 249.1416.

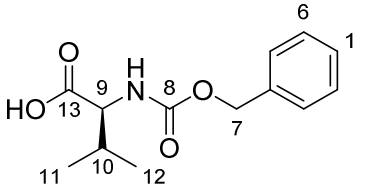
2,6-Dimethylphenyl-2-piperidin-1-ylethanethioamide 20



Based on a modified literature procedure.[253] A solution of 2,6-dimethylphenyl-2-piperidin-1-ylacetamide **11** (200 mg, 0.81 mmol, 1.0 eq) and Lawesson's reagent (220 mg, 0.54 mmol, 0.7 eq) in toluene (5.4 mL) was heated at reflux for 15 h. The solution was cooled and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (3.0 M) and washed with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined aqueous layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by flash chromatography to afford the *title compound* as a yellow solid (38 mg, 18%). **R_f** 0.65 (50% ethyl acetate / petrol); **mp** 132–134 °C; **IR** (thin film) ν_{max} / cm^{-1} 3159 (m), 2940 (m) 1487 (s), 1310 (m), 1092 (m), 993 (w), 766 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 10.7 (1H, br s, NH), 7.19 (1H, dd, *J* = 9.0, 6.0 Hz, H1), 7.14–7.12 (2H, m, H2, H6), 3.59 (2H, s, H8), 2.63

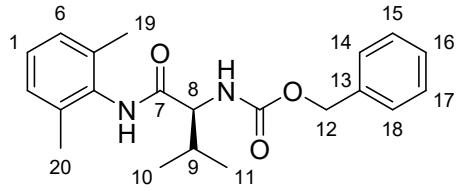
(4H, br s, H9, H13), 2.22 (6H, s, H14, H15), 1.64 (4H, quin, *J* = 5.5 Hz, H10, H12), 1.53–1.49 (2H, m, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 199.9 (C7), 135.8 (C4), 135.0 (C3, C5), 128.4 (C2, C6), 128.2 (C1), 70.2 (C8), 54.6 (C9, C13), 26.3 (C10, C12), 23.6 (C11), 18.2 (C14, C15); **HRMS** (ES^+) calc. for $C_{15}H_{23}N_2S$ $[M+H]^+$ 263.1577, found 263.1570.

[(Benzyloxy)carbonyl]-L-valine 21



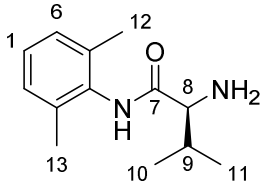
Based on a literature procedure.[420] To a solution of L-valine (1.0 g, 8.5 mmol, 1.0 eq) in aqueous NaOH (2.0 M, 5.0 mL) at 0 °C was added benzyl chloroformate (1.2 mL, 8.5 mmol, 1.0 eq) and aqueous Na_2CO_3 (2.0 M, 7.8 mL). The solution was stirred at 0 °C for 30 minutes then warmed to RT and stirred for 16 h. The solution was acidified with aqueous HCl (6.0 M) and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **21** as a colourless oil (1.85 g, 86%). **R_f** 0.45 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3324 (br, m), 2966 (w), 1709 (s), 1522 (m), 1217 (m), 1033 (w), 730 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.37–7.30 (5H, m, H1, H2, H3, H5, H6); 5.28 (1H, d, *J* = 9.0 Hz, NH), 5.12 (2H, s, H7), 4.35 (1H, dd, *J* = 9.0, 4.5 Hz, H9), 2.28–2.19 (1H, m, H10), 1.01 (3H, d, *J* = 7.0 Hz, H11), 0.93 (3H, d, *J* = 7.0 Hz, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_c 176.8 (C13), 156.4 (C8), 136.1 (C4), 128.6 (C2, C6), 128.3 (C3, C5), 128.2 (C1), 67.2 (C7), 58.9 (C9), 31.0 (C10), 19.0 (C11), 17.4 (C12); **LRMS** (ES^+) calc. for $C_{13}H_{18}NO_4$ $[M+H]^+$ 252.1, found 252.1. Data consistent with literature values.[420]

Benzyl (*S*)-{1-[(2,6-dimethylphenyl)amino]-3-methyl-1-oxobutan-2-yl}carbamate 22



Based on a modified literature procedure.[421] A solution of [(benzyloxy)carbonyl]-L-valine **21** (996 mg, 4.0 mmol, 1.0 eq), and *N*-methylmorpholine (0.44 mL, 4.0 mmol, 1.0 eq) in tetrahydrofuran (7.9 mL) was cooled to 0 °C and isobutyl chloroformate (0.51 mL, 4.0 mmol, 1.0 eq) was added. The solution was stirred for 10 minutes and 2,6-dimethylaniline (0.59 mL, 4.8 mmol, 1.2 eq) was added. The solution was warmed to RT and stirred for 16 h during which time a white precipitate formed. The solution was filtered and the precipitate was washed successively with saturated aqueous $NaHCO_3$, aqueous HCl (5% v/v), water, and ether. The precipitate was dried *in vacuo* to afford the *title compound* as a white solid (700 mg, 50%). $\mathbf{R_F}$ 0.60 (10% methanol / DCM); **mp** 194 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3284 (m), 1688 (m), 1656 (s), 1532 (w), 1248 (m), 1043 (w), 774 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.43 (1H, br s, NH), 7.36–7.32 (5H, m, H15, H16, H17, H18, H19), 7.10–7.07 (1H, m, H1), 7.04–7.02 (2H, m, H2, H6), 5.46 (1H, d, *J* = 8.5 Hz, NH), 5.14 (1H, d, *J* = 12.5 Hz, H13), 5.10 (1H, d, *J* = 12.5 Hz, H13'), 4.18 (1H, dd, *J* = 8.5, 6.5 Hz, H8), 2.34–2.28 (1H, m, H9), 2.15 (6H, s, H20, H21), 1.08 (3H, d, *J* = 6.5 Hz, H10), 1.02 (3H, d, *J* = 6.5 Hz, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.8 (C7), 135.3 (C3, C5), 133.4 (C4), 128.6 (C2), 128.3 (C14), 128.2 (C15, C16, C17, C18, C19), 128.1 (C6), 127.4 (C1), 67.2 (C13), 61.0 (C8), 30.3 (C9), 19.6 (C10), 18.5 (C20, C21), 17.9 (C11); **HRMS** (ES^+) calc. for $C_{21}H_{27}N_2O_3$ $[M+H]^+$ 355.2016, found 355.2018.

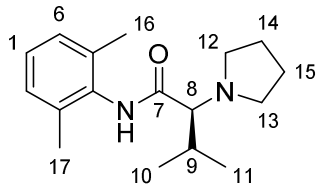
(*S*)-2,6-Dimethylphenyl-2-amino-3-methylbutanamide 23



Based on a modified literature procedure.[422] Pd (10 wt.% Pd on C, 240 mg, 0.23 mmol, 0.2 eq) was added to a solution of benzyl (*S*)-{1-[(2,6-dimethylphenyl]amino}-3-methyl-1-oxobutan-2-

yl)carbamate **22** (400 mg, 1.13 mmol, 1.0 eq) in ethanol (75 mL). H_2 was bubbled through the solution for 5 minutes and the solution was stirred under an atmosphere of H_2 for 4 h. The solution was filtered through Celite and concentrated *in vacuo* to afford **23** as a brown oil (229 mg, 92%). **R_f** 0.20 (10% methanol / DCM); **IR** (thin film) ν_{max} / cm^{-1} 3232 (w), 2965 (w), 1666 (s), 1521 (w), 1473 (w), 1372 (w), 1335 (w), 909 (m), 831 (m), 735 (s), 680 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.95 (1H, br s, NH), 7.06 (3H, s, H1, H2, H6), 3.52 (1H, s, H8), 2.53–2.45 (1H, m, H9), 2.22 (6H, s, H12, H13), 1.07 (3H, d, *J* = 7.0 Hz, H10), 0.95 (3H, d, *J* = 7.0 Hz, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.5 (C7), 134.9 (C3, C5), 134.2 (C4), 128.2 (C2, C6), 126.8 (C1), 60.4 (C8), 30.6 (C9), 20.0 (C10), 18.7 (C12, C13), 16.1 (C11); **LRMS** (ES^+) calc. for $C_{13}H_{21}N_2O$ $[M+H]^+$ 221.2, found 221.1. Data consistent with literature values.[423]

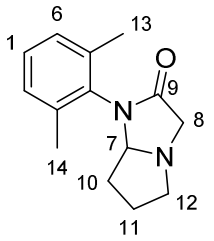
(*S*)-2,6-Dimethylphenyl-3-methyl-2-pyrrolidin-1-ylbutanamide 24



Based on a modified literature procedure.[424] A solution of (*S*)-2,6-dimethylphenyl-2-amino-3-methylbutanamide **23** (225 mg, 1.0 mmol, 1.0 eq), 1,4-dibromobutane (0.48 mL, 4.0 mmol, 4.0 eq), and *N*,*N*-diisopropylethylamine (0.59 mL, 3.4 mmol, 3.7 eq) in ethanol (20 mL) was heated at reflux for 21 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (2.0 M) and the solution was washed with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and the solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to an off-white solid. The crude product was purified by flash chromatography (100% DCM to 1% methanol / DCM) to afford the *title compound* as a white solid (100 mg, 36%). **R_f** 0.10 (5% methanol / DCM); **mp** 187 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3233 (m), 2959 (m), 1646 (s), 1519 (m), 1470 (w), 1227 (w), 767 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.16 (1H, br s, NH), 7.07 (3H, s, H1, H2, H6), 2.84 (1H, d, *J* = 4.0

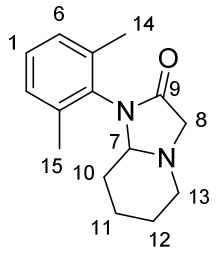
Hz, H8), 2.81–2.76 (2H, m, H12, H13), 2.68–2.63 (2H, m, H12', H13'), 2.28 (6H, s, H17, H18), 2.25–2.17 (1H, m, H9), 1.82–1.79 (4H, m, H14, H15), 1.14 (6H, dd, *J* = 7.0, 1.0 Hz, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.4 (C7), 134.4 (C3, C5), 134.1 (C4), 128.5 (C2, C6), 126.6 (C1), 75.0 (C8), 52.2 (C12, C13), 29.8 (C9), 23.2 (C14, C13), 20.4 (C10), 19.7 (C16, C17), 17.6 (C11); **HRMS** (ES^+) calc. for $C_{17}H_{27}N_2O$ $[M+H]^+$ 275.2118, found 275.2117.

1-(2,6-Dimethylphenyl)tetrahydro-1*H*-pyrrolo[1,2-a]imidazol-2(3*H*)-one 25



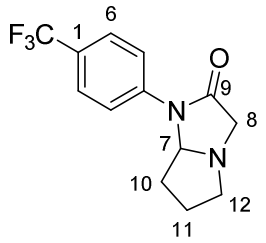
To phosphate buffer (0.2 M, pH 7.5, 167 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/FV/EV (5.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.20 µmol), 2,6-dimethylphenyl-2-pyrrolidin-1-ylacetamide **10** (4.0 mL of a 0.1 M solution in methanol, 0.4 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (89 mg, 97%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.19–7.06 (2H, m, H1, H2, H6), 5.34–5.14 (1H, m, H7), 3.83 (1H, d, *J* = 16.5 Hz, H8), 3.45 (1H, d, *J* = 16.5 Hz, H8'), 3.32–3.24 (1H, m, H12), 2.89–2.79 (1H, m, H12'), 2.23 (6H, s, H13, H14), 2.04–1.81 (3H, m, H10, H11), 1.77–1.64 (1H, m, H10'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.1 (C9), 137.5 (C3/C5), 135.6 (C5/C3), 133.3 (C4), 129.0 (C1), 128.9 (C2/C6), 128.4 (C6/C2), 82.0 (C7), 56.9 (C8), 56.0 (C12), 28.8 (C10), 24.0 (C11), 18.6 (C13, C14); **HRMS** (ES^+) calc. for $C_{14}H_{18}N_2NaO$ $[M+Na]^+$ 253.1312, found 253.1311.

1-(2,6-Dimethylphenyl)hexahydroimidazo[1,2-*a*]pyridin-2(3*H*)-one 26



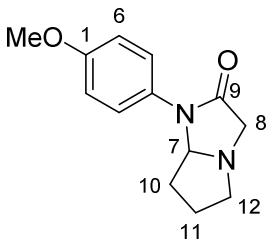
To phosphate buffer (0.2 M, pH 7.5, 167 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RT2/AP/A184I (5.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.20 µmol), 2,6-dimethylphenyl-2-piperidin-1-ylacetamide **11** (4.0 mL of a 0.1 M solution in methanol, 0.4 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (90 mg, 92%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.17–7.04 (3H, m, H1, H2, H6), 4.11–4.06 (1H, m, H7), 3.63 (1H, d, *J* = 13.5 Hz, H8), 3.20 (1H, dd, *J* = 13.5, 2.0 Hz, H8'), 3.07 (1H, dt, *J* = 11.0, 3.0 Hz, H13), 2.52–2.41 (1H, m, H13'), 2.29 (3H, s, H14), 2.23 (3H, s, H15), 1.84–1.76 (1H, m, H10), 1.74–1.62 (2H, m, H12), 1.52–1.41 (2H, m, H11), 1.41–1.29 (1H, m, H10'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 170.6 (C9), 137.9 (C4), 136.0 (C3, C5), 128.8 (C6), 128.6 (C2), 128.4 (C1), 79.5 (C7), 55.9 (C8), 49.9 (C13), 22.2 (C10), 27.6 (C11), 24.5 (C12), 19.0 (C14), 18.8 (C17); **HRMS** (ES^+) calc. for $C_{15}H_{20}N_2NaO$ $[M+H]^+$ 245.1648, found 245.1642.

1-[4-(Trifluoromethyl)phenyl]tetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 27



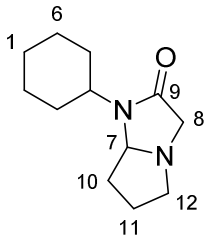
To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant KSK19/AM/E267F (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.4 µmol), 4-trifluoromethylphenyl-2-pyrrolidin-1-yl)acetamide **14** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (50% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (36 mg, 67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.63 (2H, d, *J* = 9.0 Hz, H2, H6), 7.58 (2H, d, *J* = 9.0 Hz, H3, H5), 5.44 (1H, dd, *J* = 6.5, 3.5 Hz, H7), 3.77 (1H, d, *J* = 17.5 Hz, H8), 3.50 (1H, d, *J* = 17.5 Hz, H8'), 3.26 (1H, dt, *J* = 9.5, 5.0 Hz, H12), 2.71 (1H, d, *J* = 9.5, 8.0 Hz, H12'), 2.36–2.28 (1H, m, H10), 1.93–1.86 (2H, m, H11), 1.84–1.76 (1H, m, H10'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.1 (C9), 140.1 (C4), 126.7 (q, *J* = 32.0 Hz, C1), 126.2 (q, *J* = 4.0 Hz, C2 &C6), 124.0 (q, *J* = 320.0 Hz, CF_3), 120.6 (C3, C5), 81.1 (C7), 57.3 (C8), 35.7 (C12), 31.1 (C10), 24.4 (C11); **HRMS** (ES^+) calc. for $C_{13}H_{13}N_2NaOF_3$ $[M+Na]^+$ 293.0872, found 293.0868.

1-(4-Methoxyphenyl)tetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 28



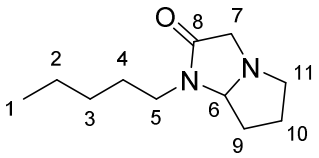
To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant KSK19/QP/F87V (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.4 µmol), 4-methoxyphenyl-2-pyrrolidin-1-ylacetamide **15** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (50% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (41 mg, 88%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.30–7.27 (2H, m, H3, H5), 6.94–6.91 (2H, m, H2, H6), 5.34 (1H, dd, *J* = 6.5, 3.0 Hz, H7), 3.80 (3H, s, OCH_3), 3.78 (1H, d, *J* = 17.0 Hz, H8), 3.45 (1H, d, *J* = 17.0 Hz, H8'), 3.26 (1H, ddd, *J* = 10.0, 6.0, 4.0 Hz, H12), 2.72 (1H, td, *J* = 9.0, 6.0 Hz, H12'), 2.18–2.12 (1H, m H10), 1.92–1.84 (2H, m, H11), 1.84–1.77 (1H, m, H10'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 176.8 (C9), 157.4 (C1), 129.7 (C4), 123.9 (C3, C5), 114.4 (C2, C6), 81.8 (C7), 57.6 (C8), 56.0 (C12), 55.5 (OCH_3), 30.7 (C10), 24.3 (C11); **HRMS** (ES^+) calc. for $C_{13}H_{16}N_2NaO$ $[M+Na]^+$ 255.1104, found 255.1010.

1-Cyclohexyltetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 29



To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/FV/EV/V78F (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), cyclohexyl-2-pyrrolidin-1-ylacetamide **16** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (20% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (27 mg, 66%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 4.82 (1H, t, *J* = 5.5 Hz, H7), 3.73 (1H, tt, *J* = 12.5, 3.5 Hz, H4), 3.60 (1H, d, *J* = 16.5 Hz, H8), 3.25 (1H, dd, *J* = 16.5, 1.5 Hz, H8'), 3.21–3.17 (1H, m, H12), 2.60–2.55 (1H, m, H12'), 2.21–2.11 (1H, m, H10), 1.95–1.90 (1H, m, H11), 1.84–1.71 (4H, m, H3, H5, H10', H11'), 1.68–1.64 (2H, m, H2, H6), 1.63–1.55 (1H, m, H3), 1.52–1.44 (1H, m, H5), 1.38–1.29 (2H,m, H2', H6'), 1.17–1.10 (2H, m, H1); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 172.0 (C9), 79.5 (C7), 57.9 (C8), 56.0 (C12), 52.2 (C4), 33.4 (C10), 31.8 (C5), 30.1 (C3), 25.8 (C6),25.5 (C1), 24.7 (C11); **HRMS** (ES^+) calc. for $C_{12}H_{21}N_2O$ $[M+H]^+$ 209.1648, found 209.1649.

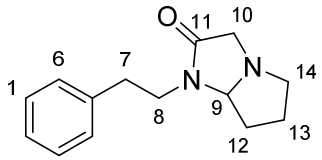
1-Pentyltetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 30



To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/FV/EV/L188Q (10.0 mL of

a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), pentyl-2-pyrrolidin-1-ylacetamide **17** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (20% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (21 mg, 55%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 4.82–4.80 (1H, m, H6), 3.63 (1H, d, *J* = 16.5 Hz, H7), 3.51 (1H, ddd, *J* = 14.0, 9.5, 7.0 Hz, H5), 3.26 (1H, d, *J* = 16.5 Hz, H7'), 3.22–3.18 (1H, m, H11), 2.97 (1H, ddd, *J* = 14.0, 9.0, 5.5 Hz, H5'), 2.58 (1H, td, *J* = 8.0, 6.0 Hz, H11'), 2.11–2.05 (1H, m, H9), 1.92–1.85 (1H, m, H10), 1.83–1.75 (2H, m, H9', H10'), 1.54–1.47 (2H, m, H4), 1.37–1.28 (4H, m, H2, H3), 0.90 (3H, t, *J* = 7.0 Hz, H1); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 172.2 (C8), 80.3 (C6), 57.3 (C7), 56.2 (C11), 40.6 (C5), 30.3 (C9), 29.2 (C2), 27.1 (C4), 24.9 (C10), 22.4 (C3), 14.0 (C1); **HRMS** (ES^+) calc. for $C_{11}H_{21}N_2O$ $[M+H]^+$ 197.1648, found 197.1649.

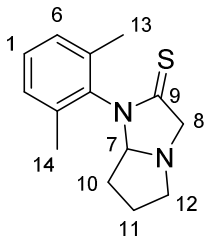
1-Phenethyltetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 31



To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant KSK19/AM/E267F (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), phenethyl-2-pyrrolidin-1-ylacetamide **18** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (50% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (32 mg, 70%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H

7.32–7.29 (2H, m, H1, H3), 7.24–7.21 (3H, m, H2, H4, H6), 4.64 (1H, dd, *J* = 6.0, 4.0 Hz, H9), 3.75 (1H, ddd, *J* = 14.0, 9.0, 7.0 Hz, H8), 3.57 (1H, d, *J* = 16.5 Hz, H10), 3.25–3.20 (2H, m, H10', H8'), 3.15 (1H, ddd, *J* = 10.0, 6.5, 4.0 Hz, H14), 2.94–2.83 (2H, m, H7), 2.54 (1H, td, *J* = 9.0, 6.5 Hz, H14'), 2.00–1.94 (1H, m, H12), 1.84–1.71 (2H, m, H13), 1.68–1.63 (1H, m, H12'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 172.6 (C11), 138.6 (C5), 128.7 (C4, C6), 128.6 (C1, C3), 126.6 (C2), 80.6 (C9), 57.2 (C10), 56.1 (C14), 42.3 (C8), 33.7 (C7), 30.1 (C12), 24.0 (C13); **HRMS** (ES^+) calc. for $C_{14}H_{18}N_2NaO$ $[M+Na]^+$ 253.1311, found 253.1307.

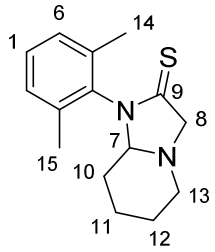
1-(2,6-Dimethylphenyl)tetrahydro-1*H*-pyrrolo[1,2-*a*]imidazole-2(3*H*)-thione 32



To phosphate buffer (0.2 M, pH 7.5, 167 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/FV/EV (5.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.20 µmol), 2,6-dimethylphenyl-2-pyrrolidin-1-ylethanethioamide **19** (4.0 mL of a 0.1 M solution in methanol, 0.4 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (90 mg, 92%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.24–7.21 (1H, m, H1), 7.17–7.14 (2H, m, H2, H6), 5.44 (1H, d, *J* = 5.0 Hz, H7), 4.27 (1H, d, *J* = 18.0 Hz, H8), 4.07 (1H, d, *J* = 18.0 Hz, H8'), 3.35–3.31 (1H, m, H12), 2.84 (1H, td, *J* = 9.5, 7.0 Hz, H12'), 2.22 (3H, s, H13), 2.19 (3H, s, H14), 2.03–1.95 (3H, m, H10, H11), 1.89–1.82 (1H, m, H10'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 198.1 (C9), 136.7 (C5), 136.7 (C4), 134.6 (C3), 129.3 (C1), 129.0

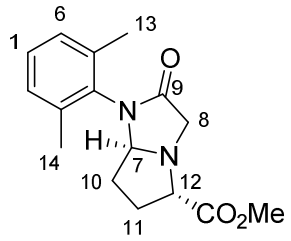
(C2, C6), 89.5 (C7), 69.0 (C8), 55.4 (C12), 28.0 (C10), 24.0 (C11), 18.5 (C13), 18.3 (C14); **HRMS** (ES^+) calc. for $C_{14}H_{19}N_2S$ $[M+H]^+$ 247.1264, found 247.1258.

1-(2,6-Dimethylphenyl)hexahydroimidazo[1,2-*a*]pyridine-2(3*H*)-thione 33



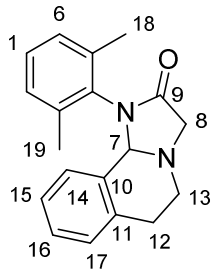
To phosphate buffer (0.2 M, pH 7.5, 169 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant KSK19/QP/FV (5.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.20 µmol), 2,6-dimethylphenyl-2-piperidin-1-ylethanethioamide **20** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (34 mg, 66%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.21–7.18 (1H, m, H1), 7.15–7.13 (1H, m, H2), 7.10 (1H, d, *J* = 7.5 Hz, H6), 4.29 (1H, dt, *J* = 9.0, 3.0 Hz, H7), 4.12 (1H, d, *J* = 15.0 Hz, H8), 3.64 (1H, dd, *J* = 15.0, 3.0 Hz, H8'), 3.05 (1H, dt, *J* = 11.0, 4.0 Hz, H13), 2.51–2.46 (1H, m, H13'), 2.28 (3H, s, H15), 2.19 (3H, s, H14), 1.80–1.74 (1H, m, H10), 1.69–1.64 (2H, m, H12), 1.57–1.51 (2H, m, H11), 1.37–1.28 (1H, m, H10'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 198.3 (C9), 136.9 (C3), 134.9 (C5), 134.6 (C4), 128.8 (C2, C6), 128.8 (C1), 84.1 (C7), 67.8 (C8), 49.4 (C13), 27.3 (C11), 24.4 (C12), 22.0 (C10), 18.6 (C14), 18.6 (C15); **HRMS** (ES^+) calc. for $C_{15}H_{20}N_2NaS$ $[M+Na]^+$ 282.1239, found 282.1233.

Methyl (5*S*,7a*R*)-1-(2,6-dimethylphenyl)-2-oxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-5-carboxylate 34



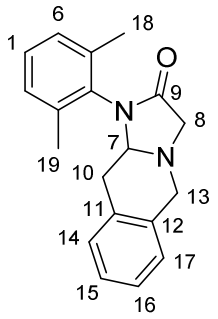
To phosphate buffer (0.2 M, pH 7.5, 162 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20mmol), $P450_{BM3}$ mutant RT2/F81W (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), methyl-{2-[(2,6-dimethylphenyl)amino]-2-oxoethyl}-L-prolinate **12** (4.0 mL of a 0.1 M solution in methanol, 0.4 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (113 mg, 98%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.21–7.07 (3H, m, H1, H2, H6), 5.39 (1H, dd, *J* = 6.0, 2.5 Hz, H7), 3.97 (1H, d, *J* = 17.0 Hz, H8), 3.80 (3H, s, OCH_3), 3.74 (1H, t, *J* = 8.0 Hz, H12), 3.57 (1H, d, *J* = 17.0 Hz, H8'), 2.40–2.30 (1H, m, H11), 2.25 (3H, s, H13), 2.23 (3H, s, H14), 2.16–2.08 (1H, m, H11'), 2.07–2.00 (1H, m, H10), 1.85–1.74 (1H, m, H10'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 173.0 ($C(O)OCH_3$), 171.5 (C9), 137.4 (C3), 135.6 (C5), 132.9 (C4), 129.2 (C2), 128.9 (C6), 128.6 (C1), 82.1 (C7), 68.6 (C12), 56.6 (C8), 52.5 (OCH_3), 28.3 (C10), 28.1 (C11), 18.7 (C13), 18.6 (C14); **HRMS** (ES^+) calc. for $C_{16}H_{20}N_2NaO_3$ $[M+Na]^+$ 311.1366, found 311.1358.

1-(2,6-Dimethylphenyl)-1,5,6,10*b*-tetrahydroimidazo[2,1-*a*]isoquinolin-2(3*H*)-one 35



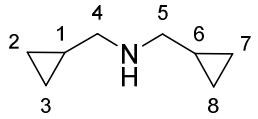
To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/H171L (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), 2,6-dimethylphenyl-2-[3,4-dihydroisoquinolin-2(1*H*)-yl]acetamide **13** (2.0 mL of a 0.1 M solution in methanol, 0.2 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (20% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (47 mg, 81%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.20–7.12 (4H, m, H1, H2, H6, H15), 6.87 (1H, d, *J* = 7.5 Hz, H14), 6.83 (1H, t, *J* = 7.5 Hz, H16), 6.18 (1H, d, *J* = 7.5 Hz, H17), 5.86 (1H, s, H7), 3.97 (1H, d, *J* = 16.0 Hz, H8), 3.54 (1H, d, *J* = 16.0 Hz, H8'), 3.30 (1H, ddd, *J* = 13.5, 9.0, 4.0 Hz, H13), 3.09–3.03 (2H, m, H12), 2.96–2.90 (1H, m, H13'), 2.37 (3H, s, H18), 1.50 (3H, s, H19); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 172.3 (C9), 139.0 (C11), 135.9 (C3/C5), 135.1 (C5/C3), 133.2 (C4), 129.4 (C10), 128.8 (C15), 128.7 (C14), 128.5 (C17), 128.5 (C2, C6), 128.4 (C16), 76.1 (C7), 58.2 (C8), 47.5 (C13), 28.0 (C12), 18.5 (C18), 18.0 (C19); **HRMS** (ES^+) calc. for $C_{19}H_{20}N_2NaO$ $[M+Na]^+$ 315.1468, found 315.1461.

1-(2,6-Dimethylphenyl)-1,5,10,10*a*-tetrahydroimidazo[1,2-*b*]isoquinolin-2(3*H*)-one 36



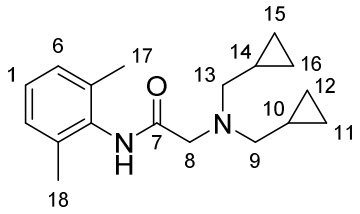
To phosphate buffer (0.2 M, pH 7.5, 164 mL) in a 500 mL beaker was added glucose (20 mL of a 1.0 M solution in 0.2 M phosphate buffer, 20 mmol), $P450_{BM3}$ mutant RP/IA/EV (10.0 mL of a 40 µM solution in 0.1 M phosphate buffer, 0.40 µmol), 2,6-dimethylphenyl-2-[3,4-dihydroisoquinolin-2(1*H*)-yl]acetamide **13** (2.0 mL of a 0.1 M solution in methanol, 0.20 mmol), and GDH (200 µL of a 2 U/µL solution in 0.2 M phosphate buffer, 400 U). $NADP^+$ monosodium salt (4.0 mL of a 4.0 mM solution in 0.2 M phosphate buffer, 16.0 µmol) was added and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (50% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (41 mg, 70%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.22–7.15 (4H, m, H2, H6, H15, H16), 7.15–7.10 (2H, m, H1, H17), 7.03 (1H, d, *J* = 7.5 Hz, H14), 4.65–4.62 (1H, m, H7), 4.17 (1H, d, *J* = 14.5 Hz, H8), 3.87–3.82 (2H, m, H8', H13), 3.43 (1H, dd, *J* = 13.5, 2.0 Hz, H13'), 2.86 (1H, dd, *J* = 14.5, 10.0 Hz, H10), 2.63 (1H, dd, *J* = 14.5, 3.5 Hz, H10'), 2.32 (3H, s, H18), 2.26 (3H, s, H19); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 170.3 (C9), 137.9 (C11), 136.0 (C3/C5), 134.0 (C5/C3), 132.7 (C12), 131.8 (C4), 129.5 (C14), 128.9 (C2/C6), 128.8 (C1), 128.6 (C6/C2), 126.9 (C15/C16), 126.7 (C16/C15), 126.7 (C17), 76.4 (C7), 55.9 (C8), 53.2 (C13), 33.0 (C10), 18.9 (C18), 18.7 (C19); **HRMS** (ES^+) calc. for $C_{19}H_{20}N_2NaO$ $[M+Na]^+$ 315.1468, found 315.1461.

Bis(cyclopropylmethyl)amine 44



Based on a literature procedure.[425] To a solution of $LiAlH_4$ (273 mg, 7.2 mmol, 1.25 eq) in tetrahydrofuran (3.4 mL) at 0 °C was added *N*-(cyclopropylmethyl)cyclopropanecarboxamide **301** (800 mg, 5.8 mmol, 1.0 eq) in tetrahydrofuran (2.3 mL) dropwise. The solution was heated at reflux for 15 h then cooled to RT. Water (0.24 mL), aqueous NaOH (4.0 M, 0.24 mL), and water (0.72 mL) were added and the solution was filtered through Celite and the residue was washed with ether. The filtrate was concentrated *in vacuo* to afford **44** as a yellow oil (522 mg, 72%). $\mathbf{R_f}$ 0.55 (50% ethyl acetate / petrol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 2.47 (4H, d, *J* = 7.0 Hz, H4, H5), 1.00–0.90 (2H, m, H3, H6), 0.47 (4H, ddd, *J* = 8.0, 6.0, 4.5 Hz, H1, H2, H7, H8), 0.11 (4H, dt, *J* = 6.0, 4.5 Hz, H1', H2' H7', H8'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 55.0 (C4, C5), 11.3 (C3, C6), 3.3 (C1, C2, C7, C8); **LRMS** not found. Data consistent with literature values.[425]

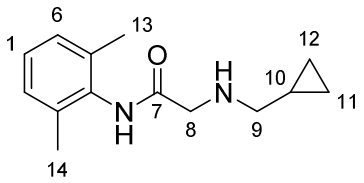
2,6-Dimethylphenyl-2-bis(cyclopropylmethyl)aminoacetamide 45



Based on a modified literature procedure.[415] A solution of 2,6-dimethylphenyl-2-chloro-acetamide **6** (237 mg, 1.2 mmol, 1.0 eq) and bis(cyclopropylmethyl)amine **44** (300 mg, 2.4 mmol, 2.0 eq) in ethanol (6.0 mL) was heated at reflux for 15 h. The solution was cooled to RT and concentrated *in vacuo*. The residue was redissolved in aqueous HCl (3.0 M) and extracted with ether. The pH of the aqueous layer was adjusted to 11 with aqueous NaOH (50% w/v) and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (30% ether / pentane to 50% ether / pentane) to afford the *title compound* as a yellow oil (104 mg, 30%). $\mathbf{R_f}$ 0.40 (50% ethyl acetate /

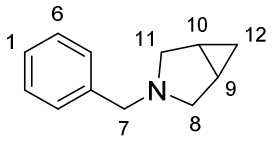
petrol); **IR** (thin film) ν_{max} / cm^{-1} 3279 (br, m), 3077 (w), 3002 (m), 2922 (w), 1682 (m), 1493 (s), 1428 (w), 1020 (w), 768 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.99 (1H, br s, NH), 7.10–7.08 (3H, m, H1, H2, H6), 3.43 (2H, s, H8), 2.58 (4H, d, *J* = 6.5 Hz, H9, H13), 2.24 (6H, s, H17, H18), 0.98–0.88 (2H, m, H10, H14), 0.59–0.54 (4H, m, H11, H12, H15, H16), 0.20–0.16 (4H, m, H11', H12', H15', H16'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.4 (C7), 135.1 (C3, C5), 134.1 (C4), 128.2 (C2, C6), 127.1 (C1), 60.5 (C9, C13), 57.8 (C8), 18.6 (C17, C18), 9.3 (C10, C14), 4.0 (C11, C12, C15, C16); **HRMS** (ES^+) calc. for $C_{18}H_{27}N_2O$ $[M+H]^+$ 287.2118, found 287.2116.

2,6-Dimethylphenyl-2-(cyclopropylmethyl)aminoacetamide 46



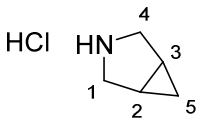
Based on a modified literature procedure.[415] A solution of 2,6-dimethylphenyl-2-chloroacetamide **6** (300 mg, 1.5 mmol, 1.0 eq) and cyclopropylmethylamine (0.26 mL, 3.0 mmol, 2.0 eq) in ethanol (2.5 mL) was heated at reflux for 15 h. The solution was concentrated *in vacuo* and aqueous HCl (3.0 M) was added at which point a precipitate formed. The solution was filtered and the residue was washed with petrol and dried *in vacuo* to afford the *title compound* as a white solid (190 mg, 39%). **R_f** 0.08 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3409 (br, s), 1656 (s), 999 (s), 824 (m), 762 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 10.19 (1H, br s, NH), 9.32 (1H, br s, NH), 7.12–7.06 (3H, m, H1, H2, H6), 4.02 (2H, t, *J* = 5.0 Hz, H8), 2.87 (2H, m, H9), 2.17 (6H, s, H13, H14), 1.16–1.06 (1H, m, H10), 0.58 (2H, ddd, *J* = 7.5, 6.5, 4.5 Hz, H11, H12), 0.40–0.37 (2H, m, H11', H12'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 164.3 (C7), 135.6 (C3, C5), 134.5 (C4), 128.2 (C2, C6), 127.3 (C1), 51.7 (C9), 47.4 (C8), 18.7 (C13, C14), 7.3 (C10), 4.4 (C11, C12); **HRMS** (ES^+) calc. for $C_{14}H_{21}N_2O$ $[M+H]^+$ 233.1648, found 233.1650.

3-Benzyl-3-azabicyclo[3.1.0]hexane 48



Based on a literature procedure.[426] 3-Benzyl-3-azabicyclo[3.1.0]hexane-2,4-dione **302** (665 mg, 3.3 mmol, 1.0 eq) was added to a solution of sodium bis(2-methoxyethoxy)aluminium hydride (60 wt.% in toluene, 4.8 mL, 14.9 mmol, 4.5 eq) in anhydrous ether (17 mL) at 0 °C. The solution was stirred at 0 °C for 30 minutes then heated at reflux for 4 h. The solution was cooled to RT and water (8.5 mL) was added. The solution was filtered through Celite and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ether / petrol) to afford **48** as a colourless oil (95 mg, 17%). $\mathbf{R_f}$ 0.45 (50% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2898 (w), 2782 (m), 1377 (w), 1172 (w), 1149 (w), 862 (m), 739 (m), 698 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.30–7.18 (5H, m, H1, H2, H3, H5, H6), 3.58 (2H, s, H7), 2.92 (2H, d, *J* = 8.5 Hz, H8, H11), 2.32 (2H, d, *J* = 8.5 Hz, H8', H11'), 1.32–1.28 (2H, m, H9, H10), 0.78 (1H, app q, *J* = 4.0 Hz, H12), 0.31 (1H, td, *J* = 7.5, 4.0 Hz, H12'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 139.8 (C4), 128.5 (C2, C6), 128.1 (C3, C5), 126.7 (C1), 59.0 (C7), 54.7 (C8, C11), 15.4 (C9, C10), 6.8 (C12); **LRMS** (ES^+) calc. for $C_{12}H_{16}N$ $[M+H]^+$ 174.1, found 174.2. Data is consistent with literature values.[427]

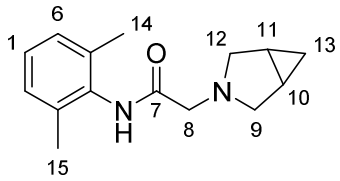
3-Azabicyclo[3.1.0]hexane hydrochloride 49



Based on a literature procedure.[427] Pd (10 wt.% on C, 66 mg, 0.06 mmol, 0.1 eq) was added to a solution of 3-benzyl-3-azabicyclo[3.1.0]hexane **48** (108 mg, 0.62 mmol, 1.0 eq) and conc. HCl (53 µL, 0.63 mmol, 1.0 eq) in methanol (1.6 mL) under an atmosphere of N_2. H_2 was bubbled through the solution for 5 minutes and the reaction was stirred under an atmosphere of H_2 for 16 h. The solution was filtered through Celite and concentrated *in vacuo* to afford **49** as an off-white solid (70 mg, 95%). $\mathbf{R_f}$ 0.10 (10% methanol / DCM); **mp** 120–123 °C (amorphous) (lit. mp 162–164 °C); **IR** (thin film) ν_{max} /

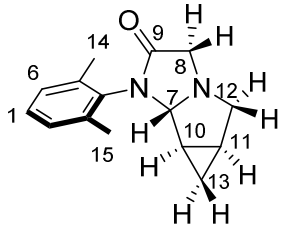
cm^{-1} 3386 (br, s), 2963 (s), 2759 (s), 2649 (m), 2559 (w), 1600 (w), 1412 (w), 1041 (w), 970 (w), 824 (m), 789 (s), 674 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 9.86 (1H, br s, NH), 9.34 (1H, br s, NH), 3.46–3.33 (4H, m, H1, H4), 1.70–1.67 (2H, m, H2, H3), 0.88–0.84 (1H, m, H5), 0.79–0.74 (1H, m, H5'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 47.3 (C1, C4), 15.4 (C2, C3), 7.1 (C5); **HRMS** (Cl^+) calc. for $C_5H_{10}N$ $[M+H]^+$ 84.0808, found 84.0809. Data is consistent with literature values.[427]

2,6-Dimethylphenyl-2-(3-azabicyclo[3.1.0]hexan-3-yl)acetamide 50



Based on a modified literature procedure.[415] A solution of 3-azabicyclo[3.1.0]hexane hydrochloride **49** (70 mg, 0.59 mmol, 1.0 eq), 2,6-dimethylphenyl-2-chloroacetamide **6** (117 mg, 0.59 mmol, 1.0 eq) and triethylamine (0.16 mL, 1.18 mmol, 2.0 eq) in ethanol was heated at reflux for 15 h. The solution was cooled to RT and aqueous HCl (3.0 M) was added. The solution was extracted with ether and the pH of the aqueous layer was adjusted to 11 with aqueous NaOH (1.0 M). The solution was extracted with DCM and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow solid (91 mg, 63%). **R_f** 0.35 (40% ethyl acetate / petrol); **mp** 74 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3280 (br, m), 2902 (w), 2798 (w), 1663 (s), 1498 (s), 1478 (m), 1176 (w), 768 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.40 (1H, br s, NH), 7.10–7.06 (3H, s, H1, H2, H6), 3.31 (2H, s, H8), 3.19 (2H, d, *J* = 8.5 Hz, H9, H12), 2.65 (2H, d, *J* = 8.5 Hz, H9', H12'), 2.23 (6H, s, H14, H15), 1.48–1.44 (2H, m, H10, H11), 0.68 (1H, app q, *J* = 4.0 Hz, H13), 0.47 (1H, td, *J* = 8.0, 4.5 Hz, H13'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.3 (C7), 135.0 (C3, C5), 133.7 (C4), 128.3 (C2, C6), 127.1 (C1), 58.2 (C8), 55.8 (C9, C12), 18.7 (C14 &C15), 15.6 (C10, C11), 6.9 (C13); **HRMS** (ES^+) calc. for $C_{15}H_{21}N_2O$ $[M+H]^+$ 245.1648, found 245.1642.

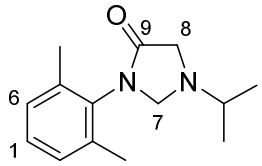
(5a*SR*,6a*RS*,6b*SR*)-1-(2,6-Dimethylphenyl)hexahydrocyclopropa[3,4]pyrrolo[1,2-*a*]imidazol-2(3*H*)-one 51



A solution containing 2,6-dimethylphenyl-2-(3-azabicyclo[3.1.0]hexan-3-ylacetamide **50** (270 µL of a 100 mM solution in isopropanol, 0.27 mmol), glucose (1.35 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.35 mmol), GDH (270 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 540 U), and $NADP^+$ monosodium salt (270 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 1.08 µmol) in 100 mM phosphate buffer (8.64 mL) was created. A 400 µL aliquot of this solution was added to each well of a 24 well plate with each well containing a different mutant $P450_{BM3}$ enzyme (100 µL of a 10 µM solution in phosphate buffer, pH 7.5). The plate was shaken for 22 h then the contents of each well were transferred to an Eppendorf tube and 300 µL of ethyl acetate was added to each tube. The tubes were shaken then centrifuged at 14,000 rpm for 2 minutes. 60 µL of the organic layer was transferred to a gas chromatography vial and the samples were analysed by gas chromatography. All tubes for which ≥27% conversion was seen were combined and extracted with ethyl acetate (see below). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (70% ethyl acetate / petrol to 100% ethyl acetate) to afford the *title compound* as a colourless oil (0.4 mg). $\mathbf{R_f}$ 0.10 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2921 (w), 2852 (w), 1707 (s), 1475 (m), 1362 (m), 1193 (w), 1111 (w), 1023 (w), 914 (w), 773 (w), 749 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.18–7.15 (1H, m, H1), 7.13–7.11 (2H, m, H2, H6), 5.11 (1H, s, H7), 3.70 (1H, d, *J* = 16.5 Hz, $H8_{exo}$), 3.41 (1H, d, *J* = 9.0 Hz, $H12_{exo}$), 3.31 (1H, d, *J* = 16.5 Hz, $H8_{endo}$), 3.01 (1H, dd, *J* = 9.0, 4.0 Hz, $H12_{endo}$), 2.29 (3H, s, H14), 2.25 (3H, s, H15), 1.66–1.62 (1H, m, H11), 1.44 (1H, ddd, *J* = 8.5, 6.0, 4.0 Hz, H10), 0.69 (1H, app q, *J* = 4.5 Hz, $H13_{exo}$), 0.59 (1H, td, *J* = 8.5, 5.0 Hz, $H13_{endo}$); **^{13}C NMR**

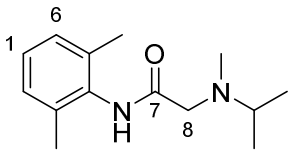
(101 MHz, $CDCl_3$) δ_C 172.7 (C9), 137.9 (C5), 135.8 (C3), 133.2 (C4), 128.9 (C2), 128.8 (C6), 128.5 (C1), 82.9 (C7), 56.9 (C12), 56.4 (C8), 18.7 (C10), 18.4 (C15), 18.3 (C14), 18.0 (C11), 7.1 (C13); **HRMS** (ES^+) calc. for $C_{15}H_{19}N_2O$ $[M+H]^+$ 243.1492, found 243.1491.

3-(2,6-Dimethylphenyl)-1-isopropylimidazolidin-4-one 55



Based on a modified literature procedure.[251] A solution of 2,6-dimethylphenyl-2-isopropylaminoacetamide **6** (600 mg, 2.72 mmol, 1.0 eq) and formaldehyde (6.1 mL of a 36 wt.% aqueous solution, 82 mmol, 30 eq) in formic acid (27.0 mL) was heated at reflux for 1 h. The solution was cooled to RT and saturated aqueous $NaHCO_3$ was added slowly and the mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a pink solid. The crude product was purified by flash chromatography (DCM + 1% Et_3N) to afford the *title compound* as a white solid (595 mg, 94%). $\mathbf{R_f}$ 0.60 (10% methanol / DCM); **mp** 60–62 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2971 (w), 1714 (s), 1477 (m), 1443 (w), 1325 (m), 1184 (w), 774 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.16 (1H, dd, *J* = 9.0, 6.5 Hz, H1), 7.11–7.09 (2H, m, H2, H6), 4.30 (2H, s, H7), 3.48 (2H, s, H8), 2.68 (1H, sept, *J* = 6.5 Hz, NCH), 2.25 (6H, s, 2×Ar-CH_3), 1.13 (6H, d, *J* = 6.5 Hz, $NCH(CH_3)_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) 170.5 (C9), 136.6 (C3, C5), 133.3 (C4), 128.7 (C1), 128.6 (C2, C6), 69.6 (C7), 54.1 (C8), 54.0 (NCH), 20.8 ($NCH(CH_3)_2$), 17.9 (2×Ar-CH_3); **HRMS** (ES^+) calc for $C_{14}H_{20}N_2NaO$ $[M+Na]^+$ 255.1468, found 255.1468.

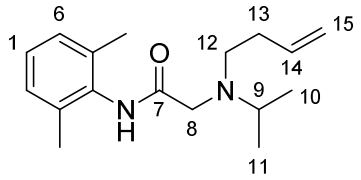
2,6-Dimethylphenyl-2-isopropyl(methyl)aminoacetamide 58



Based on a modified literature procedure.[277] To a solution of 1-isopropyl-3-phenylimidazolidin-4-one **55** (40 mg, 0.17 mmol, 1.0 eq), and $AlCl_3$ (51 mg, 0.38 mmol, 2.2 eq) in

DCM (0.7 mL) was added triethylsilane (60 µL, 0.38 mmol, 2.2 eq). The solution was stirred for 19 h. DCM was added and the solution was washed with saturated aqueous $NaHCO_3$. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a white solid (23 mg, 59%). **R_f** 0.55 (5% methanol / DCM); **mp** 78–82 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3234 (w), 2962 (m), 1715 (s), 1501 (s), 768 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.93 (1H, br s, NH), 7.09 (3H, s, H1, H2, H6), 3.18 (2H, s, H8), 2.97 (1H, sept, *J* = 6.5 Hz, NCH), 2.42 (3H, s, NCH_3), 2.24 (6H, s, 2×Ar-CH_3), 1.10 (6H, d, *J* = 6.5 Hz, NCH(CH_3)$_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.9 (C7), 135.7 (C3, C5), 133.9 (C4), 128.2 (C2, C6), 127.1 (C1), 56.7 (C8), 54.4 (NCH), 39.3 (NCH_3), 18.6 (2×Ar-CH_3), 18.5 (NCH(CH_3)$_2$); **HRMS** (ES^+) calc. for $C_{14}H_{23}N_2O$ $[M+H]^+$ 235.1805, found 235.1812.

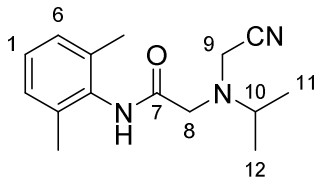
2,6-Dimethylphenyl-2-but-3-en-1-yl(isopropyl)aminoacetamide 60



To a solution of 3-(2,6-dimethylphenyl)-1-isopropylimidazolidin-4-one **55** (30 mg, 0.13 mmol, 1.0 eq) and ethylaluminium dichloride (0.26 mL of a 1.0 M solution in hexane, 0.26 mmol, 2.0 eq) in DCM (0.52 mL) was added allyltributyltin (0.20 mL, 0.65 mmol, 5.0 eq). The solution was stirred for 2 d then quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2). The solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol to 40% ethyl acetate / petrol) to afford the *title compound* as a white solid (32 mg, 90%). **R_f** 0.60 (80% ethyl acetate / petrol); **mp** 60 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3281 (w), 2959 (m), 1668 (s), 1495 (s), 1464 (m), 911 (m), 768 (m), 731 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.90 (1H, br s, NH), 7.09 (3H, s, H1, H2, H6), 5.80 (1H, ddt, *J* = 17.0, 10.5, 7.0 Hz, H14), 5.08 (1H, dq, *J* = 17.0, 1.5 Hz, $H15_{trans}$), 5.04 (1H, d, *J* = 10.5 Hz, $H15_{cis}$), 3.23 (2H, s, H8), 3.07 (1H, sept, *J* = 6.5 Hz, H9), 2.67 (2H, t, *J* = 7.0 Hz, H12), 2.30 (2H, q, *J* = 7.0 Hz, H13), 2.22 (6H, s,

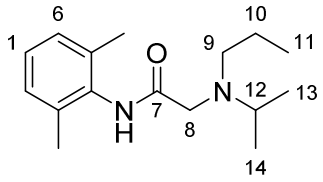
2×Ar-CH_3), 1.10 (6H, d, *J* = 6.5 Hz, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.7 (C7), 136.2 (C14), 135.2 (C3, C5), 133.9 (C4), 128.3 (C2, C6), 127.1 (C1), 116.6 (C15), 54.1 (C8), 51.5 (C9), 51.2 (C12), 33.2 (C13), 18.7 (2×Ar-CH_3), 18.3 (C10, C11); **HRMS** (ES^+) calc. for $C_{17}H_{27}N_2O$ $[M+H]^+$ 275.2118, found 275.2111.

2,6-Dimethylphenyl-2-cyanomethyl(isopropyl)aminoacetamide 61



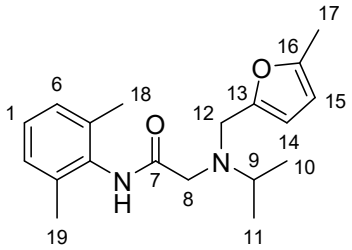
To a solution of 3-(2,6-dimethylphenyl)-1-isopropylimidazolidin-4-one **55** (30 mg, 0.13 mmol, 1.0 eq) and ethyl aluminium dichloride (0.26 mL of a 1.0 M solution in hexanes, 0.26 mmol, 2.0 eq) in DCM (0.26 mL) was added trimethylsilyl cyanide (80 µL, 0.65 mmol, 5.0 eq). The solution was stirred for 5 d then quenched with saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2). The mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (30% ethyl acetate / petrol to 70% ethyl acetate / petrol) to afford the *title compound* as a colourless oil [5 mg, 16% (20% brsm)]. **R_f** 0.40 (80% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3272 (w), 2968 (w), 2924 (w), 1664 (s), 1503 (s), 1174 (w), 1120 (w), 710 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.38 (1H, br s, NH), 7.14–7.08 (3H, m, H1, H2, H6), 3.66 (2H, s, H9), 3.43 (2H, s, H8), 3.17 (1H, sept, *J* = 6.5 Hz, H10), 2.23 (6H, s, 2×Ar-CH_3), 1.22 (6H, d, *J* = 6.5 Hz, H11, H12); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.0 (C7), 135.1 (C3, C5), 133.2 (C4), 128.4 (C2, C6), 127.5 (C1), 116.3 (CN), 54.3 (C8), 53.5 (C10), 40.5 (C9), 18.5 (C11, C12), 18.6 (2×Ar-CH_3); **HRMS** (ES^+) calc. for $C_{15}H_{22}N_3O$ $[M+H]^+$ 260.1757, found 260.1764.

2,6-Dimethylphenyl-2-isopropyl(propyl)aminoacetamide 62



To a solution of 3-(2,6-dimethylphenyl)-1-isopropylimidazolidin-4-one **55** (30 mg, 0.13 mmol, 1.0 eq) and ethyl aluminium dichloride (0.26 mL of a 1.0 M solution in hexanes, 0.26 mmol, 2.0 eq) in DCM (0.52 mL) was added thioanisole (80 µL, 0.65 mmol, 5.0 eq). The solution was stirred for 4 d then quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2). The mixture was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol to 60% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (6 mg, 13%). $\mathbf{R_f}$ 0.35 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3253 (w), 2961 (m), 2928 (m), 1662 (s), 1499 (s), 765 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.94 (1H, br s, NH), 7.09 (3H, s, H1, H2, H6), 3.21 (2H, s, H8), 3.05 (1H, sept, *J* = 6.5 Hz, H12), 2.53 (2H, t, *J* = 7.5 Hz, H9), 2.23 (6H, s, 2×Ar-CH_3), 1.56 (2H, sex, *J* = 7.5 Hz, H10), 1.09 (6H, d, *J* = 6.5 Hz, H13, H14), 0.94 (3H, t, *J* = 7.5 Hz, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.9 (C7), 135.2 (C3, C5), 134.1 (C4), 128.2 (C2, C6), 127.1 (C1), 54.3 (C8), 54.0 (C9), 51.8 (C12), 22.1 (C10), 18.6 (2×Ar-CH_3), 18.4 (C13, C14), 11.9 (C11); **HRMS** (ES^+) calc. for $C_{16}H_{27}N_2O$ $[M+H]^+$ 263.2118, found 263.2109.

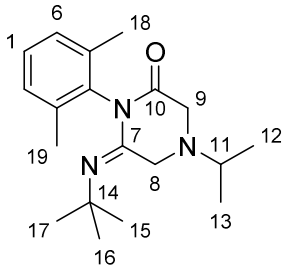
2,6-Dimethylphenyl-2-isopropyl[(5-methylfuran-2-yl)methyl]aminoacetamide 63



To a solution of 3-(2,6-dimethylphenyl)-1-isopropylimidazolidin-4-one **55** (250 mg, 1.1 mmol, 1.0 eq) and ethyl aluminium dichloride (2.2 mL of a 1.0 M solution in hexanes, 2.2 mmol, 2.0 eq) in DCM (4.4 mL) was added 2-methylfuran (0.30 mL, 3.3 mmol, 3.0 eq). The solution was stirred for 60

h. The solution was quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2) then extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ether / petrol to 40% ether / petrol) to afford the *title compound* as a yellow oil (18 mg, 5%). **R_f** 0.55 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3306 (br, m), 2965 (w), 2923 (w), 1684 (s), 1496 (s), 1464 (m), 1219 (w), 1166 (w), 1026 (w), 910 (m), 771 (m), 728 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.98 (1H, br s, NH), 7.11–7.05 (3H, m, H1, H2, H6), 6.11 (1H, d, *J* = 3.0 Hz, H14), 5.88–5.87 (1H, m, H15), 3.68 (2H, s, H12), 3.30 (2H, s, H8), 3.09 (1H, sept, *J* = 6.5 Hz, H9), 2.19 (3H, s, H17), 2.16 (6H, s, H18, H19), 1.13 (6H, d, *J* = 6.5 Hz, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.5 (C7), 152.1 (C16), 150.3 (C13), 135.2 (C3, C5), 134.0 (C4), 128.2 (C2, C6), 127.0 (C1), 109.6 (C14), 106.1 (C15), 53.0 (C8), 51.5 (C9), 48.6 (C12), 18.4 (C18, C19), 18.4 (C10, C11), 13.6 (C17); **HRMS** (Cl^+) calc. for $C_{19}H_{27}N_2O_2$ $[M+H]^+$ 315.2067, found 315.2066.

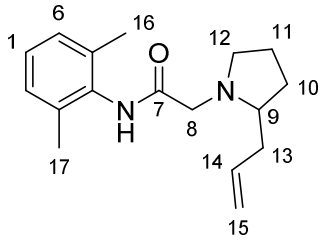
6-(*tert*-Butylimino)-1-(2,6-dimethylphenyl)-4-isopropylpiperazin-2-one 64



To a solution of 3-(2,6-dimethylphenyl)-1-isopropylimidazolidin-4-one **55** (30 mg, 0.13 mmol, 1.0 eq) and ethyl aluminium dichloride (0.26 mL of a 1.0 M solution in hexanes, 0.26 mmol, 2.0 eq) in DCM (0.52 mL) was added *tert*-butyl isocyanide (70 µL, 0.65 mmol, 5.0 eq). The solution was stirred for 19 h. The solution was quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2) then extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether / petrol to 50% ether / petrol) to afford the *title compound* as a yellow oil (18 mg, 44%). **R_f** 0.45 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2968 (w), 1703 (w), 1657 (s), 1473 (w), 1389 (w), 1364 (w), 1294 (w), 1209 (m), 1182 (m), 768 (w); **^{1}H NMR** (400 MHz,

$CDCl_3$) δ_H 7.11 (1H, dd, *J* = 8.0, 6.5 Hz, H1), 7.05–7.03 (2H, m, H2, H6), 3.56 (2H, s, H9), 3.50 (2H, s, H8), 2.87 (1H, sept, *J* = 6.5 Hz, H11), 2.04 (6H, s, H18, H19), 1.14 (6H, d, *J* = 6.5 Hz, H12, H13), 1.12 (9h, s, H15, H16, H17); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.0 (C10), 148.0 (C7), 135.9 (C4), 135.3 (C3, C5), 127.6 (C2, C6), 127.4 (C1), 53.9 (C8), 53.5 (C11), 53.1 (C14), 48.9 (C9), 30.8 (C15, C16, C17), 18.3 (C12, C13), 17.8 (C18, C19); **HRMS** Not found.

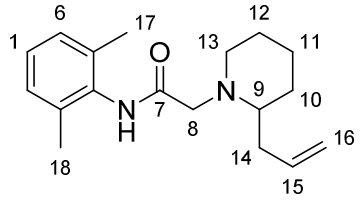
2,6-Dimethylphenyl-2-(2-allylpyrrolidin-1-yl)acetamide 65



Allyltributyltin (0.20 mL, 0.65 mmol, 5.0 eq) was added to a solution of 1-(2,6-dimethylphenyl)tetrahydro-1*H*-pyrrolo[1,2-*a*]imidazol-2(3*H*)-one **25** (30 mg, 0.13 mmol, 1.0 eq) and ethylaluminium dichloride (0.26 mL of a 1.0 M solution in hexanes, 0.26 mmol, 2.0 eq) and the solution was stirred for 22 h. The reaction was quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2). The solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol to 60% ethyl acetate / petrol) to afford the *title compound* as a yellow oil (25 mg, 71%, contains $RSnBu_3$ impurity). **R_f** 0.40 (70% ethyl acetate / petrol); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.70 (1H, br s, NH), 7.12–7.07 (3H, m, H1, H2, H6), 5.83 (1H, ddt, *J* = 17.0, 10.0, 7.0 Hz, H14), 5.11 (1H, dq, *J* = 17.0, 1.5 Hz, $H15_{trans}$), 5.08–5.05 (1H, m, $H15_{cis}$), 3.62 (1H, d, *J* = 17.0 Hz, H8), 3.33–3.26 (1H, m, H12), 3.13 (1H, d, *J* = 17.0 Hz, H8'), 2.76–2.71 (1H, m, H9), 2.48–2.38 (2H, m, H12', H13), 2.23 (6H, s, H16, H17), 2.21–2.15 (1H, m, H13'), 2.02–1.94 (1H, m, H10), 1.84–1.78 (2H, m, H11), 1.67–1.55 (2.5H, m, H10', $RSnBu_3$), 1.34 (1H, sex, *J* = 7.5 Hz, $RSnBu_3$), 1.31–1.28 (1H, m, $RSnBu_3$), 0.92 (1.5 h, t, *J* = 7.5 Hz, $RSnBu_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 169.9 (C7), 135.2 (C14), 135.1 (C3, C5), 133.8 (C4), 128.2 (C2, C6), 127.2 (C1), 117.2 (C15), 63.8 (C9), 57.9 (C8), 56.0 (C12), 39.0 (C13), 30.0 (C10), 27.9 ($RSnBu_3$), 26.9 ($RSnBu_3$), 23.0

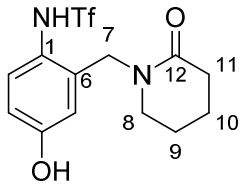
(C11), 18.6 (C16, C17), 17.6 ($RSnBu_3$), 13.6 ($RSnBu_3$); **HRMS** (ES^+) calc. for $C_{17}H_{25}N_2O$ $[M+H]^+$ 273.1961, found 273.1955.

2,6-Dimethylphenyl-2-(2-allylpiperidin-1-yl)acetamide 66



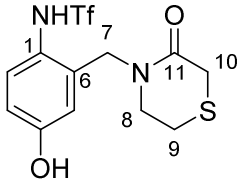
Allyltributyltin (0.19 mL, 0.60 mmol, 5.0 eq) was added to a solution of 1-(2,6-dimethylphenyl)hexahydroimidazo[1,2-*a*]pyridin-2(3*H*)-one **26** (30 mg, 0.12 mmol, 1.0 eq) and ethylaluminium dichloride (0.24 mL of a 1.0 M solution in hexanes, 0.24 mmol, 2.0 eq) and the solution was stirred for 22 h. The reaction was quenched with a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle's salt (1:2). The solution was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to an orange oil. The crude product was purified by flash chromatography (25% ethyl acetate / petrol to 60% ethyl acetate / petrol) to afford the title compound as a yellow oil (15 mg, 43%, contains $RSnBu_3$ impurity). $\mathbf{R_f}$ 0.40 (70% ethyl acetate / petrol); $^{\mathbf{1}}$**H NMR** (500 MHz, $CDCl_3$) δ_H 8.87 (1H, br s, NH), 7.12–7.07 (3H, m, H1, H2, H6), 5.82 (1H, dddd, *J* = 17.0, 10.0, 7.5, 6.5 Hz, H15), 5.12–5.05 (2H, m, H16), 3.55 (1H, d, *J* = 17.0 Hz, H8), 3.04–2.97 (2H, m, H8', H13), 2.59–2.54 (1H, m, H9), 2.46 (1H, ddd, *J* = 12.5, 9.5, 3.5 Hz, H13'), 2.39–2.27 (2H, m, H14), 2.23 (6H, s, H17, H18), 1.75–1.70 (2H, m, H10, H11), 1.69–1.53 (4H, m, H12, $RSnBu_3$), 1.46–1.40 (2H, m, H10', H11'), 1.39–1.34 (2H, m, $RSnBu_3$), 1.32–1.28 (2H, m, $RSnBu_3$), 0.92 (3H, t, *J* = 7.5 Hz, $RSnBu_3$); $^{\mathbf{13}}$**C NMR** (126 MHz, $CDCl_3$) δ_C 169.8 (C7), 135.1 (C3, C5, C15), 133.9 (C4), 128.5 (C2, C6), 127.1 (C1), 117.2 (C16), 60.3 (C9), 57.5 (C8), 53.7 (C13), 36.7 (C14), 30.3 (C10), 27.9 ($RSnBu_3$), 26.9 ($RSnBu_3$), 25.4 (C12), 22.9 (C11), 18.6 (C17, C18), 17.5 ($RSnBu_3$), 13.6 ($RSnBu_3$); **HRMS** (ES^+) calc. for $C_{18}H_{27}N_2O$ $[M+H]^+$ 287.2118, found 287.2111.

***N*-4-Hydroxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl-1,1,1-trifluoromethanesulfonamide 90**



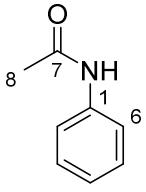
To a solution of *N*-2-[(2-oxopiperidin-1-yl)methyl]phenyl-1,1,1-trifluoromethanesulfonamide **86** (450 µL of a 0.1 M solution in ethanol, 0.045 mmol, 1.0 eq), glucose (2.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5), GDH (225 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5), $NADP^+$ monosodium salt (225 µL of a 4.0 mM solution in phosphate buffer, pH 7.5), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution) in 100 mM phosphate buffer (pH 7.5, 34 mL) was added $P450_{BM3}$ mutant RP/FV/EV (4.5 mL of a 20 µM solution in phosphate buffer, pH 7.5). The solution was stirred open to air for 23 h. The solution was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ethyl acetate / petrol to 70% ethyl acetate / petrol) to afford the *title compound* as a colourless oil [2 mg, 13% (20% brsm)]. $\mathbf{R_f}$ 0.25 (80% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3200 (br, w), 2919 (w), 1584 (w), 1504 (w), 1448 (w), 1376 (m), 1120 (s), 1145 (s), 908 (m), 732 (s); **^{1}H NMR** (500 MHz, CD_3OD) δ_H 7.15 (1H, d, *J* = 8.5 Hz, H2), 6.70 (1H, dd, *J* = 8.5, 3.0 Hz, H3), 6.67 (1H, d, *J* = 3.0 Hz, H5), 4.62 (2H, s, H7), 3.34–3.32 (2H, m, H8), 2.46–2.43 (2H, m, H11), 1.85–1.82 (4H, m, H9, H10); **^{13}C NMR** (126 MHz, CD_3OD) δ_C 171.8 (C12), 156.6 (C4), 134.3 (C1), 128.3 (C2), 125.9 (C6), 120.5 (q, *J* = 320.0 Hz, CF_3), 114.7 (C3), 114.5 (C5), 47.7 (C8), 46.5 (C7), 31.5 (C11), 22.5 (C9), 20.5 (C10); **HRMS** (ES^+) calc. for $C_{13}H_{16}F_3N_2O_4S$ $[M+H]^+$ 353.0777, found 353.0763.

***N*-4-Hydroxy-2-[(3-oxothiomorpholino)methyl]phenyl-1,1,1-trifluoromethanesulfonamide 91**



To a solution of *N*-2-[(3-oxothiomorpholino)methyl]phenyl-1,1,1-trifluoromethanesulfonamide **87** (420 µL of a 0.1 M solution in ethanol, 0.042 mmol, 1.0 eq), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5), GDH (210 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5), $NADP^+$ monosodium salt (210 µL of a 4.0 mM solution in phosphate buffer, pH 7.5), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution) in 100 mM phosphate buffer (pH 7.5, 32 mL) was added $P450_{BM3}$ mutant RP/FV/EV (4.2 mL of a 20 µM solution in phosphate buffer, pH 7.5). The solution was stirred open to air for 23 h. The solution was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (30% ethyl acetate / petrol to 100% ethyl acetate) to afford the *title compound* as a colourless oil (3 mg, 19%). $\mathbf{R_f}$ 0.60 (100% ethyl acetate); **IR** (thin film) ν_{max} / cm^{-1} 3230 (br, w), 2918 (w), 1604 (m), 1498 (m), 1373 (m), 1206 (s), 1143 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.44 (1H, d, *J* = 9.0 Hz, H2), 6.83 (1H, dd, *J* = 9.0, 3.0 Hz, H3), 6.78 (1H, d, *J* = 3.0 Hz, H5), 4.48 (2H, s, H7), 3.79 (2H, t, *J* = 6.0 Hz, H8), 3.37 (2H, s, H10), 2.86 (2H, t, *J* = 6.0 Hz, H9); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 168.3 (C11), 154.0 (C4), 130.3 (C6), 127.1 (C1), 126.5 (C2), 117.5 (C5), 116.8 (C3), 50.5 (C8), 49.7 (C7), 30.6 (C10), 26.0 (C9); **HRMS** (ES^+) calc. for $C_{12}H_{13}F_3N_2NaO_4S_2$ $[M+Na]^+$ 393.0161, found 393.0153.

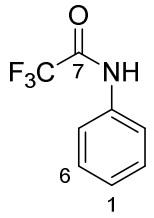
Phenylacetamide 93



Based on a literature procedure.[428] Acetic anhydride (0.33 mL, 3.5 mmol, 1.1 eq) was added dropwise to a solution of aniline (0.29 mL, 3.2 mmol, 1.0 eq) in DCM (6.4 mL) at 0 °C and the solution

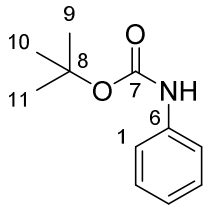
was stirred for 2 h. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with DCM. The combined organic layers were washed with aqueous HCl (3.0 M) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **93** as a yellow solid (382 mg, 88%). $\mathbf{R_f}$ 0.20 (50% ethyl acetate / petrol); **mp** 94–98 °C (amorphous) (lit. mp 112–114 °C); **IR** (thin film, ν_{max} / cm^{-1}) 3289 (m), 3136 (w), 3061 (w), 1657 (s), 1597 (s), 1539 (m), 1488 (s), 1368 (m), 1320 (s), 753 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.69 (1H. br s, NH), 7.50 (2H, d, *J* = 8.0 Hz, H2, H6), 7.30 (2H, t, *J* = 8.0 Hz, H3, H5), 7.09 (1H, t, *J* = 8.0 Hz, H4), 2.16 (3H, s, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.7 (C7), 138.0 (C1), 129.0 (C3, C5), 124.3 (C4), 120.0 (C2, C6), 24.6 (C8); **LRMS** calc. for $C_8H_{10}NO$ $[M+H]^+$ 136.1, found 136.1. Data consistent with literature values.[428]

Phenyl-2,2,2-trifluoroacetamide 94



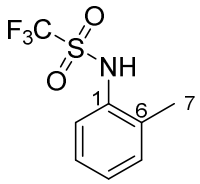
Based on a literature procedure.[429] A solution of aniline (0.59 mL, 6.4 mmol, 1.0 eq) and triethylamine (0.89 mL, 6.4 mmol, 1.0 eq) in DCM (10.7 mL) was cooled to 0 °C and trifluoroacetic anhydride (0.89 mL, 6.4 mmol, 1.0 eq) was added dropwise. The solution was warmed to RT and stirred for 1 h. DCM was added and the solution was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **94** as an off-white solid (1.14 g, 94%). $\mathbf{R_f}$ 0.45 (20% ethyl acetate / petrol); **mp** 70 °C (amorphous) (lit. mp 85–88 °C); **IR** (thin film) ν_{max} / cm^{-1} 3319 (m), 1704 (s), 1550 (m), 1286 (w), 1150 (s), 755 (m), 732 (w), 690 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.04 (1H, br s, NH), 7.60–7.58 (2H, m, H3, H5), 7.41 (2H, t, *J* = 8.0 Hz, H2, H6), 7.28–7.24 (1H, m, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 154.9 (q, *J* = 37.5 Hz, C7), 135.1 (C4), 129.4 (C2, C6), 126.4 (C1), 120.6 (C3, C5), 116.0 (q, *J* = 288.5 Hz, CF_3); **LRMS** (ES^-) calc. for $C_8H_5F_3NO$ $[M–H]^-$ 188.0, found 188.1. Data is consistent with literature values.[430]

***tert*-Butyl phenylcarbamate 95**



Based on a modified literature procedure.[431] A solution of aniline (0.20 mL, 2.2 mmol, 1.0 eq), di-*tert*-butyl dicarbonate (478 mg, 2.2 mmol, 1.0 eq), and a crystal of 4-(dimethylamino)pyridine in tetrahydrofuran (11 mL) was stirred for 1 h. Water was added and the solution was extracted with ether. The combined organic layers were washed with aqueous HCl (2.0 M), water, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a grey solid. The crude product was purified by flash chromatography (100% petrol) to afford **95** as a white solid (77 mg, 19%). $\mathbf{R_f}$ 0.50 (20% ethyl acetate / petrol); **mp** 118 °C (amorphous) (lit. mp 132 °C); **IR** (thin film) ν_{max} / cm^{-1} 3316 (m), 2983 (w), 1688 (s), 1597 (m), 1529 (s), 1440 (s), 1314 (m), 1244 (m), 1152 (s), 746 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.37–7.35 (2H, m, H1, H5), 7.31–7.27 (2H, m, H2, H4), 7.03 (1H, tt, *J* = 7.0, 1.0 Hz, H3), 6.45 (1H, br s, NH), 1.52 (9H, s, H9, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 152.8 (C7), 138.3 (C6), 129.0 (C2, C4), 123.0 (C3), 118.5 (C1, C5), 80.5 (C8) 28.4 (C9, C10, C11); **LRMS** (ES^+) calc. for $C_{11}H_{15}NNaO_2$ $[M+Na]^+$ 216.1 found 216.1. Data is consistent with literature values.[431]

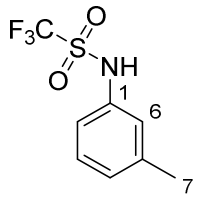
***N*-(*o*-Tolyl)-1,1,1-trifluoromethanesulfonamide 96**



Based on a modified literature procedure.[316] A solution of *o*-toluidine (0.20 mL, 1.9 mmol, 1.0 eq) in DCM (3.7 mL) was cooled to –78 °C, triethylamine (0.26 mL, 1.9 mmol, 1.0 eq) was added and the solution was stirred for 5 minutes. Trifluoromethanesulfonic anhydride (0.33 mL, 2.0 mmol, 1.1 eq) was added dropwise and the solution was stirred for 2 h. The solution was poured into ice and extracted with DCM. The combined organic layers were washed with aqueous HCl (2.0 M), and brine,

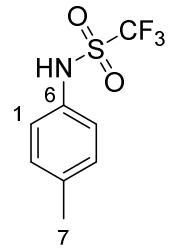
dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **96** as a brown solid (418 mg, 94%). **R_f** 0.55 (30% ethyl acetate / petrol); **mp** 42–44 °C (amorphous) (lit. mp 51–53 °C); **IR** (thin film) ν_{max} / cm^{-1} 3294 (m), 1497 (w), 1419 (m), 1363 (m), 1225 (s), 1195 (s), 1141 (s), 954 (w), 749 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.39–7.37 (1H, m, H4), 7.27–7.22 (1H, m, H2, H3, H5), 6.64 (1H, br s, NH), 2.38 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 127.3 (C2), 126.3 (C4), 119.8 (q, *J* = 320.0 Hz, CF_3), 17.8 (C7); **LRMS** (ES^-) calc. for $C_8H_7F_3NO_2S$ $[M–H]^-$ 238.0, found 238.1. Data is consistent with literature values.[432]

***N*-(*m*-Tolyl)-1,1,1-trifluoromethanesulfonamide 97**



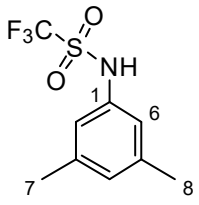
Based on a modified literature procedure.[316] A solution of *m*-toluidine (200 mg, 1.9 mmol, 1.0 eq) in DCM (3.7 mL) was cooled to –78 °C, triethylamine (0.26 mL, 1.9 mmol, 1.0 eq) was added and the solution was stirred for 5 minutes. Trifluoromethanesulfonic anhydride (0.33 mL, 2.0 mmol, 1.05 eq) was added dropwise and the solution was stirred for 1 h then poured into ice-water. The solution was extracted with DCM and the combined organic layers were washed with saturated aqueous $NaHCO_3$, aqueous HCl (2.0 M), and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **97** as a brown solid (325 mg, 73%). **R_f** 0.75 (50% ethyl acetate / petrol); **mp** 47–50 °C (amorphous) (lit. mp 64–65 °C); **IR** (thin film) ν_{max} / cm^{-1} 3257 (w), 1492 (w), 1416 (m), 1362 (m), 1227 (s), 1197 (s), 1140 (s), 964 (w), 785 (w), 692 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.27 (1H, t, *J* = 8.0 Hz, H3), 7.12 (1H, d, *J* = 8.0 Hz, H2), 7.09–7.07 (2H, m, H4, H6), 6.89 (1H, br s, NH), 2.37 (3H, s, H7); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 139.9 (C5), 133.5 (C1), 129.5 (C3), 128.5 (C2), 124.3 (C6), 120.7 (C4), 119.8 (q, *J* = 323.0 Hz, CF_3), 21.3 (C7); **LRMS** (ES^-) calc. for $C_8H_7F_3NO_2S$ $[M–H]^-$ 238.0, found 238.0. Data consistent with literature values.[433]

***N*-(*p*-Tolyl)-1,1,1-trifluoromethanesulfonamide 98**



Based on a modified literature procedure.[316] A solution of *p*-toluidine (0.51 mL, 4.7 mmol, 1.0 eq) in DCM (9.4 mL) was cooled to 0 °C and triethylamine (0.65 mL, 4.7 mmol, 1.0 eq) was added. The solution was stirred for 5 minutes and trifluoromethanesulfonic anhydride (0.82 mL, 4.9 mmol, 1.05 eq) was added dropwise and the solution was stirred for a further 4 h. The solution was poured into ice-water and extracted with DCM. The combined organic layers were washed with aqueous HCl (2.0 M) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **98** as an orange solid (772 mg, 69%). **R_f** 0.60 (60% ethyl acetate / petrol); **mp** 34–35 °C (amorphous) (lit. mp 35–37 °C); **IR** (thin film) ν_{max} / cm^{-1} 3287 (w), 1511 (w), 1408 (w), 1202 (s), 1139 (s), 952 (m), 607 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.21–7.15 (4H, m, H1, H2, H4, H5), 6.93 (1 h, br s, NH), 2.36 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 138.0 (C6), 131.0 (C3), 130.2 (C2, C4), 124.3 (C1, C5), 119.8 (q, J = 321.0 Hz, CF_3), 21.0 (C7); **LRMS** (ES^-) calc. for $C_8H_7F_3NO_2S$ $[M–H]^-$ 238.0, found 238.0. Data is consistent with literature values.[432]

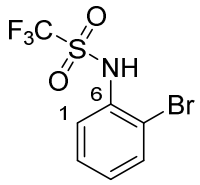
***N*-(3,5-Dimethylphenyl)-1,1,1-trifluoromethanesulfonamide 99**



Based on a modified literature procedure.[316] A solution of 3,5-dimethylaniline (400 mg, 3.3 mmol, 1.0 eq) in DCM (6.6 mL) was cooled to –78 °C and triethylamine (0.46 mL, 3.3 mmol, 1.0 eq) was added. The solution was stirred for 5 minutes and trifluoromethanesulfonic anhydride (0.58 mL, 3.5 mmol, 1.1 eq) was added dropwise. The solution was stirred for 3 h and poured into ice. The solution was extracted with DCM and the combined organic layers were washed with saturated

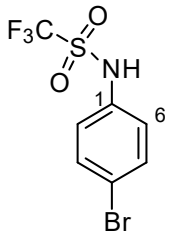
aqueous $NaHCO_3$, aqueous HCl (3.0 M), and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as an off-white solid (773 mg, 92%). $\mathbf{R_f}$ 0.25 (50% ethyl acetate / petrol); **mp** 60 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3259 (w), 1613 (w), 1599 (w), 1413 (m), 1355 (m), 1228 (m), 1190 (s), 1139 (s), 1036 (w), 968 (w), 910 (w), 847 (w), 707 (w), 609 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 6.94 (1H, s, H4), 6.87 (2H, s, H2, H6), 2.32 (6H, s, H7, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 139.6 (C3, C5), 133.4 (C1), 129.3 (C4), 121.3 (C2, C6), 119.8 (q, *J* = 323.0 Hz, CF_3), 21.2 (C7, C8); **HRMS** (ES^-) calc. for $C_9H_9F_3NO_2S$ $[M–H]^-$ 252.0312, found 252.0312.

***N*-(2-Bromophenyl)-1,1,1-trifluoromethanesulfonamide 100**



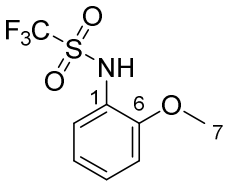
Based on a modified literature procedure.[316] A solution of 2-bromoaniline (0.39 mL, 3.5 mmol, 1.0 eq) and triethylamine (0.54 mL, 3.9 mmol, 1.1 eq) in DCM (7.0 mL) was cooled to –78 °C and trifluoromethanesulfonic anhydride (0.62 mL, 3.7 mmol, 1.05 eq) was added dropwise. The solution was stirred for 1 h then warmed to RT and stirred for a further 2 h. The solution was washed with saturated aqueous $NaHCO_3$ and aqueous HCl (2.0 M) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by flash chromatography (100% petrol to 20% ether / petrol) to afford **100** as an off-white solid (370 mg, 35%). $\mathbf{R_f}$ 0.65 (50% ethyl acetate / petrol); **mp** 57–59 °C (amorphous) (lit. mp 74–78 °C); **IR** (thin film) ν_{max} / cm^{-1} 3281 (w), 1479 (m), 1422 (m), 1367 (m), 1236 (m), 1210 (s), 1139 (s), 938 (w), 752 (w), 631 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.64 (1H, dd, *J* = 8.0, 1.5 Hz, H1), 7.62 (1H, dd, *J* = 8.0, 1.5 Hz, H4), 7.37 (1H, ddd, *J* = 8.0, 7.5, 1.5 Hz, H2), 7.17 (1H, ddd, *J* = 8.0, 7.5, 1.5 Hz, H3), 7.07 (1H, br s, NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 133.1 (C4), 132.6 (C6), 128.9 (C2), 128.3 (C3), 123.7 (C1), 116.6 (C5); **LRMS** (ES^-) calc. for $C_7H_4{}^{79}BrF_3NO_2S$ $[M–H]^-$ 301.9, found 301.9. Data is consistent with literature values.[434]

***N*-(4-Bromophenyl)-1,1,1-trifluoromethanesulfonamide 101**



Based on a modified literature procedure.[316] A solution of 4-bromoaniline (200 mg, 1.2 mmol, 1.0 eq) was cooled to –78 °C and triethylamine (0.16 mL, 1.2 mmol, 1.0 eq) was added. Trifluoromethanesulfonic anhydride (0.22 mL, 1.3 mmol, 1.1 eq) was added dropwise and the solution was stirred for 2 h. The solution was poured into ice and extracted with DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$, aqueous HCl (3.0 M), and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown oil (200 mg, 57%). **R_f** 0.60 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3284 (m), 1488 (m), 1403 (m), 1363 (w), 1201 (s), 1131 (s), 1013 (w), 949 (w), 709 (w), 631 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.52 (2H, d, *J* = 9.0 Hz, H3, H5), 7.17 (2H, d, *J* = 9.0 Hz, H2, H6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 132.8 (C3, C5), 125.2 (C2, C6), 121.3 (C1), 119.7 (q, *J* = 321.5 Hz, CF_3), 118.1 (C4); **HRMS** (ES^+) calc. for $C_7H_4{}^{79}BrF_3NO_2S$ $[M–H]^–$ 301.9104, found 301.9106.

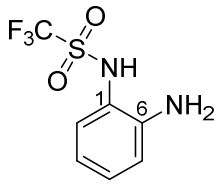
***N*-(2-Methoxyphenyl)-1,1,1-trifluoromethanesulfonamide 102**



Based on a modified literature procedure.[316] A solution of *o*-anisidine (0.14 mL, 1.2 mmol, 1.0 eq) in DCM (2.4 mL) was cooled to –78 °C and triethylamine (0.17 mL, 1.2 mmol, 1.0 eq) was added. The solution was stirred for 5 minutes and trifluoromethanesulfonic anhydride (0.23 mL, 1.3 mmol, 1.1 eq) was added dropwise. The solution was stirred for 2 h then poured into ice-water and extracted with DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$, aqueous HCl (3.0 M), and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **102**

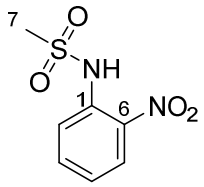
as a brown solid (207 mg, 66%). **R_f** 0.65 (40% ethyl acetate / petrol); **mp** 44 °C (amorphous) (lit. mp 54–56 °C); **IR** (thin film) ν_{max} / cm^{-1} 3278 (w), 1502 (m), 1449 (m), 1370 (m), 1199 (s), 1138 (s), 1114 (m), 949 (w), 750 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.51 (1H, dd, *J* = 8.0, 1.5 Hz, H2), 7.22 (td, *J* = 8.0, 1.5 Hz, H4), 7.13 (1H, br s, NH), 6.97 (1H, td, *J* = 8.0, 1.5 Hz, H3), 6.94 (1H, dd, *J* = 8.0, 1.5 Hz, H5), 3.90 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 150.1 (C6), 127.2 (C4), 123.5 (C1), 121.8 (C2), 121.2 (C3), 119.8 (q, *J* = 323.5 Hz, CF_3), 110.9 (C5), 55.9 (C7); **LRMS** (ES^-) calc. for $C_8H_7F_3NO_3S$ $[M–H]^-$ 254.0, found 254.0. Data consistent with literature values.[432]

***N*-(2-Aminophenyl)-1,1,1-trifluoromethanesulfonamide 104**



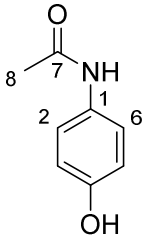
Based on a modified literature procedure.[316] A solution of *o*-phenylenediamine (400 mg, 3.7 mmol, 1.0 eq) in DCM (7.4 mL) was cooled to –78 °C and triethylamine (0.51 mL, 3.7 mmol, 1.0 eq) was added. The solution was stirred for 5 minutes and trifluoromethanesulfonic anhydride (0.68 mL, 4.1 mmol, 1.1 eq) was added dropwise. The solution was stirred for 2 h, poured into ice, and extracted with DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether / petrol to 60% ether / petrol) to afford **104** as a yellow oil (33 mg, 4%). **R_f** 0.55 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3272 (br, w), 1625 (w), 1510 (w), 1365 (m), 1196 (s), 1136 (s), 953 (w), 749 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.20–7.16 (2H, m, H4, H5), 6.83–6.78 (2H, m, H2, H3), 4.95 (3H, br s, NH, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 143.2 (C6), 130.2 (C4), 129.0 (C3), 119.9 (C2), 119.8 (q, *J* = 320.5 Hz, CF_3), 119.4 (C1), 117.8 (C5); **LRMS** (ES^-) calc. for $C_7H_6F_3N_2O_2S$ $[M–H]^-$ 239.0, found 239.0. Data consistent with literature values.[435]

_N_-(2-Nitrophenyl)methanesulfonamide 107



Based on a literature procedure.[436] Methanesulfonyl chloride (0.46 mL, 6.0 mmol, 1.7 eq) was added to a solution of 2-nitroaniline (500 mg, 3.6 mmol, 1.0 eq) in pyridine (1.2 mL) and the solution was stirred for 16 h. The solution was poured onto ice and filtered. The precipitate was redissolved in 2:3 tetrahydrofuran/ aqueous NaOH (1.0 M, 12 mL) and stirred for 2 h. The pH was adjusted to 7 with aqueous HCl (3.0 M) and the solution was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow solid. The crude product was purified by flash chromatography (10% ether / petrol to 30% ether / petrol) to afford **107** as a yellow solid (193 mg, 25%). **R_f** 0.25 (30% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3263 (m), 1613 (m), 1578 (w), 1529 (m), 1404 (m), 1323 (s), 1267 (s), 1168 (s), 1144 (s), 966 (s), 911 (m), 845 (s), 735 (s); **mp** 86–88 °C (amorphous) (lit. mp 102–103 °C); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 9.76 (1H, br s, NH), 8.27 (1H, dd, *J* = 8.5, 1.5 Hz, H5), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H2), 7.67 (1H, ddd, *J* = 8.5, 7.5, 1.5 Hz, H3), 7.24 (1H, ddd, *J* = 8.5, 7.5, 1.5 Hz, H4), 3.16 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 136.4 (C3), 136.3 (C6), 134.3 (C1), 126.7 (C5), 123.6 (C4), 119.5 (C2), 40.8 (C7); **LRMS** (ES^-) calc. for $C_7H_7N_2O_4S$ $[M–H]^-$ 215.0, found 214.9. Data is consistent with literature values.[437]

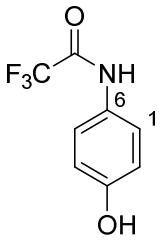
4-Hydroxyphenylacetamide 108



Two 500 mg paracetamol tablets (Galpharm Healthcare Ltd) were ground in a pestle and mortar then stirred in ethyl acetate (60 mL) for 1 h. The solution was filtered and concentrated *in*

vacuo to afford **108** as a white solid (330 mg). $\mathbf{R_f}$ 0.35 (100% ethyl acetate); **mp** 152–154 °C (amorphous) (lit. mp 166–168 °C); **IR** (thin film) ν_{max} / cm^{-1} 3270 (br, m), 1638 (m), 1605 (m), 1548 (m), 1509 (s), 1372 (m), 1322 (m), 1238 (m), 839 (m), 796 (w); **^{1}H NMR** (400 MHz, CD_3CN) δ_H 8.15 (1H, br s, NH), 7.35–7.31 (2H, m, H2, H6), 6.91 (1H, br s, OH), 6.75–6.72 (2H, m, H3, H5), 2.01 (3H, s, H8); **^{13}C NMR** (101 MHz, CD_3CN) δ_C 169.2 (C7), 154.0 (C4), 132.3 (C1), 122.3 (C2, C6), 116.0 (C3, C5), 24.0 (C8); **LRMS** (ES^-) calc. for $C_8H_8NO_2$ $[M–H]^-$ 150.1, found 150.1. Data consistent with literature values.[438]

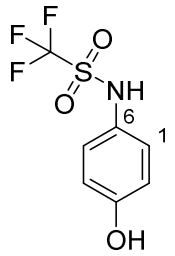
***N*-(4-Hydroxyphenyl)-2,2,2-trifluoroacetamide 110**



To a solution of *N*-phenyl-2,2,2-trifluoroacetamide **94** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (4.0 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 4.0 mmol), GDH (198 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 396 U), and $P450_{BM3}$ mutant KSK19/AI/AI (1.5 mL of a 50 µM solution in phosphate buffer, 0.075 µmol) in 100 mM phosphate buffer (pH 7.5, 30 mL) was added $NADP^+$ monosodium salt (198 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.79 µmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by flash chromatography (30% ether / petrol to 50% ether / petrol) to afford **110** as a colourless oil (8 mg, 51%). $\mathbf{R_f}$ 0.60 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3315 (br, m), 1693 (s), 1355 (m), 1515 (m), 1188 (s), 834 (m), 698 (w); **^{1}H NMR** (500 MHz, $(CD_3)_2CO$) δ_H 10.05 (1H, br s, NH), 8.48 (1H, s, OH), 7.55–7.52 (2H, m, H1, H5), 6.88–6.85 (2H, m, H2, H4); **^{13}C NMR** (126 MHz, $(CD_3)_2CO$) δ_C 155.3 (C3), 154.4 (q, *J* = 36.5 Hz, $C(O)CF_3$), 128.4 (C6), 122.6 (C1, C5), 116.3 (q, *J* = 287.5 Hz, CF_3), 115.4 (C2,

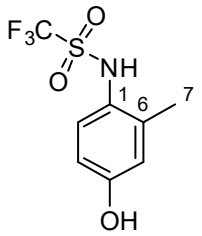
C4); **LRMS** (ES^-) calc. for $C_8H_5F_3NO_2$ $[M–H]^-$ 204.0, found 204.0. Data consistent with literature values.[439]

***N*-(4-Hydroxyphenyl)-1,1,1-trifluoromethanesulfonamide 111**



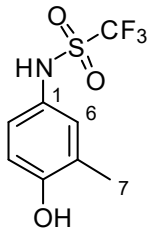
To a solution of *N*-phenyl-1,1,1-trifluoromethanesulfonamide **98** (670 µL of a 100 mM solution in methanol, 0.067 mmol), glucose (3.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.3 mmol), GDH (165 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 330 U) and $NADP^+$ monosodium salt (165 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.66 µmol), in 100 mM phosphate buffer (pH 7.5, 25 mL) was added $P450_{BM3}$ mutant RP/FV/EV/L437LV (3.3 mL of a 20 µM solution in phosphate buffer, 0.066 µM). The solution was stirred open to air for 20 h. Methanol was added and the solution was centrifuged at 9,500 g and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ether / petrol to 60% ether / petrol) to afford **111** as a colourless oil (9 mg, 56%). $\mathbf{R_f}$ 0.20 (30% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3528 (br, m), 3283 (br, m), 1495 (m), 1409 (m), 1361 (w), 1205 (s), 1136 (s), 1011 (w), 950 (w), 711 (w), 630 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.20–7.16 (2H, m, H1, H5), 6.85–6.82 (2H, m, H2, H4); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 155.6 (C3), 127.6 (C1, C5), 125.9 (C6), 116.3 (C2, C4); **LRMS** (ES^-) calc. for $C_7H_5F_3NO_3S$ $[M–H]^-$ 240.0, found 240.0. Data consistent with literature values.[440]

***N*-(4-Hydroxy-2-methylphenyl)-1,1,1-trifluoromethanesulfonamide 112**



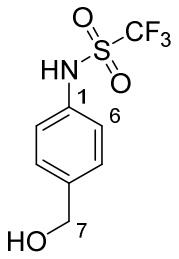
To a solution of *N*-(*o*-tolyl)-1,1,1-trifluoromethanesulfonamide **96** (1.0 mL of a 100 mM solution in isopropanol, 0.1 mmol), glucose (1.7 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.7 mmol), GDH (260 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 520 U), and $P450_{BM3}$ mutant KSK19/AI/AI (5.2 mL of a 20 µM solution in phosphate buffer, pH 7.5, 0.10 µmol) in 100 mM phosphate buffer (pH 7.5, 43 mL) was added $NADP^+$ monosodium salt (260 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 1.0 µmol). The solution was stirred open to air for 18 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether / petrol to 40% ether / petrol) to afford the *title compound* as a colourless oil (9 mg, 34%). **R_f** 0.25 (30% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3488 (m, br), 3288 (m, br), 1591 (w), 1417 (w), 1359 (w), 1197 (s), 1140 (s), 931 (w), 623 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.20 (1H, d, *J* = 8.5 Hz, H2), 6.73 (1H, d, *J* = 3.0 Hz, H5), 6.67 (1H, dd, *J* = 8.5, 3.0 Hz, H3), 2.33 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 155.8 (C4), 137.6 (C1), 129.5 (C2), 124.5 (C6), 117.7 (C5), 113.9 (C3); **HRMS** (ES^-) calc. for $C_8H_7F_3NO_3S$ $[M–H]^-$ 254.0104, found 254.0105.

***N*-(4-Hydroxy-3-methylphenyl)-1,1,1-trifluoromethanesulfonamide 113**



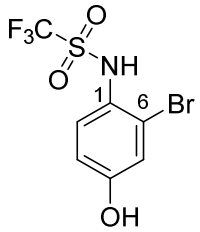
To a solution of *N*-(*m*-tolyl)-1,1,1-trifluoromethanesulfonamide **97** (0.63 mL of a 100 mM solution in isopropanol, 0.063 mmol), glucose (3.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.1 mmol), GDH (155 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 310 U), and $P450_{BM3}$ mutant RP/EV (3.1 mL of a 20 µM solution in phosphate buffer, 0.062 µmol) in 100 mM phosphate buffer (pH 7.5, 23 mL) was added $NADP^+$ monosodium salt (155 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.62 µmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether / petrol to 40% ether / petrol) to afford **113** as a colourless oil (5 mg, 32%). $\mathbf{R_f}$ 0.55 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3524 (br, m), 3286 (br, m), 1511 (w), 1411 (m), 1349 (m), 1201 (s), 1140 (m), 971 (w), 631 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.07 (1H, d, *J* = 2.5 Hz, H6), 7.01 (1H, dd, *J* = 8.5, 2.5 Hz, H2), 6.76 (1H, d, *J* = 8.5 Hz, H3), 2.25 (3H, s, H7); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 153.9 (C4), 128.6 (C6), 125.5 (C5), 125.4 (C1), 124.8 (C2), 119.8 (q, *J* = 320.5 Hz, CF_3), 115.6 (C3), 15.8 (C7); **LRMS** (ES^-) calc. for $C_8H_7F_3NO_3S$ $[M–H]^-$ 254.0, found 254.0. Data consistent with literature values.[441]

***N*-[4-(Hydroxymethyl)phenyl]-1,1,1-trifluoromethanesulfonamide 14**



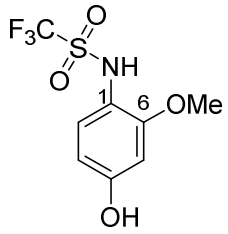
To a solution of *N*-(*p*-tolyl)1,1,1-trifluoromethanesulfonamide **98** (0.63 mL of a 100 mM solution in isopropanol, 0.063 mmol), glucose (3.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.1 mmol), GDH (155 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 310 U), and $P450_{BM3}$ mutant RP/IA/EV (1.5 mL of a 50 µM solution in phosphate buffer, 0.075 µmol) in 100 mM phosphate buffer (pH 7.5, 23 mL) was added $NADP^+$ monosodium salt (155 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.62 µmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (15% ether / petrol to 50% ether / petrol) to afford the *title compound* as a colourless oil (8 mg, 47%). **R_f** 0.25 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3279 (br, w), 2921 (w), 1514 (w), 1417 (w), 1374 (m), 1212 (s), 1142 (s), 958 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.39–7.37 (2H, m, H3, H5), 7.25–7.23 (2H, m, H2, H6), 7.14 (1H, br s, NH), 4.71 (2H, s, H7), 1.87 (1H, br s, OH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 140.5 (C4), 132.9 (C1), 128.1 (C3, C5), 124.1 (C2, C6), 119.8 (q, *J* = 320.5 Hz, CF_3), 64.5 (C7); **HRMS** (ES^-) calc. for $C_8H_7F_3NO_3S$ $[M–H]^-$ 254.0104, found 254.0104.

***N*-(2-Bromo-4-hydroxyphenyl)-1,1,1-trifluoromethanesulfonamide 115**



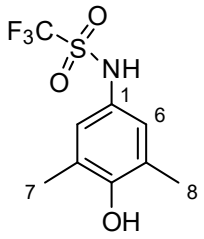
To a solution of *N*-(2-bromophenyl)-1,1,1-trifluoromethanesulfonamide **100** (0.49 mL of a 100 mM solution in isopropanol, 0.049 mmol), glucose (0.8 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 0.80 mmol), GDH (125 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 250 U), and $P450_{BM3}$ mutant RPEV (4.5 mL of a 20 µM solution in phosphate buffer, 0.090 µmol) in 100 mM phosphate buffer (pH 7.5, 20 mL) was added $NADP^+$ monosodium salt (125 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.50 µmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether / petrol to 80% ether / petrol) to afford the *title compound* as a colourless oil (6 mg, 35%). $\mathbf{R_f}$ 0.45 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3488 (br, w), 3282 (br, w), 1606 (w), 1495 (m), 1419 (w), 1365 (w), 1231 (m), 1203 (s), 1139 (m), 954 (w), 886 (w), 623 (m); ^{1}**H NMR** (500 MHz, $CDCl_3$) δ_H 7.44 (1H, d, *J* = 9.0 Hz, H2), 7.13 (1H, d, *J* = 3.0 Hz, H5), 6.82 (1H, dd, *J* = 9.0, 3.0 Hz, H3); ^{13}**C NMR** (126 MHz, $CDCl_3$) δ_C 155.5 (C4), 127.1 (C2), 125.3 (C1), 119.8 (C5), 119.7 (q, *J* = 322.0 Hz, CF_3), 119.4 (C6), 115.8 (C3); **HRMS** (ES^-) calc. for $C_7H_4{}^{79}BrF_3NO_3S$ $[M–H]^-$ 317.9053, found 317.9054.

***N*-(4-Hydroxy-2-methoxyphenyl)-1,1,1-trifluoromethanesulfonamide 116**



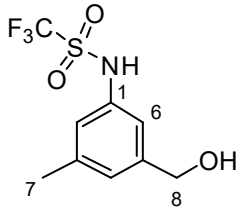
To a solution of *N*-(2-methoxyphenyl)-1,1,1-trifluoromethanesulfonamide **102** (0.78 mL of a 100 mM solution in isopropanol, 0.078 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.30 mmol , 390 U), GDH (195 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5), and $P450_{BM3}$ mutant RP/IA/EV (1.1 mL of a 71 µM solution in phosphate buffer, pH 7.5, 0.078 µmol) in 100 mM phosphate buffer (pH 7.5, 31 mL) was added $NADP^+$ monosodium salt (195 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 µmol). The solution was stirred open to air for 24 h and aqueous HCl (3.0 M) was added. The solution was centrifuged at 9,500 g, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether / petrol to 30% ether / petrol) to afford the *title compound* as a colourless oil (6 mg, 27%). $\mathbf{R_f}$ 0.30 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3470 (br, m), 3262 (br, m), 2924 (w), 1610 (w), 1512 (m), 1369 (m), 1231 (m), 1200 (s), 1140 (m), 949 (w), 836 (w), 640 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.30 (1H, d, *J* = 8.5 Hz, H2), 6.73 (1H, br s, NH), 6.46 (1H, d, *J* = 2.5 Hz, H5), 6.39 (1H, dd, *J* = 8.5, 2.5 Hz, H3), 5.01 (1H, br s, OH), 3.86 (3H, s, OCH_3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 155.8 (C4), 153.0 (C6), 125.5 (C2), 119.9 (q, *J* = 322.5 Hz, CF_3), 116.0 (C1), 107.3 (C3), 99.4 (C5), 56.0 (OCH_3); **HRMS** (ES^+) calc. for $C_8H_7F_3NO_4S$ $[M–H]^–$ 270.0053, found 270.0051.

***N*-(4-Hydroxy-3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide 117**



To a solution of *N*-(3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide **99** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.30 mmol), GDH (195 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 390 U), and $P450_{BM3}$ mutant KSK19/AI/AI (1.6 mL of a 50 µM solution in phosphate buffer, pH 7.5, 0.079 µmol) in 100 mM phosphate buffer (pH 7.5, 35 mL) was added $NADP^+$ monosodium salt (195 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 µmol). The solution was stirred open to air for 17 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (5% ether / petrol) to afford **117** as a colourless oil (5 mg, 24%). $\mathbf{R_f}$ 0.55 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3544 (br, w), 3282 (br, w), 2925 (w), 1682 (w), 1489 (m), 1413 (w), 1361 (w), 1195 (s), 1139 (m), 1032 (w), 915 (w), 612 (m); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 6.92 (2H, s, H2, H6), 2.24 (6H, s, H7, H8); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 151.2 (C4), 125.0 (C2, C6), 123.9 (C1), 123.3 (C3, C5), 118.8 (q, J = 322.0 Hz, CF_3), 14.9 (C7, C8); **LRMS** (ES^-) calc. for $C_9H_9F_3NO_3S$ $[M–H]^-$ 268.0, found 268.0. Data consistent with literature values.[441]

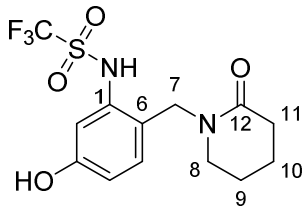
***N*-[3-(Hydroxymethyl)-5-methylphenyl]-1,1,1-trifluoromethanesulfonamide 118**



To a solution of *N*-(3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide **99** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate

buffer, pH 7.5, 1.30 mmol), GDH (195 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 390 U), and $P450_{BM3}$ mutant KSK19/AI/FV (1.0 mL of a 78 µM solution in phosphate buffer, pH 7.5, 0.078 µmol) in 100 mM phosphate buffer (pH 7.5, 35 mL) was added $NADP^+$ monosodium salt (195 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 µmol). The solution was stirred open to air for 21 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (40% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (12 mg, 58%). **R_f** 0.45 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3566 (br, m), 3288 (br, m), 2980 (m), 1603 (w), 1372 (m), 1229 (s), 1195 (s), 1141 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.07 (1H, s, H4), 7.01 (1H, s, H2), 7.00 (1H, s, H6), 4.65 (2H, s, H8), 2.34 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 141.9 (C5), 140.1 (C1), 134.1 (C3), 126.7 (C4), 123.3 (C6), 119.8 (q, *J* = 320.0 Hz, CF_3), 119.1 (C2), 64.6 (C8), 21.3 (C7); **HRMS** (ES^-) calc. for $C_9H_9F_3NO_3S$ $[M–H]^-$ 268.0261, found 268.0260.

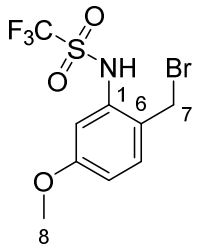
***N*-{5-Hydroxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl}-1,1,1-trifluoromethanesulfonamide 119**



Based on a modified literature procedure.[442] A solution of *N*-{5-methoxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl}-1,1,1-trifluoromethanesulfonamide **130** (265 mg, 0.72 mmol, 1.0 eq) in DCM (2.9 mL) was cooled to 0 °C and BBr_3 (2.2 mL of a 1.0 M solution in DCM, 2.2 mmol, 3.0 eq) in DCM (4.2 mL) was added dropwise. The solution was warmed to RT and stirred for 3 h then neutralised with saturated aqueous $NaHCO_3$. The solution was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a brown solid (62 mg, 27%). **R_f** 0.25 (50% ethyl acetate / petrol); **mp** 212–214 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3400 (br, m), 2490 (br, w), 1586 (m), 1499 (w), 1225 (m), 1194

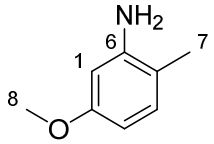
(s), 1144 (m); **^{1}H NMR** (400 MHz, CD_3OD) δ_H 7.03 (1H, d, *J* = 8.5 Hz, H5), 6.86 (1H, d, *J* = 2.5 Hz, H2), 6.54 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 4.57 (2H, s, H7), 3.33–3.32 (2H, m, H8), 2.42–2.39 (2H, m, H2), 1.81–1.77 (4H, m, H9, H10); **^{13}C NMR** (101 MHz, CD_3OD) δ_C 171.8 (C12), 157.2 (C3), 139.7 (C6), 129.7 (C5), 121.3 (C1), 121.0 (q, *J* = 325.0 Hz, CF_3), 111.4 (C4),111.3 (C2), 47.3 (C8), 46.2 (C7), 31.4 (C11), 22.4 (C9), 20.5 (C10); **HRMS** calc. for $C_{13}H_{16}F_3N_2O_4S$ $[M+H]^+$ 353.0777, found 353.0777.

***N*-[2-(Bromomethyl)-5-methoxyphenyl]-1,1,1-trifluoromethanesulfonamide 120**



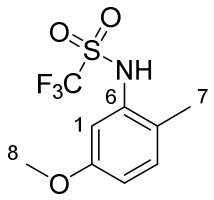
Based on a modified literature procedure.[443] PBr_3 (40 µL, 0.41 mmol, 1.1 eq) was added to a solution of *N*-[2-(hydroxymethyl)-5-methoxyphenyl]-1,1,1-trifluoromethanesulfonamide **129** (100 mg, 0.37 mmol, 1.0 eq) in DCM (3.7 mL) at 0 °C and the solution was stirred for 4 h. The solution was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow oil (83 mg, 64%). **R_f** 0.16 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3290 (br, w), 1614 (m), 1509 (m), 1417 (w), 1364 (w), 1194 (s), 1137 (s), 1035 (w), 980 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.31 (1H, d, *J* = 8.5 Hz, H5), 7.12 (1H, br s, NH), 7.07 (1H, d, *J* = 2.5 Hz, H3), 6.83 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 4.53 (2H, s, H7), 3.83 (3H, s, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 161.1 (C3), 134.0 (C1), 131.6 (C5), 123.7 (C6), 119.7 (q, *J* = 320.5 Hz, CF_3), 113.8 (C4), 112.2 (C3), 55.7 (C8), 30.5 (C7); **HRMS** (ES^-) calc. for $C_9H_8{}^{79}BrF_3NO_3S$ $[M–H]^-$ 345.9366, found 345.9370.

5-Methoxy-2-methylaniline 122



Pd (10 wt.% Pd on C, 640 mg, 0.6 mmol, 0.1 eq) was added to a solution of 4-methyl-2-nitroanisole (1.0 g, 6.0 mmol, 1.0 eq) in methanol (27 mL) and H_2 was bubbled through the solution for 5 minutes. The solution was stirred for 16 h under an atmosphere of H_2, filtered through Celite, and concentrated *in vacuo* to afford **122** as a red oil (772 mg, 94%). $\mathbf{R_f}$ 0.20 (30% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3462 (w), 3378 (w), 1621 (m), 1586 (m), 1511 (s), 1214 (s), 1167 (m), 1031 (w), 908 (m), 728 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 6.95 (1H, d, *J* = 8.0 Hz, H4), 6.30–6.26 (2H, m, H1, H3), 3.75 (3H, s, H8), 3.60 (2H, br s, NH_2), 2.11 (3H, s, H7); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 159.0 (C2), 145.5 (C6), 131.0 (C4), 114.8 (C5), 103. 6 (C3), 101.0 (C1), 55.2 (C8), 16.5 (C7); **LRMS** (ES^+) calc. for $C_8H_{12}NO$ $[M+H]^+$ 138.1, found 138.1.

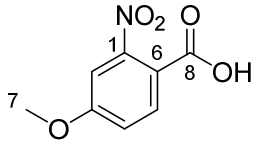
***N*-(5-Methoxy-2-methylphenyl)-1,1,1-trifluoromethanesulfonamide 123**



A solution of 5-methoxy-2-methylaniline **122** (410 mg, 3.0 mmol, 1.0 eq) in DCM (6 mL) was cooled to –78 °C, triethylamine (0.42 mL, 3.0 mmol, 1.0 eq) was added, and the solution was stirred for 5 minutes. Trifluoromethanesulfonic anhydride (0.53 mL, 3.1 mmol, 1.05 eq) was added dropwise and the solution was stirred for 3 h then poured onto ice. The solution was extracted with DCM and the combined organic layers were washed with aqueous HCl (2.0 M) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as an off-white solid (706 mg, 87%). $\mathbf{R_f}$ 0.50 (30% ethyl acetate / petrol); **mp** 66 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3291 (m), 1618 (w), 1508 (m), 1415 (w), 1306 (w), 1231 (m), 1196 (s), 1142 (s), 909 (m), 734 (m), 609 (m); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.15 (1H, d, *J* = 8.5 Hz, H4), 6.98 (1H, d, *J* = 2.5 Hz, H1), 6.81 (1H, dd, *J* = 8.5, 2.5

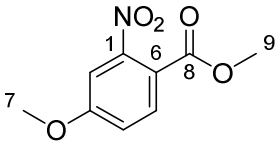
Hz, H3), 6.55 (1H, br s, NH), 3.79 (3H, s, H8), 2.29 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 158.5 (C2), 132.6 (C6), 131.7 (C4), 124.7 (C5), 119.7 (q, *J* = 324.0 Hz, CF_3), 114.0 (C3), 111.3 (C1), 55.5 (C8), 16.9 (C7); **HRMS** (ES^-) calc. for $C_9H_9F_3NO_3S$ $[M–H]^-$ 268.0261, found 268.0264.

4-Methoxy-2-nitrobenzoic acid 125



Based on a modified literature procedure.[444] A solution of 4-methyl-3-nitroanisole (1.2 mL, 9.0 mmol, 1.0 eq) and $KMnO_4$ (5.0 g, 31 mmol, 3.5 eq) in water (90 mL) was heated at reflux for 48 h. The solution was cooled and filtered. The pH of the solution was adjusted to 2 with aqueous HCl (3.0 M). The solution was filtered and the solid was washed with petrol to afford **125** as a white solid (1.12 g, 63%). **R_f** 0.30 (50% ethyl acetate / petrol); **mp** 188 °C (amorphous) (lit. mp 194 °C); **IR** (thin film) ν_{max} / cm^{-1} 3447 (br, m), 1697 (m), 1609 (s), 1536 (s), 1363 (m), 1289 (s), 1246 (s), 1070 (w), 916 (w), 615 (m); **^{1}H NMR** (400 MHz, CD_3OD) δ_H 7.88 (1H, d, *J* = 8.5 Hz, H5), 7.30 (1H, d, *J* = 2.5 Hz, H2), 7.21 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 3.92 (3H, s, H7); **^{13}C NMR** (101 MHz, CD_3OD) δ_C 165.7 (C3), 162.8 (C8), 151.5 (C1), 132.1 (C5), 117.4 (C6), 116.4 (C4), 108.9 (C2), 55.4 (C7); **LRMS** (ES^-) calc. for $C_8H_6NO_5$ $[M–H]^-$ 196.0, found 196.0. Data consistent with literature values.[445]

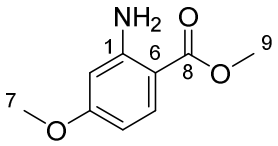
Methyl 4-methoxy-2-nitrobenzoate 126



Based on a modified literature procedure.[446] To a solution of 4-methoxy-2-nitrobenzoic acid **125** (365 mg, 1.9 mmol, 1.0 eq) in dimethylformamide (3.7 mL) at 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (0.55 mL, 3.7 mmol, 2.0 eq) and iodomethane (0.35 mL, 3.7 mmol, 3.0 eq) and the solution was stirred at 0 °C for 15 minutes. The solution was warmed to RT, stirred for 16 h and poured into water. The solution was extracted with ethyl acetate and the combined organic layers were washed with saturated aqueous $NaHCO_3$, saturated aqueous NH_4Cl, and brine,

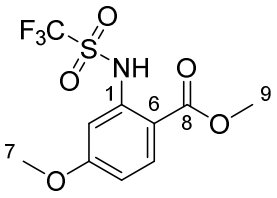
dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **126** as a yellow solid (375 mg, 96%). **R_f** 0.35 (30% ethyl acetate / petrol); **mp** 52 °C (amorphous) (lit. mp 68 °C); **IR** (thin film) ν_{max} / cm^{-1} 2955 (w), 1725 (s), 1614(m), 1537 (s), 1434 (m), 1272 (s), 1246 (m), 1124 (s), 1027 (m), 825 (w), 801 (w), 724 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.78 (1H, d, *J* = 8.5 Hz, H5), 7.23 (1H, d, *J* = 2.5 Hz, H2), 7.10 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 3.90 (3H, s, H9), 3.87 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 165.0 (C3), 162.3 (C8), 150.8 (C1), 132.0 (C5), 118.0 (C6), 117.4 (C2), 109.2 (C4), 56.2 (C7), 53.0 (C9); **LRMS** (ES^+) calc. for $C_9H_{10}NO_5$ $[M+H]^+$ 212.1, found 212.2. Data consistent with literature values.[446]

Methyl 2-amino-4-methoxybenzoate 127



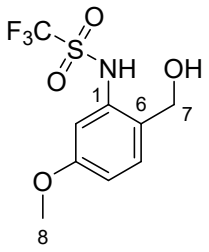
Based on a modified literature procedure.[446] A solution of methyl 4-methoxy-2-nitrobenzoate **126** (66 mg, 0.31 mmol, 1.0 eq) in methanol (3.1 mL) was degassed by bubbling N_2 through the solution and $Pd(OH)_2$ (20 wt.% on carbon, 21 mg, 0.03 mmol, 0.1 eq) was added. H_2 was bubbled through the solution for 5 minutes and the solution was stirred under an atmosphere of H_2 for 16 h. The solution was filtered through Celite and concentrated *in vacuo* to afford **127** as a yellow solid (56 mg, quant.). **R_f** 0.55 (50% ethyl acetate / petrol); **mp** 58–62 °C (amorphous) (lit. mp 73–75 °C); **IR** (thin film) ν_{max} / cm^{-1} 3477 (w), 3367 (w), 2951 (w), 1682 (m), 1616 (s), 1593 (m), 1436 (w), 1244 (s), 1218 (s), 1027 (m), 770 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.78 (1H, d, *J* = 9.0 Hz, H5), 6.23 (1H, dd, *J* = 9.0, 2.5 Hz, H4), 6.11 (1H, d, *J* = 2.5 Hz, H2), 5.79 (2H, br s, NH), 3.83 (3H, s, H9), 3.79 (3H, s, H7); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 168.8 (C8), 168.3 (C3), 152.5 (C1), 133.1 (C5), 104.5 (C4), 104.4 (C6), 99.4 (C2), 55.2 (C7), 51.3 (C9); **LRMS** (ES^+) calc. for $C_9H_{12}NO_3$ $[M+H]^+$ 182.1, found 182.1. Data consistent with literature values.[446]

Methyl 4-methoxy-2-[(trifluoromethyl)sulfonamido]benzoate 128



Based on a modified literature procedure.[316] A solution of methyl 2-amino-4-methoxybenzoate **127** (240 mg, 1.3 mmol, 1.0 eq) in DCM (2.6 mL) was cooled to –78 °C, triethylamine (0.18 mL, 1.3 mmol, 1.0 eq) was added, and the solution was stirred for 15 minutes. Trifluoromethanesulfonic anhydride (0.23 mL, 1.4 mmol, 1.1 eq) was added dropwise and the solution was stirred at –78 °C for 3 h. The reaction mixture was poured into ice and extracted with DCM. The combined organic layers were washed with aqueous HCl (2.0 M), saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow solid (313 mg, 77%). $\mathbf{R_f}$ 0.65 (50% ethyl acetate / petrol); **mp** 77–80 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2958 (w), 1686 (m), 1619 (m), 1151 (m), 1410 (m), 1285 (m), 1205 (s), 1140 (s), 1102 (m), 1041 (m), 961 (m), 908 (m), 772 (m), 625 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 11.60 (1H, br s, NH), 7.99 (1H, d, *J* = 9.0 Hz, H5), 7.28 (1H, d, *J* = 2.5 Hz, H2), 6.71 (1H, dd, *J* = 9.0, 2.5 Hz, H4), 3.93 (3H, s, H9), 3.87 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.7 (C8), 164.6 (C3), 140.7 (C1), 133.0 (C5), 119.8 (q, *J* = 321.5 Hz, CF_3), 110.9 (C4), 108.7 (C6), 103.6 (C2), 55.7 (C7), 52.7 (C9); **HRMS** (ES^-) calc. for $C_{10}H_9F_3NO_5S$ $[M–H]^-$ 312.0159, found 312.0166.

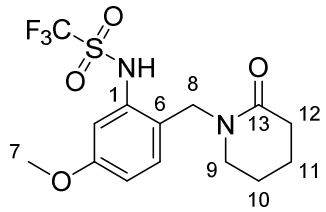
***N*-[2-(Hydroxymethyl)-5-methoxyphenyl]-1,1,1-trifluoromethanesulfonamide 129**



Based on a modified literature procedure.[447] A solution of methyl 4-methoxy-2-[(trifluoromethyl)sulfonamido]benzoate **128** (270 mg, 0.9 mmol, 1.0 eq) in tetrahydrofuran (0.57 mL) was added dropwise to a solution of $LiAlH_4$ (67 mg, 1.8 mmol, 2.05 eq) in tetrahydrofuran (0.57 mL)

and the solution was heated at reflux for 1 h. The solution was cooled, aqueous HCl (3.0 M) was added, and the solution was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow oil (88 mg, 36%). **R_f** 0.25 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3292 (br, s), 3080 (w), 1635 (s), 1549 (s), 1436 (w), 1337 (w), 993 (m), 914 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.70 (1H, br s, NH), 7.14 (1H, d, *J* = 2.5 Hz, H2), 7.10 (1H, d, *J* = 8.5 Hz, H5), 6.72 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 4.75 (2H, s, H7), 3.81 (3H, s, H8); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 160.4 (C3), 135.9 (C1), 129.8 (C5), 122.9 (C6), 119.9 (q, *J* = 322.0 Hz, CF_3), 111.7 (C4), 108.7 (C2), 63.8 (C7), 55.5 (C8); **HRMS** (ES^-) calc. for $C_9H_9F_3NO_4S$ $[M–H]^-$ 284.0210, found 284.0211.

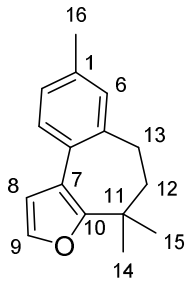
***N*-{5-Methoxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl}-1,1,1-trifluoromethanesulfonamide 130**



Based on a modified literature procedure.[448] 4-Methylbenzenesulfonic acid monohydrate (39 mg, 0.21 mmol, 0.5 eq) and δ-valerolactam (48 mg, 0.49 mmol, 1.2 eq) were added to a solution of 1,1,1-trifluoro-*N*-[2-(hydroxymethyl)-5-methoxyphenyl]methanesulfonamide **129** (117 mg, 0.41 mmol, 1.0 eq) in toluene (2.1 mL) and the solution was heated at reflux for 21 h. The solution was cooled to RT and poured into water. The solution was extracted with chloroform and the combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether / petrol to 50% ether / petrol) to afford the *title compound* as a yellow oil (39 mg, 26%). **R_f** 0.20 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2952 (w), 1618 (m), 1594 (m), 1504 (m), 1378 (s), 1229 (s), 1192 (s), 1145 (s), 1113 (m), 991 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.17–7.15 (2H, m, H3, H5), 6.70 (1H, dd, *J* = 8.5, 2.5 Hz, H4), 4.40 (2H, s, H8), 3.79 (3H, s, H7), 3.41 (2H, t, *J* = 5.5 Hz, H9), 2.41 (2H, *J* = 6.0 Hz, H12), 1.83–1.75 (4H, m, H10, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.1

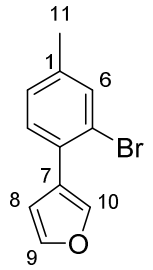
(C13), 160.6 (C5), 136.2 (C1), 132.2 (C5), 120.1 (q, J = 322.0 Hz, CF_3), 120.1 (C6), 111.6 (C4), 108.5 (C2), 55.5 (C7), 48.7 (C8), 48.2 (C9), 31.7 (C12), 22.7 (C10), 20.5 (C11); **HRMS** (ES^+) calc. for $C_{14}H_{18}F_3N_2O_4S$ $[M+H]^+$ 367.0934, found 367.0931.

4,4,8-Trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan 143



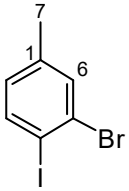
Based on a modified literature procedure.[449] $NH_2NH_2{\cdot}H_2O$ (0.25 mL, 5.22 mmol, 9.3 eq) and KOH (94 mg, 1.68 mmol, 3.0 eq) were added to a solution of 4,4,8-trimethyl-4,6-dihydro-5*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5-one **192** (135 mg, 0.56 mmol, 1.0 eq) in ethylene glycol (0.84 mL). The solution was heated at 130 °C for 2 h then heated at 180 °C for a further 20 h. The solution was cooled to RT and water was added. The solution was extracted with hexane and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford the *title compound* as a white solid (101 mg, 80%). $\mathbf{R_f}$ 0.65 (40% ether / petrol); **mp** 60–62 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2962 (m), 2923 (s), 2857 (w), 1525 (m), 1455 (w), 1154 (m), 1068 (w), 931 (w), 891 (w), 820 (m), 756 (w), 736 (m); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.39 (1H, d, J = 8.0 Hz, H3), 7.36 (1H, d, J = 2.0 Hz, H9), 7.04 (1H, d, J = 8.0 Hz, H2), 6.96 (1H, s, H6), 6.58 (1H, d, J = 2.0 Hz, H8), 2.78–2.76 (2H, m, H13), 2.32 (3H, s, H16), 1.84–1.81 (2H, m, H12), 1.36 (6H, s, H14, H15); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 156.6 (C10), 140.4 (C5), 139.4 (C9), 134.5 (C1), 128.6 (C6), 127.9 (C4), 125.9 (C3), 125.8 (C2), 116.7 (C7), 109.0 (C8), 39.6 (C12), 37.0 (C11), 30.2 (C13), 28.4 (C14, C15), 19.9 (C16); **HRMS** (EI^+) calc. for $C_{16}H_{18}O$ $[M^+]$ 226.1358, found 226.1353.

3-(2-Bromo-4-methylphenyl)furan 145



Based on a modified literature procedure.[450] Ar was bubbled through a solution of water (1.7 mL) and dimethoxyethane (11 mL) for 5 minutes. Furan-3-ylboronic acid (223 mg, 2.0 mmol, 1.2 eq), 2-bromo-1-iodo-4-methylbenzene **146** (493 mg, 1.7 mmol, 1.0 eq), and K_2CO_3 (574 mg, 1.0 mmol, 2.5 eq) were added and Ar was bubbled through the solution for 5 minutes. $(Ph_3P)_2PdCl_2$ (58 mg, 0.08 mmol, 5 mol%) was added and the solution was heated at 80 °C for 19 h. The solution was cooled to RT and water was added. The solution was extracted with ether and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (100% petrol) to afford the *title compound* as a colourless oil (268 mg, 68%). $\mathbf{R_f}$ 0.20 (100% petrol); **IR** (thin film) ν_{max} / cm^{-1} 2923 (m), 1511 (m), 1165 (m), 1064 (m), 1036 (m), 1015 (m), 874 (s), 824 (m), 789 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.73 (1H, dd, *J* = 1.5, 1.0 Hz, H10), 7.46 (1H, br s, H6), 7.45 (1H, t, *J* = 1.5 Hz, H9), 7.25 (1H, d, *J* = 8.0 Hz, H3), 7.10 (1H, ddd, *J* = 8.0, 1.5, 0.5 Hz, H2), 6.67 (1H, dd, *J* = 1.5, 1.0 Hz, H8), 2.33 (3H, s, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 142.3 (C9), 140.7 (C10), 138.7 (C1), 134.0 (C6), 130.4 (C3), 128.3 (C2), 124.9 (C7), 122.2 (C5), 111.5 (C8), 20.7 (C11); **HRMS** (Cl^+) calc. for $C_{11}H_{10}{}^{79}BrO$ $[M+H]^+$ 236.9910, found 236.9915.

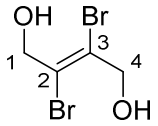
2-Bromo-1-iodo-4-methylbenzene 146



Based on a literature procedure.[451] A solution of conc. HCl (21 mL) in water (50 mL) was added to a solution of 2-bromo-4-methylaniline (5.0 mL, 40.3 mmol, 1.0 eq) in acetonitrile (73 mL).

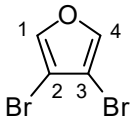
$NaNO_2$ (3.3 g, 48.4 mmol, 1.2 eq) in water (50 mL) was added. The solution was stirred for 30 minutes and a solution of KI (7.0 g, 60.5 mmol, 1.5 eq) in water (50 mL) was added. The solution was stirred at RT for 15 h then poured into water and extracted with DCM. The combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by Kugelrohr distillation (27 Torr, 50 °C setting) to afford **146** as a brown oil (10.2 g, 85%). $\mathbf{R_f}$ 0.50 (100% petrol); **IR** (thin film) ν_{max} / cm^{-1} 1458 (s), 1370 (w), 1097 (w), 1006 (m), 838 (m), 806 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.70 (1H, d, *J* = 8.0 Hz, H3), 7.46 (1H, d, *J* = 2.0 Hz, H6), 6.80 (1H, dd, *J* = 8.0, 2.0 Hz, H2), 2.28 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 139.9 (C1), 139.9 (C3), 133.4 (C6), 129.6 (C2), 129.4 (C5), 96.9 (C4), 20.8 (C7); **HRMS** (EI^+) calc. for $C_7H_6{}^{79}Br^{127}I$ $[M]^+$ 295.8698, found 295.8693. Data consistent with literature values.[451]

(*E*)-2,3-dibromobut-2-ene-1,4-diol 149



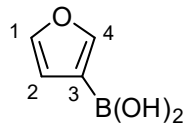
Based on a literature procedure.[452] A solution of 2-butyn-1,4-diol (3.5 g, 41 mmol, 1.0 eq) in water (82 mL) was cooled to 0 °C and Br_2 (2.7 mL, 53 mmol, 1.3 eq) was added dropwise. The solution was stirred at 0 °C for 30 minutes then warmed to RT and stirred for a further 90 minutes after which time saturated aqueous $Na_2S_2O_3$ was added. The solution was neutralised with aqueous NaOH (1.0 M) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **149** as a white solid (7.0 g, 70%). $\mathbf{R_f}$ 0.25 (50% ethyl acetate / petrol); **mp** 93–95 °C (amorphous) (lit. mp 115–116 °C); **IR** (thin film) ν_{max} / cm^{-1} 3257 (s), 2970 (w), 2919 (w), 1069 (s), 1012 (m); **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ_H 5.54 (2H, t, *J* = 6.0 Hz, OH), 4.31 (4H, d, *J* = 6.0 Hz, H1, H4); **^{13}C NMR** (101 MHz, $(CD_3)_2SO$) δ_C 123.3 (C2, C3), 65.9 (C1, C4); **HRMS** (CI^+) calc. for $C_4H_{10}{}^{79}Br_2NO_2$ $[M+NH_4]^+$ 261.9073, found 261.9070. Data is consistent with literature values.[452]

3,4-Dibromofuran 150



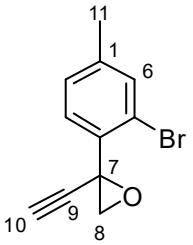
Method A: Based on a literature procedure.[453] A solution of (*E*)-2,3-dibromobut-2-ene-1,4-diol **149** (6.65 g, 27.0 mmol, 1.0 eq) in H_2SO_4 (7.5% aqueous solution, 16 mL) and hexane (54 mL) was heated to 85 °C and $K_2Cr_2O_7$ (8.0 g, 27.0 mmol, 1.0 eq) in conc. H_2SO_4 (5.4 mL) and water (27 mL) was added dropwise over 10 minutes. The reaction was heated at 85 °C for 6 h then cooled to RT and separated. The aqueous layer was extracted with hexane and the combined organic layers were washed with saturated aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **150** as a yellow oil (1.0 g, 17%). **Method B:** Based on a literature procedure.[347] A 2-necked round bottom flash with dropping funnel and distillation apparatus attached containing (*E*)-2,3-dibromobut-2-ene-1,4-diol **149** (7.0 g, 28.5 mmol, 1.0 eq) in H_2SO_4 (7% aqueous solution, 18 mL) was heated to 120 °C until distillation began and a solution of $K_2Cr_2O_7$ (5.0 g, 17.1 mmol, 0.6 eq) and conc. H_2SO_4 (5.6 mL) in water (57 mL) was added dropwise over 1 h. Distillation was continued for a further 1 h and the distillate was extracted with petrol. The combined organic layers were washed with saturated aqueous Na_2CO_3, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **150** as a colourless oil (2.3 g, 36%). $\mathbf{R_f}$ 0.50 (100 % petrol); **IR** (thin film) ν_{max} / cm^{-1} 3149 (w), 1770 (m), 1612 (w), 1260 (w), 1138 (w), 1028 (m), 985 (m), 971 (s), 864 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.45 (2H, s, H1, H4); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 141.6 (C1, C4), 104.0 (C2, C3); **HRMS** (EI^+) calc. for $C_4H_2{}^{79}Br_2O$ $[M]^+$ 223.8472, found 223.8462. Data consistent with literature values.[347]

Furan-3-ylboronic acid 151



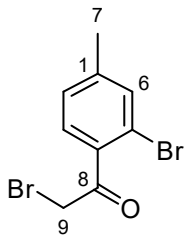
Based on literature procedures.[348,349] A solution of 3,4-dibromofuran **150** (1.61 g, 7.1 mmol, 1.0 eq) in anhydrous ether (16 mL) was cooled to –78 °C and butyllithium (2.5 M in hexanes, 2.9 mL, 7.1 mmol, 1.0 eq) was added dropwise. The solution was stirred for 10 minutes and water (13 mL) was added. The solution was extracted with ether and the combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. Ether was removed carefully *in vacuo* and the residue was redissolved in tetrahydrofuran (10.8 mL) and triisopropyl borate (1.7 mL, 7.5 mmol 1.05 eq) was added. The solution was cooled to –78 °C and butyllithium (2.5 M in hexanes, 3.0 mL, 7.5 mmol, 1.05 eq) was added dropwise over 40 minutes. The solution was stirred at –78 °C for 40 minutes then warmed to 0 °C over 20 minutes. Aqueous HCl (3.0 M, 10 mL) was added to the solution and it was stirred for 15 minutes then extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by flash chromatography (30% ethyl acetate / petrol) to afford **151** as an off-white solid (225 mg, 28% over two-steps). **R_f** 0.35 (50% ethyl acetate / petrol); **mp** 110 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3206 (s), 2925 (m), 1705 (w), 1560 (w), 1454 (br, s), 1197 (m), 1153 (m), 1011 (m), 811 (s), 740 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.04 (1H, dd, *J* = 1.5, 0.5 Hz, H4), 7.55 (1H, t, *J* = 1.5 Hz, H1), 6.78 (1H, dd, *J* = 1.5, 0.5 Hz, H2); **^{13}C NMR** δ_C 153.5 (C4), 143.5 (C1), 113.2 (C2); **LRMS** (ES^-) calc. for $C_4H_4{}^{11}BO_3$ $[M–H]^-$ 111.0, found 111.0. Data consistent with literature values.[349]

2-(2-Bromo-4-methylphenyl)-2-ethynyloxirane 157



Based on a modified literature procedure.[454] Ethynylmagnesium bromide (2.2 mL of a 0.5 M solution in tetrahydrofuran, 0.30 mmol, 1.2 eq) was added dropwise to a solution of 2-bromo-1-(2-bromo-4-methylphenyl)ethan-1-one **158** (270 mg, 0.92 mmol, 1.0 eq) in tetrahydrofuran (1.8 mL) at 0 °C. The solution was heated at reflux for 2 h. The reaction was cooled to RT, saturated aqueous NH_4Cl was added, and the solution was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (100% petrol to 2% ether / petrol) to afford the *title compound* as a colourless oil (100 mg, 46%). **R_f** 0.50 (15% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3313 (s), 2962 (w), 1507 (w), 1385 (w), 1165 (m), 1123 (w), 1045 (m), 1013 (s), 870(s), 813 (s), 796 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.40–7.39 (1H, m, H6), 7.38 (1H, d, *J* = 8.0 Hz, H3), 7.14–7.11 (1H, m, H2), 3.41 (1H, d, *J* = 6.0 Hz, H8), 2.95 (1H, d, *J* = 6.0 Hz, H8'), 2.41 (1H, s, H10), 2.33 (3H, s, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 140.5 (C1), 134.1 (C4), 133.1 (C6), 128.5 (C3), 128.3 (C2), 122.0 (C5), 81.3 (C9), 71.7 (C10), 56.3 (C8), 51.5 (C7), 20.9 (C11); **HRMS** Not found.

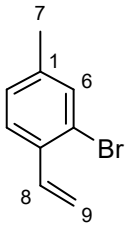
2-Bromo-1-(2-bromo-4-methylphenyl)ethan-1-one 158



Based on a modified literature procedure.[455] A homogenous mixture of pyridinium chlorochromate (698 mg, 3.2 mmol, 3.0 eq) and silica gel (698 mg) was added to a solution of 2-bromo-1-(2-bromo-4-methylphenyl)ethan-1-ol **303** (317 mg, 1.1 mmol, 1.0 eq) in DCM (5.4 mL).

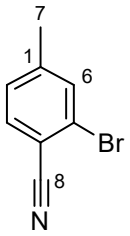
The solution was stirred for 17 h, filtered through a pad of silica, and concentrated *in vacuo* to afford the *title compound* as a colourless oil (272 mg, 86%). **R_f** 0.35 (16% ether / petrol; **IR** (thin film) ν_{max} / cm^{-1} 2923 (w), 1695 (s), 1600 (s), 1384 (w), 1286 (s), 1192 (m), 1054 (m), 822 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.46 (1H, s, H6), 7.42 (1H, d, *J* = 8.0 Hz, H3), 7.21–7.18 (1H, m, H2), 4.49 (2H, s, H9), 2.38 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 194.3 (C8), 143.8 (C1), 135.3 (C4), 134.4 (C6), 130.0 (C3), 128.4 (C2), 119.5 (C5), 34.0 (C9), 21.1 (C7); **HRMS** (ES^+) calc. for $C_9H_8{}^{79}Br_2O$ $[M]^+$ 289.8942, found 289.8932.

2-Bromo-4-methyl-1-vinylbenzene 162



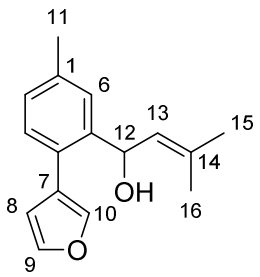
Based on a modified literature procedure.[456] 2-Bromo-1-iodo-4-methylbenzene **146** (3.95 g, 13.3 mmol, 1.0 eq), vinyltrimethoxysilane (2.4 mL, 16.0 mmol, 1.2 eq), and tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 16.0 mL, 16.0 mmol, 1.2 eq) were added to a solution of $PdCl_2$ (47 mg, 0.27 mmol, 2 mol%) in tetrahydrofuran (67 mL). The solution was heated at reflux for 14 h then cooled to RT. Petrol and water were added and the solution was extracted with petrol. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (100% pentane) to afford the *title compound* as a colourless oil (2.15 g, 82%). **R_f** 0.45 (100% petrol); **IR** (thin film) ν_{max} / cm^{-1} 2920 (w), 1625 (w), 1603 (w), 1487 (s), 1458 (m), 1043 (m), 1028 (m), 987 (m), 913 (m), 821 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.46 (1H, d, *J* = 8.0 Hz, H3), 7.38 (1H, s, H6), 7.10–7.08 (1H, m, H2), 7.02 (1H, dd, *J* = 17.5, 11.0 Hz, H8), 5.66 (1H, dd, *J* = 17.5, 1.0 Hz, $H9_{trans}$), 5.31 (1H, dd, *J* = 11.0, 1.0 Hz, $H9_{cis}$), 2.32 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 139.4 (C1), 135.6 (C8), 134.5 (C4), 133.2 (C6), 128.4 (C2), 126.4 (C3), 123.4 (C5), 115.7 (C9), 20.8 (C7); **HRMS** (Cl^+) calc. for $C_9H_{10}{}^{79}Br$ $[M+H]^+$ 196.9960, found 196.9957.

2-Bromo-4-methylbenzonitrile 166



Based on a literature procedure.[457] A solution of 2-bromo-4-methylaniline (1.3 mL, 10.7 mmol, 1.0 eq) in aqueous HCl (1.0 M, 50 mL) was cooled to 0 °C and $NaNO_2$ (1.1 g, 16.1 mmol, 1.5 eq) in water (11 mL) was added. The solution was stirred for 30 minutes then neutralised with solid $NaHCO_3$. The solution was added to a solution of NaCN (2.4 g, 49.2 mmol, 4.6 eq) and CuCN (2.1 g, 23.5 mmol, 2.2 eq) in water (50 mL) and the mixture was heated at 60 °C for 2 h. The solution was cooled to RT and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by Kugelrohr distillation (33 Torr, 150 °C setting) to afford **166** as an orange solid (1.36 g, 65%). $\mathbf{R_f}$ 0.35 (30% ether / petrol); **mp** 34–36 °C (amorphous) (lit. mp 55 °C); **IR** (thin film) ν_{max} / cm^{-1} 2220 (s), 1601 (s), 1486 (s), 1447 (w), 1042 (m), 866 (w), 821 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.52 (1H, d, *J* = 8.0 Hz, H3), 7.50 (1H, s, H6), 7.21 (1H, d, *J* = 8.0 Hz, H2), 2.40 (3H, s, H7); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 145.4 (C1), 134.0 (C3), 133.7 (C6), 128.5 (C3), 125.1 (C5), 117.4 (C8), 112.7 (C4), 21.6 (C7); **LRMS** (ES^+) calc. for $C_8H_7{}^{79}BrN$ $[M+H]^+$ 196.0, found 196.0. Data is consistent with literature values.[458]

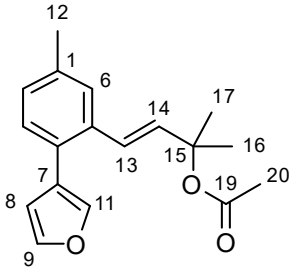
1-[2-(Furan-3-yl)-5-methylphenyl]-3-methylbut-2-en-1-ol 170



Based on a modified literature procedure.[459] A solution of 3-(2-bromo-4-methylphenyl)furan **170** (500 mg, 2.1 mmol, 1.0 eq) in tetrahydrofuran (14 mL) was cooled to –78 °C and butyllithium (2.5

M in hexanes, 0.93 mL, 2.3 mmol, 1.1 eq) was added dropwise. The solution was stirred for 10 minutes and 3-methylcrotonaldehyde (0.22 mL, 2.3 mmol, 1.1 eq) was added dropwise. The solution was stirred at –78 °C for 1 h then warmed to RT and quenched with saturated aqueous $NaHCO_3$. The solution was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to an orange oil. The crude product was purified by flash chromatography (10% ether / petrol to 20% ether / petrol) to afford the *title compound* as a colourless oil (455 mg, 89%). **R_f** 0.15 (20% ether / petroleum ether); **IR** (thin film) ν_{max} / cm^{-1} 3343 (br, m), 2980 (w), 2916 (w), 1509 (w), 1448 (w), 1377 (w), 1160 (m), 1083 (m), 1014 (m), 873 (s), 823 (m), 790 (s); **^{1}H NMR** (400 MHz, C_6D_6) δ_H 7.63 (1H, s, H6), 7.37 (1H, dd, *J* = 1.5, 1.0 Hz, H10), 7.14 (1H, t, *J* = 1.5 Hz, H9), 7.13 (1H,d, *J* = 8.0 Hz, H3), 6.94–6.91 (1H, m, H2), 6.37 (1H, dd, *J* = 1.5, 1.0 Hz, H8), 5.61 (1H, d, *J* = 8.5 Hz, H12), 5.48–5.44 (1H, m, H13), 2.19 (3H, s, H11), 1.48 (3H, d, *J* = 1.5 Hz, H15), 1.36 (3H, d, *J* = 1.0 Hz, H16), 1.30 (1H, br s, OH); **^{13}C NMR** (101 MHz, C_6D_6) δ_C 142.8 (C5), 142.4 (C10), 140.2 (C9), 137.2 (C1), 134.2 (C14), 129.9 (C3), 129.0 (C13), 128.4 (C4), 127.8 (C2), 127.4 (C6), 124.9 (C7), 112.2 (C8), 67.5 (C12), 25.3 (C15), 21.0 (C11), 17.7 (C16); **HRMS** (ES^+) calc. for $C_{16}H_{18}NaO_2$ $[M+Na]^+$ 265.1199, found 265.1198.

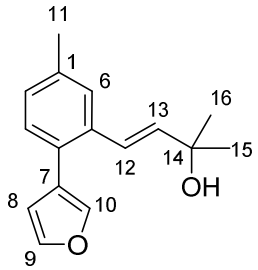
(*E*)-4-[2-(furan-3-yl)-5-methylphenyl]-2-methylbut-3-en-2-yl acetate 177



Acetic anhydride (0.10 mL, 1.11 mmol 2.0 eq) was added to a solution of 1-[2-(furan-3-yl)-5-methylphenyl]-3-methylbut-2-en-1-ol **170** (134 mg, 0.55 mmol, 1.0 eq) and 4-(dimethylamino)pyridine (67 mg, 0.55 mmol, 1.0 eq) in DCM (5.5 mL) and the reaction was stirred for 1 h. Silica (240 mg) was added and the solution was stirred for 2 h, filtered through Celite, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (5% ether / petrol) to afford the *title compound* as a yellow oil (95 mg, 60%). **R_f** 0.45 (30% ether / petrol);

IR (thin film) ν_{max} / cm^{-1} 2980 (m), 1732 (s), 1509 (w), 1367 (m), 1249 (s), 1161 (w), 1122 (w), 1049 (w), 1016 (w), 874 (w), 791 (w); **^{1}H NMR** (400 MHz, $(CD_3)_2CO$) δ_H 7.73 (1H, dd, *J* = 1.5, 1.0 Hz, H10), 7.64 (1H, t, *J* = 1.5 Hz, H9), 7.35 (1H, s, H6), 7.29 (1H, d, *J* = 7.5 Hz, H3), 7.14–7.12 (1H, m, H2), 6.75 (1H, d, *J* = 16.0 Hz, H12), 6.71 (1H, dd, *J* = 1.5, 1.0 Hz, H8), 6.32 (1H, d, *J* = 16.0 Hz, H13), 2.35 (3H, s, H11), 1.96 (3H, s, H18), 1.59 (6H, s, H15, H16); **^{13}C NMR** (101 MHz, $(CD_3)_2CO$) δ_C 169.3 (C17), 143.0 (C9), 140.8 (C10), 136.8 (C1) 135.3 (C13), 135.2 (C5), 129.0 (C3), 128.6 (C7), 128.4 (C2), 127.4 (C12), 127.3 (C6), 124.9 (C4), 111.5 (C8), 79.9 (C14), 26.2 (C15, C16), 21.2 (C18), 20.2 (C11); **HRMS** (ES^+) calc. for $C_{18}H_{20}NaO_3$ $[M+Na]^+$ 307.1305, found 307.1309.

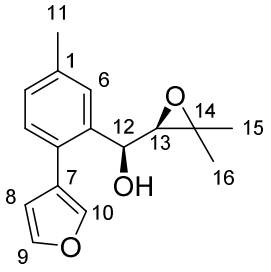
(*E*)-4-[2-(furan-3-yl)-5-methylphenyl]-2-methylbut-3-en-2-ol 178



Acetic anhydride (60 µL, 0.63 mmol 3.0 eq) was added to a solution of 1-[2-(furan-3-yl)-5-methylphenyl]-3-methylbut-2-en-1-ol **170** (50 mg, 0.21 mmol, 1.0 eq) and 4-(dimethylamino)pyridine (26 mg, 0.21 mmol, 1.0 eq) in DCM (2.1 mL) and the reaction was stirred for 8 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (1% ether / petrol to 15% ether / petrol) to afford the *title compound* as a colourless oil (11 mg, 22%). **R_f** 0.15 (30% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3366 (br, m), 2972 (m), 2925 (w), 1509 (w), 1359 (w), 1160 (s), 1049 (m), 976 (m), 873 (s), 788 (s); **^{1}H NMR** (400 MHz, $(CD_3)_2CO$) δ_H 7.65 (1H, d, *J* = 1.5, 1.0 Hz, H10), 7.62 (1H, t, *J* = 1.5 Hz, H9), 7.38 (1H, s, H6), 7.27 (1H, d, *J* = 8.0 Hz, H3), 7.09 (1H, dd, *J* = 8.0, 1.5 Hz, H2), 6.86 (1H, d, *J* = 16.0 Hz, H12), 6.68 (1H, dd, *J* = 1.5, 1.0 Hz, H8), 6.32 (1H, d, *J* = 16.0 Hz, H13), 3.75 (1H, s, OH), 2.34 (3H, s, H11), 1.36 (6H, s, H15, H16); **^{13}C NMR** (101 MHz, $(CD_3)_2CO$) δ_C 142.9 (C9), 140.5 (C10), 140.1 (C13),

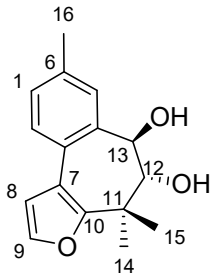
136.7 (C1), 135.8 (C5), 129.1 (C3), 128.3 (C7), 127.9 (C2), 127.1 (C6), 125.0 (C4), 124.8 (C12), 111.6 (C8), 69.8 (C14), 29.5 (C15, C16), 20.2 (C11); **HRMS** Not found.

(*SR*)-[(*RS*)-3,3-Dimethyloxiran-2-yl][2-(furan-3-yl)-5-methylphenyl]methanol 182



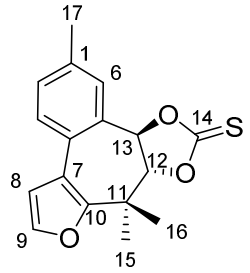
Based on a modified literature procedure.[460] A solution of 1-[2-(furan-3-yl)-5-methylphenyl]-3-methylbut-2-en-1-ol **170** (532 mg, 2.20 mmol, 1.0 eq) in DCM (22 mL) was cooled to 0 °C and 3-chloroperbenzoic acid (523 mg, 2.31 mmol, 1.05 eq) was added. The solution was stirred for 1 h then quenched with aqueous K_2CO_3 (10% w/v) and extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ether / petrol) to afford the *title compound* as a yellow oil (457 mg, 80%). $\mathbf{R_f}$ 0.10 (30% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3427 (br, m), 2964 (w), 1510 (w), 1379 (w), 1167 (m), 1125 (w), 1048 (m), 1015 (s), 874 (s), 813 (s), 794 (s); **^{1}H NMR** (400 MHz, C_6D_6) δ_H 7.65 (1H, s, H6), 7.29 (1H, dd, *J* = 1.5, 1.0 Hz, H10), 7.11 (1H, d, *J* = 8.0 Hz, H3), 7.06 (1H, t, *J* = 1.5 Hz, H9), 6.93–6.90 (1H, m, H2), 6.28 (1H, dd, *J* = 1.5, 1.0 Hz, H8), 4.85 (1H, dd, *J* = 6.5, 3.5 Hz, H12), 2.96 (1H, d, *J* = 6.5 Hz, H13), 2.38 (1H, d, *J* = 3.5 Hz, OH), 2.15 (3H, s, H11), 0.93 (3H, s, H15), 0.89 (3H, s, H16); **^{13}C NMR** (101 MHz, C_6D_6) δ_C 142.7 (C9), 140.0 (C10), 139.6 (C1), 137.5 (C5), 130.1 (C3), 128.9 (C4), 128.5 (C2), 127.8 (C6), 124.9 (C7), 112.0 (C8), 68.6 (C12), 67.7 (C13), 59.6 (C14), 24.2 (C15), 20.9 (C11), 18.4 (C16); **HRMS** not found.

(5*RS*,6*RS*)-4,4,8-Trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5,6-diol 183



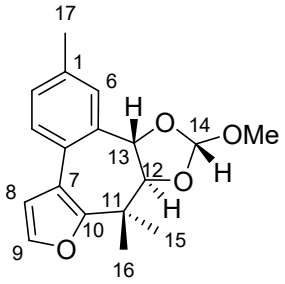
Based on a modified literature procedure.[461] Chlorotriisopropoxytitanium (IV) (1.0 M in hexanes, 6.0 mL, 6.0 mmol, 3.0 eq) was added to a solution of (*SR*)-[(*RS*)-3,3-dimethyloxiran-2-yl][2-(furan-3-yl)-5-methylphenyl]methanol **182** (520 mg, 2.0 mmol, 1.0 eq) in DCM (20 mL). The solution was stirred for 1 h then quenched with aqueous HCl (1.0 M, 20 mL), stirred for ten minutes, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether / petrol) to afford the *title compound* as a white solid (321 mg, 62%). **R_f** 0.30 (40% ether / petrol); **mp** 115 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3384 (br, m), 2975 (m), 2934 (m), 1524 (m), 1499 (w), 1167 (s), 1071 (s), 1055 (s), 990 (m), 822 (m), 750 (m), 733 (w); **^{1}H NMR** (400 MHz, C_6D_6) δ_H 7.80 (1H, s, H6), 7.36 (1H, d, *J* = 8.0 Hz, H3), 7.10 (1H, d, *J* = 2.0 Hz, H9), 7.03–7.00 (1H, m, H2), 6.40 (1H, d, *J* = 2.0 Hz, H8), 4.49 (1H, d, *J* = 8.5 Hz, H13), 3.38 (1H, d, *J* = 8.5 Hz, H12), 2.77 (1H, br s, OH), 2.44 (1H, br s, OH), 2.27 (3H, s, H16), 1.47 (3H, s, H14), 1.36 (3H, s, H15); **^{13}C NMR** (101 MHz, C_6D_6) δ_C 154.8 (C10), 141.3 (C9), 140.6 (C1), 136.0 (C5), 127.5 (C2), 126.8 (C3), 125.9 (C4), 124.0 (C6), 117.4 (C7), 110.1 (C8), 77.6 (C12), 68.9 (C13), 42.2 (C11), 27.5 (C15), 21.1 (C16), 20.6 (C14), **HRMS** (ES^+) calc. for $C_{16}H_{19}O_3$ $[M+H]^+$ 259.1329, found 259.1328.

(4b*RS*,7a*RS*)-3,8,8-Trimethyl-4b,7a-dihydro-8*H*-benzo[3,4]furo[3',2':5,6]cyclohepta[1,2-*d*][1,3]dioxole-6-thione 184

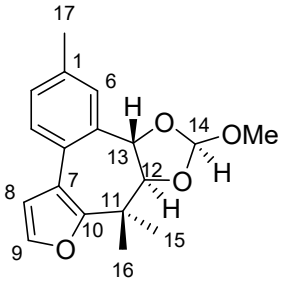


Based on a modified literature procedure.[366] 1,1'-Thiocarbonyldiimidazole (267 mg, 1.50 mmol, 3.0 eq) and 4-(dimethylamino)pyridine (6 mg, 0.05 mmol, 0.1 eq) were added to a solution of (5*RS*,6*RS*)-4,4,8-trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5,6-diol **183** (130 mg, 0.50 mmol, 1.0 eq) in toluene (5.0 mL). The solution was heated at reflux for 4 h then cooled to RT and concentrated *in vacuo*. The crude product was purified by flash chromatography (10% ether / petrol) to afford the *title compound* as a yellow solid (135 mg, 90%). **R_f** 0.55 (40% ether / petrol); **mp** 102–104 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2925 (w), 1523 (w), 1340 (m), 1278 (s), 1164 (w), 1004 (w); **^{1}H NMR** (400 MHz, C_6D_6) δ_H 7.38 (1H, s, H6), 7.14 (1H, d, *J* = 8.0 Hz, H3), 6.92–6.89 (2H, m, H2, H9), 6.18 (1H, d, *J* = 2.0 Hz, H8), 5.02 (1H, d, *J* = 11.0 Hz, H13), 3.79 (1H, d, *J* = 11.0 Hz, H12), 2.08 (3H, s, H17), 1.12 (3H, s, H16), 1.09 (3H, s, H15); **^{13}C NMR** (101 MHz, C_6D_6) δ_C 190.3 (C14), 151.7 (C10), 141.8 (C9), 137.1 (C1), 132.1 (C5), 128.9 (C2), 127.2 (C3), 124.7 (C4), 123.5 (C6), 117.3 (C7), 110.1 (C8), 89.4 (C12), 81.5 (C13), 39.3 (C11), 26.3 (C16), 21.0 (C15), 20.7 (C17); **HRMS** (ES^+) calc. for $C_{17}H_{17}O_3S$ $[M+H]^+$ 301.0893, found 301.0898.

(4b*SR*,6*SR*,7a*SR*)-6-methoxy-3,8,8-trimethyl-4b,7a-dihydro-8*H*-benzo[3,4]furo[3',2':5,6]cyclohepta[1,2-*d*][1,3]dioxole (A) 189 and (4b*SR*,6*RS*,7a*SR*)-6-methoxy-3,8,8-trimethyl-4b,7a-dihydro-8*H*-benzo[3,4]furo[3',2':5,6]cyclohepta[1,2-*d*][1,3]dioxole (B) 189



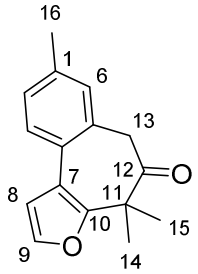
A



B

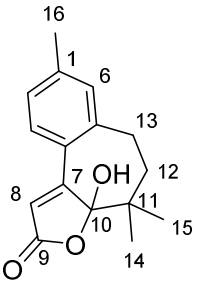
Based on a modified literature procedure.[462] A solution of (4b*RS*,7a*RS*)-3,8,8-trimethyl-4b,7a-dihydro-8*H*-benzo[3,4]furo[3',2':5,6]cyclohepta[1,2-*d*][1,3]dioxole-6-thione **184** (133 mg, 0.44 mmol, 1.0 eq) in trimethyl phosphite (2.2 mL) was heated at reflux for 14 h then cooled to RT and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (1% ether / petrol) to afford a mixture of the *title compounds* as a colourless oil (86 mg, 1:1 **A**:**B**, 44%, 70% pure). $\mathbf{R_f}$ 0.50 (20% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2980 (m), 2889 (w), 1524 (w), 1394 (w), 1382 (w), 1134 (s), 1069 (s), 1034 (m), 983 (m), 959 (m), 823 (w), 753 (w), 734 (w); **^{1}H NMR** (400 MHz, CD_3CN) δ_H 7.51–7.50 (2H, m, H9A, H9B), 7.48 (2H, d, *J* = 8.0 Hz, H3A, H3B), 7.43 (1H, s, H6B), 7.42 (1H, s, H6A), 7.17–7.14 (2H, m, H2A, H2B), 6.72–6.71 (2H, m, H8A, H8B), 6.05 (1H, s, H14A), 6.00 (1H, s, H14B), 5.05 (1H, d, *J* = 9.0 Hz, H13B), 4.95 (1H, d, *J* = 9.0 Hz, H13A), 3.99 (1H, d, *J* = 9.0 Hz, H12A), 3.87 (1H, d, *J* = 9.0 Hz, H12B), 3.40 (3H, s, OCH_3-B), 3.35 (3H, s, OCH_3-A), 2.38–2.36 (6H, m, H17A, H17B), 1.47 (3H, s, H16B), 1.43 (3H, s, H15A), 1.42 (3H, s, H16A), 1.42 (3H, s, H15B); **^{13}C NMR** (101 MHz, CD_3CN) δ_C 154.4 (C10A, C10B), 142.5 (C9A/B), 142.4 (C9B/A), 137.2 (C1A/B), 137.2 (C1B/A), 137.1 (C5B), 136.2 (C5A), 128.7 (C2A/B), 128.6 (C2B/A), 127.7 (C3A, C3B), 126.0 (C4A, C4B), 124.1 (C6A/B), 124.1 (C6B/A), 117.0 (C14A), 116.4 (C14B), 110.9 (C8A/B), 110.8 (C8B/A), 86.1 (C12B), 84.6 (C12A), 77.8 (C13A), 75.9 (C13B), 51.2 (OCH_3-B), 50.9 (OCH_3-A), 40.4 (C11B), 40.4 (C11A), 27.6 (C16B), 27.3 (C16A), 22.1 (C15B), 21.5 (C15A), 20.9 (C17A/B), 20.8 (C17B/A); **HRMS** (Cl^+) calc. for $C_{18}H_{21}O_4$ $[M+H]^+$ 301.1434, found 301.1404.

4,4,8-Trimethyl-4,6-dihydro-5*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5-one 192



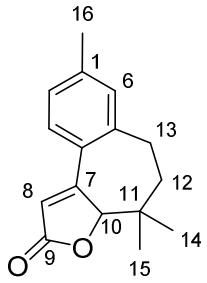
Based on a modified literature procedure.[370] Triphenylphosphine (986 mg, 3.76 mmol, 4.0 eq) and imidazole (256 mg, 3.76 mmol, 4.0 eq) were added to a solution of (5*RS*,6*RS*)-4,4,8-trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5,6-diol **183** (243 mg, 0.94 mmol, 1.0 eq) in toluene (24 mL) and the solution was stirred for thirty minutes. I_2 (716 mg, 2.82 mmol, 3.0 eq) was added and the solution was heated at reflux for 90 minutes. The solution was cooled to RT and poured into a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$. The solution was extracted with toluene and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow solid. The crude product was purified by flash chromatography (100% petrol to 2% ether / petrol) to afford the *title compound* as a white solid (148 mg, 65%). $\mathbf{R_f}$ 0.10 (2% ether / petrol); **mp** 92–94 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2981 (w), 2930 (w), 1713 (s), 1521 (w), 1285 (w), 1164 (w), 1070 (m), 821 (m), 756 (m); **^{1}H NMR** (400 MHz, CD_3CN) δ_H 7.58 (1H, d, *J* = 2.0 Hz, H9), 7.42 (1H, s, *J* = 8.0 Hz, H3), 7.19 (1H, d, *J* = 8.0 Hz, H2), 7.13 (1H, s, H6), 6.72 (1H, d, *J* = 2.0 Hz, H8), 3.79 (2H, s, H13), 2.34 (3H, s, H16), 1.38 (6H, s, H14, H15); **^{13}C NMR** (101 MHz, CD_3CN) δ_C 206.7 (C12), 153.1 (C10), 143.0 (C9), 138.4 (C1), 131.4 (C5), 130.6 (C6), 128.9 (C2), 128.8 (C4), 127.5 (C3), 119.4 (C7), 111.0 (C8), 50.7 (C11), 47.9 (C13), 24.7 (C14, C15), 20.6 (C16); **HRMS** (ES^+) calc. for $C_{16}H_{17}O_2$ $[M+H]^+$ 241.1223, found 241.1223.

3a-Hydroxy-4,4,8-trimethyl-3a,4,5,6-tetrahydro-2*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-2-one 195



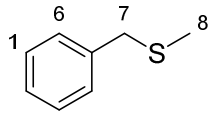
Based on a modified literature procedure.[463] *N*,*N*-Diisopropylethylamine (70 µL, 0.40 mmol, 5.0 eq) and Rose Bengal (spatula tip) were added to a solution of 4,4,8-trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan **143** (20 mg, 0.08 mmol, 1.0 eq) in DCM (8.3 mL). O_2 was bubbled through the solution and the solution was cooled to 0 °C. The solution was irradiated for 5 minutes then bubbling of O_2 was stopped and the solution was irradiated under an atmosphere of O_2 for 150 minutes. The solution was washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a pink oil. The crude product was purified by flash chromatography (20% ether / petrol to 30% ether / petrol) to afford the *title compound* as an off-white solid (14 mg, 69%). **R_f** 0.15 (40% ether / petrol); **mp** 154–156 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3240 (br, m), 2978 (w), 2935 (w), 1731 (s), 1623 (w), 1609 (w), 1266 (m), 1201 (m), 1176 (m), 1053 (m), 949 (m), 909 (w), 870 (m), 821 (m), 731 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.24 (1H, d, *J* = 7.5 Hz, H3), 7.07 (1H, d, *J* = 7.5 Hz, H2), 7.02 (1H, s, H6), 6.05 (1H, s, H8), 3.63 (1H, br s, OH), 3.00 (1H, t, *J* = 14.5 Hz, H13), 2.67 (1H, dd, *J* = 16.0, 7.0 Hz, H13'), 2.34 (3H, s, H16), 2.25 (1H, t, *J* = 14.5 Hz, H12), 1.57 (1H, dd, *J* = 14.5, 7.0 Hz, H12'), 1.27 (3H, s, H14), 1.09 (3H, s, H15); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.7 (C9), 169.6 (C7), 141.3 (C5), 140.9 (C1), 131.4 (C6), 130.8 (C3), 127.5 (C2), 127.5 (C4), 116.9 (C8), 110.5 (C10), 41.2 (C11), 38.1 (C12), 31.5 (C13), 24.8 (C14), 21.3 (C16), 21.1 (C15); **HRMS** (EI^+) calc. for $C_{16}H_{18}O_3$ [M+] 258.1256, found 258.1253.

4,4,8-Trimethyl-3a,4,5,6-tetrahydro-2*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-2-one 196



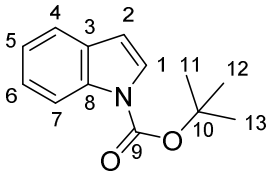
Based on a modified literature procedure.[464] $NaBH_4$ (8 mg, 0.20 mmol, 4.0 eq) was added to a solution of 3a-hydroxy-4,4,8-trimethyl-3a,4,5,6-tetrahydro-2*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-2-one **195** (13 mg, 0.05 mmol, 1.0 eq) and $CeCl_3 \cdot 7H_2O$ (2 mg, 5 µmol, 0.1 eq) in methanol (0.63 mL) at 0 °C. The solution was stirred at this temperature for 80 minutes then quenched with conc. HCl (5.0 mL). The solution was extracted with chloroform. The combined organic layers were washed with H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ether / petrol to 20% ether / petrol) to afford the *title compound* as a colourless oil (7 mg, 61%). **R_f** 0.25 (40% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2920 (w), 1755 (s), 1662 (w), 1455 (w), 1315 (w), 1166 (w), 1025 (w), 917 (w), 854 (w), 819 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.33 (1H, d, *J* = 8.0 Hz, H3), 7.08 (1H, d, *J* = 8.0 Hz, H2), 7.00 (1H, s, H6), 6.18 (1H, d, *J* = 1.5 Hz, H8), 4.87 (1H, d, *J* = 1.5 Hz, H10), 2.93 (1H, dd, *J* = 16.0, 11.5 Hz, H13), 2.72 (1H, ddd, *J* = 16.0, 8.0, 1.5 Hz, H13'), 2.35 (3H, s, H16), 1.94 (1H, ddd, *J* = 14.5, 11.5, 1.5 Hz, H12), 1.72 (1H, ddd, *J* = 14.5, 8.0, 1.5 Hz, H12'), 1.28 (3H, s, H14), 0.93 (3H, s, H15); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 172.1 (C9), 167.8 (C7), 140.7 (C5), 140.1 (C1), 130.4 (C6), 129.0 (C3), 126.9 (C4), 126.6 (C2), 114.4 (C8), 89.0 (C10), 38.9 (C12), 37.1 (C11), 30.1 (C13), 28.7 (C14), 20.2 (C16), 17.7 (C15); **HRMS** (ES^+) calc. for $C_{16}H_{19}O_2$ $[M+H]^+$ 243.1380, found 243.1380.

Benzyl methyl sulfide 202



Based on a literature procedure.[465] A solution of benzyl mercaptan (0.47 mL, 4.00 mmol, 1.0 eq) and iodomethane (0.27 mL, 4.30 mmol, 1.1 eq) in benzene (1.8 mL) was added to a solution of NaOH (269 mg, 6.70 mmol, 1.7 eq) and tetrabutylammonium iodide (45 mg, 0.10 mmol, 0.03 eq) in water (2.2 mL) and the solution was stirred for 17 h. The solution was extracted with ether and the combined organic layers were washed with aqueous NaOH (1.0 M) and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **202** as a yellow oil (500 mg, 90%). $\mathbf{R_f}$ 0.70 (40% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3028 (w), 2914 (w), 1494 (w), 1453 (w), 910 (w), 725 (m), 698 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.33 (4H, m, H1, H3, H4, H6), 7.30–7.26 (1H, m, H2), 3.71 (2H, s, H7), 2.03 (3H, s, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 138.3 (C5), 128.9 (C4, C6), 128.5 (C1, C3), 127.0 (C2), 38.4 (C7), 14.9 (C8); **HRMS** (FI^+) calc. for $C_8H_{10}S$ $[M]^+$ 138.0503, found 138.0505. Data consistent with literature values.[465]

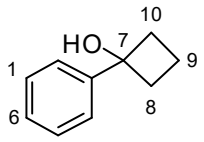
***tert*-Butyl 1*H*-indole-1-carboxylate 204**



Based on a literature procedure.[466] Di-*tert*-butyl-dicarbonate (819 mg, 3.75 mmol, 1.1 eq) and 4-(dimethylamino)pyridine (42 mg, 0.34 mmol, 0.1 eq) were added to a solution of indole (400 mg, 3.41 mmol, 1.0 eq) in DCM (8.5 mL) and the solution was stirred for 14 h. The solution was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **204** as a yellow oil (741 mg, quant). $\mathbf{R_f}$ 0.80 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2979 (w), 1731 (s), 1451 (m), 1379 (w), 1346 (s), 1330 (s), 1250 (s), 1211 (w), 1155 (s), 1117 (s), 1079 (m), 1022 (w), 767 (m), 744 (s), 724 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.15 (1H, d, *J* = 8.0 Hz, H7), 7.64 (1H, d, *J* = 3.5 Hz, H1), 7.56 (1H, d, *J* = 8.0 Hz, H4), 7.33–7.29 (1H,

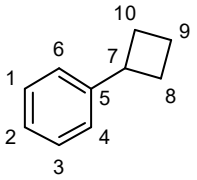
m, H6), 7.25–7.21 (1H, m, H5), 6.57 (1H, d, *J* = 3.5 Hz, H2), 1.68 (9H, s, H11, H12, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 149.8 (C9), 135.2 (C8), 130.6 (C3), 125.9 (C1), 124.2 (C6), 122.6 (C5), 120.9 (C4), 115.2 (C7), 107.3 (C2), 83.7 (C10), 28.2 (C11, C12, C13); **LRMS** (ES^+) calc. for $C_{13}H_{16}NO_2$ $[M+H]^+$ 218.1, found 218.1. Data consistent with literature values.[467]

1-Phenylcyclobutan-1-ol 206



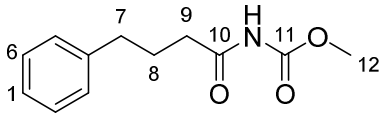
Based on a literature procedure.[468] Butyllithium (3.1 mL of a 2.5 M solution in hexane, 7.8 mmol, 1.1 eq) was added dropwise to a solution of iodobenzene (0.87 mL, 7.8 mmol, 1.1 eq) in tetrahydrofuran (18 mL) at –78 °C. The solution was stirred for 15 minutes and cyclobutanone (0.53 mL, 7.1 mmol, 1.0 eq) in tetrahydrofuran (3.1 mL) was added. The solution was warmed to RT and water was added. The solution was extracted with ether and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ether / petrol to 20% ether / petrol) to afford **206** as a white solid (836 mg, 72%). **R_f** 0.30 (30% ether / petrol); **mp** 32–35 °C (amorphous) (lit. mp 37 °C); **IR** (thin film) ν_{max} / cm^{-1} 3367 (m), 2987 (w), 2946 (w), 1133 (w), 907 (m), 731 (s), 699 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.52–7.49 (2H, m, H2, H4), 7.41–7.36 (2H, m, H1, H5), 7.30 (1H, tt, *J* = 7.5, 1.5 Hz, H6), 2.62–2.54 (2H, m, H8, H10), 2.42–2.33 (2H, m, H8', H10'), 2.07 (1H, s, OH), 2.05–1.98 (1H, m, H9), 1.75–1.64 (1H, m H9'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 146.3 (C3), 128.5 (C1, C5), 127.3 (C6), 125.0 (C2, C4), 77.3 (C7), 36.8 (C8, C10), 12.0 (C9); **LRMS** (ES^+) calc. for $C_{10}H_{13}O$ $[M+H]^+$ 149.1, found 149.1. Data consistent with literature values.[468]

Cyclobutylbenzene 207



Based on a literature procedure.[469] Pd/C (10 wt.% Pd, 361 mg, 0.34 mmol, 0.1 eq) was added to a solution of 1-phenylcyclobutan-1-ol **206** (500 mg, 3.4 mmol, 1.0 eq) in ethanol (6.8 mL) and H_2 was bubbled through the solution for 5 minutes. The solution was then stirred under an atmosphere of H_2 for 17 h. The solution was filtered through Celite and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (100% petrol) to afford **207** as a colourless oil (82 mg, 18%). $\mathbf{R_f}$ 0.55 (100% petrol); **IR** (thin film) ν_{max} / cm^{-1} 3027 (w), 2972 (w), 1494 (w), 1447 (w), 731 (m), 696 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.32–7.28 (2H, m, H1, H3), 7.24–7.22 (2H, m, H4, H6), 7.20–7.16 (1H, m, H2), 3.56 (1H, quin, *J* = 9.0 Hz, H7), 2.39–2.31 (2H, m, H8, H10), 2.21–2.11 (2H, m, H8', H10'), 2.08–1.96 (1H, m, H9), 1.90–1.82 (1H, m, H9'); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 146.3 (C5), 128.2 (C1, C3), 126.3 (C4, C6), 125.7 (C2), 40.4 (C7), 29.8 (C8, C10), 18.3 (C9); **LRMS** (ES^-) calc. for $C_{10}H_{11}$ $[M–H]^-$ 131.1, found 131.1. Data consistent with literature values.[469]

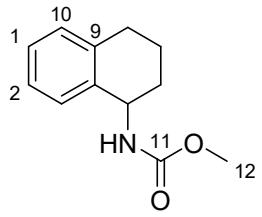
Methyl (4-phenylbutanoyl)carbamate 209



Based on a literature procedure.[470] To a solution of 4-phenylbutanoyl chloride **303** (500 mg, 2.7 mmol, 1.0 eq) in toluene (1.0 mL) was added methyl carbamate (399 mg, 5.3 mmol, 1.9 eq). The solution was heated at 80 °C for 14 h then cooled to RT. Ethyl acetate was added and the solution was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a white powder. The crude product was purified by flash chromatography (10% ether / petrol to 30% ether / petrol) to afford **209** as a white solid (167 mg, 28%). $\mathbf{R_f}$ 0.25 (30% ethyl acetate / petrol); **mp** 103 °C (amorphous) (lit. mp 122 °C); **IR** (thin film, ν_{max} / cm^{-1}) 3252 (w), 3184 (w), 1756 (s), 1694 (w), 1520 (m), 1229 (m), 1172 (m), 1036 (w), 700 (w); ^{1}**H NMR** (400 MHz,

$CDCl_3$) δ_H 7.68 (1H, br s, NH), 7.30–7.26 (2H, m, H2, H6), 7.20–7.17 (3H, m, H1, H3, H6), 3.76 (3H, s, H12) 2.78 (2H, t, *J* = 7.5 Hz, H9), 2.61 (2H, t, *J* = 7.5 Hz, H7), 1.99 (2H, quin, *J* = 7.5 Hz, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.4 (C10), 152.3 (C11), 141.5 (C4), 128.5 (C3, C5), 128.4 (C2, C6), 126.0 (C1), 53.0 (C12), 35.4 (C9), 35.1 (C7), 25.8 (C8); **LRMS** (ES^+) calc. for $C_{12}H_{15}NO_3$ $[M+H]^+$ 222.1, found 222.1. Data consistent with literature values.[470]

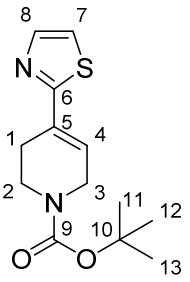
Methyl (1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 210



Based on a literature procedure.[470] To a solution of methyl (4-phenylbutanoyl)carbamate **209** (130 mg, 0.59 mmol, 1.0 eq) in DCM at –78 °C was added diisobutylaluminium hydride (1.30 mL of a 1.0 M solution in cyclohexane, 1.30 mmol, 2.2 eq). The solution was stirred for 90 minutes and methanol (0.60 mL) was added. The dry-ice bath was removed and florisil (2.0 g) and brine (0.60 mL) were added. The solution was diluted with ethyl acetate (6.0 mL) and stirred for 15 minutes. $MgSO_4$ (2.0 g) was added and the solution was stirred for 15 minutes. The solution was filtered and concentrated *in vacuo* affording methyl (1-hydroxy-4-phenylbutyl)carbamate as a white solid. To a solution of methyl (1-hydroxy-4-phenylbutyl)carbamate in DCM (2.6 mL) at –78 °C was added $TiCl_4$ (0.89 mL of a 1.0 M solution in DCM, 0.89 mmol, 1.5 eq). The solution was stirred for 1 h and quenched with saturated aqueous $NaHCO_3$. The solution was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ether / petrol) to afford **210** as a white solid (77 mg, 64% over 2 steps). **R_f** 0.45 (30% ethyl acetate / petrol); **mp** 58–61 °C (amorphous) (lit. mp 76–77 °C); **IR** (thin film) ν_{max} / cm^{-1} 3317 (w), 2941 (w), 1698 (s), 1529 (s), 1451 (w), 1243 (m), 1071 (m), 772 (w), 744 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.34–7.32 (1H, m, H3), 7.19–7.15 (2H, m, H1, H2), 7.11–7.08 (1H, m, H10), 4.90 (2H, br s, H5, NH), 3.71 (3H, s, H12), 2.81 (1H, dt, *J* = 16.5, 6.5 Hz, H8), 2.74 (1H, dt, *J* = 16.5, 6.5 Hz, H8'), 2.07–2.03 (1H, m, H6), 1.86–1.82 (3H,

m, H6', H7); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 156.6 (C11), 137.5 (C9), 136.8 (C4), 129.2 (C10), 128.9 (C3), 127.3 (C2), 126.2 (C1), 52.1 (C12), 49.3 (C5), 30.5 (C6), 29.2 (C8), 19.9 (C7); **LRMS** (ES^+) calc. for $C_{12}H_{15}NNaO_2$ $[M+Na]^+$ 228.1, found 228.1. Data consistent with literature values.[470]

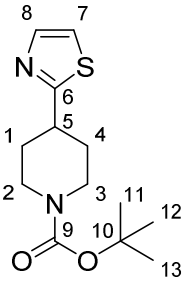
***tert*-Butyl 4-(thiazol-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate 213**



Based on modified literature procedures.[471,472] A solution of diisopropylamine (0.10 mL, 0.69 mmol, 1.1 eq) in tetrahydrofuran (0.9 mL) was cooled to –78 °C and butyllithium (0.28 mL of a 2.5 M solution in hexanes, 0.69 mmol, 1.1 eq) was added. The solution was stirred for 15 minutes and *tert*-butyl 4-oxo-1-piperidinecarboxylate (125 mg, 0.63 mmol, 1.0 eq) in tetrahydrofuran (0.90 mL) was added. The solution was stirred for a further 15 minutes and *N*-phenyl-bis(trifluoromethanesulfonimide) (250 mg, 0.69 mmol, 1.1 eq) in tetrahydrofuran (0.90 mL) was added. The solution was warmed to 0 °C and stirred at this temperature for 3 h then concentrated *in vacuo.* The crude product was purified by flash chromatography (5% ethyl acetate / petrol to 20% ethyl acetate / petrol) to afford *tert*-butyl 4-{[(trifluoromethyl)sulfonyl]oxy}-3,6-dihydropyridine-1(2*H*)-carboxylate **212** (56 mg, 0.17 mmol). This compound was redissolved in tetrahydrofuran (0.56 mL) and thiazol-2-ylzinc(II) bromide (0.85 mL of a 0.25 M solution in tetrahydrofuran, 0.21 mmol, 1.25 eq) and $Pd(PPh_3)_4$ (20 mg, 0.02 mmol, 10 mol%) were added. The solution was heated at reflux for 16 h then cooled to RT. Brine and ethyl acetate were added, the layers were separated, and the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether / petrol to 70% ether / petrol) to afford **213** as a yellow oil (16 mg, 10% over 2 steps). **R_f** 0.35 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2977 (w), 1687 (s), 1418 (m), 1366 (m), 1239 (m), 1164 (s), 1114 (m), 909 (w), 728 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.75 (1H, d, *J* = 3.5 Hz, H8), 7.21 (1H, d, *J* = 3.5 Hz, H7), 6.55 (1H, br s, H4), 4.12–

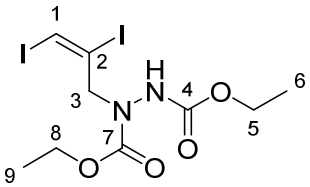
4.10 (2H, m, H3), 3.63 (2H, t, *J* = 5.5 Hz, H2), 2.76 (2H, br s, H1), 1.48 (9H, s, H11, H12, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 168.9 (C6), 154.8 (C9), 143.0 (C8), 131.1 (C5), 125.2 (C4), 117.9 (C7), 80.0 (C10), 43.1 (C3), 39.2 (C2), 28.5 (C11, C12, C13), 26.7 (C1); **LRMS** (ES^+) calc. for $C_{13}H_{19}N_2O_2S$ $[M+H]^+$ 267.1, found 267.1. Data consistent with literature values.[473]

***tert*-Butyl 4-(thiazol-2-yl)piperidine-1-carboxylate 214**



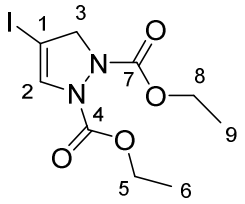
Based on a modified literature procedure.[474] A solution of *tert*-butyl-4-(thiazol-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate **213** (48 mg, 0.18 mmol, 1.0 eq) and $Pd(OH)_2$ on C (15 mg of a 20% w/w mixture, 0.02 mmol, 12 mol%) in methanol (6.0 mL) was purged with N_2. H_2 gas was bubbled through the solution for 10 minutes then the solution was stirred under an atmosphere of H_2 for 14 h. The solution was filtered through Celite then concentrated *in vacuo* to a green oil. The crude product was purified by flash chromatography (10% ether / petrol to 50% ether / petrol) to afford the *title compound* as a white solid (24 mg, 50%). **R_f** 0.20 (30% ethyl acetate / petrol); **mp** 39–42 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 2974 (w), 2929 (w), 1688 (s), 1447 (m), 1365 (m), 1233 (m), 1167 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.68 (1H, d, *J* = 3.5 Hz, H8), 7.21 (1H, d, *J* = 3.5 Hz, H7), 4.19 (2H, br s, H2, H3), 3.16 (1H, tt, *J* = 11.5, 4.0 Hz, H5), 2.87 (2H, t, *J* = 11.5 Hz, H2', H3'), 2.11–2.07 (2H, m, H1, H4), 1.73 (2H, qd, *J* = 12.5, 4.0 Hz, H1', H4'), 1.45 (9H, s, H11, H12, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.7 (C6), 154.7 (C9), 142.2 (C8), 117.8 (C7), 79.6 (C10), 43.3 (C2, C3), 40.5 (C5), 32.4 (C1, C4), 28.5 (C11, C12, C13); **HRMS** calc. for $C_{13}H_{21}N_2O_2S$ $[M+H]^+$ 269.1318, found 269.1314.

Diethyl (*E*)-1-(2,3-diiodoallyl)hydrazine-1,2-dicarboxylate 216



Based on a literature procedure.[475] A solution of (*E*)-2,3-diiodoprop-2-en-1-ol **305** (1.0 g, 3.20 mmol, 1.0 eq) and triphenylphosphine (1.7 g, 6.40 mmol, 2.0 eq) in tetrahydrofuran (26 mL) was cooled to 0 °C and diethyl azodicarboxylate (1.0 mL, 6.40 mmol, 2.0 eq) was added dropwise. The solution was warmed to RT, stirred for 24 h, and concentrated *in vacuo* to an orange solid. The crude product was purified by flash chromatography (100% petrol to 30% ether / petrol) to afford **216** as a white solid (811 mg, 54%). $\mathbf{R_f}$ 0.20 (40% ether / petrol); **mp** 38–40 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3302 (w), 2983 (w), 1709 (s), 1381 (w), 1213 (m), 1059 (m), 907 (s), 726 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.14 (1H, s, H1), 6.71–6.61 (1H, m, NH), 4.50–4.45 (2H, m, H3), 4.28–4.17 (4H, m, H5, H8), 1.33–1.24 (6H, m, H6, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 155.8 (C4, C7), 98.1 (C2), 83.2 & 82.6 (C1), 63.3 & 63.1 (C8), 62.2 (C5), 61.1 & 60.1 (C3), 14.6 (C6, C9); **LRMS** (ES+) calc. for $C_9H_{15}{}^{127}I_2N_2O_4$ $[M+H]^+$ 468.9, found 468.9. Data consistent with literature values.[475]

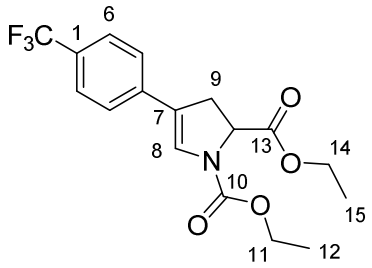
Diethyl 4-iodo-1*H*-pyrazole-1,2(3*H*)-dicarboxylate 217



Based on a literature procedure.[475] To a solution of diethyl (*E*)-1-(2,3-diiodoallyl)hydrazine-1,2-dicarboxylate **216** (600 mg, 1.30 mmol, 1.0 eq), CuI (73 mg, 0.40 mmol, 0.3 eq), and Cs_2CO_3 (834 mg, 2.60 mmol, 2.0 eq) in tetrahydrofuran (12 mL) was added DMEDA (80 µL, 0.80 mmol, 0.6 eq) dropwise. The solution was heated at reflux for 22 h then cooled to RT and filtered through Celite, silica, and $MgSO_4$ sequentially using ethyl acetate to wash the residue. The solution was concentrated *in vacuo* to afford **217** as a yellow solid (428 mg, 98%). $\mathbf{R_f}$ 0.35 (30% ethyl acetate / petrol); **mp** 68 °C (amorphous) (lit. mp 98–99 °C); **IR** (thin film) ν_{max} / cm^{-1} 3117 (w), 2982 (w), 1710

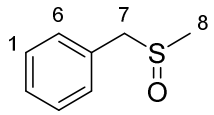
(s), 1372 (m), 1312 (s), 1214 (s), 1149 (m), 1051 (m), 757 (m); **^{1}H NMR** (200 MHz, $CDCl_3$) δ_H 6.89 (1H, t, *J* = 2.0 Hz, H2), 4.46 (2H, m, H3), 4.26 (4H, q, *J* = 7.0 Hz, H5, H8), 1.32 (3H, t, *J* = 7.0 Hz, H6), 1.31 (3H, t, *J* = 7.0 Hz, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 157.7 (C7), 152.9 (C4), 133.4 (C2), 64.0 (C1), 63.3 (C5), 63.1 (C8), 60.9 (C3), 14.4 (C6, C9); **LRMS** (ES^+) calc. for $C_9H_{14}{}^{127}IN_2O_4$ $[M+H]^+$ 341.0, found 341.0. Data consistent with literature values.[475]

Diethyl 4-[4-(trifluoromethyl)phenyl]-1*H*-pyrazole-1,2(3*H*)-dicarboxylate 218



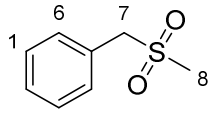
Based on a modified literature procedure.[476] A solution of diethyl 4-iodo-1*H*-pyrazole-1,2(3*H*)-dicarboxylate **217** (59 mg, 0.17 mmol, 1.0 eq), 4-(trifluoromethyl)phenylboronic acid (40 mg, 0.21 mmol, 1.2 eq), and $Pd(PPh_3)_4$ (8 mg, 0.007 mmol, 0.04 eq) in toluene (3.4 mL), ethanol (1.7 mL), and aqueous Na_2CO_3 (2.0 M, 1.1 mL) was heated at 80 °C for 15 h then cooled to RT. The solution was diluted with ethyl acetate then washed with brine. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ether / pentane to 40% ether / pentane) to afford the *title compound* as a yellow oil (38 mg, 63%). **R_f** 0.40 (30% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2985 (w), 1749 (s), 1708 (s), 1615(m), 1322 (s), 1224 (m), 1163 (m), 1111 (s), 1068 (m), 825 (m), 754 (m); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.59 (2H, d, *J* = 8.5 Hz, H2, H6) 7.37 (2H, d, *J* = 8.5 Hz, H3, H5), 7.25 (1H, t, *J* = 2.0 Hz, H8), 4.81 (2H, br s, H9), 4.33–4.27 (4H, m, H11, H14), 1.35 (3H, t, *J* = 7.0 Hz, H12), 1.33 (3H, t, *J* = 7.0 Hz, H15); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 158.0 (C10), 153.3 (C13), 134.7 (C4), 129.4 (q, *J* = 33.0 Hz, C1), 125.8 (q, *J* = 4.0 Hz, C2, C6), 125.0 (C3, C5), 124.5 (C8), 124.0 (q, *J* = 272.0 Hz, CF_3), 118.9 (C7), 63.3 (C11), 63.2 (C14), 54.6 (C9), 14.5 (C12), 14.4 (C15); **HRMS** (ES^+) calc. for $C_{16}H_{17}F_3N_2NaO_4$ $[M+Na]^+$ 381.1033, found 381.0675.

[(Methylsulfinyl)methyl]benzene 230



Based on a literature procedure.[477] Benzyl methyl sulfide **202** (200 mg, 1.5 mmol, 1.0 eq) was added to *para*-toluenesulfonic acid (19 mg, 0.10 mmol, 0.07 eq) and H_2O_2 (0.27 mL of a 35 wt.% aqueous solution, 3.2 mmol, 2.2 eq) and the solution was stirred for 40 minutes. The solution was extracted with DCM and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ether / petrol to 5% methanol / ether) to afford **230** as a brown solid (115 mg, 51%); $\mathbf{R_f}$ 0.10 (50% ethyl acetate / petrol); **mp** 48–50 °C (lit. mp 53–54 °C); **IR** (thin film) ν_{max} / cm^{-1} 1496 (w), 1455 (w), 1029 (s), 911 (m), 766 (m), 727 (m), 698 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.41–7.35 (3H, m, H2, H4, H6), 7.30–7.27 (2H, m, H1, H3), 4.06 (1H, d, *J* = 13.0 Hz, H7), 3.92 (1H, d, *J* = 13.0 Hz, H7'), 2.45 (3H, s, H8); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 130.1 (C1, C3), 129.6 (C5), 129.0 (C4, C6), 128.5 (C2), 60.3 (C7), 37.3 (C8); **LRMS** (ES^+) calc. for $C_8H_{11}OS$ $[M+H]^+$ 155.1, found 155.1. Data consistent with literature values.[477]

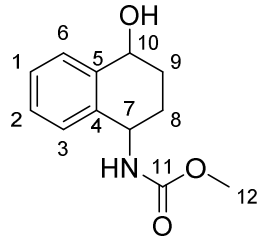
[(Methylsulfonyl)methyl]benzene 231



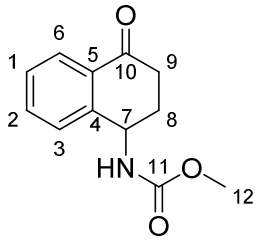
Based on a literature procedure.[478] A solution of 3-chloroperbenzoic acid (<77%, 650 mg, 2.90 mmol, 2.0 eq) in DCM (4.8 mL) was added to a solution of benzyl methyl sulfide **202** (200 mg, 1.45 mmol, 1.0 eq) in DCM (9.7 mL) and the solution was stirred for 50 minutes. The solution was washed with aqueous NaOH (40% w/v) and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **231** as a white solid (214 mg, 87%). $\mathbf{R_f}$ 0.30 (40% ethyl acetate / petrol); **mp** 103–106 °C (amorphous) (lit. mp 123–124 °C); **IR** (thin film) ν_{max} / cm^{-1} 1302 (s), 1251 (m), 1167 (w), 1117 (s), 968 (w), 886 (m), 788 (m), 700 (s); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ_H 7.41 (5H, s, H1, H2, H3, H4, H6), 4.25 (2H, s, H7), 2.75 (3H, s, H8); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ_C 130.5 (C1, C3), 130.0 (C5), 129.2 (C4, C6), 128.3

(C2), 61.4 (C7), 39.0 (C8); **LRMS** (ES^+) calc. for $C_8H_{10}NaO_2S$ $[M+Na]^+$ 193.0, found 193.0. Data consistent with literature values.[478]

Methyl (4-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 232 and Methyl (4-oxo-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 233



232

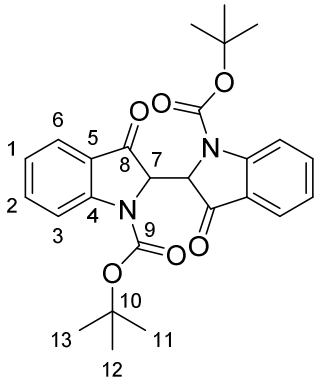


233

To a solution of methyl (1,2,3,4-tetrahydronaphthalen-1-yl)carbamate **210** (1.0 mL of a 100 mM solution in methanol, 0.10 mmol), glucose (4.8 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 4.80 mmol), GDH (240 µL of a 2.0 U/µL solution in 100 mM phosphate buffer, pH 7.5, 480 U), $NADP^+$ monosodium salt (240 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.96 µmol), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500 U) in 100 mM phosphate buffer (pH 7.5, 36 mL) was added $P450_{BM3}$ mutant RT2/IP (4.8 mL of a 10 µM solution in phosphate buffer, 0.048 µmol). The solution was stirred open to air for 17 h. Methanol was added, the solution was centrifuged at 9,500 g, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow solid. The crude product was purified by flash chromatography (20% ether to 70% ether) to afford **232** as a colourless oil (13 mg, 59%) and **233** as a colourless oil (5 mg, 24%). **232**: $\mathbf{R_f}$ 0.10 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3311 (m), 2948 (w), 1693 (s), 1532 (m), 1251 (m), 1049 (m), 766 (m), 732 (m); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.48–7.46 (1H, m, H6), 7.36–7.31 (1H, m, H3), 7.30–7.27 (2H, m, H1, H2), 4.94–4.87 (2H, m, H7, NH), 4.78 (1H, t, *J* = 5.5 Hz, H10), 3.70 (3H, s, H12), 2.32–2.26 (1H, m, H8), 2.19–2.13 (1H, m, H9), 1.87–1.80 (1H, m, H9'), 1.78–1.72 (1H, m, H8'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 156.7 (C11), 139.3 (C4), 136.8 (C5), 128.3 (C6), 128.3 (C1), 128.2 (C3), 128.1 (C2), 67.9 (C10), 52.2 (C12), 49.2 (C7), 29.7 (C9), 26.9 (C8); **HRMS** (ES^+) calc. for $C_{12}H_{15}NNaO_3$ $[M+Na]^+$ 244.0944, found 244.0944. **233**: $\mathbf{R_f}$ 0.25 (40% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3322

(w), 2952 (w), 1687 (s), 1531 (m), 1250 (m), 771 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.05 (1H, dd, *J* = 8.0, 1.0 Hz, H6), 7.58 (1H, td, *J* = 8.0, 1.0 Hz, H2), 7.47 (1H, d, *J* = 8.0 Hz, H3), 7.42 (1H, t, *J* = 8.0 Hz, H1), 5.14–5.10 (1H, m, H7), 4.95 (1H, d, *J* = 6.5 Hz, NH), 3.76 (3H, s, H12), 2.83 (1H, ddd, *J* = 17.0, 6.5, 4.5 Hz, H9), 2.69 (1H, ddd, *J* = 17.0, 11.0, 4.5 Hz, H9'), 2.46–2.40 (1H, m, H8), 2.18–2.10 (1H, m, H8'); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 196.8 (C10), 156.7 (C11), 143.4 (C4), 134.2 (C2), 128.3 (C1), 127.5 (C6), 127.1 (C3), 52.5 (C12), 49.5 (C7), 36.4 (C9), 30.3 (C8); **HRMS** (ES^+) calc. for $C_{12}H_{13}NNaO_3$ $[M+Na]^+$ 242.0788, found 242.0789.

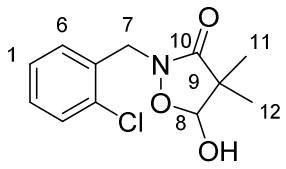
Di-*tert*-butyl 3,3'-dioxo-[2,2'-biindoline]-1,1'-dicarboxylate 238



To a solution of *tert*-butyl 1*H*-indole-1-carboxylate **204** (1.15 mL of a 100 mM solution in isopropanol, 0.12 mmol), glucose (1.90 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.90 mmol), GDH (290 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 580 U), and $P450_{BM3}$ mutant RP/FV (3.80 mL of a 30 µM solution in phosphate buffer, pH 7.5, 0.11 µmol) in 100 mM phosphate buffer (pH 7.5, 51 mL) was added $NADP^+$ monosodium salt (290 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 1.16 µmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a red oil. The crude product was purified by flash chromatography (10% ether / petrol to 30% ether / petrol) to afford the *title compound* as a purple oil (5 mg, 18%). **R_f** 0.60 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3429 (w), 1715 (s), 1609 (w), 1470 (m), 1369 (m), 1295 (m), 1256 (m), 1158 (m), 1093 (w), 1018 (w), 756 (w); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.74 (2H, d, *J* = 7.5 Hz, H6), 7.64 (2H, t, *J* = 7.5 Hz,

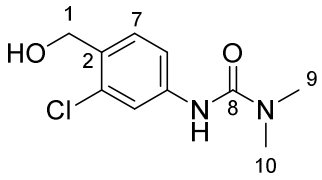
H2), 7.15 (2H, t, *J* = 7.5 Hz, H1), 5.45 (2H, s, H7), 1.64 (18H, s, H11, H12, H13); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 194.1 (C8), 151.8 (C9), 151.6 (C4), 138.0 (C2), 124.9 (C6), 123.5 (C1), 121.9 (C5), 116.3 (C3), 83.9 (C10), 81.0 (C7), 28.4 (C11, C12, C13); **HRMS** (ES^+) calc. for $C_{26}H_{28}N_2NaO_6$ $[M+Na]^+$ 487.1840, found 487.1839.

2-(2-Chlorobenzyl)-5-hydroxy-4,4-dimethylisoxazolidin-3-one 247



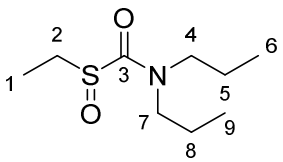
To a solution of 2-(2-chlorobenzyl)-4,4-dimethyloxazolidin-3-one **219** (420 µL of a 0.10 M solution in methanol, 0.042 mmol, 1.0 eq), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 2.10 mmol), GDH (110 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 220 U), $NADP^+$ monosodium salt (110 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.44 µmol), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500 U) in 100 mM phosphate buffer (pH 7.5, 15 mL) was added $P450_{BM3}$ mutant KSK19/IA (540 µL of a 39 µM solution in phosphate buffer, pH 7.5, 0.021 µmol). The solution was stirred open to air for 17 h. The solution was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (5% ethyl acetate / petrol to 30% ethyl acetate / petrol) to afford the *title compound* as a white solid (3 mg, 32%). **R_f** 0.40 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3302 (m), 2924 (w), 1675 (s), 1469 (m), 1443 (m), 1105 (m), 915 (w), 689 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.40–7.36 (2H, m, H3, H6), 7.27–7.24 (2H, m, H1, H2), 5.26 (1H, s, H8), 4.92 (1H, d, *J* = 16.5 Hz, H7), 4.85 (1H, d, *J* = 16.5 Hz, H7'), 3.14 (1H, br s, OH), 1.29 (3H, s, H11), 1.25 (3H, s, H12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 171.8 (10), 132.3 (C5), 131.7 (C4), 128.5 (C6), 128.4 (C3), 128.1 (C2), 126.0 (C1), 101.7 (C8), 45.6 (C9), 45.5 (C7), 21.2 (C11), 15.5 (C12); **HRMS** (ES^+) calc. for $C_{12}H_{15}O_3N^{35}Cl$ $[M+H]^+$ 256.0735, found 256.0734.

3-[3-Chloro-4-(hydroxymethyl)phenyl]-1,1-dimethylurea 248



To a solution of 3-(3-chloro-4-methylphenyl)-1,1-dimethylurea **220** (470 µL of a 0.10 M solution in ethanol, 0.047 mmol, 1.0 eq), glucose (2.4 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 2.40 mmol), GDH (120 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 240 U), $NADP^+$ monosodium salt (120 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.48 µmol), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500 U) in 100 mM phosphate buffer (pH 7.5, 15 mL) was added $P450_{BM3}$ mutant RP/FV/EV (2.4 mL of a 20 µM solution in phosphate buffer, pH 7.5, 0.048 µmol). The solution was stirred open to air for 24 h. The solution was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ethyl acetate / petrol to 80% ethyl acetate / petrol) to afford the *title compound* as a colourless oil (3 mg, 28%). $\mathbf{R_f}$ 0.25 (80% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3320 (m), 2917 (w), 1649 (s), 1588 (m), 1522 (s), 1397 (m), 1241 (w), 1185 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.56 (1H, d, *J* = 2.5 Hz, H4), 7.34 (1H, d, *J* = 8.5 Hz, H7), 7.22 (1H, dd, *J* = 8.5, 2.5 Hz, H6), 6.33 (1H, br s, NH), 4.71 (2H, d, *J* = 6.5 Hz, H1), 3.04 (6H, s, H9, H10), 1.95 (1H, t, *J* = 6.5 Hz, OH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 155.3 (C8), 139.7 (C3), 133.2 (C2), 132.5 (C5), 129.2 (C7), 120.6 (C4), 118.0 (C6), 62.7 (C1), 36.5 (C9, C10); **HRMS** (ES^+) calc. for $C_{10}H_{14}N_2O_2{}^{35}Cl$ $[M+H]^+$ 229.0738, found 229.0736.

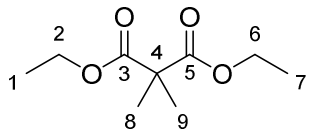
1-(Ethylsulfinyl)-*N*,*N*-dipropylmethanamide 253



To a solution of *S*-ethyl dipropylcarbamothioate **221** (1.1 mL of a 100 mM solution in ethanol, 0.11 mmol, 1.0 eq), glucose (5.5 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 5.50 mmol), GDH

(275 µL of a 2.0 U/µL solution in phosphate buffer, pH 7.5, 550 U), $NADP^+$ monosodium salt (275 µL of a 4.0 mM solution in phosphate buffer, pH 7.5, 1.10 µmol), and catalase (from *micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500 U) in 100 mM phosphate buffer (pH 7.5, 42 mL) was added $P450_{BM3}$ mutant KSK19/AI (2.8 mL of a 20 µM solution in phosphate buffer, pH 7.5, 0.055 µmol). The reaction was stirred open to air for 20 h. Methanol was added and the solution was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (20% ethyl acetate / petrol to 40% ethyl acetate / petrol) to afford **253** as a colourless oil (8 mg, 34%). $\mathbf{R_f}$ 0.20 (80% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2967 (m), 2936 (m), 2877 (w), 1695 (s), 1416 (m), 1067 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 3.55 (1H, ddd, *J* = 14.5, 9.0, 6.0 Hz, H7), 3.46–3.36 (2H, m, H7', H4), 3.29 (1H, ddd, *J* = 13.5, 8.5, 6.5 Hz, H4'), 3.03–2.94 (2H, m, H2), 1.75–1.55 (4H, m, H5, H8), 1.36 (3H, t, *J* = 7.5 Hz, H1), 0.93 (6H, dd, *J* = 8.5, 7.5 Hz, H6, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 167.9 (C3), 49.9 (C5), 47.8 (C4), 45.3 (C2), 22.5 (C8), 20.6 (C7), 11.2 (C6), 10.9 (C9), 6.9 (C1); **LRMS** (ES^+) calc. for $C_9H_{19}NNaO_2S$ $[M+Na]^+$ 228.3, found 228.1. Data is consistent with literature values.[479]

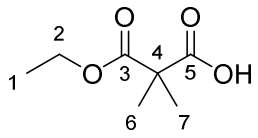
Diethyl 2,2-dimethylmalonate 285



Based on a literature procedure.[480] A solution of NaH (60% in mineral oil, 1.10 g, 26.3 mmol, 2.1 eq) in tetrahydrofuran (12.5 mL) was cooled to 0 °C and diethyl malonate (1.9 mL, 12.5 mmol, 1.0 eq) in tetrahydrofuran (5 mL) was added. The solution was warmed to RT and stirred for 30 minutes. The solution was cooled to 0 °C and iodomethane (1.7 mL, 27.5 mmol, 2.2 eq) was added. The solution was heated at reflux for 18 h then poured into ice-water. The mixture was extracted with ethyl acetate and the combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **285** as a yellow oil (1.91 g, 81%). $\mathbf{R_f}$ 0.65 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2984 (w), 2928 (w), 1730 (s), 1276

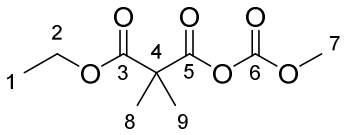
(m), 1179 (m), 1133 (m), 910 (m), 733 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 4.15 (4H, q, *J* = 7.0 Hz, H2, H6), 1.40 (6H, s, H8, H9), 1.22 (6H, t, *J* = 7.0 Hz, H1, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.9 (C3, C5), 61.2 (C2, C6), 50.0 (C4), 22.7 (C8, C9), 14.0 (C1, C7); **LRMS** (ES^+) calc. for $C_9H_{17}O_4$ $[M+H]^+$ 189.1, found 189.1. Data consistent with literature values.[480]

3-Ethoxy-2,2-dimethyl-3-oxopropanoic acid 286



Based on a literature procedure.[481] KOH (328 mg, 5.8 mmol, 1.0 eq) was added to a solution of diethyl 2,2-dimethylmalonate **285** (1.10 g, 5.8 mmol, 1.0 eq) in ethanol (5.8 mL). The solution was stirred for 17 h then concentrated *in vacuo*. Water was added to the residue and the solution was extracted with ether. The pH of the aqueous layer was adjusted to 4 with aqueous HCl (6.0 M) and the solution was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **286** as a yellow oil (622 mg, 67%). **R_f** 0.40 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3100 (br, w), 1712 (s), 1471 (w), 1269 (m), 1143 (m), 909 (m), 731 (s); **^{1}H NMR** (200 MHz, $CDCl_3$) δ_H 4.21 (2H, q, *J* = 7.0 Hz, H2), 1.47 (6H, s, H6, H7), 1.27 (3H, t, *J* = 7.0 Hz, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 178.9 (C5), 172.6 (C3), 61.7 (C2), 49.8 (C4), 22.7 (C6, C7), 14.0 (C1); **LRMS** (ES^+) calc. for $C_7H_{13}O_4$ $[M+H]^+$ 161.1, found 161.1. Data is consistent with literature values.[481]

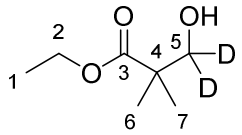
3-Ethoxy-2,2-dimethyl-3-oxopropanoic (methyl carbonic) anhydride 287



Based on a modified literature procedure.[482] A solution of 3-ethoxy-2,2-dimethyl-3-oxopropanoic acid **286** (300 mg, 1.9 mmol, 1.0 eq) in tetrahydrofuran (19 mL) was cooled to 0 °C and triethylamine (0.29 mL, 2.1 mmol, 1.1 eq) and methyl chloroformate (0.22 mL, 2.8 mmol, 1.5 eq) were added. The solution was stirred at 0 °C for 4 h and saturated aqueous NH_4Cl was added. The

solution was extracted with ether and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the *title compound* as a yellow oil (378 mg, 93%). **R_f** 0.70 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2988 (w), 1826 (m), 1735 (s), 1470 (w), 1389 (w), 1263 (m), 1154 (m), 1004 (s), 859 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 4.21 (2H, q, *J* = 7.0 Hz, H2), 3.89 (3H, s, H7), 1.49 (6H, s, H8, H9), 1.25 (3H, t, *J* = 7.0 Hz, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 171.3 (C3), 167.1 (C5), 149.3 (C6), 61.9 (C2), 56.0 (C7), 50.4 (C4), 22.3 (C8, C9), 13.9 (C1); **HRMS** (ES^+) calc. for $C_9H_{14}NaO_6$ [M+Na]+ 241.0683, found 241.0683.

Ethyl 3-hydroxy-2,2-dimethylpropanoate-3,3-d_2 288

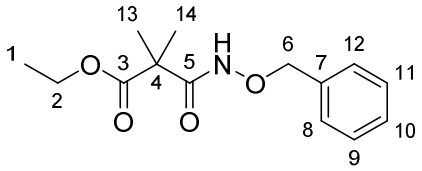


Based on a modified literature procedure.[483] A solution of 3-ethoxy-2,2-dimethyl-3-oxopropanoic (methyl carbonic) anhydride **287** (1.39 g, 6.4 mmol, 1.0 eq) in ethanol (25 mL) was cooled to 0 °C and $NaBD_4$ (587 mg, 14.0 mmol, 2.2 eq) was added portionwise. The reaction was warmed to RT and stirred for 45 minutes. The reaction was quenched with aqueous HCl (3.0 M) and the solution was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ether / petrol to 40% ether / petrol) to afford **288** as a yellow oil (29 mg, 3%).

Based on a modified literature procedure.[484] 2-Bromoisobutyrate (2.8 mL, 18.8 mmol, 1.5 eq) was added to a solution of In (1.4 g, 12.5 mmol, 1.0 eq) in tetrahydrofuran (25 mL) and the solution was sonicated for 20 minutes. Paraformaldehyde-d_2 (400 mg, 12.5 mmol, 1.0 eq) was added and the solution was sonicated for a further 23 h. The solution was quenched with saturated aqueous $NaHCO_3$ and extracted with ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (30% ether / petrol) to afford **288** as a colourless oil (1.11 g, 60%). **R_f** 0.40 (50% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3467 (br, m), 2978 (w), 1714 (s), 1281 (s), 1174 (s),

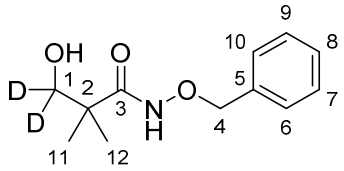
1153 (s), 1106 (s), 1077 (m), 1026 (w), 979 (w), 915 (w), 731 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 4.13 (2H, q, *J* = 7.0 Hz, H2), 2.51 (1H, br s, OH), 1.24 (3H, t, *J* = 7.0 Hz, H1), 1.16 (6H, s, H6, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 177.7 (C3), 69.5–68.7 (m, C5), 60.7 (C2), 43.9 (C4), 22.0 (C6, C7), 14.1 (C1); **LRMS** (ES^+) calc. for $C_7H_{12}{}^2H_2NaO_3$ $[M+H]^+$ 171.1, found 171.1.

Ethyl 3-[(benzyloxy)amino]-2,2-dimethyl-3-oxopropanoate 289



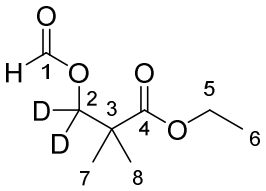
Based on a modified literature procedure.[421] Isobutyl chloroformate (0.82 mL, 6.3 mmol, 1.0 eq) was added dropwise to a solution of 3-ethoxy-2,2-dimethyl-3-oxopropanoic acid **286** (1.00 g, 6.3 mmol, 1.0 eq) and triethylamine (1.9 mL, 13.9 mmol, 2.2 eq) in tetrahydrofuran (12.6 mL) at 0 °C. The solution was stirred for 30 minutes at 0 °C and *O*-benzylhydroxylamine hydrochloride (1.20 g, 7.6 mmol, 1.2 eq) was added in one portion. The solution was allowed to warm to RT overnight and water was added. The solution was extracted with ethyl acetate and the combined organic layers were washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (15% ether / petrol to 60% ether / petrol) to afford the *title compound* as a white solid (1.10 g, 67%). **R_f** 0.35 (30% ethyl acetate / petrol); **mp** 30 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3240 (br, w), 2984 (w), 1732 (m), 1661 (m), 1470 (w), 1261 (m), 1147 (s), 1026 (m), 911 (m), 731 (s), 698 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 9.13 (1H, br s, NH), 7.39–7.32 (5H, m, H8–H12), 4.88 (2H, s, H6), 4.11 (2H, q, *J* = 7.0 Hz, H2), 1.41 (6H, s, H13, H14), 1.21 (3H, t, *J* = 7.0 Hz, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.2 (C3), 169.9 (C5), 135.1 (C7), 129.3 (C9, C11), 128.8 (C10), 128.6 (C8, C12), 78.0 (C6), 61.8 (C2), 49.1 (C5), 23.6 (C13, C14), 13.9 (C1); **HRMS** (ES^+) calc. for $C_{14}H_{19}NNaO_4$ $[M+Na]^+$ 288.1206, found 288.1205.

***N*-(Benzyloxy)-3-hydroxy-2,2-dimethylpropanamide-3,3-d_2 290**



Based on a modified literature procedure.[483] A solution of ethyl 3-[(benzyloxy)amino]-2,2-dimethyl-3-oxopropanoate **289** (810 mg, 3.0 mmol, 1.0 eq) in ethanol (12.1 mL) was cooled to 0 °C and $NaBD_4$ (278 mg, 6.6 mmol, 2.2 eq) was added portionwise. The solution was stirred for 2 h then quenched with aqueous HCl (1.0 M). The solution was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (10% ethyl acetate / petrol to 60% ethyl acetate / petrol) to afford the *title compound* as a white solid [430 mg, 63% (81% brsm)]. **R_f** 0.15 (50% ethyl acetate / petrol); **mp** 54 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3259 (br, m), 2933 (w), 1652 (s), 1470 (m), 1039 (w), 748 (m), 699 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.73 (1H, br s, NH), 7.41–7.36 (5H, m, H6–H10), 4.92 (2H, s, H4), 1.55 (1H, s, H1), 114 (6H, s, H11, H12); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 175.4 (C3), 135.2 (C5), 129.4 (C7, C9), 128.8 (C8), 128.6 (C6, C10), 78.0 (C4), 42.5 (C2), 22.3 (C11, C12); **HRMS** (ES^-) calc. for $C_{12}H_{14}{}^2H_2NO_3$ $[M–H]^-$ 224.1261, found 224.1258.

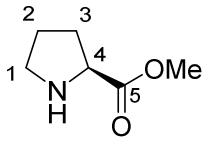
Ethyl 3-(formyloxy)-2,2-dimethylpropanoate-3,3-d_2 295



Based on a modified literature procedure.[485] A solution of oxalyl chloride (0.45 mL, 5.3 mmol, 2.1 eq) in DCM (12.7 mL) was cooled to 0 °C and dimethylformamide (0.43 mL, 5.6 mmol, 2.2 eq) was added dropwise. The solution was stirred at RT for 10 minutes then cooled to 0 °C and ethyl 3-hydroxy-2,2-dimethylpropanoate-3,3-d_2 **288** (376 mg, 2.5 mmol, 1.0 eq) in DCM (13 mL) was added. The solution was heated at reflux for 15 h then cooled to RT. The solution was washed with saturated aqueous $NaHCO_3$ and the combined aqueous layers were extracted with DCM. The

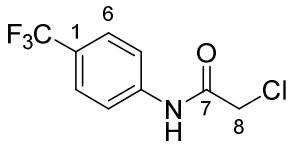
combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether / petrol to 25% ether / petrol) to afford the *title compound* as a yellow oil (248 mg, 55%). **R_f** 0.50 (40% ether / petrol); **IR** (thin film) ν_{max} / cm^{-1} 2932 (w), 1597 (m), 1494 (m), 1235 (m), 1220 (m), 1131 (w), 1025 (w), 916 (w), 753 (s), 691 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.06 (1H, s, H1), 4.15 (2H, q, *J* = 7.0 Hz, H5), 1.25 (3H, t, *J* = 7.0 Hz, H6), 1.23 (6H, s, H7, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 175.3 (C4), 160.7 (C1), 60.9 (C5), 42.2 (C3), 22.2 (C7, C8), 14.1 (C6); **HRMS** (CI^+) calc. for $C_8H_{16}{}^2H_2NO_4$ $[M+NH_4]^+$ 194.1356, found 194.1360.

Methyl-L-prolinate 297



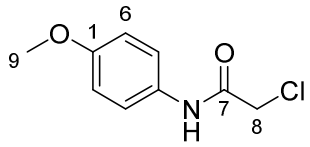
Based on a literature procedure.[486] To a solution of L-proline (3.0 g, 26.1 mmol, 1.0 eq) in methanol (19 mL) at 0 °C was added thionyl chloride (2.1 mL, 28.9 mmol, 1.1 eq) dropwise over 5 minutes. The solution was stirred for 3 h. The solution was concentrated *in vacuo*. The residue was redissolved in DCM and the solution was neutralised with 50% w/w aqueous NaOH. The organic layer was separated then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **297** as a colourless oil (1.32 g, 39%). **R_f** 0.10 (70% ethyl acetate / petrol); **IR** (thin film) ν_{max} / cm^{-1} 3353 (w), 2954 (w), 2876 (w), 1734 (s), 1663 (w), 1438 (w), 1214 (m), 1176 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 3.76 (1H, dd, *J* = 9.0 Hz, 5.5 Hz, H4), 3.71 (3H, s, OCH_3), 3.06 (1H, dt, *J* = 10.0, 6.5 Hz, H1), 2.90 (1H, dt, *J* = 10.0, 6.5 Hz, H1'), 2.33 (1H, br s, NH), 2.16–2.07 (1H, m, H3), 1.88–1.80 (1H, m, H3'), 1.78–1.70 (2H, m, H2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 175.9 (C5), 60.0 (C4), 52.1 (OCH_3), 47.0 (C1), 30.2 (C3), 25.5 (C2); **LRMS** (ES^+) calc. for $C_6H_{12}NO_2$ $[M+H]^+$ 130.1, found 130.1.

4-Trifluoromethylphenyl-2-chloroacetamide 298



Based on a modified literature procedure.[415] To a solution of 4-trifluoromethylaniline hydrochloride (500 mg, 2.5 mmol, 1.0 eq) in acetic acid (2.5 mL) was added chloroacetyl chloride (0.23 mL, 2.8 mmol, 1.1 eq) dropwise. The solution was stirred for 1 h and NaOAc (410 mg, 5.0 eq, 2.0 eq) in water (2.4 mL) was added slowly. The solution was stirred for 45 minutes and water (2.9 mL) was added. The solution was stirred for 1 h. The solution was filtered and the solid was washed with water and dried *in vacuo* to afford **298** as a white solid (481 mg, 81%). $\mathbf{R_f}$ 0.70 (60% ethyl acetate / petrol); **mp** 143–145 °C (amorphous) (lit. mp 146–148 °C); **IR** (thin film) ν_{max} / cm^{-1} 3280 (w), 3141 (w), 1613 (s), 1556 (s), 1403 (m), 1319 (s), 1114 (s), 1066 (s), 843 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.37 (1H, br s, NH), 7.70 (2H, d, *J* = 8.5 Hz, H3, H5), 7.62 (2H, d, *J* = 8.5 Hz, H2, H6), 4.21 (2H, s, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 164.1 (C7), 139.7 (C4), 127.3 (q, *J* = 33.0 Hz, C1), 126.4 (q, *J* = 4.0 Hz, C2, C6), 124.0 (q, *J* = 271.0 Hz, CF_3), 119.7 (C3, C5), 42.8 (C8); **LRMS** (ES^+) calc. for $C_9H_6{}^{35}ClF_3NO$ $[M–H]^–$ 236.0, found 236.0. Data consistent with literature values.[487]

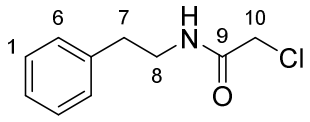
4-Methoxyphenyl-2-chloroacetamide 299



Based on a modified literature procedure.[415] To a solution of *p*-anisidine (500 mg, 4.1 mmol, 1.0 eq) in acetic acid (4.1 mL) was added chloroacetyl chloride (0.36 mL, 4.6 mmol, 1.1 eq) dropwise. The solution was stirred for 1 h and NaOAc (673 mg, 8.2 mmol, 2.0 eq) in water (3.9 mL) was added slowly. The solution was stirred for 45 minutes and water (4.8 mL) was added. The solution was stirred for 1 h. The solution was filtered and the solid was washed with water and dried *in vacuo* to afford **299** as a white solid (639 mg, 78%). $\mathbf{R_f}$ 0.60 (60% ethyl acetate / petrol); **mp** 102–104 °C (amorphous) (lit. mp 118–120 °C); **IR** (thin film) ν_{max} / cm^{-1} 3293 (w), 3010 (w), 1663 (s), 1541 (m),

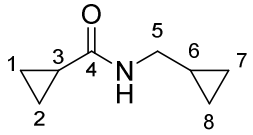
1180 (m), 1031 (m), 831 (m), 716 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.18 (1H, br s, NH), 7.45–7.42 (2H, m, H3, H5), 6.90–6.87 (2H, m, H2, H6), 4.17 (2H, s, H8), 3.79 (3H, s, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 163.8 (C7), 157.1 (C1), 129.7 (C4), 122.1 (C3, C5), 114.3 (C2, C6), 55.5 (C9), 42.9 (C8); **LRMS** (ES^+) calc. for $C_9H_{11}{}^{35}ClNO_2$ $[M+H]^+$ 200.0, found 200.1. Data consistent with literature values.[488]

Phenethyl-2-chloroacetamide 300



Based on a literature procedure.[489] To a solution of 2-phenethylamine (0.78 mL, 6.2 mmol, 1.0 eq) and $NaHCO_3$ (521 mg, 6.2 mmol, 1.0 eq) in DCM (10 mL) at 0 °C was added chloroacetyl chloride (0.59 mL, 7.4 mmol, 1.2 eq) dropwise. The solution was warmed to RT and stirred for 3 h. Water was added and the mixture was extracted with DCM. The combined organic layers were washed with aqueous HCl (10% v/v), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **300** as an off-white solid (952 mg, 78%). **R_f** 0.20 (70% ethyl acetate / petrol); **mp** 58–60 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3293 (w), 1656 (s), 1536 (m), 909 (w), 730 (s), 699 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.34 (2H, m, H4, H6), 7.30 (3H, m, H1, H2, H3), 6.70 (1H, br s, NH), 4.04 (2H, s, H9), 3.59 (2H, 2H, q, *J* = 6.5 Hz, H8), 2.88 (2H, t, *J* = 7.0 Hz, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 165.9 (C9), 138.4 (C5), 128.7 (C1, C3, C4, C6), 126.7 (C2), 42.7 (C10), 41.0 (C8), 35.5 (C7); **LRMS** (ES^+) calc. for $C_{10}H_{13}{}^{35}ClNO$ $[M+H]^+$ 198.1, found 198.1. Data is consistent with literature values.[489]

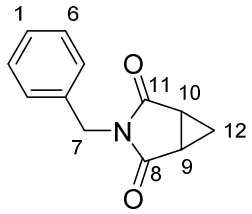
***N*-(Cyclopropylmethyl)cyclopropanecarboxamide 301**



Based on a literature procedure.[490] To a solution of (cyclopropylmethyl)amine (0.91 mL, 10.5 mmol, 1.0 eq) and triethylamine (2.9 mL, 21.0 mmol, 2.0 eq) in tetrahydrofuran (21 mL) at 0 °C was added cyclopropanecarbonyl chloride (1.4 mL, 15.8 mmol, 1.5 eq) dropwise. The solution was stirred for 3 h. Ethyl acetate and saturated aqueous $NaHCO_3$ was added and the mixture was extracted with

ethyl acetate. The combined organic layers were washed with saturated aqueous NH_4Cl, water, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **301** as an orange solid (1.20 g, 84%). $\mathbf{R_f}$ 0.45 (60% ethyl acetate / petrol); **mp** 50 °C (amorphous) (lit. mp 56–58 °C); **IR** (thin film) ν_{max} / cm^{-1} 3289 (m), 3082 (w), 3009 (w), 1641 (s), 1547 (s), 1243 (m), 946 (m), 826 (w), 686 (w); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 5.72 (1H, br s, NH), 3.13 (2H, dd, *J* = 7.5, 5.5 Hz, H5), 1.33 (1H, tt, *J* = 8.0, 4.5 Hz, H3), 1.00–0.93 (3H, m, H1, H2, H6), 0.72 (2H, dt, *J* = 7.5, 4.0 Hz, H1', H2'), 0.53–0.49 (2H, m, H7, H8), 0.20 (2H, dt, *J* = 6.0, 4.5 Hz, H7', H8'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.4 (C4), 44.6 (C5), 14.7 (C3), 10.8 (C6), 7.0 (C1, C2), 2.4 (C7, C8); **LRMS** (ES^+) calc. for $C_8H_{14}NO$ $[M+H]^+$ 140.1, found 140.1. Data consistent with literature values.[491]

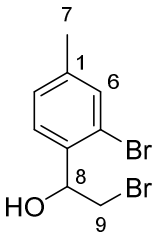
3-Benzyl-3-azabicyclo[3.1.0]hexane-2,4-dione 302



Based on a literature procedure.[427] 4-(Dimethylamino)pyridine (55 mg, 0.45 mmol, 0.10 eq) and benzylamine (0.49 mL, 4.5 mmol, 1.0 eq) were added to a solution of 3-oxabicyclo[3.1.0]hexane-2,4-dione (500 mg, 4.5 mmol, 1.0 eq) in acetic acid (6.7 mL) and the solution was stirred at 100 °C for 2 d then cooled to RT and concentrated *in vacuo*. The residue was redissolved in ethyl acetate and the solution was washed with saturated aqueous $NaHCO_3$, saturated aqueous NH_4Cl, and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **302** as an off-white solid (669 mg, 74%). $\mathbf{R_f}$ 0.15 (50% ether / petrol); **mp** 72–74 °C (amorphous) (lit. mp 90–91 °C); **IR** (thin film) ν_{max} / cm^{-1} 1772 (w), 1699 (s), 1392 (m), 1346 (w), 1170 (w), 928 (w), 719 (w), 697 (w), 630 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.30–7.26 (5H, m, H1, H2, H3, H5, H6), 4.50 (2H, s, H7), 2.47 (2H, dd, *J* = 8.0, 3.5 Hz, H9, H10), 1.49 (1H, td, *J* = 8.0, 4.5 Hz, H12), 1.30 (1H, td, *J* = 4.5, 3.5 Hz, H12'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.8 (C8, C11), 136.0 (C4), 128.7 (C2, C6), 128.5 (C3, C5), 127.9 (C1), 41.6 (C7), 20.5 (C11), 20.2

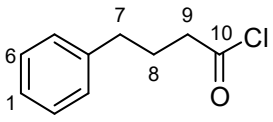
(C9, C10); **LRMS** (ES^+) calc. for $C_{12}H_{11}NNaO_2$ $[M+Na]^+$ 224.1, found 224.0. Data is consistent with literature values.[427]

2-Bromo-1-(2-bromo-4-methylphenyl)ethan-1-ol 303



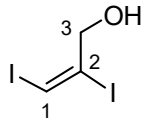
Based on a modified literature procedure.[492] *N*,*N*-Dibromo-4-methylbenzenesulfonamide (3.34 g, 10.2 mmol, 2.2 eq) was added to a solution of 2-bromo-4-methyl-1-vinylbenzene **162** (910 mg, 4.6 mmol, 1.0 eq) in acetone/water (14.5 mL, 30:1). The solution was stirred for 1 h and $Na_2S_2O_5$ was added and the solution was stirred for a further 10 minutes. Water was added and the solution was extracted with ethyl acetate. The combined organic layers were washed with aqueous NaOH (1.0 M) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to a brown oil. The crude mixture was purified by flash chromatography (1% ether / petrol) to afford the *title compound* as a white solid (339 mg, 25%). $\mathbf{R_f}$ 0.15 (16% diethyl ether / petroleum ether); **mp** 52 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3390 (br, m), 1606 (w), 1487 (w), 1418 (w), 1219 (w), 1070 (s), 1038 (s), 908 (m), 825 (s), 782 (w), 731 (s), 674 (m), 642 (s); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.49 (1H, d, *J* = 8.0 Hz, H3), 7.37 (1H, s, H6), 7.18–7.16 (1H, m, H2), 5.23 (1H, ddd, *J* = 9.0, 3.5, 3.0 Hz, H8), 3.78 (1H, dd, *J* = 10.5, 3.0 Hz, H9), 3.41 (1H, dd, *J* = 10.5, 9.0 Hz, H9'), 2.71 (1H, d, *J* = 3.5 Hz, OH), 2.33 (3H, s, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 140.1 (C4), 136.2 (C1), 133.3 (C6), 128.7 (C2), 1273 (C2), 121.7 (C5), 72.5 (C8), 38.9 (C9), 20.8 (C7); **HRMS** (Cl^+) calc. for $C_9H_{14}{}^{79}Br_2NO$ $[M+NH_4]^+$ 309.9437, found 309.9430.

4-Phenylbutanoyl chloride 304



Based on a literature procedure.[493] To a solution of 4-phenylbutanoic acid (1.0 g, 6.1 mmol, 1.0 eq) in DCM (15 mL) was added oxalyl chloride (2.2 mL, 25.6 mmol, 4.2 eq). The solution was stirred until gas evolution ceased then concentrated *in vacuo* to afford **304** as a yellow oil (1.07 g, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.35 (2H, m, H2, H6), 7.30–7.28 (1H, m, H1), 7.24–7.22 (2H, m, H3, H5), 2.94 (2H, t, *J* = 7.5 Hz, H9), 2.74 (2H, t, *J* = 7.5 Hz, H7), 2.09 (2H, quin, *J* = 7.5 Hz, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.7 (C10), 140.4 (C4), 128.6 (C2, C6), 128.5 (C3, C5), 126.4 (C1), 46.3 (C9), 34.3 (C7), 26.6 (C8); **LRMS** not found. Data consistent with literature values.[493]

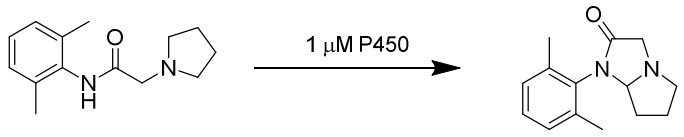
(*E*)-2,3-Diiodoprop-2-en-1-ol 305



Based on a literature procedure.[494] To a solution of propargyl alcohol (1.0 g, 17.8 mmol, 1.0 eq) in tetrahydrofuran (17 mL) was added H_2O_2 (7.7 mL of a 35% w/w aqueous solution, 89.0 mmol, 5.0 eq) and I_2 (4.5 g, 17.8 mmol, 1.0 eq). The solution was stirred for 17 h and ether was added. The layers were separated and the organic layer was washed with saturated aqueous $Na_2S_2O_3$ and water then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **305** as an off-white solid (3.96 g, 72%). **R_f** 0.55 (30% ethyl acetate / petrol); **mp** 39–41 °C (amorphous); **IR** (thin film) ν_{max} / cm^{-1} 3298 (s), 3062 (m), 2909 (m), 1442 (w), 1212 (s), 1041 (s), 1008 (s), 952 (m), 780 (m); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.04 (1H, s, H1), 4.28 (2H, d, *J* = 1.0 Hz, H3), 2.15 (1H, br s, OH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_c 104.1 (C2), 79.9 (C1), 70.8 (C3); **LRMS** (ES^+) calc. for $C_3H_4{}^{127}I_2NaO$ $[M+Na]^+$ 332.8, found 332.1. Data is consistent with literature values.[494]

Summary of Screening Data

Table S1: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-pyrrolidin-1-ylacetamide **10**



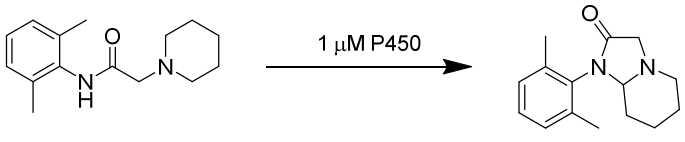
10
2 mM

25

No.	Enzyme	Conversion	TTN[a]
1	RP/FV/EV	99%	1980
2	KSK19/I263A	35%	700
3	RP/FV/EV/A184I	25%	500
4	KSK19/QP	24%	480
5	RT2/IP	19%	380
6	KSK19	16%	320
7	RP/IA/EV	12%	240
8	RT2/AP/A184I	4%	80
9	RP/E267V	3%	60
10	KSK19/AI/A184I	3%	60
11	WT	3%	60
12	RT2/AP/A82M	2%	40
13	RP/FV	2%	40
14	RT2/AP/V78I	2%	40
15	KSK19/AI/Y51L	2%	40
16	RT2/AP	2%	40
17	RT2/I401P/A330P	1%	20
18	RP/AP	1%	20
19	RT2/A330W	1%	20
20	KSK19/AI/F42L	1%	20
21	RP/A330P/A82M	1%	20
22	KSK19/AI/V78I	1%	20
23	RLYF/KSK19/AI	1%	20
24	KSK19/AI	1%	20
25	RT2	1%	20
26	KSK19/A82M	1%	20
27	RP/F81W	1%	20

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S2: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-piperidin-1-ylacetamide **11**



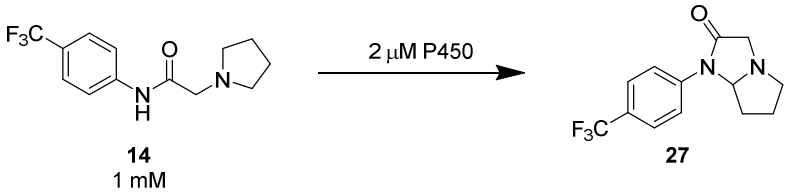
11
2 mM

26

No.	Enzyme	Conversion	TTN[a]
1	RT2/AP/A184I	94%	1880
2	RP/FV/EV	77%	1540
3	RT2/IP	32%	640
4	RP/FV/EV/A184I	27%	540
5	RP/IA/EV	24%	480
6	RP/AP	22%	440
7	RP/EV	9%	180
8	RP/FV/EV/L437LV	9%	180
9	KSK19/AI/AI	8%	160
10	RP	8%	160
11	KSK19/IA	8%	160
12	RT2AP/V78I	7%	140
13	RP/FV	6%	120
14	KSK19/AI/FV	6%	120
15	RP/FV/EV/L437VL	4%	80
16	KSK19/AI/Y51L	4%	80
17	RT2/AP/AM	3%	60
18	KSK19/A82M	3%	60
19	RT2	3%	60
20	KSK19/AI	2%	40
21	KSK19/AI/F42L	2%	40
22	RLYF/KSK19/AI	2%	40
23	RT2/AP/IP	2%	40
24	RT2/AP	2%	40
25	RP/AP/AM	1%	20
26	KSK19/AI/VI	1%	20
27	WT	1%	20

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S3: Substrate conversion for the initial screen of the oxidation of 4-trifluoromethylphenyl-2-pyrrolidin-1-ylacetamide **14**



14
1 mM

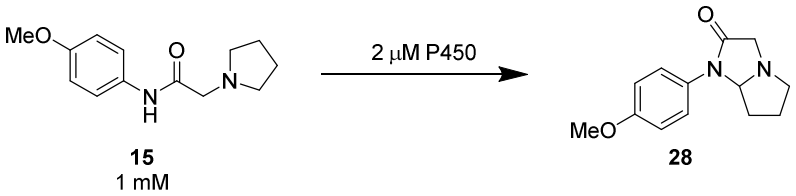
27

No.	Enzyme	Conversion	Cyclisation Product (27)	Other Products	TTN[a]
1	RP/FV/EV/V78F	99%	44%	55%	495
2	RP/FV/EV/L188Q	97%	60%	37%	485
3	RT2/F81W	90%	69%	21%	450
4	RP/FV/EV	87%	60%	27%	435
5	KSK19/A82M/E267F	84%	70%	14%	420
6	KSK19/A82M	72%	55%	17%	360
7	RT2/IP/F87V	71%	23%	48%	355
8	RP/H171L	71%	38%	33%	355
9	RP/IA/EV	66%	32%	34%	330
10	RP/FV/EV/F81W	66%	53%	13%	330
11	RP	65%	42%	23%	325
12	KSK19/A82M/I401M	64%	53%	11%	320
13	RP/FV	63%	32%	31%	315
14	RP/E267M	59%	28%	31%	295
15	RP/FV/EV/L437VL	58%	37%	21%	290
16	RP/F81W	57%	30%	27%	285
17	RP/EV	47%	28%	19%	235
18	RP/E267I	46%	30%	16%	230
19	RP/FV/EV/L437LV	44%	32%	12%	220
20	KSK19/QP/F87V	44%	21%	23%	220
21	RT2/IP	43%	23%	20%	215
22	RT2/AP/PV	41%	36%	5%	205
23	RT2/I263W	41%	35%	6%	205
24	RT2	39%	31%	7%	195
25	RT2/IP/A184I	39%	6%	33%	195
26	R19	39%	36%	3%	195
27	QP/A82M	39%	31%	8%	195
28	EV/VI/LI	38%	22%	16%	190
29	RP/FA	38%	29%	9%	190
30	RP/AM/IA	37%	33%	4%	185
31	RLYF/KSK19	35%	28%	7%	175
32	KT2/E267F	34%	26%	8%	170
33	KT2/E267L	33%	19%	14%	165
34	RP/IA	32%	18%	14%	160
35	KT2/A82G	28%	20%	8%	140
36	RT2/AP/A82F	26%	4%	22%	130
37	KT2	26%	23%	3%	130
38	KSK19/AI/F87V	25%	25%	0 %	125
39	KSK19/QP	21%	14%	7%	105
40	F87A/I401P	21%	17%	4%	105
41	RP/F87A/A328I	20%	20%	0%	100

No.	Enzyme	Conversion	Cyclisation Product (27)	Other Products	TTN[a]
42	KSK19	20%	16%	4%	100
43	RT2/F81W/A328I	20%	16%	4%	100
44	RQA	20%	20%	0%	100

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S4: Substrate conversion for the initial screen of the oxidation of 4-methoxyphenyl-2-pyrrolidin-1-ylacetamide **15**



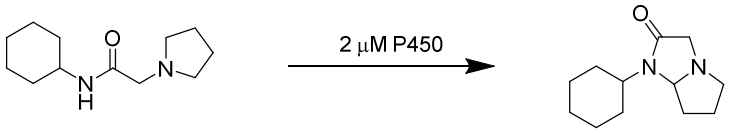
15
1 mM

28

No.	Enzyme	Conversion	Cyclisation Product (28)	Other Products	TTN[a]
1	RP/FV/EV	100%	72%	28%	500
2	KSK19/QP/F87V	95%	92%	3%	475
3	KSK19/A82M/E267F	91%	89%	2%	455
4	KSK19/A82M	90%	90%	0%	450
5	RP/FV/EV/V78F	89%	82%	7%	445
6	RP/FV/EV/F81W	78%	70%	8%	390
7	KSK19/A82M/I401M	76%	75%	1%	380
8	RP/H171L	70%	59%	11%	350
9	RP/E267M	68%	64%	4%	340
10	RP/IA/EV	64%	59%	5%	320
11	RP/E267I	61%	59%	2%	305
12	RP/FV/EV/L437VL	60%	59%	1%	300
13	RT2/IP/F87V	50%	48%	2%	250
14	RP	49%	49%	0%	245
15	KT2/E267L	49%	49%	0%	245
16	RP/EV	48%	43%	5%	240
17	EV/VI/LI	48%	46%	2%	240
18	RP/FV	48%	45%	3%	240
19	RP/FV/EV/L437LV	44%	40%	4%	220
20	GVQ	43%	43%	0%	215
21	QP/A82M	41%	40%	1%	205
22	RP/FA	34%	34%	0%	170
23	KT2/E267F	33%	33%	0%	165
24	RT2/AP/A82L	28%	25%	3%	140
25	RT2/IP	26%	23%	3%	130
26	KSK19/QP	23%	23%	0%	115
27	KT2/A82G	23%	23%	0%	115
28	RP/AM/IA	22%	22%	0%	110
29	RT2/I263W	22%	22%	0%	110
30	R19	21%	20%	1%	105

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S5: Substrate conversion for the initial screen of the oxidation of cyclohexyl-2-pyrrolidin-1-ylacetamide **16**



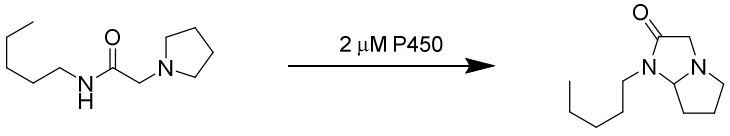
16
1 mM

29

No.	Enzyme	Conversion	Cyclisation Product (29)	Other Products	TTN[a]
1	RP/FV/EV/V78F	93%	75%	18%	465
2	RT2/F81W	90%	55%	35%	450
3	RP/IA/EV	81%	18%	63%	405
4	RP/FV/EV/L188Q	80%	63%	17%	400
5	RP/EV	73%	19%	54%	365
6	RP/FV/EV	70%	52%	18%	350
7	RP/E267I	67%	26%	41%	335
8	RP/FV/EV/L437LV	66%	47%	19%	330
9	RP/H171L	52%	42%	10%	260
10	RP/FV/EV/L437VL	52%	47%	5%	260
11	RP/FV/EV/F81W	51%	44%	7%	255
12	KT2/E267L	45%	31%	14%	225
13	RP/E267M	43%	27%	16%	215
14	EV/VI/LI	43%	28%	15%	215
15	RT2/IP/F87V	36%	29%	7%	180
16	RP/FV	30%	24%	6%	150
17	RP/FV/EV/A330W	28%	23%	5%	140
18	KSK19/QP/F87V	25%	22%	3%	125
19	RP/FV/EV/A330W	24%	21%	3%	120
20	RT2/IP/A184I	17%	11%	6%	85
21	RT2/FV/EV/IA/A330W	16%	16%	0%	80
22	KSK19/A82M/E267F	15%	15%	0%	75

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S6: Substrate conversion for the initial screen of the oxidation of pentyl-2-pyrrolidin-1-ylacetamide **17**



17
1 mM

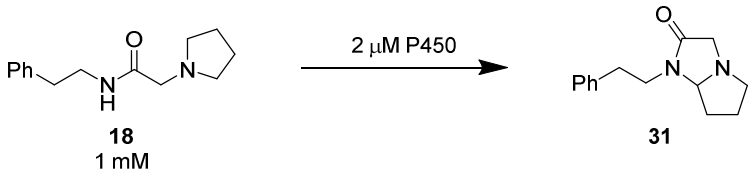
30

No.	Enzyme	Conversion	Cyclisation Product (30)	Other Products	TTN[a]
1	RT2/F81W	86%	56%	30%	430
2	RP/FV/EV/L188Q	72%	59%	13%	360
3	RP/FV/EV	68%	54%	14%	340
4	RP/FV/EV/V78F	68%	57%	11%	340

No.	Enzyme	Conversion	Cyclisation Product (30)	Other Products	TTN[a]
5	RP/H171L	61%	53%	8%	305
6	RP/E267I	56%	40%	16%	280
7	RP/EV	45%	23%	22%	225
8	RP/FV/EV/L437VL	40%	40%	0%	200
9	RP/FV/EV/F81W	40%	40%	0%	200
10	RP/FV/EV/L437LV	39%	39%	0%	195
11	RT2/IP/F87V	27%	27%	0%	135
12	EV/VI/LI	27%	27%	0%	135
13	KSK19/A82M/E267F	26%	26%	0%	130
14	KT2/E267L	25%	25%	0%	125
15	RP/E267M	23%	23%	0%	115
16	RP/IA/EV/H171L	23%	23%	0%	115
17	RT2/IP/A184I	23%	23%	0%	115
18	RT2/AP/AM/AI	21%	21%	0%	105
19	RP/FV/EV/A330W	21%	21%	0%	105
20	RT2/FV/EV/IA/A330W	21%	21%	0%	105
21	RP/FV	21%	21%	0%	105

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S7: Substrate conversion for the initial screen of the oxidation of phenethyl-2-pyrrolidin-1-ylacetamide **18**



18
1 mM

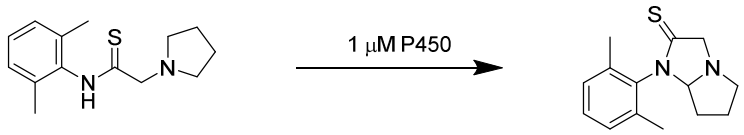
31

No.	Enzyme	Conversion	Cyclisation Product (31)	Other Products	TTN[a]
1	KSK19/A82M/E267F	79%	74%	5%	395
2	RLYF/KSK19	71%	56%	15%	355
3	KSK19	70%	59%	11%	350
4	KSK19/QP	59%	41%	18%	295
5	RT2/F87A/A330W	42%	42%	0%	210
6	RP/H171L	41%	40%	1%	205
7	RP/FV/EV/V78F	39%	37%	2%	195
8	RP/E267I	36%	21%	15%	180
9	RP/FV/EV/F81W	36%	33%	3%	180
10	RP/IA/EV	35%	27%	8%	175
11	KSK19/A82M	33%	31%	2%	165
12	RP/FA	33%	24%	9%	165
13	RP/E267M	33%	33%	0%	165
14	RP/FV/EV/L437VL	30%	30%	0%	150
15	RP/FV/EV/L188Q	30%	28%	2%	150
16	KT2/E267L	29%	27%	2%	145
17	RP/FV/EV	29%	27%	2%	145
18	KSK19/AI/AI	28%	28%	0%	140
19	KT2/E267F	26%	25%	1%	130
20	RP	25%	25%	0%	125
21	RP/EV	25%	25%	0%	125

No.	Enzyme	Conversion	Cyclisation Product (31)	Other Products	TTN[a]
22	EV/VI/LI	24%	24%	0%	120
23	RP/FV/EV/L437LV	24%	23%	1%	120
24	KSK19/A82M/I401M	24%	23%	1%	120
25	KSK19/QP/F87V	22%	21%	1%	110
26	RT2/F81W	22%	20%	2%	110
27	RP/IA	20%	20%	0%	100
28	RP/FV	20%	18%	2%	100
29	RT2/IP/F87V	20%	15%	5%	100

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S8: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-pyrrolidin-1-ylethanethioamide **19**



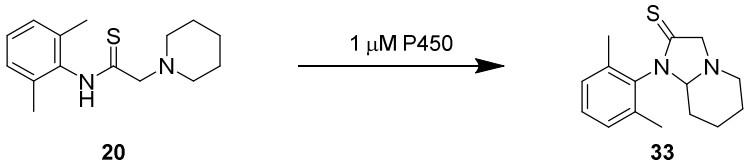
19
2 mM

32

No.	Enzyme	Conversion	TTN[a]
1	RP/FV/EV	95%	1900
2	RP/FV/EV/L437VL	90%	1800
3	RT2/AP	42%	840
4	KSK19/AI/F87V	38%	760
5	RP/IA/EV	29%	580
6	RT2/IP	26%	520
7	RT2/AP/A184I	25%	500
8	RP/QP	24%	480
9	RT2	19%	380
10	RT2/AP/A82L	16%	320
11	RP/AP	16%	350
12	RP/AP/AM	15%	300
13	RP/FV/EV/A184I	14%	280
14	RP/EV	14%	280
15	RT2/AP/AM	14%	280
16	RT2/AP/I401M	13%	260
17	RP/FV	13%	260
18	RP	13%	260
19	R19	12%	240
20	RT2/AP/V78I	10%	200
21	KSK19/I263A	9%	180
22	KSK19/AI/AI	8%	160
23	RLYF/KSK19/AI	8%	160
24	KSK19/AI/V78I	8%	160
25	KSK19/AI	7%	140
26	KSK19/AI/F42L	6%	120
27	KSK19/AI/Y51L	4%	80
28	KSK19/A82M	3%	60

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S9: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-piperidin-1-ylethanethioamide **20**



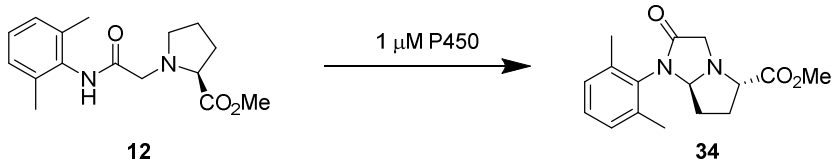
20
1 mM

33

No.	Enzyme	Conversion	Cyclisation Product (33)	Other Products	TTN[a]
1	KSK19/QP/F87V	84%	69%	15%	840
2	GVQ	56%	48%	8%	560
3	RT2/AP/E267L	55%	49%	6%	550
4	RP/FV/EV/L188Q	54%	41%	13%	540
5	RT2/F81W	53%	32%	21	530
6	KSK19/AI/F87V	51%	51%	0%	510
7	RT2/AP/A82F	49%	44%	5%	490
8	RP/E267M	44%	40%	4%	440
9	RP/FV/EV/V78F	42%	33%	9%	420
10	RT2/IP/A184I	40%	22%	18%	400
11	RP/FV/EV	33%	26%	7%	330
12	RT2/AP/E267F	32%	30%	2%	320
13	EV/VI/LI	32%	28%	4%	320
14	F87A/I401P	31%	14%	17%	310
15	RP/F81W	31%	18%	13%	310
16	RL/YF/KSK19	28%	8%	20%	280
17	KSK19/A82M	28%	21%	7%	280
18	KSK19	27%	10%	17%	270
19	RP/FV	27%	22%	5%	270
20	RP/EV	25%	23%	2%	250
21	KSK19/QP	25%	10%	15%	250
22	RP/FV/EV/F81W	24%	19%	5%	240
23	RP/F87A/A328I	24%	15%	9%	240
24	RT2/A330W/A82M	23%	23%	0%	230
25	KSK19/A82M/I401M	23%	13%	10%	230
26	RT2/F81W/A328I	23%	23%	0%	230
27	RT2/AP/AM	23%	23%	0%	230
28	RT2/AP/A82L	22%	22%	0%	220
29	RT2/AP/A184I/F81W	21%	21%	0%	210

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S10: Substrate conversion for the initial screen of the oxidation of methyl-{2-[(2,6-dimethylphenyl)amino]-2-oxoethyl}-L-prolinate **12**



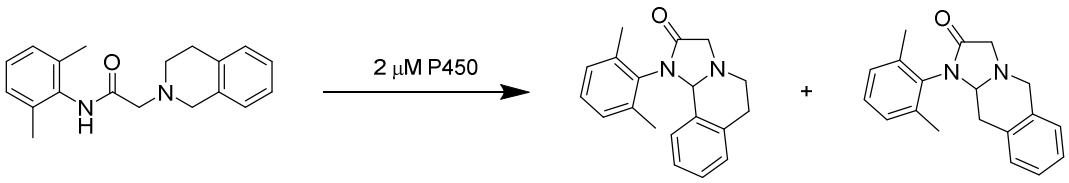
12
1 mM

34

No.	Enzymes	Conversion	Cyclisation Product (34)	Other Products	TTN[a]
1	RT2/F81W	100%	100%	0%	1000
2	RP/E267I	100%	98%	2%	1000
3	RP/EV	86%	84%	2%	860
4	RP/FV/EV	85%	84%	4%	850
5	RP/FV/EV/L188Q	76%	75%	1%	760
6	EV/VI/LI	70%	69%	1%	700
7	RP/IA/EV	69%	67%	2%	690
8	RT2/IP/A184I	66%	65%	1%	660
9	KSK19/A82M	53%	52%	4%	530
10	KSK19/A82M/I401M	48%	46%	2%	480
11	RT2/IP/F87V	43%	41%	2%	430
12	KSK19/QP/F87V	39%	37%	2%	390
13	RP/FV/EV/V78F	38%	37%	1%	380
14	KT2/E267L	38%	37%	1 %	380
15	RP/E267M	35%	33%	2%	350
16	RT2/AP/A82F	28%	17%	11%	280
17	KSK19/QP	27%	26%	1%	270
18	RT2/AP/E267L	24%	23%	1%	240
19	RP/FV	23%	23%	0%	230
20	KSK19/A82M/E267F	21%	20%	1%	210
21	RP/FV/EV/F81W	20%	19%	1%	190

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S11: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-[3,4-dihydroisoquinolin-2(1*H*)-yl]acetamide **13**



13
1 mM

35

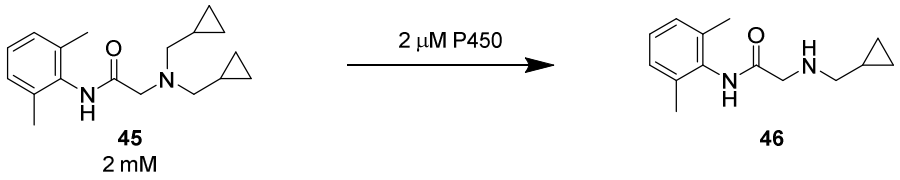
36

No.	Enzyme	Conversion	Benzylic Product (35)	Non-benzylic Product (36)	Other Products	TTN[a]
1	RP/H171L	97%	83%	10%	4%	485
2	RP/IA/EV	94%	13%	72%	9%	470
3	RP/FV/EV	92%	20%	63%	9%	460

No.	Enzyme	Conversion	Benzylic Product (35)	Non-benzylic Product (36)	Other Products	TTN[a]
4	RT2/IP/F87V	84%	36%	35%	14%	420
5	RP/FV/EV/L188Q	69%	10%	52%	7%	345
6	RT2/F81W	58%	23%	24%	11%	290
7	EV/VI/LI	58%	20%	24%	14%	290
8	RT2/IP	45%	2%	24%	19%	225
9	RP/FV/EV/V78F	42%	2%	15%	25%	210
10	RP/FV/EV/F81W	41%	3%	30%	8%	205
11	RT2/AP/E267L	36%	9%	24%	3%	180
12	RP/E267M	32%	0%	27%	5%	160
13	KSK19/AI	31%	0%	0%	100%	155
14	RP/F81W	25%	0%	24%	1%	125
15	RP/FV/EV/L437VL	24%	10%	8%	6%	120
16	RT2/AP/A82F	23%	1%	4%	18%	115
17	RP/FV/EV/L437LV	22%	2%	4%	16%	110
18	IP/QP	22%	21%	0%	1%	110
19	KT2/E267F	20%	2%	18%	0%	100
20	KSK19/AI/AI	18%	6%	12%	0%	90
21	RP	16%	0%	8%	8%	80
22	RT2/AP/A82L	16%	0%	2%	14%	80
23	RP/FV	15%	3%	12%	0%	75
24	KSK19/A82M/E267F	15%	1%	11%	3%	75
25	GVQ	15%	1%	12%	2%	75

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S12: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-bis(cyclopropylmethyl)aminoacetamide **45**



45
2 mM

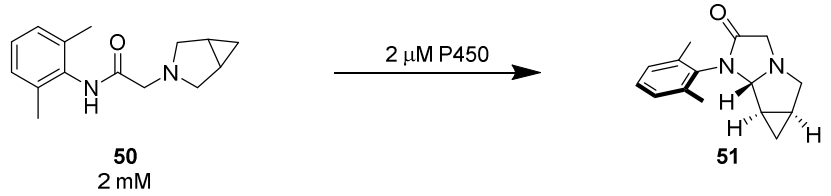
46

No.	Enzyme	Conversion	Dealkylation Product (46)	Other Products	TTN[a]
1	KSK19/A82M	67%	66%	1%	670
2	KSK19/AI/AI	26%	25%	1%	260
3	RP/IA/EV	18%	17%	1%	180
4	RP/FV/EV	17%	16%	1%	170
5	RP/EV	15%	14%	1%	150
6	RT2/IP	9%	9%	0%	90
7	RP/FV/EV/L437LV	7%	6%	1%	70
8	RT2/AP/AM	6%	6%	0%	60
9	RT2/F81W	6%	5%	1%	60
10	RP/FV/EV/L437VL	3%	3%	0%	30
11	RT2/AP/A184I	2%	2%	0%	20
12	RP/AP	2%	2%	0%	20
13	RT2/AP/I401M	1%	1%	0%	10
14	KSK19/I263A	1%	1%	0%	10
15	RT2	1%	1%	0%	10

No.	Enzyme	Conversion	Dealkylation Product (46)	Other Products	TTN[a]
16	RP	1%	1%	0.0%	10

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S13: Substrate conversion for the initial screen of the oxidation of 2,6-dimethylphenyl-2-(3-azabicyclo[3.1.0]hexan-3-yl)acetamide **50**



50
2 mM

51

No.	Enzyme	Conversion	TTN[a]
1	RP/EV	100%	1000
2	RT2/IP	99%	990
3	RP/IA/EV	99%	990
4	RP/FV	97%	970
5	RT2/F81W	81%	810
6	RP	79%	790
7	RP/FV/EV	73%	730
8	RP/FV/EV/L437LV	58%	580
9	RT2	53%	530
10	RT2/AP	36%	360
11	RP/FV/EV/L437VL	35%	350
12	RT2/AP/I401M	33%	330
13	RT2/AP/A184I	33%	330
14	KSK19/AI/F87V	27%	270
15	RP/AP	20%	200
16	KSK19/AI/Y51L	19%	190
17	KSK19/AM	18%	180
18	RT2/AP/AM	18%	180
19	KSK19/I263A	17%	170
20	KSK19/AI/AI	14%	140
21	RLYF/KSK19/AI	9%	90
22	RT2/AP/V78I	9%	90
23	KSK19/AI/V78I	3%	30
24	WT	3%	30

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S14: Substrate conversion for the initial screen of the oxidation of phenylacetamide **93**



93
2 mM

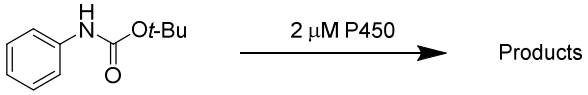
108

No.	Enzyme	Conversion	4-OH Product (108)	Other Products	TTN[a]
1	KSK19/AI/AI	17%	3%	14%	170

No.	Enzyme	Conversion	4-OH Product (108)	Other Products	TTN[a]
2	RP/IA/EV	2%	2%	0%	20
3	RT2/AP/A184I	2%	0%	2%	20
4	RT2	1%	0%	1%	10
5	RT2/I401P	1%	1%	0%	10
6	RP	1%	0%	1%	10

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S15: Substrate conversion for the initial screen of the oxidation of *tert*-butyl phenylcarbamate **95**

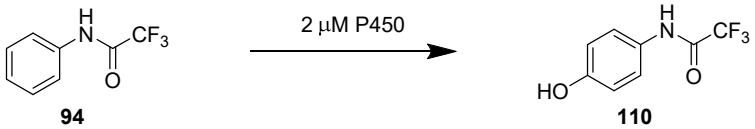


95
2 mM

No.	Enzyme	Conversion	rt 1.99	rt 7.07	rt 8.65	Other Products	TTN[a]
1	RT2/AP/AM	93%	39%	0%	0%	54%	930
2	RP/FV/EV/L437VL	84%	82%	0%	2%	0%	840
3	RP/FV/EV/L437LV	83%	43%	15%	20%	5%	830
4	RP/AP	77%	72%	0%	3%	2%	770
5	RT2/AP/A184I	72%	69%	0%	2%	1%	720
6	RP/EV	67%	49%	4%	1%	13%	670
7	RP/FV/EV	65%	51%	6%	0%	8%	650
8	RP	61%	59%	0%	0%	2%	610
9	RT2/AP/V78I	46%	2%	15%	0%	29%	460
10	KSK19/AI/Y51L	41%	0%	34%	0%	7%	410
11	RT2/AP/I401M	40%	33%	0%	2%	5%	400
12	RT2	38%	5%	16%	0%	17%	380
13	RP/FV	37%	25%	6%	4%	2%	370
14	RT2/IP	32%	29%	0%	3%	0%	320
15	RT2/F81W	31%	29%	0%	0%	2%	310
16	KSK19/AM	24%	24%	0%	0%	0%	240
17	RT2/AP	16%	13%	2%	0%	1%	160
18	KSK19/AI/AI	9%	3%	5%	0%	1%	90
19	KSK19/I263A	8%	4%	0%	2%	2%	80
20	WT	5%	0%	0%	0%	5%	50
21	RLYF/KSK19/AI	2%	2%	0%	0%	0%	20
22	RP/IA/EV	2%	2%	0%	0%	0%	20

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S16: Substrate conversion for the initial screen of the oxidation of 2,2,2-trifluoro-*N*-phenylacetamide **94**



94
2 mM

110

No.	Enzyme	Conversion	4-OH Product (110)	Other Products	TTN[a]
1	KSK19/AI/AI	100%	100%	0%	1000
2	RP/FV	98%	84%	14%	980
3	RP/FV/EV/L437LV	96%	96%	0%	960
4	RLYF/KSK19/AI	90%	90%	0%	900
5	RT2/AP/A184I	84%	74%	10%	840
6	KSK19/AM	81%	81%	0%	810
7	RP/FV/EV	74%	74%	0%	740
8	RT2/AP/A82L	70%	64%	6%	700
9	KSK19/AI/Y51L	67%	67%	0%	670
10	KSK19/AI/F87V	67%	67%	0%	670
11	RT2/AP/AM	49%	23%	26%	490
12	RP/FV/EV/L437VL	45%	45%	0%	450
13	RT2/IP	43%	31%	12%	430
14	RP/AP	43%	32%	10%	430
15	RP/AP/AM	42%	11%	31%	420
16	KSK19/AI/V78I	40%	31%	8%	400
17	RT2	37%	17%	19%	370
18	KSK19/I263A	28%	20%	10%	280
19	R19	26%	15%	11%	260
20	RP	23%	23%	0%	230
21	RT2/AP/V78I	23%	15%	8%	230
22	RP/EV	8%	0%	8%	8

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S17: Substrate conversion for the initial screen of the oxidation of *N*-phenyl-1,1,1-trifluoromethanesulfonamide **92**



92
2 mM

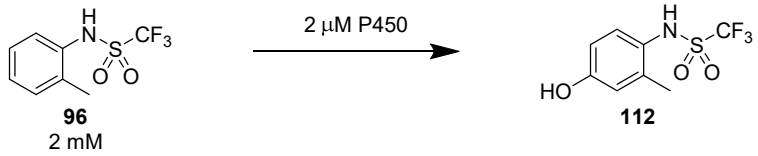
111

No.	Enzyme	Conversion	4-OH Product (111)	Other Products	TTN[a]
1	RP/FV/EV/L437LV	100%	100%	0%	1000
2	RP/IA/EV	100%	100%	0%	1000
3	RP/EV	97%	97%	0%	970
4	KSK19/AI/AI	78%	76%	2%	780
5	RP/FV/EV/L437VL	77%	77%	0%	770
6	RT2/AP/A184I	69%	69%	0%	690

No.	Enzyme	Conversion	4-OH Product (111)	Other Products	TTN[a]
7	KSK19/AM	66%	39%	27%	660
8	KSK19/AI/F87V	56%	45%	12%	560
9	RLYF/KSK19/AI	48%	36%	12%	480
10	RT2/IP	46%	46%	0%	460
11	RP	43%	43%	0%	430
12	RP/AP	23%	23%	0%	230
13	KSK19/AI/Y51L	16%	16%	0%	160

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S18: Substrate conversion for the initial screen of the oxidation of *N*-(*o*-tolyl)-1,1,1-trifluoromethanesulfonamide **96**



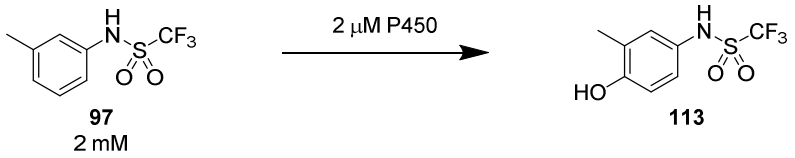
96
2 mM

112

No.	Enzyme	Conversion	4-OH Product (112)	Other Products	TTN[a]
1	RP/IA/EV	100%	100%	0%	1000
2	RP/FV/EV	99%	22%	77%	990
3	RP/FV/EV/L188Q	99%	22%	77%	990
4	RP/H171L/I263G	96%	96%	0%	960
5	RP/EV	94%	94%	0%	940
6	KSK19/AM	83%	0%	83%	830
7	RP/FV/EV/L437LV	72%	72%	0%	720
8	RP/E267M	72%	72%	0%	720
9	RP/FV	60%	60%	0%	600
10	RP/FV/EV/V78F	57%	54%	3%	570
11	RT2/IP	49%	49%	0%	490
12	GVQ	48%	48%	0%	480
13	RT2	41%	41%	0%	410
14	KSK19/AI/AI	37%	37%	0%	370
15	RP/H171L	36%	36%	0%	360
16	KSK19/AI/F87V	34%	34%	0%	340
17	KSK19/AM/E267F	34%	34%	0%	340
18	KSK19/QP/F87V	33%	33%	0%	330
19	RP/FV/EV/L437VL	27%	27%	0%	270
20	RT2/F81W	27%	27%	0%	270
21	RT2/AP/A184I	24%	24%	0%	240
22	RP	20%	20%	0%	200
23	RP/F81W	18%	18%	0%	180
24	RT2/AP/PV	14%	14%	0%	140
25	RT2/AP/VI/AI	14%	14%	0%	140
26	RP/AP	10%	10%	0%	100
27	RP/AM/IA	10%	10%	0%	100
28	RT2/AP	7%	7%	0%	70
29	RLYF/KSK19	5%	5%	0%	50
30	RT2/AP/I401M	4%	4%	0%	40
31	RT2/S72W/A330W	3%	3%	0%	30

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S19: Substrate conversion for the initial screen of the oxidation of *N*-(*m*-tolyl)-1,1,1-trifluoromethanesulfonamide **97**



97
2 mM

113

No.	Enzyme	Conversion	4-OH Product (113)	Other Products	TTN[a]
1	RP/FV/EV/L437LV	100%	64%	36%	1000
2	RP/EV	99%	85%	14%	990
3	RT2/F81W	97%	0%	97%	970
4	RP/IA/EV	97%	65%	32%	970
5	RP/FV/EV	93%	72%	21%	930
6	KSK19/AI/AI	91%	75%	16%	910
7	RT2/IP	86%	72%	14%	860
8	KSK19/AM	64%	25%	39%	640
9	RP	62%	36%	26%	620
10	RT2/AP/AM	61%	20%	41%	610
11	RT2/AP/A184I	58%	52%	6%	580
12	RT2/AP	37%	22%	15%	370
13	RP/AP	34%	34%	0%	340
14	KSK19/AI/F87V	24%	24%	0%	240
15	RP/FV/EV/L437VL	11%	11%	0%	110
16	RP/FV	11%	11%	0%	110
17	KSK19/AI/Y51L	10%	10%	0%	100
18	RLYF/KSK19/AI	3%	3%	0%	30
19	RT2/AP/I401M	3%	3%	0%	30

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S20: Substrate conversion for the initial screen of the oxidation of *N*-(*p*-tolyl)-1,1,1-trifluoromethanesulfonamide **98**



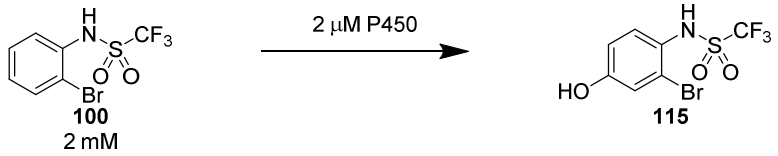
98
2 mM

114

No.	Enzyme	Conversion	Benzylic Alcohol (114)	Other Products	TTN[a]
1	RP/IA/EV	79%	77%	2%	790
2	RP/EV	63%	37%	26%	630
3	RP/FV/EV/L437VL	52%	0%	52%	520
4	RP/FV/EV/L437LV	30%	30%	0%	300
5	WT	28%	28%	0%	280
6	KSK19/AI/AI	19%	0%	19%	190
7	RP/FV/EV	18%	18%	0%	180
8	KSK19/AI/F87V	6%	6%	0%	60

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

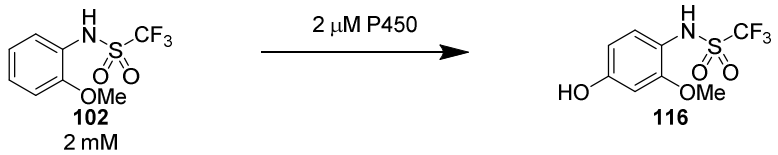
Table S21: Substrate conversion for the initial screen of the oxidation of *N*-(2-bromophenyl)-1,1,1-trifluoromethanesulfonamide **100**



No.	Enzyme	Conversion	4-OH Product (115)	Other Products	TTN[a]
1	RP/IA/EV	100%	44%	56%	1000
2	RP/EV	85%	40%	45%	850
3	RP/FV/EV	81%	19%	62%	810
4	RP/FV/EV/L437LV	78%	43%	35%	780
5	RT2/AP/I401M	60%	0%	60%	600
6	RP/FV	31%	17%	14%	310
7	RT2/AP/AM	30%	0%	30%	300
8	RT2/IP	28%	17%	11%	280
9	RT2/F81W	21%	9%	12%	210
10	KSK19/AIAI	16%	0%	16%	160
11	RP/FV/EV/L437VL	15%	5%	10%	150
12	RT2	12%	5%	7%	120
13	RP	10%	0%	10%	100
14	RP/AP	10%	0%	10%	100
15	RT2/AP	6%	2%	4%	60
16	KSK19/AM	5%	0%	5%	50
17	KSK19/AI/F87V	5%	0%	5%	50
18	RT2/AP/A184I	5%	1%	4%	50
19	RT2/AP/V78I	5%	0%	5%	50
20	RLYF/KSK19/AI	2%	0%	2%	20
21	WT	2%	0%	2%	20

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S22: Substrate conversion for the initial screen of the oxidation of *N*-(2-methoxyphenyl)-1,1,1-trifluoromethanesulfonamide **102**

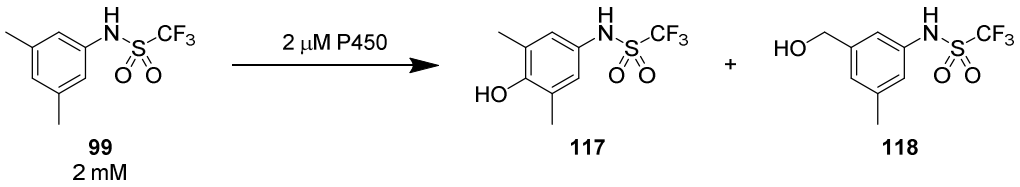


No.	Enzyme	Conversion	4-OH Product (116)	Other Products	TTN[a]
1	RP/FV/EV	97%	6%	91%	970
2	RP/IA/EV	81%	80%	1%	810
3	RP/EV	49%	48%	1%	490
4	KSK19/AM	30%	4%	26%	300
5	RT2/F81W	13%	13%	0%	130
6	RT2/IP	12%	12%	0%	120
7	RT2	9%	9%	0%	90

No.	Enzyme	Conversion	4-OH Product (116)	Other Products	TTN[a]
8	RP/FV/EV/L437VL	7%	7%	0%	70
9	RT2/AP/I401M	3%	3%	0%	30
10	RP/AP	3%	3%	0%	30
11	RLYF/KSK19/AI	2%	2%	0%	20
12	KSK19/AI/Y51L	2%	2%	0%	20

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S23: Substrate conversion for the initial screen of the oxidation of *N*-(3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide **99**



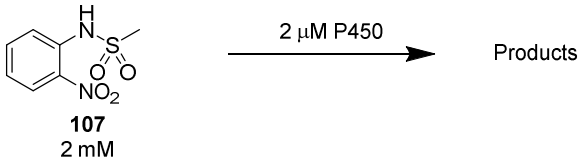
99
2 mM

117 **118**

No.	Enzyme	Conversion	4-(OH) Product (117)	Benzylic Alcohol (118)	Other Products	TTN[a]
1	RP/IA/EV	100%	85%	9%	6%	1000
2	KSK19/AM	99%	12%	18%	69%	990
3	KSK19/AI/F87V	99%	3%	96%	0%	990
4	RP/EV	97%	28%	63%	6%	970
5	KSK19/AI/AI	90%	54%	36%	0%	900
6	KSK19/AI/Y51L	80%	37%	43%	0%	800
7	RT2	80%	3%	71%	6%	800
8	RT2/F81W	68%	25%	43%	0%	680
9	RP/FV/EV	50%	19%	31%	0%	500
10	KSK19/AI/V78I	50%	35%	15%	0%	500
11	RLYF/KSK19/AI	45%	25%	20%	0%	450
12	RP	43%	13%	30%	0%	430
13	RT2/IP	41%	18%	23%	0%	410
14	RT2/AP/A184I	26%	26%	0%	0%	260
15	RP/FV/EV/L437LV	13%	9%	4%	0%	130
16	RT2/AP/AM	10%	7%	3%	0%	100
17	RT2/AP/I401M	7%	7%	0%	0%	70
18	WT	7%	2%	5%	0%	70
19	RP/FV/EV/L437VL	3%	3%	0%	0%	30
20	RT2/AP	3%	3%	0%	0%	30
21	RP/AP	3%	3%	0%	0%	30
22	RP/FV	3%	2%	1%	0%	30

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

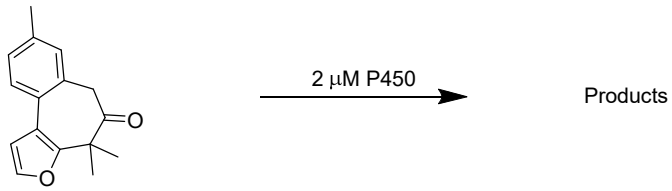
Table S24: Substrate conversion for the initial screen of the oxidation of *N*-(2-nitrophenyl)methanesulfonamide **107**



No.	Enzyme	Conversion	rt 7.83	rt 8.61	Other Products	TTN[a]
1	RT2/AP/I401M	35%	2%	33%	0%	350
2	RT2/IP	28%	8%	15%	5%	280
3	RP/FV/EV/L437VL	21%	3%	18%	0%	210
4	RP/FV/EV	13%	0%	0%	13%	130
5	RP/EV	10%	3%	6%	1%	100
6	RT2/AP/V78I	9%	3%	6%	0%	90
7	KSK19/AI/V78I	8%	2%	6%	0%	80
8	KSK19/AM	7%	0%	0%	7%	70
9	KSK19/AI/F87V	1%	1%	0%	0%	10
10	RP/FV/EV/L437LV	1%	1%	0%	0%	10

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S25: Substrate conversion for the initial screen of the oxidation of 4,4,8-trimethyl-4,6-dihydro-5*H*-benzo[3,4]cyclohepta[1,2-*b*]furan-5-one **192**

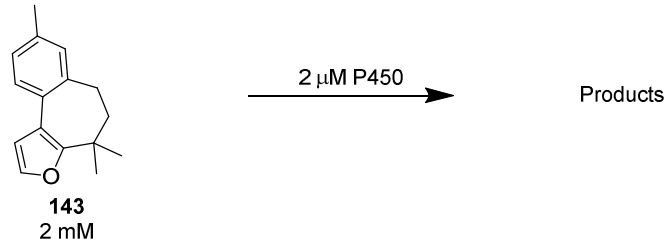


No.	Enzyme	Conversion	rt 7.76	rt 8.85	Other Products	TTN[a]
1	RP/H171L/I263G	95%	34%	15%	46%	950
2	RLYF/KSK19	93%	0%	43%	50%	930
3	RP/F81W	91%	77%	0%	14%	910
4	KSK19/QP/F87V	70%	0%	19%	51%	700
5	RP/H171L	63%	49%	12%	2%	630
6	RT2/AP/PV	46%	9%	20%	17%	460
7	GVQ	42%	0%	16%	26%	420
8	RP/AM/IA	42%	4%	13%	25%	420
9	KSK19/AI/IA	41%	22%	10%	9%	410
10	RT2/F81W/A328I	38%	2%	15%	21%	380
11	RP/E267M	32%	0%	12%	20%	320
12	RT2/V78F	26%	5%	8%	13%	260
13	RP/FV/EV/V78F	25%	0%	13%	12%	250
14	RT2/AP/VI/AI	24%	13%	10%	1%	240
15	RT2/AP/AI/S72A	20%	1%	11%	8%	200
16	RP/FV/EV/L188Q	18%	8%	9%	1%	180

No.	Enzyme	Conversion	rt 7.76	rt 8.85	Other Products	TTN[a]
17	RT2/A330W	13%	0%	8%	5%	130
18	F87A	13%	0%	10%	3%	130
19	KSK19/AM/E267F	13%	0%	13%	0%	130
20	RT2/S72W/A330W	12%	5%	7%	0%	120
21	RP/E267G	11%	0%	11%	0%	110
22	RLYF	9%	0%	9%	0%	90
23	A330P	7%	0%	6%	1%	70
24	KSK48	7%	0%	7%	0%	70

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S26: Substrate conversion for the initial screen of the oxidation of 4,4,8-trimethyl-5,6-dihydro-4*H*-benzo[3,4]cyclohepta[1,2-*b*]furan **143**

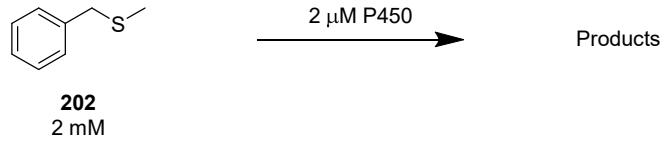


143
2 mM

No.	Enzyme	Conversion	rt 7.91	Other Products	TTN[a]
1	KSK19/AI/F87V	89%	53%	36%	890
2	KSK19/AI/Y51L	83%	59%	24%	830
3	KSK19/AI/AI	83%	60%	23%	830
4	RP/FV/EV/L437VL	82%	60%	22%	820
5	RLYF/KSK19/AI	61%	9%	52%	610
6	RT2/IP	55%	40%	15%	550
7	RP/FV	39%	30%	9%	390
8	RT2/AP/A184I	34%	26%	8%	340
9	RP/IA/EV	30%	0%	30%	300
10	RP	28%	19%	9%	280
11	RT2/AP/I401M	25%	20%	5%	250
12	RT2/AP/AM	23%	17%	5%	230
13	RT2/AP	22%	16%	6%	220
14	RP/EV	21%	17%	4%	210
15	RT2/F81W	19%	5%	14%	190
16	RP/FV/EV	10%	10%	0%	100
17	RP/FV/EV/L437LV	8%	5%	3%	80
18	RT2/AP/V78I	7%	5%	2%	70
19	RT2	7%	5%	2%	70
20	KSK19/AI/V78I	6%	4%	2%	60
21	RP/AP	5%	2%	3%	50

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S27: Product formation in the initial screen of the oxidation of benzyl methyl sulfide **202**

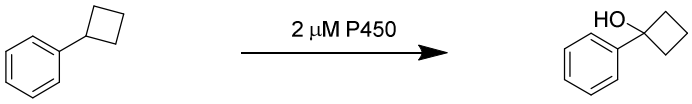


202
2 mM

No.	Enzyme	rt 2.29	rt 2.41	rt 2.63
1	RLYF/KSK19/AI	75%	24%	1%
2	KSK19/A82M	0%	100%	0%
3	KSK19/AI/Y51L	64%	23%	13%
4	KSK19/AI/AI	10%	81%	9%
5	RP/FV/EV/L437VL	1%	99%	0%
6	KSK19/IA	73%	20%	7%
7	RP	2%	97%	1%
8	RP/EV	0%	100%	0%
9	RP/AP/AM	19%	80%	1%
10	RP/FV/EV	0%	100%	0%
11	KSK9/AI/F87V	27%	70%	3%
12	RP/FV/EV/L437LV	1%	99%	0%
13	KSK19/AI/V78I	71%	22%	7%
14	RT2/AP/AM	4%	95%	1%
15	RT2/IP	5%	95%	0%
16	RT2/AP	13%	86%	1%
17	RT2/AP/V78I	55%	39%	6%
18	RT2	20%	78%	2%
19	RP/IA/EV	0%	100%	0%
20	RP/AP	53%	40%	7%
21	RP/FV	0%	100%	0%
22	RT2/AP/A82L	22%	76%	2%
23	R19	23%	74%	3%

a. Conversion not shown due to overlap of solvent peaks with starting material peak

Table S28: Substrate conversion for the initial screen of the oxidation of cyclobutylbenzene **207**



207
2 mM

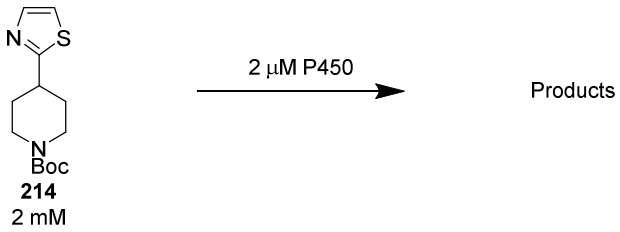
206

No.	Enzyme	Conversion	Benzylic alcohol (206)	Other Products	TTN[a]
1	RLYF/KSK19/AI	100%	100%	0%	1000
2	KSK19/AM	100%	89%	11%	1000
3	KSK19/AI/Y51L	100%	77%	23%	1000
4	KSK19/AI/AI	100%	100%	0%	1000
5	RP/FV/EV/L437VL	100%	12%	88%	1000
6	KSK19/I263A	100%	100%	0%	1000
7	RP	100%	64%	36%	1000
8	RP/EV	100%	73%	27%	1000
9	RT2/F81W	100%	90%	10%	1000
10	RP/FV/EV	100%	7%	93%	1000
11	KSK19/AI/F87V	100%	26%	74%	1000

No.	Enzyme	Conversion	Benzylic alcohol (206)	Other Products	TTN[a]
12	RP/FV/EV/L437LV	100%	0%	100%	1000
13	KSK19/AI/V78I	100%	69%	31%	1000
14	RT2/AP/I401M	100%	21%	79%	1000
15	RT2/AP/AM	100%	77%	23%	1000
16	RT2/IP	100%	81%	9%	1000
17	RT2/AP	100%	42%	58%	1000
18	RT2/AP/A184I	100%	10%	90%	1000
19	RT2/AP/V78I	100%	21%	79%	1000
20	RT2	100%	64%	36%	1000
21	RP/IA/EV	100%	72%	28%	1000
22	WT	100%	20%	80%	1000
23	RP/AP	100%	16%	84%	1000
24	RP/FV	100%	0%	100%	1000

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S29: Substrate conversion for the initial screen of the oxidation of *tert*-butyl 4-(thiazol-2-yl)piperidine-1-carboxylate **214**

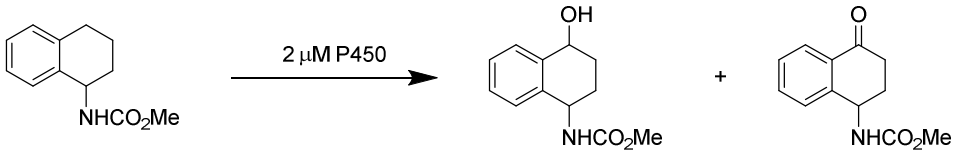


214
2 mM

No.	Enzyme	Conversion	rt 5.21	rt 5.65	rt 8.64	TTN[a]
1	KSK19	61%	17%	43%	0%	610
2	RP/FV/EV	33%	0%	0%	33%	330
3	KSK19/IA	7%	3%	4%	0%	70
4	KSK19/A82M	4%	0%	4%	0%	40

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S30: Substrate conversion for the initial screen of the oxidation of methyl (1,2,3,4-tetrahydronaphthalen-1-yl)carbamate **210**



210
2 mM

232

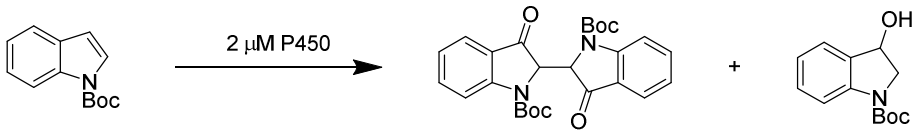
233

No.	Enzyme	Conversion	Alchohol Product (232)	Ketone Product (233)	Other Products	TTN[a]
1	RP	100%	76%	24%	0%	1000
2	RP/FV/EV/A184I	100%	34%	25%	41%	1000
3	RT2/IP	100%	78%	22%	0%	1000
4	GVQ	99%	85%	13%	2%	990

No.	Enzyme	Conversion	Alchohol Product (232)	Ketone Product (233)	Other Products	TTN[a]
5	RT2/AP/AM	98%	55%	38%	5%	980
6	KSK19	96%	71%	16%	9%	960
7	KSK19/AI/FV	94%	50%	15%	29%	940
8	RP/IA/EV	93%	61%	32%	0%	930
9	KSK19/A82M	91%	64%	14%	13%	910
10	RT2/FW	91%	61%	19%	11%	910
11	RT2/IP/V78I	88%	52%	36%	0%	880
12	KSK19/AI/AI	86%	34%	25%	27%	860
13	RP/AP/AM	77%	45%	32%	0%	770
14	RP/FA	61%	43%	16%	2%	610
15	RP/AP	58%	34%	24%	0%	580
16	KSK19/AI	57%	14%	9%	34%	570
17	RP/FV/EV	38%	31%	6%	1%	380
18	RP/FV/EV/L437LV	26%	26%	0%	0%	260
19	F87A	18%	17%	0%	1%	180
20	KSK19/AI/VI	15%	9%	3%	3%	150
21	KSK19/IA	5%	5%	0%	0%	50

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S31: Substrate conversion for the initial screen of the oxidation of *tert*-butyl 1*H*-indole-1-carboxylate **204**



204
2 mM

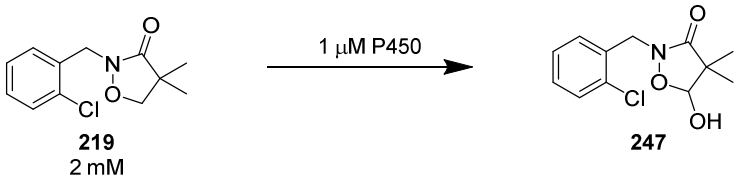
238 **239**

No.	Enzyme	Conversion	TTN[a]
1	KSK19	100%	1000
2	RP/IA/EV	100%	1000
3	RT2/A330W	100%	1000
4	RT2/AP	100%	1000
5	RP/FV	91%	910
6	RT2/IP	91%	910
7	RT2/AP/A184I	90%	900
8	RT2/FW	88%	880
9	RT2/V78F	85%	850
10	RP	85%	850
11	KSK19/IA	76%	760
12	RT2/A330W/I263A	71%	710
13	RT2/AP/AM	41%	410
14	RP/FV/EV	34%	340
15	RT2/A330W/E267L	30%	300
16	KSK19/AI/F87V	19%	190
17	RT2/IP/V78I	14%	140
18	KSK19/A82M	12%	120
19	KSK19/AI/VI	10%	100
20	A330P/F87A	10%	100

No.	Enzyme	Conversion	TTN[a]
21	KSK19/AI	10%	100
22	RP/FV/EV/A184I	9%	90
23	KSK19/AI/AI	8%	80
24	RP/FV/EV/L437LV	5%	50

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S32: Substrate conversion for the initial screen of the oxidation of 2-(2-chlorobenzyl)-4,4-dimethyloxazolidin-3-one **219**



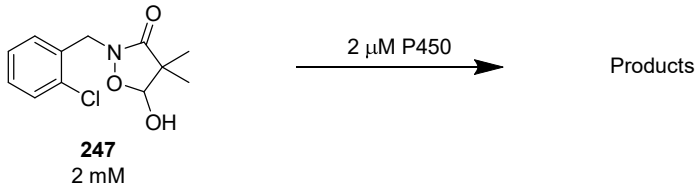
219
2 mM

247

No.	Enzyme	Conversion	Hemiacetal Product (247)	Other Products	TTN[a]
1	KSK19	100%	100%	0%	2000
2	GVQ	100%	100%	0%	2000
3	KSK19/I263A	96%	96%	0%	1920
4	F87A	87%	80%	7%	1740
5	RP/FA	78%	78%	0%	1560
6	KSK19/A82M	58%	58%	0%	1160
7	RP/IA/EV	40%	37%	3%	800
8	RP/FV/EV	25%	25%	0%	500
9	RT2/IP	23%	22%	1%	460
10	RP	14%	4%	10%	280
11	RP/AP	14%	4%	10%	280
12	RP/AP/AM	13%	5%	8%	260
13	RT2/FW	12%	12%	0%	140
14	KSK19/AI/AI	11%	11%	0%	220
15	RT2/AP/VI	11%	11%	0%	220
16	RP/FV/EV/A184I	10%	10%	0%	200
17	RT2/IP/V78I	10%	9%	1%	200
18	KSK19/AI	8%	6%	2%	160
19	RP/FV/EV/L437LV	8%	8%	0%	160
20	KSK19/AI/FV	5%	0%	5%	100
21	KSK19/AI/VI	2%	2%	0%	40

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S33: Substrate conversion for the initial screen of the oxidation of 2-(2-chlorobenzyl)-5-hydroxy-4,4-dimethylisoxazolidin-3-one **247**

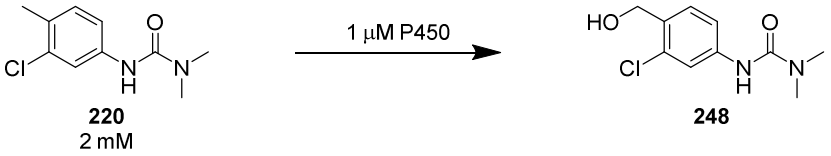


247
2 mM

No.	Enzyme	Conversion	rt 6.06	rt 8.61	Other Products	TTN[a]
1	RP	44%	0%	44%	0%	440
2	RP/FV/EV/L437LV	43%	0%	0%	43%	430
3	KSK19/IA	30%	0%	0%	30%	300
4	KSK19/AI/F87V	24%	0%	24%	0%	240
5	RT2/AP/A184I	24%	24%	0%	0%	240
6	RP/AP/AM	8%	8%	0%	0%	80
7	RT2/AP/AM	7%	7%	0%	0%	70
8	RP/EV	6%	0%	0%	6%	60

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S34: Substrate conversion for the initial screen of the oxidation of 3-(3-chloro-4-methylphenyl)-1,1-dimethylurea **220**



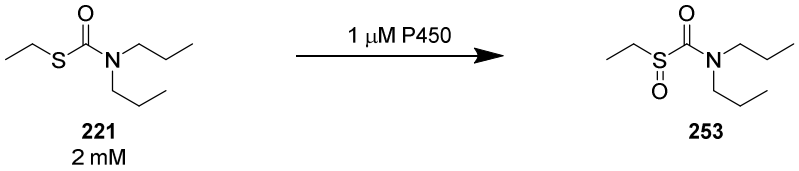
220
2 mM

248

No.	Enzyme	Conversion	Benzylic Alcohol (248)	Other Products	TTN[a]
1	RP/IA/EV	86%	14%	72%	1720
2	RP/FA	73%	0%	73%	1460
3	RT2/FW	73%	55%	18%	1460
4	RT2/IP	71%	31%	40%	1420
5	RP/FV/EV	66%	40%	26%	1320
6	KSK19/AI/AI	43%	43%	0%	860
7	F87A	41%	0%	41%	820
8	KSK19/AI/F87V	28%	28%	0%	560
9	GVQ	27%	0%	27%	540
10	RP/FV/EV/A184I	24%	0%	24%	480
11	RP/FV/EV/L437LV	24%	0%	24%	480
12	RP/AP	15%	0%	15%	300
13	RT2/AP/AM	11%	0%	11%	220

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S35: Substrate conversion for the initial screen of the oxidation of *S*-ethyl dipropylcarbamothioate **221**



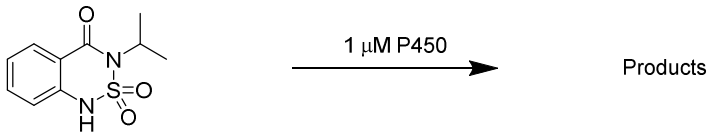
221
2 mM

253

No.	Enzyme	Conversion	Sulfoxide (253)	Other Products	TTN[a]
1	KSK19/AI	100%	88%	12%	2000
2	KSK19/AI/AI	100%	68%	32%	2000
3	KSK19/A82M	100%	62%	38%	2000
4	KSK19	100%	66%	34%	2000
5	KSK19/AI/VI	100%	73%	27%	2000
6	KSK19/AI/F87V	100%	69%	31%	2000
7	RP/FV/EV/A184I	100%	58%	42%	2000
8	RP/FA	100%	38%	62%	2000
9	RT2/IP	100%	50%	50%	2000
10	GVQ	100%	33%	67%	2000
11	RP/AP	98%	14%	84%	1960
12	F87A	96%	43%	53%	1920
13	RP/IA/EV	93%	50%	43%	1860
14	RP	90%	12%	78%	1800
15	RP/FV/EV	81%	7%	74%	1620
16	RT2/IP/V78I	64%	37%	27%	1280
17	RT2/AP/AM	51%	19%	32%	1020
18	RP/FV/EV/L437LV	22%	15%	7%	440
19	RP/AP/AM	16%	6%	10%	320
20	KSK19/I263A	9%	2%	7%	180

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S36: Substrate conversion for the initial screen of the oxidation of 3-isopropyl-1*H*-benzo[*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide **222**

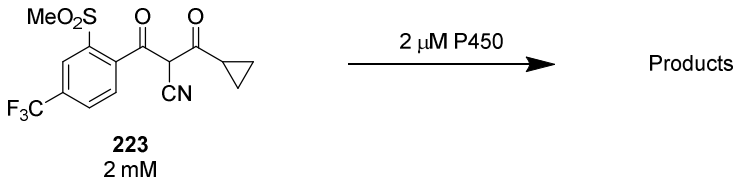


222
2 mM

No.	Enzyme	Conversion	rt 6.60	Other Products	TTN[a]
1	KSK19/AI	80%	4%	76%	1600
2	KSK19/IA	72%	0%	72%	1440
3	RP	71%	71%	0%	1420
4	RP/FV/EV/A184I	37%	37%	0%	740
5	KSK19/AI/F87V	34%	34%	0%	680
6	RT2/IP/V78I	34%	34%	0%	680
7	RT2/FW	6%	6%	0%	120

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S37: Substrate conversion for the initial screen of the oxidation of 2-(cyclopropanecarbonyl)-3-[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]-3-oxopropanenitrile **223**

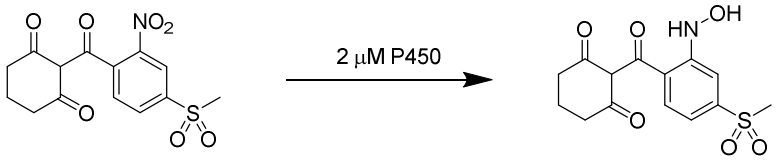


223
2 mM

No.	Enzyme	Conversion	rt 2.65	rt 8.62	TTN[a]
1	RP/EV	100%	100%	0%	1000
2	R19	87%	0%	87%	870

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S38: Substrate conversion for the initial screen of the oxidation of 2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohexane-1,3-dione **225**



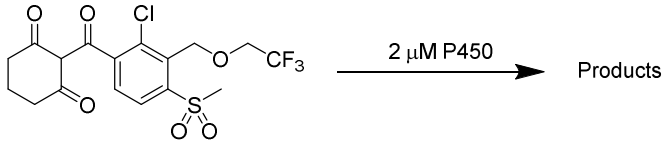
225
2 mM

273

No.	Enzyme	Conversion	TTN[a]
1	RT2/AP/A184I	49%	490
2	RT2/IP	47%	470
3	RP/FV	33%	330
4	RP/FV/EV/A184I	32%	320
5	RP/FV/EV/L437LV	25%	250
6	KSK19/IA	17%	170
7	RT2/V78F	13%	130
8	RT2/A330W/I263A	11%	110
9	KSK19/AI/F87V	10%	100
10	RP	10%	100
11	RP/FV/EV	7%	70
12	RT2/AP/AM	7%	70
13	KSK19/AI	7%	70
14	RT2/A330W	6%	60
15	RT2/AP	6%	60
16	KSK19/A82M	5%	50
17	KSK19/AI/AI	5%	50

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S39: Substrate conversion for the initial screen of the oxidation of 2-(2-chloro-4-{methylsulfonyl}-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl)cyclohexane-1,3-dione **226**



226
2 mM

No.	Enzyme	Conversion	rt 1.06	rt 2.06	TTN[a]
1	KSK19/AI/AI	9%	9%	0%	90
2	RT2/A330W/E267L	8%	0%	8%	80
3	KSK19	7%	7%	0%	70
4	RT2/IP	7%	0%	7%	70

a. TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

References

1 J. B. Jones, *Tetrahedron*, 1986, **42**, 3351–3403.

2 G. M. Whitesides and C. H. Wong, *Angew. Chem Int. Ed.*, 1985, **24**, 617–718.

3 B. G. Davis and V. Boyer, *Nat. Prod. Rep.*, 2001, **18**, 618–640.

4 K. M. Koeller and C. H. Wong, *Nature*, 2001, **409**, 232–240.

5 N. J. Turner, *Nat. Chem. Biol.*, 2009, **5**, 567–573.

6 G. A. Strohmeier, H. Pichler, O. May and M. Gruber-Khadjawi, *Chem. Rev.*, 2011, 111, 4141–4164.

7 A. S. Bommarius and B. R. Riebel-Bommarius, *Biocatalysis: Fundamentals and Applications*, John Wiley & Sons , 2004.

8 C. M. Clouthier and J. N. Pelletier, *Chem. Soc. Rev.*, 2012, **41**, 1585–1605.

9 U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore and K. Robins, *Nature*, 2012, **485**, 185–194.

10 M. T. Reetz, *J. Am. Chem. Soc.*, 2013, **135**, 12480–12496.

11 J. M. Choi, S. S. Han and H. S. Kim, *Biotechnol. Adv.*, 2015, 33, 1443–1454.

12 A. S. Bommarius, *Annu. Rev. Chem. Biomol. Eng.*, 2015, **6**, 319–345.

13 K. Faber, W.-D. Fessner and N. J. Turner, Eds., *Biocatalysis in Organic Synthesis 1*, Thieme, Stuttgart, 2015.

14 A. M. Bezborodov and N. A. Zagustina, *Appl. Biochem. Microbiol.*, 2016, **52**, 237–249.

15 G. M. Whitesides and C. H. Wong, *Aldrichimica Acta*, 1983, **16**, 27–34.

16 *Enzyme Nomenclature*, Academic Press, New York, 1979.

17 A. Ghanem and H. Y. Aboul-Enein, *Chirality*, 2005, 17, 1–15.

18 M. Rachwalski, N. Vermue and F. P. J. T. Rutjes, *Chem. Soc. Rev.*, 2013, **42**, 9268–9282.

19 O. Verho and J. E. Bäckvall, *J. Am. Chem. Soc.*, 2015, **137**, 3996–4009.

20 R. Lihammar, R. Millet and J. E. Bäckvall, *J. Org. Chem.*, 2013, **78**, 12114–12120.

21 K. Nakamura, R. Yamanaka, T. Matsuda and T. Harada, *Tetrahedron: Asymmetry*, 2003, 14,

2659–2681.

22 T. Matsuda, R. Yamanaka and K. Nakamura, *Tetrahedron: Asymmetry*, 2009, **20**, 513–557.

23 K. Nakamura and T. Matsuda, *Curr. Org. Chem.*, 2006, **10**, 1217–1246.

24 M. D. Mihovilovic, B. Müller and P. Stanetty, *Eur J. Org. Chem.*, 2002, **2002**, 3711–3730.

25 V. Alphand, G. Carrea, R. Wohlgemuth, R. Furstoss and J. M. Woodley, *Trends Biotechnol.*, 2003, 21, 318–323.

26 M. D. Mihovilovic, G. Chen, S. Wang, B. Kyte, F. Rochon, M. M. Kayser and J. D. Stewart, *J. Org. Chem.*, 2001, **66**, 733–738.

27 N. J. Turner, *Chem. Rev.*, 2011, **111**, 4073–4087.

28 J.-S. Shin and B.-G. Kim, *Biosci. Biotechnol. Biochem.*, 2001, **65**, 1782–1788.

29 J. Ward and R. Wohlgemuth, *Curr. Org. Chem.*, 2010, **14**, 1914–1927.

30 D. Koszelewski, K. Tauber, K. Faber and W. Kroutil, *Trends Biotechnol.*, 2010, **28**, 324–332.

31 S. T. Ahmed, F. Parmeggiani, N. J. Weise, S. L. Flitsch and N. J. Turner, *ACS Catal.*, 2015, **5**, 5410–5413.

32 J. Hioe and H. Zipse, *Org. Biomol. Chem.*, 2010, **8**, 3609–3617.

33 M. Hudlicky, *Oxidations in Organic Chemistry*, American Chemical Society, 1990.

34 T. Newhouse and P. S. Baran, *Angew. Chem Int. Ed.*, 2011, **50**, 3362–3374.

35 J. Muzart, *Chem. Rev.*, 1992, **92**, 113–140.

36 J. Młochowski and H. Wójtowicz-Młochowska, *Molecules*, 2015, **20**, 10205–10243.

37 W. J. Mijs and C. R. H. I. de Jonge, *Organic syntheses by oxidation with metal compounds*, Springer Science & Business Media, 2013.

38 A. Nakamura and M. Nakada, *Synthesis.*, 2013, **45**, 1421–1451.

39 K. Lee and J. K. Cha, *J. Am. Chem. Soc.*, 2001, **123**, 5590–5591.

40 K. C. Nicolaou, Q.-Y. Toh and D. Y.-K. Chen, *J. Am. Chem. Soc.*, 2008, **130**, 11292–11293.

41 A. Nickel, T. Maruyama, H. Tang, P. D. Murphy, B. Greene, N. Yusuff and J. L. Wood, *J. Am. Chem. Soc.*, 2004, **126**, 16300–16301.

42 P. Mackiewicz and R. Furstoss, *Tetrahedron*, 1978, **34**, 3241–3260.

43 K. Chen, J. M. Richter and P. S. Baran, *J. Am. Chem. Soc.*, 2008, **130**, 7247–7249.

44 D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, 1960, **82**, 2641.

45 D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, 1961, **83**, 4083–4089.

46 K. A. Hollister, E. S. Conner, M. L. Spell, K. Deveaux, L. Maneval, M. W. Beal and J. R. Ragains, *Angew. Chem Int. Ed.*, 2015, **54**, 7837–7841.

47 D. Yang, M.-K. Wong, X.-C. Wang and Y.-C. Tang, *J. Am. Chem. Soc.*, 1998, **120**, 6611–6612.

48 R. Giri, B.-F. Shi, K. M. Engle, N. Maugel and J.-Q. Yu, *Chem. Soc. Rev.*, 2009, **38**, 3242–3272.

49 Z. Chen, B. Wang, J. Zhang, W. Yu, Z. Liu and Y. Zhang, *Org. Chem. Front.*, 2015, **2**, 1107–1295.

50 T. W. Lyons and M. S. Sanford, *Chem. Rev.*, 2010, **110**, 1147–1169.

51 J. E. Baldwin, R. H. Jones, C. Najera and M. Yus, *Tetrahedron*, 1985, **41**, 699–711.

52 L. V. Desai, K. L. Hull and M. S. Sanford, *J. Am. Chem. Soc.*, 2004, **126**, 9542–9543.

53 Y.-F. Liang, X. Wang, Y. Yuan, Y. Liang, X. Li and N. Jiao, *ACS Catal.*, 2015, **5**, 6148–6152.

54 Z. Wang, Y. Kuninobu and M. Kanai, *Org. Lett.*, 2014, **16**, 4790–4793.

55 L. Zhou and W. Lu, *Org. Lett.*, 2014, **16**, 508–511.

56 R. K. Rit, M. R. Yadav and A. K. Sahoo, *Org. Lett.*, 2012, **14**, 3724–3727.

57 L. V. Desai, K. L. Hull and M. S. Sanford, *J. Am. Chem. Soc.*, 2004, **126**, 9542–9543.

58 D.-H. Wang, X.-S. Hao, D.-F. Wu and J.-Q. Yu, *Org. Lett.*, 2006, **8**, 3387–3390.

59 R. Giri, J. Liang, J. G. Lei, J. J. Li, D. H. Wang, X. Chen, I. C. Naggar, C. Guo, B. M. Foxman and J.-Q. Yu, *Angew. Chem Int. Ed.*, 2005, **44**, 7420–7424.

60 P. Liu, J. Han, C. P. Chen, D. Q. Shi and Y. S. Zhao, *RSC Adv.*, 2015, **5**, 28430–28434.

61 B. D. Dangel, J. A. Johnson and D. Sames, *J. Am. Chem. Soc.*, 2001, **123**, 8149–8150.

62 M. C. White, *Science*, 2012, **335**, 807–810.

63 M. S. Chen and M. C. White, *Science*, 2010, **327**, 566–71.

64 S. J. Blanksby and G. B. Ellison, *Acc. Chem. Res.*, 2003, **36**, 255–263.

65 R. Curci, A. Dinoi and M. F. Rubino, *Pure Appl. Chem*, 1995, **67**, 811–822.

66 Z. Yang, P. Yu and K. N. Houk, *J. Am. Chem. Soc.*, 2016, **138**, 4237–4242.

67 R. Curci, A. Dinoi, C. Fusco and M. A. Lillo, *Tetrahedron Lett.*, 1996, **37**, 249–252.

68 A. Bravo, F. Fontana, G. Fronza, F. Minisci and L. Zhao, *J. Org. Chem.*, 1998, **63**, 254–263.

69 R. D. Bach, *J. Phys. Chem. A*, 2016, **120**, 840–850.

70 R. Mello, M. Fiorentino, C. Fusco and R. Curci, *J. Am. Chem. Soc.*, 1989, **111**, 6749–6757.

71 R. Curci, L. D'Accolti and C. Fusco, *Acc. Chem. Res.*, 2006, **39**, 1–9.

72 P. Bovicelli, P. Lupattelli, E. Mincione, T. Prencipe and R. Curci, *J. Org. Chem.*, 1992, **57**, 5052–5054.

73 B. H. Brodsky and J. Du Bois, *J. Am. Chem. Soc.*, 2005, **127**, 15391–15393.

74 S. Lee and P. L. Fuchs, *J. Am. Chem. Soc.*, 2002, **124**, 13978–13979.

75 E. McNeill and J. Du Bois, *J. Am. Chem. Soc.*, 2010, **132**, 10202–10204.

76 C. Kim, K. Chen, J. Kim and L. Que, *J. Am. Chem. Soc.*, 1997, **119**, 5964–5965.

77 T. Okuno, S. Ito, S. Ohba and Y. Nishida, *J. Chem. Soc. Dalt. Trans*, 1997, 3547–3551.

78 K. Chen and L. Que Jr., *Chem. Commun.*, 1999, 1375–1376.

79 M. S. Chen and M. C. White, *Science*, 2007, **318**, 783–787.

80 P. E. Gormisky and M. C. White, *J. Am. Chem. Soc.*, 2013, **135**, 14052–14055.

81 J. C. Lewis, P. S. Coelho and F. H. Arnold, *Chem. Soc. Rev.*, 2011, **40**, 2003–2021.

82 R. Bernhardt, *J. Biotechnol.*, 2006, **124**, 128–145.

83 W. J. H. van Berkel, N. M. Kamerbeek and M. W. Fraaije, *J. Biotechnol.*, 2006, **124**, 670–689.

84 C. D. Reeve, M. A. Carvert and D. J. Hopper, *Biochem. J*, 1990, **269**, 815–819.

85 R. P. Hausinger, *Crit. Rev. Biochem. Mol. Biol.*, 2004, **39**, 21–68.

86 C. Loenarz and C. J. Schofield, *Nat. Chem. Biol.*, 2008, **4**, 152–156.

87 H. Mori, T. Shibasaki, Y. Uozaki, K. Ochiai and A. Ozaki, *Appl Env. Microbiol*, 1996, **62**, 1903–1907.

88 M. H. Sazinsky and S. J. Lippard, *Acc. Chem. Res.*, 2006, **39**, 558–566.

89 D. E. Torres Pazm No, M. Winkler, A. Glieder and M. W. Fraaije, *J. Biotechnol.*, 2010, **146**, 9–24.

References

90 E. Levin, B. Levenberg and S. Kaufman, *J. Biol. Chem.*, 1960, **235**, 2080–2086.

91 Z. Gou, X. H. Xing, M. Luo, H. Jiang, B. Han, H. Wu, L. Wang and F. Zhang, *FEMS Microbiol. Lett.*, 2006, **263**, 136–141.

92 M. Klingenberg, *Arch. Biochem. Biophys.*, 1958, **75**, 376–386.

93 D. Garfinkel, *Arch. Biochem. Biophys.*, 1958, **77**, 493–509.

94 T. Omura and R. Sato, *J. Biol. Chem.*, 1964, **239**, 2370–2378.

95 T. Omura and R. Sato, *J. Biol. Chem.*, 1964, **239**, 2379.

96 P. B. Danielson, *Curr. Drug Metab.*, 2002, **3**, 561–597.

97 D. C. Lamb, L. Lei, A. G. S. Warrilow, G. I. Lepesheva, J. G. L. Mullins, M. R. Waterman and S. L. Kelly, *J. Virol.*, 2009, **83**, 8266–8269.

98 K. Uchida, T. Akashi and T. Aoki, *Plant Biotechnol.*, 2015, **32**, 205–213.

99 R. L. Wright, K. Harris, B. Solow, R. H. White and P. J. Kennelly, *FEBS Lett.*, 1996, 235–239.

100 R. H. Wickramasinghe and C. A. Villee, *Nature*, 1975, **256**, 509–511.

101 I. Hanukoglu, *Adv. Mol. Cell Biol.*, 1996, **14**, 29–56.

102 C. J. C. Whitehouse, S. G. Bell and L. L. Wong, *Chem. Soc. Rev.*, 2012, **41**, 1218–1260.

103 J. Rittle and M. T. Green, *Science*, 2010, **330**, 933–937.

104 F. Ogliaro, S. P. de Visser and S. Shaik, *J. Inorg. Biochem.*, 2002, **91**, 554–567.

105 T. H. Yosca, J. Rittle, C. M. Krest, E. L. Onderko, A. Silakov, J. C. Calixto, R. K. Behan and M. T. Green, *Science*, 2013, **342**, 825–829.

106 R. K. Behan and M. T. Green, *J. Inorg. Biochem.*, 2006, **100**, 448–459.

107 J. A. McIntosh, T. Heel, A. R. Buller, L. Chio and F. H. Arnold, *J. Am. Chem. Soc.*, 2015, **137**, 13861–13865.

108 D. C. Haines, D. R. Tomchick, M. Machius and J. A. Peterson, *Biochemistry*, 2001, **40**, 13456–13465.

109 K. Kumaki, M. Sato, H. Kon and D. W. Nebert, *J. Biol. Chem.*, 1978, **253**, 1048–2058.

110 S. G. Sligar, D. L. Cinti, G. G. Gibson and J. B. Schenkman, *Biochem. Biophys. Res. Commun.*,

1979, **90**, 925–932.

111 F. P. Guengerich, *Biochemistry*, 1983, **22**, 2811–2820.

112 A. Luthra, I. G. Denisove and S. G. Sligar, *Arch. Biochem. Biophys.*, 2011, **507**, 26–35.

113 M. Sono, M. P. Roach, E. D. Coulter and J. H. Dawson, *Chem. Rev.*, 1996, **96**, 2841–2888.

114 S. Shaik, M. Filatov, D. Schröder and H. Schwarz, *Chem. Eur. J.*, 1998, **4**, 193–199.

115 N. Harris, S. Cohen, M. Filatov, F. Ogliaro and S. Shaik, *Angew. Chem Int. Ed.*, 2000, **39**, 2003–2007.

116 H. B. Dunford, A. M. Lambeir, M. A. Kashem and M. Pickard, *Arch. Biochem. Biophys.*, 1987, **252**, 292–302.

117 O. Shoji and Y. Watanabe, *J. Biol. Inorg. Chem.*, 2014, **19**, 529–39.

118 H. Onoda, O. Shoji and Y. Watanabe, *Dalton Trans.*, 2015, **44**, 15316–15323.

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

120 P. D. Josephy, F. P. Guengerich and J. O. Miners, *Drug Metab. Rev.*, 2005, **37**, 575–580.

121 F. P. Guengerich, *Chem. Res. Toxicol.*, 2001, **14**, 611–650.

122 L. S. Kaminsky and Z.-Y. Zhang, *Pharmacol. Ther.*, 1997, **73**, 67–74.

123 D. Projean, P.-E. Morin, T. M. Tu and J. Ducharme, *Xenobiotica*, 2003, **33**, 841–854.

124 D. S. Bhakuni, A. N. Singh, S. Tewari and R. S. Kapil, *J. Chem. Soc. Perkin Trans. 1*, 1977, 1662.

125 W.-M. Chou and T. M. Kutchan, *Plant J.*, 1998, **15**, 289–300.

126 M. R. Waterman and D. S. Keeney, *Horm. Res.*, 1992, **38**, 217–221.

127 I. Hanukoglu, *J. Steroid Biochem. Molec. Biol*, 1992, **43**, 779–804.

128 R. Kaspera and R. Croteau, *Phytochem Rev.*, 2006, **5**, 433–444.

129 J. A. Del Rio, A. Ortuno, D. Garcia-Puig, I. Porras, A. Garcia-Lidon and F. Sabater, *J. Agric. Food Chem.*, 1992, **40**, 1488–1490.

130 M. A. Fraatz, R. G. Berger and H. Zorn, *Appl Microbiol Biotechnol*, 2009, **83**, 35–41.

131 C. Weitzel and H. T. Simonsen, *Phtyochem Rev.*, 2015, **14**, 7–24.

References

132 Y. J. Zhao, Q. Q. Cheng, P. Su, X. Chen, X. J. Wang, W. Gao and L. Q. Huang, *Appl. Microbiol. Biotechnol.*, 2014, 98, 2371–2383.

133 D. D. Stiff, J. T. Robicheau and M. A. Zemaitis, *Xenobiotica*, 1992, **22**, 1–11.

134 C. J. Weitz, K. F. Faull and A. Goldstein, *Nature*, 1987, **330**, 674–677.

135 T. Amann, P. H. Roos, H. Huh and H. Meinhart, *Heterocycles*, 1995, **40**, 425–440.

136 P. S. Vary, R. Biedendieck, T. Fuerch, F. Meinhardt, M. Rohde, W. D. Deckwer and D. Jahn, *Appl. Microbiol. Biotechnol.*, 2007, 76, 957–967.

137 J. Fulco and Y. Miura, *J. Biol. Chem.*, 1974, **249**, 1880–1888.

138 R. S. Hare and A. J. Fulco, *Biochem. Biophys. Res. Commun.*, 1975, **65**, 665–672.

139 A. J. Fulco, *Annu. Rev. Pharmacol. Toxicol.*, 1991, **31**, 177–203.

140 J. H. Capdevila, S. Wei, C. Helvig, J. R. Falck, Y. Belosludtsev, G. Truan, S. E. Graham-Lorence and J. A. Peterson, *J. Biol. Chem.*, 1996, **271**, 22663–22671.

141 L. O. Narhi and A. J. Fulco, *J. Biol. Chem.*, 1986, **261**, 7160–7169.

142 L. O. Narhi and A. J. Fulco, *J. Biol. Chem.*, 1987, **262**, 6683–6690.

143 J. S. Miles, A. W. Munro, B. N. Rospendowski, W. E. Smith, J. Mcknight and A. J. Thomson, *Biochem. J*, 1992, **288**, 503–509.

144 S. S. Boddupalli, R. W. Estabrook and J. A. Peterson, *J. Biol. Chem.*, 1990, **265**, 4233–4239.

145 A. W. Munro, H. M. Girvan and K. J. McLean, *Biochim. Biophys. Acta*, 2007, **1770**, 345–359.

146 S. S. Boddupalli, C. A. Hasemann, K. G. Ravichandran, J.-Y. Lu, E. J. Goldsmith, J. Deisenhofer and J. A. Peterson, *Biochemistry*, 1992, **89**, 5567–5571.

147 K. G. Ravichandran, S. S. Boddupalli, C. A. Hasermann, J. A. Peterson and J. Deisenhofer, *Science*, 1993, **261**, 731–736.

148 C. F. Oliver, S. Modi, M. J. Sutcliffe, W. U. Primrose, L. Y. Lian and G. C. K. Roberts, *Biochemistry*, 1997, **36**, 1567–1572.

149 H. Li and T. L. Poulos, *Nat. Struct. Biol.*, 1997, **4**, 140–146.

150 L. A. Cowart, J. R. Falck and J. H. Capdevila, *Arch. Biochem. Biophys.*, 2001, **387**, 117–124.

151 H. Yeom and S. G. Sligar, *Arch. Biochem. Biophys.*, 1997, **337**, 209–216.

152 J. P. Clark, C. S. Miles, C. G. Mowat, M. D. Walkinshaw, G. A. Reid, S. N. Daff and S. K. Chapman, *J. Inorg. Biochem.*, 2006, **100**, 1075–1090.

153 E. M. J. Gillam, *Chem. Res. Toxicol.*, 2008, **21**, 220–231.

154 A. Celik, D. Sperandio, R. E. Speight and N. J. Turner, *Org. Biomol. Chem.*, 2005, **3**, 2688–2690.

155 M. Dietrich, T. A. Do, R. D. Schmid, J. Pleiss and V. B. Urlacher, *J. Biotechnol.*, 2009, **139**, 115–117.

156 J. D. Bloom, M. M. Meyer, P. Meinhold, C. R. Otey, D. MacMillan and F. H. Arnold, *Curr. Opin. Struct. Biol.*, 2005, 15, 447–452.

157 S. Lutz, *Curr. Opin. Biotechnol.*, 2011, **21**, 734–743.

158 U. Schwaneberg, C. Schmidt-Dannert, J. Schmitt and R. D. Schmid, *Anal. Biochem.*, 1999, **269**, 359–366.

159 B. M. A. Lussenburg, L. C. Babel, N. P. E. Vermeulen and J. N. M. Commandeur, *Anal. Biochem.*, 2005, **341**, 148–155.

160 Q.-S. Li, U. Schwaneberg, P. Fischer and R. D. Schmid, *Chem. Eur. J.*, 2000, **6**, 1531–1536.

161 L. S. Zhang and S. S. Davies, *Genome Med.*, 2016, **8**, 46–64.

162 Q. S. Li, J. Ogawa, R. D. Schmid and S. Shimizu, *Appl Env. Microbiol*, 2001, **67**, 5735–5739.

163 F. H. Arnold and G. Georgiou, in *Methods in Molecular Biology*, Humana Press, New Jersey, 2003, vol. 230.

164 K. Lan Tee and U. Schwaneberg, *Comb. Chem. High Throughput Screen.*, 2007, **10**, 197–217.

165 G.-D. Roiban and M. T. Reetz, *Chem Commun*, 2015, **51**, 2208–2224.

166 S. T. Jung, R. Lauchli and F. H. Arnold, *Curr. Opin. Biotechnol.*, 2011, **22**, 809–817.

167 S. A. Maves, H. Yeom, M. A. McLean and S. G. Sligar, *FEBS Lett.*, 1997, **414**, 213–218.

168 A. Glieder, E. T. Farinas and F. H. Arnold, *Nat. Biotechnol.*, 2002, **20**, 1135–1139.

169 M. W. Peters, P. Meinhold, A. Glieder and F. H. Arnold, *J. Am. Chem. Soc.*, 2003, **125**, 13442–13450.

170 M. Landwehr, L. Hochrein, C. R. Otey, A. Kasrayan, J. E. Bäckvall and F. H. Arnold, *J. Am. Chem. Soc.*, 2006, **128**, 6058–6059.

171 F. E. Zilly, J. P. Acevedo, W. Augustyniak, A. Deege and M. T. Reetz, *Angew. Chem Int. Ed.*, 2011, **50**, 2720–2724.

172 N. Kawakami, O. Shoji and Y. Watanabe, *Chem. Sci.*, 2013, **4**, 2344–2348.

173 Z. Cong, O. Shoji, C. Kasai, N. Kawakami, H. Sugimoto, Y. Shiro and Y. Watanabe, *ACS Catal.*, 2015, **5**, 150–156.

174 M. T. Reetz, *Angew. Chem Int. Ed.*, 2011, 50, 138–174.

175 S. Kille, F. E. Zilly, J. P. Acevedo and M. T. Reetz, *Nat. Chem.*, 2011, **3**, 738–743.

176 S. Graham-Lorence, G. Truan, J. A. Peterson, J. R. Falck, S. Wei, C. Helvig and J. H. Capdevila, *J. Biol. Chem.*, 1997, **272**, 1127–1135.

177 S. A. Maves, H. Yeom, M. A. McLean and S. G. Sligar, *FEBS Lett.*, 1997, **414**, 213–218.

178 C. F. Oliver, S. Modi, W. U. Primrose, L. Y. Lian and G. C. Roberts, *Biochem. J*, 1997, **327**, 537–544.

179 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**, 371–379.

180 A. B. Carmichael and L. L. Wong, *Eur. J. Biochem.*, 2001, **268**, 3117–3125.

181 C. J. C. Whitehouse, S. G. Bell, H. G. Tufton, R. J. P. Kenny, L. C. I. Ogilvie and L. L. Wong, *Chem Commun*, 2008, 966–968.

182 C. J. C. Whitehouse, N. H. Rees, S. G. Bell and L. L. Wong, *Chem. Eur. J.*, 2011, **17**, 6862–6868.

183 V. B. Urlacher, A. Makhsumkhanov and R. D. Schmid, *Appl. Microbiol. Biotechnol.*, 2006, **70**, 53–59.

184 Y. Watanabe, S. Laschat, M. Budde, O. Affolter, Y. Shimada and V. B. Urlacher, *Tetrahedron*, 2007, **63**, 9413–9422.

185 J. A. Dietrich, Y. Yoshikuni, K. J. Fisher, F. X. Woolard, D. Ockey, D. J. McPhee, N. S. Renninger, M. C. Y. Chang, D. Baker and J. D. Keasling, *ACS Chem. Biol.*, 2009, **4**, 261–267.

186 T. W. B. Ost, C. S. Miles, J. Murdoch, Y. F. Cheung, G. A. Reid, S. K. Chapman and A. W. Munro, *FEBS Lett.*, 2000, **486**, 173–177.

187 W. C. Huang, A. C. G. Westlake, J. D. Maréchal, M. G. Joyce, P. C. E. Moody and G. C. K. Roberts, *J. Mol. Biol.*, 2007, **373**, 633–651.

188 W.-C. Huang, P. M. Cullis, E. L. Raven and G. C. K. Roberts, *Metallomics*, 2011, **3**, 410–416.

189 C. J. C. Whitehouse, S. G. Bell and L. L. Wong, *Chem. Eur. J.*, 2008, **14**, 10905–10908.

190 C. J. C. Whitehouse, W. Yang, J. A. Yorke, B. C. Rowlatt, A. J. F. Strong, C. F. Blanford, S. G. Bell, M. Bartlam, L. L. Wong and Z. Rao, *ChemBioChem*, 2010, **11**, 2549–2556.

191 E. W. Svastits, J. H. Dawson, R. Breslow and S. H. Gellman, *J. Am. Chem. Soc.*, 1985, **107**, 6427–6428.

192 P. S. Coelho, E. M. Brustad, A. Kannan and F. H. Arnold, *Science*, 2013, **339**, 307–310.

193 P. S. Coelho, Z. J. Wang, M. E. Ener, S. A. Baril, A. Kannan, F. H. Arnold and E. M. Brustad, *Nat. Chem. Biol.*, 2013, **9**, 485–487.

194 J. A. McIntosh, P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown and F. H. Arnold, *Angew. Chem Int. Ed.*, 2013, **52**, 9309–9312.

195 Z. J. Wang, H. Renata, N. E. Peck, C. C. Farwell, P. S. Coelho and F. H. Arnold, *Angew. Chem Int. Ed.*, 2014, **53**, 6810–6813.

196 R. Singh, M. Bordeaux and R. Fasan, *ACS Catal.*, 2014, **4**, 546–552.

197 R. Singh, J. N. Kolev, P. A. Sutera and R. Fasan, *ACS Catal.*, 2015, **5**, 1685–1691.

198 P. C. Cirino, Y. Tang, K. Takahashi, D. A. Tirrell and F. H. Arnold, *Biotechnol. Bioeng.*, 2003, **83**, 729–734.

199 J. N. Kolev, J. M. Zaengle, R. Ravikumar and R. Fasan, *ChemBioChem*, 2014, **15**, 1001–1010.

200 E. W. Reynolds, M. W. McHenry, F. Cannac, J. G. Gober, C. D. Snow and E. M. Brustad, *J. Am. Chem. Soc.*, 2016, **138**, 12451–12458.

201 S. T. Jung, R. Lauchli and F. H. Arnold, *Curr. Opin. Biotechnol.*, 2011, 22, 809–817.

202 E. O'Reilly, V. Köhler, S. L. Flitsch and N. J. Turner, *Chem. Commun.*, 2011, **47**, 2490–501.

References

203 V. B. Urlacher and M. Girhard, *Trends Biotechnol.*, 2012, **30**, 26–36.

204 K. L. Davis-Bruno and A. Atrakchi, *Chem. Res. Toxicol.*, 2006, **19**, 1561–1563.

205 C. R. Otey, G. Bandara, J. Lalonde, K. Takahashi and F. H. Arnold, *Biotechnol. Bioeng.*, 2006, **93**, 494–499.

206 X. Ren, J. A. Yorke, E. Taylor, T. Zhang, W. Zhou and L. L. Wong, *Chem. Eur. J.*, 2015, **21**, 15039–15047.

207 H. Venkataraman, M. C. A. Verkade-Vreeker, L. Capoferri, D. P. Geerke, N. P. E. Vermeulen and J. N. M. Commandeur, *Bioorg. Med. Chem.*, 2014, **22**, 5613–5620.

208 G. Di Nardo and G. Gilardi, *Int. J. Mol. Sci.*, 2012, **13**, 15901–15924.

209 A. Rentmeister, T. R. Brown, C. D. Snow, M. N. Carbone and F. H. Arnold, *ChemCatChem*, 2011, **3**, 1065–1071.

210 A. M. Sawayama, M. M. Y. Chen, P. Kulanthaivel, M. S. Kuo, H. Hemmerle and F. H. Arnold, *Chem. Eur. J.*, 2009, **15**, 11723–11729.

211 M. C. Damsten, B. M. A. van Vugt-Lussenburg, T. Zeldenthuis, J. S. B. de Vlieger, J. N. M. Commandeur and N. P. E. Vermeulen, *Chem. Biol. Interact.*, 2008, **171**, 96–107.

212 B. M. A. van Vugt-Lussenburg, E. Stjernschantz, J. Lastdrager, C. Oostenbrink, N. P. E. Vermeulen and J. N. M. Commandeur, *J. Med. Chem.*, 2007, **50**, 455–461.

213 C. F. Butler, C. Peet, K. J. McLean, M. T. Baynham, R. T. Blankley, K. Fisher, S. E. J. Rigby, D. Leys, M. W. Voice and A. W. Munro, *Biochem. J.*, 2014, **460**, 247–59.

214 H. Venkataraman, E. M. te Poele, K. Z. Rosłoniec, N. P. E. Vermeulen, J. N. M. Commandeur, R. van der Geize and L. Dijkhuizen, *Appl. Microbiol. Biotechnol.*, 2014, **99**, 4713–4721.

215 K. Zhang, B. M. Shafer, M. D. Demars, H. A. Stern and R. Fasan, *J. Am. Chem. Soc.*, 2012, **134**, 18695–18704.

216 P. M. O'Neill and G. H. Posner, *J. Med. Chem.*, 2004, 47, 2945–2964.

217 A. Rentmeister, F. H. Arnold and R. Fasan, *Nat. Chem. Biol.*, 2009, **5**, 26–28.

218 J. A. Dietrich, Y. Yoshikuni, K. J. Fisher, F. X. Woolard, D. Ockey, D. J. McPhee, N. S. Renninger,

M. C. Y. Chang, D. Baker and J. D. Keasling, *ACS Chem. Biol.*, 2009, **4**, 261–267.

219 N. Acton and R. J. Roth, *J. Org. Chem.*, 1992, **57**, 3610–3614.

220 R. J. Sowden, S. Yasmin, N. H. Rees, S. G. Bell and L.-L. Wong, *Org. Biomol. Chem.*, 2005, **3**, 57–64.

221 K. Zhang, S. El Damaty and R. Fasan, *J. Am. Chem. Soc.*, 2011, **133**, 3242–3245.

222 C. K. J. Chen, R. E. Berry, T. K. Shokhireva, M. B. Murataliev, H. Zhang and F. A. Walker, *J. Biol. Inorg. Chem.*, 2010, **15**, 159–174.

223 L. C. Chiou, J. Y. Ling and C. C. Chang, *Eur. J. Pharmacol. Environ. Toxicol.*, 1995, **292**, 151–156.

224 N. Misawa, M. Nodate, T. Otomatsu, K. Shimizu, C. Kaido, M. Kikuta, A. Ideno, H. Ikenaga, J. Ogawa, S. Shimizu and K. Shindo, *Appl. Microbiol. Biotechnol.*, 2011, **90**, 147–157.

225 T. Wriessnegger, P. Augustin, M. Engleder, E. Leitner, M. Müller, I. Kaluzna, M. Schürmann, D. Mink, G. Zellnig, H. Schwab and H. Pichler, *Metab. Eng.*, 2014, **24**, 18–29.

226 M. Furusawa, T. Hashimoto, Y. Noma and Y. Asakawa, *Chem. Pharm. Bull*, 2005, **53**, 1513–1514.

227 R. J. Sowden, S. Yasmin, N. H. Rees, S. G. Bell and L. L. Wong, *Org. Biomol. Chem.*, 2005, **3**, 57–64.

228 Y. Cao and L. L. Wong, *Unpublished Results*, 2016.

229 R. Agudo, G. D. Roiban and M. T. Reetz, *ChemBioChem*, 2012, **13**, 1465–1473.

230 G. D. Roiban, R. Agudo and M. T. Reetz, *Tetrahedron*, 2013, **69**, 5306–5311.

231 G.-D. Roiban, R. Agudo and M. T. Reetz, *Angew. Chem*, 2014, **126**, 8803–8807.

232 G.-D. Roiban, R. Agudo, A. Ilie, R. Lonsdale and M. T. Reetz, *Chem Commun*, 2014, **50**, 14310–14313.

233 A. Ilie, R. Agudo, G. D. Roiban and M. T. Reetz, *Tetrahedron*, 2015, **71**, 470–475.

234 R. Agudo, G. D. Roiban, R. Lonsdale, A. Ilie and M. T. Reetz, *J. Org. Chem.*, 2015, **80**, 950–956.

235 A. Li, A. Ilie, Z. Sun, R. Lonsdale, J.-H. Xu and M. T. Reetz, *Angew. Chem*, 2016, **55**, 12026–12029.

236 C. A. Denard, M. J. Bartlett, Y. Wang, L. Lu, J. F. Hartwig and H. Zhao, *ACS Catal.*, 2015, **5**, 3817–3822.

237 C. J. C. Whitehouse, S. G. Bell, W. Yang, J. A. Yorke, C. F. Blanford, A. J. F. Strong, E. J. Morse, M. Bartlam, Z. Rao and L. L. Wong, *ChemBioChem*, 2009, **10**, 1654–1656.

238 C. J. C. Whitehouse, W. Yang, J. A. Yorke, H. G. Tufton, L. C. I. Ogilvie, S. G. Bell, W. Zhou, M. Bartlam, Z. Rao and L. L. Wong, *Dalton Trans.*, 2011, **40**, 10383–10396.

239 *19th WHO Model List of Essential Medicines*, 2015.

240 R. Kawai, S. Fujita and T. Suzuki, *J. Pharm. Sci.*, 1985, **74**, 1219–1224.

241 Y. Oda, S. Imaoka, Y. Nakahira, A. Asada, M. Fujimori, S. Fujita and Y. Funae, *Biochem. Pharmacol.*, 1989, **38**, 4439–4444.

242 S. Imaoka, K. Enomoto, Y. Oda, A. Asada, M. Fujimori, T. Shimada, S. Fujita, F. P. Guengerich and Y. Funae, *J. Pharmacol. Exp. Ther.*, 1990, **255**, 1385–1391.

243 G. D. Breck and W. F. Trager, *Science*, 1971, **173**, 544–546.

244 S. D. Nelson, G. D. Breck and W. F. Trager, *J. Med. Chem.*, 1973, **16**, 1106–1112.

245 A. R. Katritzky, H. He and J. Wang, *J. Org. Chem.*, 2002, **67**, 4951–4956.

246 O. Tsuge, H. Watanabe, K. Masuda and M. M. Yousif, *J. Org. Chem.*, 1979, **44**, 4543–4547.

247 M. K. Sinha, K. Khoury, E. Herdtweck and A. Dömling, *Org. Biomol. Chem.*, 2013, **11**, 4792–4796.

248 L. Vasvari-Debreczy, A. H. Beckett and W. Vutthikongsirigool, *Tetrahedron*, 1981, **37**, 4337–4342.

249 M. Pinza, C. Farina, A. Cerri, U. Pfeiffer, M. T. Riccaboni, S. Banfi, R. Biagetti, O. Pozzi, M. Magnani and L. Dorigotti, *J. Med. Chem.*, 1993, **36**, 4214–4220.

250 M. Iwami, O. Nakayama, H. Terano, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1987, **40**, 612–622.

251 M. Ziółkowski, Z. Czarnocki, A. Leniewski and J. K. Maurin, *Tetrahedron: Asymmetry*, 1999, **10**, 3371–3380.

252 US4920116, 1990.

253 E. Miserazzi, M. A. Spotti, R. Profeta, S. Spada, A. Nalin, E. Moro and D. Andreotti, *Tetrahedron Lett.*, 2011, **52**, 448–452.

254 K. B. Wiberg and D. J. Rush, *J. Am. Chem. Soc.*, 2001, **123**, 2038–2046.

255 P. D. Morse and D. A. Nicewicz, *Chem. Sci.*, 2014, **6**, 270–274.

256 W. Walter, M. Steffen and K. Heyns, *Chem. Ber.*, 1961, **94**, 2462–2469.

257 W. Walter, *Angew. Chem Int. Ed.*, 1967, **6**, 468–468.

258 G. Han, M. C. McIntosh and S. M. Weinreb, *Tetrahedron Lett.*, 1994, **35**, 5813–5816.

259 Q. Chen, J. Zhou, Y. Wang, C. Wang, X. Liu, Z. Xu, L. Lin and R. Wang, *Org. Lett.*, 2015, **17**, 4212–4215.

260 E. Shirakawa, T. Yoneda, K. Moriya, K. Ota, N. Uchiyama, R. Nishikawa and T. Hayashi, *Chem. Lett.*, 2011, **40**, 1041–1043.

261 L. Ma, W. Chen and D. Seidel, *J. Am. Chem. Soc.*, 2012, **134**, 15305–15308.

262 G. Lahm and T. Opatz, *Org. Lett.*, 2014, **16**, 4201–4203.

263 Y. Kang, W. Chen, M. Breugst and D. Seidel, *J. Org. Chem.*, 2015, **80**, 9628–9640.

264 E. Stjernschantz, B. M. A. van Vugt-Lussenburg, A. Bonifacio, S. B. A. De Beer, G. Van Der Zwan, C. Gooijer, J. N. M. Commandeur, N. P. E. Vermeulen and C. Oostenbrink, *Proteins Struct. Funct. Bioinforma.*, 2008, **71**, 336–352.

265 X. Ren, J. A. O'Hanlon, M. Morris, J. Robertson and L. L. Wong, *ACS Catal.*, 2016, **6**, 6833–6837.

266 R. N. Austin, D. Deng, Y. Jiang, K. Luddy, J. B. Van Beilen, P. R. Ortiz De Montellano and J. T. Groves, *Angew. Chem Int. Ed.*, 2006, **45**, 8192–8194.

267 D. Griller and K. U. Ingold, *Acc. Chem. Res.*, 1976, **9**, 13–19.

268 V. W. Bowry and K. U. Ingold, *J. Am. Chem. Soc.*, 1991, **113**, 5699–5707.

269 K. Auclair, Z. Hu, D. M. Little, P. R. Ortiz de Montellano and J. T. Groves, *J. Am. Chem. Soc.*, 2002, **124**, 6020–6027.

270 M. Newcomb, R. Shen, S. Y. Choi, P. H. Toy, P. F. Hollenberg, A. D. N. Vaz and M. J. Coon, *J. Am.*

Chem. Soc., 2000, **122**, 2677–2686.

271 R. K. Boeckman, J. P. Sabatucci, S. W. Goldstein, D. M. Springer and P. F. Jackson, *J. Org. Chem.*, 1986, **51**, 3740–3742.

272 M. Hannoun, M. Žinić, D. Kolbah, F. Kajfež and N. Blažević, *J. Heterocyclic Chem.*, 1981, **18**, 963–965.

273 R. Fitzi and D. Seebach, *Tetrahedron*, 1988, **44**, 5277–5292.

274 R. Poltt and D. Seebach, *J. Am. Chem. Soc.*, 1989, **111**, 2622–2632.

275 D. M. Shendage, R. Frohlich, K. Bergander and G. Haufe, *Eur J. Org. Chem.*, 2005, **2005**, 719–727.

276 W. Schlenk and W. Schlenk, *Chem. Ber.*, 1929, **62**, 920–924.

277 S. Zhang, T. Govender, T. Norström and P. I. Arvidsson, *J. Org. Chem.*, 2005, **70**, 6918–6920.

278 H. Mayr, B. Kempf and A. R. Ofial, *Acc. Chem. Res.*, 2003, **36**, 66–77.

279 K. George, M. Jura, W. Levason, M. E. Light and G. Reid, *Dalton Trans.*, 2014, **43**, 3637–3648.

280 K. C. Nicolaou, Y. Wang, M. Lu, D. Mandal, M. R. Pattanayak, R. Yu, A. A. Shah, J. S. Chen, H. Zhang, J. J. Crawford, L. Pasunoori, Y. B. Poudel, N. S. Chowdari, C. Pan, A. Nazeer, S. Gangwar, G. Vite and E. N. Pitsinos, *J. Am. Chem. Soc.*, 2016, **138**, 8235–8246.

281 A. G. Myers, M. E. Fraley, N. J. Tom, S. B. Cohen and D. J. Madar, *Chem. Biol.*, 1995, **2**, 33–43.

282 M. D. Shair, T. Y. Yoon, K. K. Mosny, T. C. Chou and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1996, **118**, 9509–9525.

283 O. Hara, K. Sugimoto and Y. Hamada, *Tetrahedron*, 2004, **60**, 9381–9390.

284 E. E. Boros, I. Kaldor and P. S. Turnbull, *J. Heterocyclic Chem.*, 2011, **48**, 733–736.

285 I. Sosič, H. Barreteau, M. Simčič, R. Šink, J. Cesar, A. Zega, S. G. Grdadolnik, C. Contreras-Martel, A. Dessen, A. Amoroso, B. Joris, D. Blanot and S. Gobec, *Eur. J. Med. Chem.*, 2011, **46**, 2880–2894.

286 C.-W. Yang, Y.-X. Bai, N.-T. Zhang, C.-C. Zeng, L.-M. Hu and H.-Y. Tian, *Tetrahedron*, 2012, **68**, 10201–10208.

287 S. K. Jackson, S. C. Banfield and M. A. Kerr, *Org. Lett.*, 2005, **7**, 1215–1218.

288 M. D. Ganton and M. A. Kerr, *J. Org. Chem.*, 2007, **72**, 574–582.

289 K. A. Parker and T. L. Mindt, *Org. Lett.*, 2002, **4**, 4265–4268.

290 US 7887887B2, 2008.

291 T. B. Shah, R. S. Shiny, R. B. Dixit and B. C. Dixit, *J. Saudi Chem. Soc.*, 2014, **18**, 985–992.

292 A. Tsai, M.-J. Hsu and K. K. Wu, *Biochim. Biophys. Acta* , 1989, **993**, 74–82.

293 S. Yan, R. Huang, C. Wang, Y. Zhou, J. Wang, B. Fu, X. Weng and X. Zhou, *Chem. Asian J.*, 2012, **7**, 2782–2785.

294 Toshiro Konoike, Katsuo Oda, A. Masaaki Uenaka and K. Takahashi, *Org. Process Res. Dev.*, 1999, **3**, 347–351.

295 D. Ménard, I. Niculescu-Duvaz, H. P. Dijkstra, D. Niculescu-Duvaz, B. M. J. M. Suijkerbuijk, A. Zambon, A. Nourry, E. Roman, L. Davies, H. A. Manne, F. Friedlos, R. Kirk, S. Whittaker, A. Gill, R. D. Taylor, R. Marais and C. J. Springer, *J. Med. Chem.*, 2009, **52**, 3881–3891.

296 C. Dugave and P. Kessler, *Tetrahedron Lett.*, 1994, **35**, 9557–9560.

297 Y. Sugano and J. A. Katzenellenbogen, *Bioorganic Med. Chem. Lett.*, 1996, **6**, 361–366.

298 L. Zhu, Z. Miao, C. Sheng, W. Guo, J. Yao, W. Liu, X. Che, W. Wang, P. Cheng and W. Zhang, *Eur. J. Med. Chem.*, 2010, **45**, 2726–2732.

299 W. Guo, W. Liu, L. Zhu, Y. Zhang, P. Cheng, G. Dong, C. Zhuang, J. Yao, C. Sheng, Z. Miao and W. Zhang, *Chem. Biodivers.*, 2011, **8**, 1539–1549.

300 G. V. De Lucca, U. T. Kim, C. Johnson, B. J. Vargo, P. K. Welch, M. Covington, P. Davies, K. A. Solomon, R. C. Newton, G. L. Trainor, C. P. Decicco and S. S. Ko, *J. Med. Chem.*, 2002, **45**, 3794–3804.

301 R. W. Hoffmann, *Synthesis.*, 2006, 21, 3531–3541.

302 I. S. Young and P. S. Baran, *Nat. Chem.*, 2009, **1**, 193–205.

303 S. M. Inamdar, V. K. More and S. K. Mandal, *Tetrahedron Lett.*, 2013, **54**, 530–532.

304 S. Bombek, F. Požgan, M. Kočevar and S. Polanc, *J. Org. Chem.*, 2004, **69**, 2224–2227.

References

305 J.-C. Jacquesy, M.-P. Jouannetaud, G. Morellet and Y. Vidal, *Tetrahedron Lett.*, 1984, **25**, 1479–1482.

306 P. B. Kokil, S. D. Patil, T. Ravindranathan and P. M. Nair, *Tetrahedron Lett.*, 1979, **20**, 989–992.

307 N. Itoh, T. Sakamoto, E. Miyazawa and Y. Kikugawa, *J. Org. Chem.*, 2002, **67**, 7424–7428.

308 H. Liu, X. Wang and Y. Gu, *Org. Biomol. Chem.*, 2011, **9**, 1614–20.

309 A. Pialat, B. Liégault and M. Taillefer, *Org. Lett.*, 2013, **15**, 1764–1767.

310 S. Wang, Z. Ni, Y. Wang, Y. Pang and Y. Pan, *Tetrahedron*, 2014, **70**, 6879–6884.

311 J. M. Wiśniewska-knypl and J. K. Jabłońska, *Xenobiotica*, 1975, **5**, 511–519.

312 G. Di Nardo, A. Fantuzzi, A. Sideri, P. Panicco, C. Sassone, C. Giunta and G. Gilardi, *J. Biol. Inorg. Chem.*, 2007, **12**, 313–323.

313 Y. Li and L. L. Wong, *Unpublished Results*, 2016.

314 J. Reinen, G. Vredenburg, K. Klaering, N. P. E. Vermeulen, J. N. M. Commandeur, M. Honing and J. C. Vos, *J. Mol. Catal. B Enzym.*, 2015, **121**, 64–74.

315 EP2336104 A4, 2011.

316 K. S. L. Chan, M. Wasa, L. Chu, B. N. Laforteza, M. Miura and J.-Q. Yu, *Nat. Chem.*, 2014, **6**, 146–150.

317 R. Ballini, E. Marcantoni and M. Petrini, *Tetrahedron Lett.*, 1992, **33**, 4835–4838.

318 J. Biochem and F. Fukuoka, *J. Biochem.*, 1980, **87**, 1539–1554.

319 S. P. De Visser and S. Shaik, *J. Am. Chem. Soc.*, 2003, **125**, 7413–7424.

320 M. Asaka and H. Fujii, *J. Am. Chem. Soc.*, 2016, **138**, 8048–8051.

321 G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop and S. Udenfriend, *Science*, 1967, **157**, 1524.

322 D. M. Jerina and J. W. Daly, *Science*, 1974, **185**, 573–582.

323 J. W. Daly, D. M. Jerina and B. Witkop, *Experientia*, 1972, **28**, 1129–1149.

324 D. R. Boyd, J. W. Daly and D. M. Jerina, *Biochemistry*, 1972, **11**, 1961–1966.

325 S. Iwasaki, O. Yonemitsu, Y. Okuno, Y. Kanaoka and B. Witkop, *J. Am. Chem. Soc.*, 1968, **90**, **288**

6523–6525.

326 H. Sato and F. P. Guengerich, *J. Am. Chem. Soc.*, 2000, 122, 8099–8100.

327 K. R. Korzekwa, D. C. Swinney and W. F. Trager, *Biochemistry*, 1989, **28**, 9019–9027.

328 J. E. Tomaszewski, D. M. Jerina and J. W. Daly, *Biochemistry*, 1975, **14**, 2024–2031.

329 R. P. Hanzlik and K. J. Ling, *J. Am. Chem. Soc.*, 1993, **115**, 93639370.

330 T. Cernak, K. D. Dykstra, S. Tyagarajan, P. Vachal and S. W. Krska, *Chem. Soc. Rev.*, 2016, **45**, 546–576.

331 B. Yang, G. Y. Chen, X. P. Song, L. Q. Yang, X. Y. Wu, C. R. Han, W. H. Chen, B. Y. Zou and X. M. Li, *Bioorganic Med. Chem. Lett.*, 2013, **23**, 5748–5751.

332 B. D. Lin, M. L. Han, Y. C. Ji, H. D. Chen, S. P. Yang, S. Zhang, M. Y. Geng and J. M. Yue, *J. Nat. Prod.*, 2010, **73**, 1301–1305.

333 B. Yang, G.-Y. Chen, X.-P. Song, L.-Q. Yang, C.-R. Han, X.-Y. Wu, C.-J. Zheng, X. Ran and R.-F. Tang, *Tetrahedron Lett.*, 2013, **54**, 6434–6438.

334 M. H. Tankard and J. S. Whitehurst, *Tetrahedron*, 1974, **30**, 451–454.

335 S. P. Chavan, P. K. Zubaidha and N. R. Ayyangar, *Tetrahedron Lett.*, 1992, **33**, 4605–4608.

336 E. Krawczyk, M. Koprowski and J. Luczak, *Tetrahedron: Asymmetry*, 2007, **18**, 1780–1787.

337 D. W. Knight, *Contemp. Org. Synth.*, 1994, **1**, 287–315.

338 T. Montagnon, D. Noutsias, I. Alexopoulou, M. Tofi and G. Vassilikogiannakis, *Org. Biomol. Chem.*, 2011, **9**, 2031.

339 B. Feringa, *Recuel des Trav. Chim. des Pays-Bas*, 1987, **106**, 469–488.

340 T. Montagnon, D. Kalaitzakis, M. Triantafyllakis, M. Stratakis and G. Vassilikogiannakis, *Chem Commun*, 2014, **50**, 15480–15498.

341 J. C. Lee, S. J. Jin and J. K. Cha, *J. Org. Chem.*, 1998, **63**, 2804–2805.

342 J. C. Lee and J. K. Cha, *Tetrahedron*, 2000, **56**, 10175–10184.

343 M. Cano, C. Rojas, W. Hidalgo, J. Sáez, J. Gil, B. Schneider and F. Otálvaro, *Tetrahedron Lett.*, 2013, **54**, 351–354.

344 D. Pflästerer, S. Schumacher, M. Rudolph and A. S. K. Hashmi, *Chem. Eur. J.*, 2015, **21**, 11585–11589.

345 S. D. Munday, O. Shoji, Y. Watanabe, L. L. Wong and S. G. Bell, *Chem Commun*, 2015, **52**, 1036–1039.

346 G. A. Kraus and X. Wang, *Synth. Commun.*, 1998, **28**, 1093–1096.

347 S. P. H. Mee, V. Lee, J. E. Baldwin and A. Cowley, *Tetrahedron*, 2004, **60**, 3695–3712.

348 C. Alvarez-lbarra, M. L. Quiroga and E. Toledano, *Tetrahedron*, 1996, **52**, 4065–4078.

349 P. L. Beaulieu, J. Gillard, D. Bykowski, C. Brochu, N. Dansereau, J. S. Duceppe, B. Haché, A. Jakalian, L. Lagacé, S. LaPlante, G. McKercher, E. Moreau, S. Perreault, T. Stammers, L. Thauvette, J. Warrington and G. Kukolj, *Bioorganic Med. Chem. Lett.*, 2006, **16**, 4987–4993.

350 A. Blanc, K. Tenbrink, J. M. Weibel and P. Pale, *J. Org. Chem.*, 2009, **74**, 4360–4363.

351 T. Chatterjee, R. Dey and B. C. Ranu, *New J. Chem.*, 2011, **35**, 1103.

352 M. H. Shinde and U. A. Kshirsagar, *Green Chem.*, 2015, **18**, 1455–1458.

353 Q. Jiang, W. Sheng and C. Guo, *Green Chem.*, 2013, **15**, 2175.

354 A. M. Sanjuán, A. Martínez, P. García-García, M. A. Fernández-Rodríguez and R. Sanz, *Beilstein J. Org. Chem.*, 2013, **9**, 2242–2249.

355 C. Wang, T. Tobrman, Z. Xu and E. I. Negishi, *Org. Lett.*, 2009, **11**, 4092–4095.

356 K. F. Eidman and P. J. Nichols, in *Encyclopedia of Reagents for Organic Synthesis*, John Wiley & Sons, Ltd, Chichester, UK, 2004, pp. 1–5.

357 S. P. Tanis and P. M. Herrinton, *J. Org. Chem.*, 1985, **50**, 3988–3996.

358 H. Burton, *J. Chem. Soc.*, 1929, 455–458.

359 J. Kenyon, S. M. Partridge and H. Phillips, *J. Chem. Soc.*, 1937, 207–218.

360 R. Dehn, Y. Katsuyama, A. Weber, K. Gerth, R. Jansen, H. Steinmetz, G. Höfle, R. Müller and A. Kirschning, *Angew. Chem Int. Ed.*, 2011, **50**, 3882–3887.

361 H. Le, A. Batten and J. P. Morken, *Org. Lett.*, 2014, **16**, 2096–2099.

362 F.-Q. Yuan, L.-X. Gao and F.-S. Han, *Chem. Commun.*, 2011, **47**, 5289–5291.

363 S. P. Tanis and P. M. Herrinton, *J. Org. Chem.*, 1983, **48**, 4572–4580.

364 E. J. Corey and R. A. E. Winter, *J. Am. Chem. Soc.*, 1963, **85**, 2677–2678.

365 Y. Hagooly and S. Rozen, *J. Org. Chem.*, 2008, **73**, 6780–6783.

366 I. Shiina and M. Hashizume, *Tetrahedron*, 2006, **62**, 7934–7939.

367 C. Tindall and D. Horton, *J. Org. Chem.*, 1970, 35, 3558–3559.

368 E. J. Corey, F. A. Carey and R. A. E. Winter, *J. Am. Chem. Soc.*, 1965, **87**, 934.

369 M. E. Squillacote, J. DeFellipis and Q. Shu, *J. Am. Chem. Soc.*, 2005, **127**, 15983–15988.

370 P. J. Garegg and B. Samuelsson, *Synthesis.*, 1979, **6**, 469–470.

371 H. Ohrui, K. Nagihashi and H. Meguro, *Biosci. Biotechnol. Biochem.*, 1992, **56**, 1721–1724.

372 N. J. Kishner, *J. Russ. Phys. Chem. Soc.*, 1911, **43**, 582.

373 L. Wolff, *Justus Liebig's Ann. der Chemie*, 1912, **394**, 86–108.

374 B. Siminszky, *Phytochem. Rev.*, 2006, **5**, 445–458.

375 G. Bellucci, C. Chiappe, L. Pucci and P. G. Gervasi, *Chem. Res. Toxicol.*, 1996, **9**, 871–874.

376 E. M. J. Gillam, L. M. Notley, H. Cai, J. J. De Voss and F. P. Guengerich, *Biochemistry*, 2000, **39**, 13817–13824.

377 S. F. El-Naggar, R. W. Creekmore, M. J. Schocken, R. T. Rosen and R. A. Robinson, *J. Agric. Food Chem.*, 1992, **40**, 880–883.

378 H. Yasuor, W. Zou, V. V Tolstikov, R. S. Tjeerdema and A. J. Fischer, *Plant Physiol.*, 2010, **153**, 319–326.

379 Y. Ferhatoglu, S. Avdiushko and M. Barrett, *Pestic. Biochem. Physiol.*, 2005, **81**, 59–70.

380 S. Y. Liu, M. Shocken and J. P. N. Rosazza, *J. Agric. Food Chem.*, 1996, **44**, 313–319.

381 R. J. F. Branco, A. Seifert, M. Budde, V. B. Urlacher, M. J. Ramos and J. Pleiss, *Proteins Struct. Funct. Bioinforma.*, 2008, **73**, 597–607.

382 M. W. Peters, P. Meinhold, A. Glieder and F. H. Arnold, *J. Am. Chem. Soc.*, 2003, **125**, 13442–13450.

383 Z. Mehmood, D. E. Kelly and S. L. Kelly, *Chemosphere*, 1995, **31**, 4515–4529.

References

384 R. Kebeish, E. Azab, C. Peterhaensel and R. El-Basheer, *Environ. Sci. Pollut. Res.*, 2014, **21**, 8224–8232.

385 N. Shiota, H. Inui and H. Ohkawa, *Pestic. Biochem. Physiol.*, 1996, **54**, 190–198.

386 R. Fonné-Pfister and K. Kreuz, *Phytochemistry*, 1990, **29**, 2793–2796.

387 I. Nagy, G. Schoofs, F. Compernolle, P. Proost, J. Vanderleyden and R. De Mot, *J. Bacteriol.*, 1995, **177**, 676–687.

388 Y. S. Chen and J. E. Casida, *J. Agric. Food. Chem.*, 1978, **26**, 263–267.

389 R. M. Behki and S. U. Khan, *Chemosphere*, 1990, **21**, 1457–1463.

390 A. Mine and S. Matsunaka, *Pestic. Biochem. Physiol.*, 1975, **5**, 444–450.

391 T. M. Sterling and N. E. Balke, *Pestic. Biochem. Physiol.*, 1989, **34**, 39–48.

392 T. M. Sterling and N. E. Balke, *Pestic. Biochem. Physiol.*, 1990, **38**, 66–75.

393 J. Rouchaud, O. Neus, H. Eelen and R. Bulcke, *Arch. Environ. Contam. Toxicol.*, 2002, **42**, 280–285.

394 R. N. Lerch, C. H. Lin and N. D. Leigh, *J. Agric. Food Chem.*, 2007, **55**, 1893–1899.

395 K. E. Pallett, J. P. Little, M. Sheekey and P. Veerasekaran, *Pest. Biochem. Physiol.*, 1998, **62**, 113–124.

396 K. E. Diehl, E. W. Stoller and M. Barrett, *Pestic. Biochem. Physiol.*, 1995, **51**, 137–149.

397 T. R. Roberts, *Metabolic pathways of agrochemicals. Part 1, Herbicides and plant growth regulators*, Royal Society of Chemistry, Information Services, 1998.

398 S. O. Duke and K. C. Vaughn, *Physiol. Plant.*, 1982, **54**, 381–385.

399 A. R. Lax, H. S. Shepherd and J. V Edwards, *Weed Technol.*, 1988, **2**, 540–544.

400 M. Delaforge, F. Andre, F. Cavelier, J. Verducci, J. L. Aubagnac and B. Liebermann, *Eur. J. Biochem.*, 1997, **250**, 150–157.

401 B. Liebermann, R. Ellinger and E. Pinet, *Phytochemistry*, 1996, **42**, 1537–1540.

402 G. Mitchell, D. W. Bartlett, T. E. M. Fraser, T. R. Hawkes, D. C. Holt, J. K. Townson and R. A. Wichert, in *Pest Management Science*, John Wiley & Sons, Ltd., 2001, vol. 57, pp. 120–128.

403 G. Pinke, K. Tóth, A. J. Kovács, G. Milics, Z. Varga, K. Blazsek, K. E. Gál and Z. Botta-Dukát, *Int. J. Pest Manag.*, 2014, **60**, 187–195.

404 P. Alferness and L. Wiebe, *J. Agric. Food Chem.*, 2002, **50**, 3926–3934.

405 I. Batisson, M. Sancelme, C. Mallet and P. Besse-Hoggan, *Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol.*, 2010, 287–294.

406 H. Ahrens, G. Lange, T. Müller, C. Rosinger, L. Willms and A. Van Almsick, *Angew. Chem Int. Ed.*, 2013, 52, 9388–9398.

407 N. Harada and T. Omura, *J. Biochem*, 1980, **87**, 1539–1554.

408 M. I. Blake, H. L. Crespi and J. J. Katz, *J. Pharm. Sci.*, 1975, **64**, 367–91.

409 K. Sanderson, *Nature*, 2009, **458**, 269.

410 A. Katsnelson, *Nat. Med.*, 2013, **19**, 656.

411 G. S. Timmins, *Expert Opin. Ther. Pat.*, 2014, **24**, 1067–75.

412 T. G. Gant, *J. Med. Chem.*, 2014, **57**, 3595–3611.

413 A. Mullard, *Nat. Rev. Drug Discov.*, 2016, **15**, 219–221.

414 S. Wolfe and C.-C. Shaw, *Can. J. Chem.*, 1982, **60**, 144–153.

415 US4920116, 1990.

416 S. Aalla, G. Gilla, R. R. Anumula, S. Kurella, P. R. Padi and P. R. Vummenthala, *Org. Process Res. Dev.*, 2012, **16**, 748–754.

417 WO20050025498, 2005.

418 T. Yoshimitsu, K. Matsuda, H. Nagaoka, K. Tsukamoto and T. Tanaka, *Org. Lett.*, 2007, **9**, 5115–5118.

419 WO20140122474, 2014.

420 R. P. Robinson, E. R. Laird, K. M. Donahue, L. L. Lopresti-Morrow, P. G. Mitchell, M. R. Reese, L. M. Reeves, A. I. Rouch, E. J. Stam and S. A. Yocum, *Bioorganic Med. Chem. Lett.*, 2001, **11**, 1211–1213.

421 WO20060008303, 2006.

References

422 A. Gaeta, F. Molina-Holgado, X. L. Kong, S. Salvage, S. Fakih, P. T. Francis, R. J. Williams and R. C. Hider, *Bioorg Med. Chem.*, 2011, **19**, 1285–1297.

423 Y. Tanimura, K. Yasunaga and K. Ishimaru, *Eur J. Org. Chem.*, 2013, **2013**, 6535–6539.

424 H. Sun, J. A. Stuckey, Z. Nikolovska-Coleska, D. Qin, J. L. Meagher, S. Qiu, J. Lu, C. Y. Yang, N. G. Saito and S. Wang, *J. Med. Chem.*, 2008, **51**, 7169–7180.

425 EP1935883, 2006.

426 US201300109661, 2013.

427 WO20150164643, 2015.

428 A. Bunescu, T. Piou, Q. Wang and J. Zhu, *Org. Lett.*, 2015, **17**, 334–337.

429 J. P. Gotteland, A. Delhon, D. Junquéro, P. Oms and S. Halazy, *Bioorganic Med. Chem. Lett.*, 1996, **6**, 533–538.

430 A. Augurusa, M. Mehta, M. Perez, J. Zhu and D. W. Stephan, *Chem Commun*, 2016, **52**, 12195–12198.

431 E. Tayama and Y. Toma, *Tetrahedron*, 2015, **71**, 554–559.

432 D. Qiu, J. He, X. Yue, J. Shi and Y. Li, *Org. Lett.*, 2016, **18**, 3130–3133.

433 N. Kamigata, K. Yamamoto, O. Kawakita, K. Hikita, H. Matsuyama, M. Yoshida and M. Kobayashi, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 3601–3602.

434 WO20150031295, 2015.

435 R. D. Trepka, J. K. Harrington, J. W. McConville, K. T. McGurran, A. Mendel, D. R. Pauly, J. E. Robertson and J. T. Waddington, *J. Agric. Food Chem.*, 1974, **22**, 1111–1119.

436 WO20090112490, 2009.

437 B. Kilpatrick, M. Heller and S. Arns, *Chem. Commun.*, 2013, **49**, 514–516.

438 P. S. Mahajan, V. T. Humne, S. D. Tanpure and S. B. Mhaske, *Org. Lett.*, 2016, **18**, 3450–3453.

439 N. Haga, Y. Endo, K. Kataoka, K. Yamaguchi and K. Shudo, *J. Am. Chem. Soc.*, 1992, **114**, 9795–9806.

440 R. J. Bergeron and P. G. Hoffman, *J. Org. Chem.*, 1979, **44**, 1835–1839.

441 A. P. Avdeenko, S. A. Konovalova, O. N. Mikhailichenko, S. V. Shelyazhenko, V. V. Pirozhenko and L. M. Yagupol'skii, *Russ. J. Org. Chem.*, 2011, **47**, 510–519.

442 G. Spadoni, A. Bedini, S. Bartolucci, D. Pala, M. Mor, T. Riccioni, F. Borsini, W. Cabri, D. Celona, M. Marzi, G. Tarzia, S. Rivara and P. Minetti, *Eur. J. Med. Chem.*, 2014, **80**, 8–35.

443 L. Feng, K. Lv, M. Liu, S. Wang, J. Zhao, X. You, S. Li, J. Cao and H. Guo, *Eur. J. Med. Chem.*, 2012, **55**, 125–136.

444 US5665791, 1995.

445 B. Danieli, G. Palmisano, G. Rainoldi and G. Russo, *Phytochemistry*, 1974, **13**, 1603–1606.

446 WO2010/065134, 2010.

447 A. T. Hewson, K. Hughes, S. K. Richardson, D. A. Sharpe and A. H. Wadsworth, *J. Chem. Soc. Perkin Trans. 1*, 1991, **2**, 1565–1569.

448 EP2336104, 2011.

449 S. P. Chavan and H. S. Khatod, *Tetrahedron: Asymmetry*, 2012, **23**, 1410–1415.

450 T. H. Jepsen, M. Larsen, M. Jørgensen, K. A. Solanko, A. D. Bond, A. Kadziola and M. B. Nielsen, *Eur J. Org. Chem.*, 2011, **2011**, 53–57.

451 J. Lv, Q. Liu, J. Tang, F. Perdih and K. Kranjc, *Tetrahedron Lett.*, 2012, **53**, 5248–5252.

452 S. Karabiyikoglu, R. G. Iafe and C. A. Merlic, *Org. Lett.*, 2015, **17**, 5248–5251.

453 G. A. Kraus and X. Wang, *Synth. Commun.*, 1998, **28**, 1093–1096.

454 V. V. Pagar, A. M. Jadhav and R. S. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 20728–20731.

455 X. Kou, Y. Li, L. Wu, X. Zhang, G. Yang and W. Zhang, *Org. Lett.*, 2015, **17**, 5566–5569.

456 T. Chatterjee, R. Dey and B. C. Ranu, *New J. Chem.*, 2011, **35**, 1103–1110.

457 EP2322176A1, 2009.

458 B. Du, X. Jiang and P. Sun, *J. Org. Chem.*, 2013, **78**, 2786–2791.

459 A. M. Sanjuán, A. Martínez, P. García-García, M. A. Fernández-Rodríguez and R. Sanz, *Beilstein J. Org. Chem.*, 2013, **9**, 2242–2249.

460 C. A. Fan, X. D. Hu, Y. Q. Tu, B. M. Wang and Z. L. Song, *Chem. Eur. J.*, 2003, **9**, 4301–4310.

References

461 S. P. Tanis and P. M. Herrinton, *J. Org. Chem.*, 1983, **48**, 4572–4580.

462 Y. Fang and H. Park, *Synth. Commun.*, 2015, **45**, 137–142.

463 C. L. Hugelshofer and T. Magauer, *J. Am. Chem. Soc.*, 2015, **137**, 3807–3810.

464 O. Miyata, R. Iba, J. Hashimoto and T. Naito, *Org. Biomol. Chem.*, 2003, **1**, 772–774.

465 P. Renaud, T. Bourquard, P. A. Carrupt and M. Gerster, *Helv. Chim. Acta*, 1998, **81**, 1048–1063.

466 L. F. Silva, M. V. Craveiro and M. T. P. Gambardella, *Synthesis.*, 2007, **2007**, 3851–3857.

467 H. C. Hsu and D. R. Hou, *Tetrahedron Lett.*, 2009, **50**, 7169–7171.

468 S. Celebi, S. Leyva, D. A. Modarelli and M. S. Platz, *J. Am. Chem. Soc.*, 1993, **115**, 8613–8620.

469 R. C. Hahn, T. F. Corbin and H. Shechter, *J. Am. Chem. Soc.*, 1968, **90**, 3404–3415.

470 M. P. DeNinno, C. Eller and J. B. Etienne, *J. Org. Chem.*, 2001, **66**, 6988–6993.

471 J.-H. Lee, S. H. Seo, E. J. Lim, N.-C. Cho, G. Nam, S. B. Kang, A. N. Pae, N. Jeong and G. Keum, *Eur. J. Med. Chem.*, 2014, **74**, 246–257.

472 US200500176727, 2005.

473 M. V Patel, T. Kolasa, K. Mortell, M. A. Matulenko, A. A. Hakeem, J. J. Rohde, S. L. Nelson, M. D. Cowart, M. Nakane, L. N. Miller, M. E. Uchic, M. A. Terranova, O. F. El-Kouhen, D. L. Donnelly-Roberts, M. T. Namovic, P. R. Hollingsworth, R. Chang, B. R. Martino, J. M. Wetter, K. C. Marsh, R. Martin, J. F. Darbyshire, G. Gintant, G. C. Hsieh, R. B. Moreland, J. P. Sullivan, J. D. Brioni and A. O. Stewart, *J Med Chem*, 2006, **49**, 7450–7465.

474 US200600069123, 2006.

475 M. J. Brown, G. J. Clarkson, G. G. Inglis and M. Shipman, *Org. Lett.*, 2011, **13**, 1686–1689.

476 Y. Kim, Y. D. Hong, Y. H. Joo, B. Y. Woo, S.-Y. Kim, H. J. Koh, M. Park, K. H. Byoun and S. S. Shin, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2807–2810.

477 A. Rostami, F. Hassanian, A. Ghorbani-Choghamarani and S. Saadati, *Phosphorus Sulfur Silicon Relat. Elem.*, 2013, **188**, 833–838.

478 EP0257921, 1988.

479 C. K. Tseng and J. F. Below, *J. Agric. Food. Chem.*, 1977, **25**, 1383–1385.

480 J. Kim, S. Matsuyama and T. Suzuki, *J. Label. Compd. Radiopharm.*, 2004, **47**, 921–934.

481 US20110071196, 2010.

482 Y. Namiki, T. Fujii and M. Nakada, *Tetrahedron: Asymmetry*, 2014, **25**, 718–724.

483 J. Z. Huang, X. Wu and L. Z. Gong, *Adv. Synth. Catal.*, 2013, **355**, 2531–2537.

484 R. G. Soengas and A. M. Estevez, *Synlett*, 2010, **17**, 2625–2627.

485 S. Chénard, C. Barberis, F. Otis, J.-F. Paquin, J. Martel, C. Banville and N. Voyer, *Tetrahedron Lett.*, 2012, **53**, 409–411.

486 T. K. Beng and R. E. Gawley, *J. Am. Chem. Soc*, 2010, **132**, 12216–12217.

487 R. Kumar, M. Kaur, M. S. Bahia and O. Silakari, *Eur. J. Med. Chem.*, 2014, **80**, 83–91.

488 L.-W. Liu, Z.-Z. Wang, H.-H. Zhang, W.-S. Wang, J.-Z. Zhang and Y. Tang, *Chem. Commun.*, 2015, **51**, 9531–9534.

489 S. A. L. Laurent, J. Boissier, F. Coslédan, H. Gornitzka, A. Robert and B. Meunier, *Eur J. Org. Chem.*, 2008, **2008**, 895–913.

490 EP2141150, 2008.

491 WO2007032371, 2008.

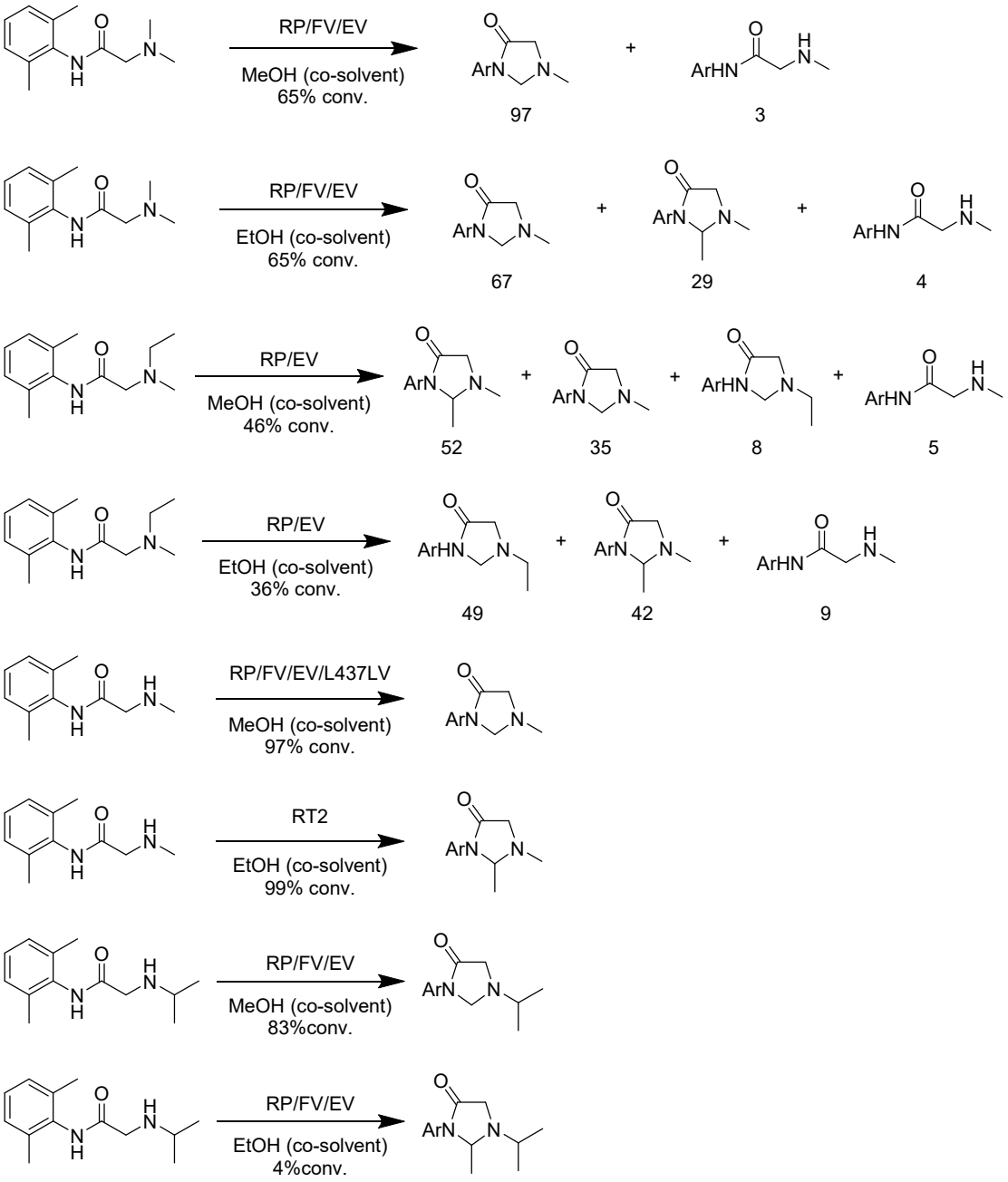
492 K. K. Rajbongshi, D. Hazarika and P. Phukan, *Tetrahedron Lett.*, 2015, **56**, 356–358.

493 US20040092598, 2004.

494 A. O. Terent'ev, D. A. Borisov, I. B. Krylov and G. I. Nikishin, *Synth. Commun.*, 2007, **37**, 3151–3164.

Appendix A – Synthesis of monocyclic imidazolidin-4-ones

As shown by the oxidation of lidocaine (discussed in Chapter 1), monocyclic imidazolidin-4-ones are also accessible *via* CYP102A1 mutant catalysed transformation; trisubstituted and disubstituted amine side chains were viable substrates for synthesis of imidazolidin-4-ones either by the aforementioned oxidative cyclisation or by incorporation of formaldehyde or acetaldehyde which were formed by oxidation of the co-solvent (methanol or ethanol respectively). Examples of this reaction are shown in the scheme below.



Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Table S40: Summary of substrate conversion and product ratios for $P450_{BM3}$ mutants screened

Enzyme	Substrate (conv., product ratio)
A328P	S3: 14 (5%, 27 5%, Other 0%)
	S4: 15 (2%, 28 2%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S10: 12 (2%, 34 1%, Other 1%)
A328V	S3: 14 (4%, 27 4%, Other 0%)
	S4: 15 (1%, 28 1%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S10: 12 (2%, 34 0%, Other 2%)
A330P	S3: 14 (1%, 27 1%, Other 0%)
	S7: 18 (1%, 31 1%, Other 0%)
	S9: 20 (3%, 33 3%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (1%, 35 0.5%, 36 0.5%, Other 0%)
	S25: 192 (7%)
A330W	S3: 14 (1%, 27 1%, Other 0%)
	S7: 18 (1%, 31 0.5%, Other 0.5%)
	S10: 12 (1%, 34 0%, Other 1%)
EV/VI/LI	S3: 14 (38%, 27 22%, Other 16%)
	S4: 15 (48%, 28 46%, Other 2%)
	S5: 16 (43%, 29 28%, Other 15%)
	S6: 17 (27%, 30 27%, Other 0%)
	S7: 18 (24%, 31 24%, Other 0%)
	S9: 20 (32%, 33 28%, Other 4%)
	S10: 12 (70%, 34 69%, Other 1%)
	S11: 13 (58%, 35 20%, 36 24%, Other 14%)
F87A	S3: 14 (2%, 27 2%, Other 0%)
	S7: 18 (7%, 31 6%, Other 1%)
	S9: 20 (5%, 33 5%, Other 0%)
	S10: 12 (1.3%, 34 0%, Other 1%)
	S25: 192 (13%)
	S30: 210 (18%, 232 17%, 233 0%, Other 1%)
	S32: 219 (87%, 247 80%, Other 7%)
	S34: 220 (41%, 248 0%, Other 41%)
	S35: 221 (96%, 253 43%, Other 53%)
F87A/I401P	S3: 14 (21%, 27 17%, Other 4%)
	S4: 15 (2.4%, 28 2%, Other 0%)
	S7: 18 (14%, 31 14%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (4%, 30 4%, Other 0%)
	S9: 20 (31%, 33 14%, Other 17%)
	S10: 12 (11%, 34 11%, Other 0%)
	S11: 13 (4%, 35 4%, 36 0%, Other 0%)
F87A/T88A/I401P	S3: 14 (2%, 27 2%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (2%, 31 1%, Other 1%)
	S9: 20 (4%, 33 4%, Other 0%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
F87A/T88A/I401P	S10: 12 (1%, 34 0%, Other 1%)
F87P/I401P	S3: 14 (2%, 27 2%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (2%, 31 1%, Other 1%)
	S9: 20 (5%, 33 5%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
GVQ	S3: 14 (18%, 27 11%, Other 7%)
	S4: 15 (43%, 28 43%, Other 0%)
	S5: 16 (11%, 29 11%, Other 0%)
	S6: 17 (13%, 30 13%, Other 0%)
	S7: 18 (18%, 31 18%, Other 0%)
	S9: 20 (56%, 33 48%, Other 8%)
	S10: 12 (14%, 34 13%, Other 1%)
	S11: 13 (15%, 35 1%, 36 12%, Other 2%)
	S18: 96 (48%, 112 48%, Other 0%)
	S25: 192 (42%)
	S30: 210 (99%, 232 85%, 233 13%, Other 2%)
	S32: 219 (100%, 247 100%, Other 0%)
	S34: 220 (27%, 248 0%, Other 27%)
	S35: 221 (100%, 253 33%, Other 67%)
I263F	S3: 14 (4%, 27 4%, Other 0%)
	S7: 18 (1%, 31 1%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (1%, 35 1%, 36 0%, Other 0%)
IP/QP	S3: 14 (5%, 27 5%, Other 0%)
	S4: 15 (1%, 28 1%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S10: 12 (1%, 34 0.5%, Other 0.5%)
	S11: 13 (22%, 35 21%, 36 0.5%, Other 0.5%)
KSK19	S1: 10 (16%, 25)
	S3: 14 (20%, 27 16%, Other 4%)
	S4: 15 (3%, 28 3%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (7%, 30 7%, Other 0%)
	S7: 18 (70%, 31 59%, Other 11%)
	S9: 20 (27%, 33 10%, Other 17%)
	S10: 12 (11%, 34 11%, Other 0%)
	S11: 13 (2%, 35 0%, 36 2%, Other 0%)
	S29: 214 (61%)
	S30: 210 (96%, 232 71%, 233 16%, Other 9%)
	S31: 204 (100%)
	S32: 219 (100%, 247 100%, Other 0%)
	S35: 221 (100%, 253 66%, Other 34%)
	S39: 226 (7%)
KSK19/A82M	S1: 10 (1%, 25)
	S2: 11 (3%, 26)
	S3: 14 (72%, 27 55%, Other 17%)
	S4: 15 (90%, 28 90%, Other 0%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (7%, 30 7%, Other 0%)
	S7: 18 (33%, 31 31%, Other 2%)
	S8: 19 (3%, 32)

Enzyme	Substrate (conv., product ratio)
KSK19/A82M	S9: 20 (28%, 33 21%, Other 7%)
	S10: 12 (56%, 34 52%, Other 4%)
	S11: 13 (3%, 35 1.5%, 36 1.5%, Other 0%)
	S12: 45 (67%, 46 66%, Other 1%)
	S13: 50 (18%, 51)
	S15: 95 (24%)
	S16: 94 (81%, 110 81%, Other 0%)
	S17: 92 (66%, 111 39%, Other 27%)
	S18: 96 (83%, 112 0%, Other 83%)
	S19: 97 (64%, 113 25%, Other 39%)
	S21: 100 (5%, 115 0%, Other 5%)
	S22: 102 (30%, 116 4%, Other 26%)
	S23: 99 (99%, 117 12%, 118 18%, Other 69%)
	S24: 107 (7%)
	S28: 207 (100%, 206 89%, Other 11%)
	S29: 214 (4%)
	S30: 210 (91%, 232 64%, 233 14%, Other 13%)
	S31: 204 (12%)
	S32: 219 (58%, 247 58%, Other 0%)
	S35: 221 (100%, 253 62%, Other 38%)
	S38: 225 (5%, 273)
KSK19/A82M/E267F	S3: 14 (84%, 27 70%, Other 14%)
	S4: 15 (91%, 28 89%, Other 2%)
	S5: 16 (15%, 29 15%, Other 0%)
	S6: 17 (26%, 30 26%, Other 0%)
	S7: 18 (79%, 31 74%, Other 5%)
	S10: 12 (21%, 34 20%, Other 1%)
	S11: 13 (15%, 35 1%, 36 11%, Other 3%)
	S18: 96 (34%, 112 34%, Other 0%)
	S25: 192 (13%)
KSK19/A82M/I401M	S3: 14 (64%, 27 53%, Other 11%)
	S4: 15 (76%, 28 75%, Other 1%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (10%, 30 10%, Other 0%)
	S7: 18 (24%, 31 23%, Other 1%)
	S9: 20 (23%, 33 13%, Other 10%)
	S10: 12 (48%, 34 46%, Other 2%)
	S11: 13 (1%, 35 0%, 36 1%, Other 0%)
KSK19/AI	S1: 10 (1%, 25)
	S2: 11 (2%, 26)
	S3: 14 (2%, 27 2%, Other 0%)
	S4: 15 (2%, 28 1%, Other 1%)
	S7: 18 (2%, 31 1%, Other 1%)
	S8: 19 (7%, 32)
	S9: 20 (7%, 33 7%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (31%, 35 0%, 36 0%, Other 31%)
	S30: 210 (57%, 232 14%, 233 9%, Other 34%)
	S31: 204 (10%)
	S32: 219 (8%, 247 6%, Other 2%)
	S35: 221 (100%, 253 88%, Other 12%)
	S36: 222 (80%)
	S38: 225 (7%, 273)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
KSK19/AI/AI	S2: 11 (8%, 26)
	S3: 14 (12%, 27 12%, Other 0%)
	S4: 15 (14%, 28 14%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (4%, 30 4%, Other 0%)
	S7: 18 (28%, 31 28%, Other 0%)
	S8: 19 (8%, 32)
	S9: 20 (7%, 33 7%, Other 0%)
	S10: 12 (13%, 34 11%, Other 2%)
	S11: 13 (18%, 35 6%, 36 12%, Other 0%)
	S12: 45 (26%, 46 25%, Other 1%)
	S13: 50 (14%, 51)
	S14: 93 (17%, 108 3%, Other 14%)
	S15: 95 (9%)
	S16: 94 (100%, 110 100%, Other 0%)
	S17: 92 (78%, 111 76%, Other 2%)
	S18: 96 (37%, 112 37%, Other 0%)
	S19: 97 (91%, 113 75%, Other 16%)
	S20: 98 (19%, 114 0%, Other 19%)
	S21: 100 (16%, 115 0%, Other 16%)
	S23: 99 (90%, 117 54%, 118 36%, Other 0%)
	S26: 143 (83%)
	S28: 207 (100%, 206 100%, Other 0%)
	S30: 210 (86%, 232 34%, 233 25%, Other 27%)
	S31: 204 (8%)
	S32: 219 (11%, 247 11%, Other 0%)
	S34: 220 (43%, 248 43%, Other 0%)
	S35: 221 (100%, 253 68%, Other 32%)
	S38: 225 (5%, 273)
	S39: 226 (9%)
KSK19/AI/F87V	S2: 11 (6%, 26)
	S3: 14 (25%, 27 25%, Other 0%)
	S4: 15 (16%, 28 93.9%, Other 6.1%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (3%, 30 3%, Other 0%)
	S7: 18 (6%, 31 6%, Other 0%)
	S8: 19 (38%, 32)
	S9: 20 (51%, 33 51%, Other 0%)
	S10: 12 (3.4%, 34 55.1%, Other 44.9%)
	S11: 13 (2%, 35 1%, 36 1%, Other 0%)
	S13: 50 (27%, 51)
	S16: 94 (67%, 110 67%, Other 0%)
	S17: 92 (56%, 111 45%, Other 12%)
	S18: 96 (34%, 112 34%, Other 0%)
	S19: 97 (24%, 113 24%, Other 0%)
	S20: 98 (6%, 114 6%, Other 0%)
	S21: 100 (5%, 115 0%, Other 5%)
	S23: 99 (99%, 117 3%, 118 96%, Other 0%)
	S24: 107 (1%)
	S26: 143 (89%)
	S28: 207 (100%, 206 26%, Other 74%)
	S30: 210 (94%, 232 50%, 233 15%, Other 29%)
	S31: 204 (19%)

Enzyme	Substrate (conv., product ratio)
KSK19/AI/F87V	S32: 219 (5%, 247 0%, Other 5%)
	S33: 247 (24%, 247 0%, Other 0%)
	S34: 220 (28%, 248 28%, Other 0%)
	S35: 221 (100%, 253 69%, Other 31%)
	S36: 222 (34%)
	S38: 225 (10%, 273)
KSK19/AI/IA	S3: 14 (2%, 27 2%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (7%, 31 5%, Other 2%)
	S9: 20 (9%, 33 2%, Other 7%)
	S10: 12 (2%, 34 0%, Other 2%)
	S11: 13 (1%, 35 0%, 36 1%, Other 0%)
	S25: 192 (41%)
KSK19/AI/V78I	S1: 10 (1%, 25)
	S2: 11 (1%, 26)
	S3: 14 (2%, 27 2%, Other 0%)
	S4: 15 (2%, 28 1%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S8: 19 (8%, 32)
	S9: 20 (4%, 33 4%, Other 0%)
	S10: 12 (2%, 34 0%, Other 2%)
	S11: 13 (5%, 35 3%, 36 2%, Other 0%)
	S13: 50 (3%, 51)
	S16: 94 (40%, 110 31%, Other 8%)
	S23: 99 (50%, 117 35%, 118 15%, Other 0%)
	S24: 107 (8%)
	S26: 143 (6%)
	S28: 207 (100%, 206 69%, Other 31%)
	S30: 210 (15%, 232 9%, 233 3%, Other 3%)
	S31: 204 (10%)
	S32: 219 (2%, 247 2%, Other 0%)
	S35: 221 (100%, 253 73%, Other 27%)
KSK19/AI/Y51L	S1: 10 (2%, 25)
	S2: 11 (4%, 26)
	S3: 14 (7%, 27 7%, Other 0%)
	S4: 15 (5%, 28 3%, Other 2%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (8%, 31 6%, Other 2%)
	S8: 19 (4%, 32)
	S9: 20 (8%, 33 6%, Other 2%)
	S10: 12 (5%, 34 2%, Other 3%)
	S13: 50 (19%, 51)
	S15: 95 (41%)
	S16: 94 (67%, 110 67%, Other 0%)
	S17: 92 (16%, 111 16%, Other 0%)
	S19: 97 (10%, 113 10%, Other 0%)
	S22: 102 (2%, 116 2%, Other 0%)
	S23: 99 (80%, 117 37%, 118 43%, Other 0%)
	S26: 143 (83%)
	S28: 207 (100%, 206 77%, Other 23%)
KSK19/F87S	S3: 14 (8%, 27 8%, Other 0%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
KSK19/F87S	S4: 15 (3%, 28 3%, Other 0%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (9%, 31 8%, Other 1%)
	S9: 20 (13%, 33 7%, Other 6%)
	S10: 12 (2%, 34 1%, Other 1%)
	S11: 13 (1%, 35 0%, 36 0%, Other 1%)
KSK19/I263A	S1: 10 (35%, 25)
	S2: 11 (8%, 26)
	S3: 14 (3%, 27 3%, Other 0%)
	S4: 15 (2%, 28 2%, Other 0%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (11%, 31 11%, Other 0%)
	S8: 19 (9%, 32)
	S9: 20 (5%, 33 3%, Other 2%)
	S10: 12 (3%, 34 2%, Other 1%)
	S11: 13 (1%, 35 0%, 36 1%, Other 0%)
	S12: 45 (1%, 46 1%, Other 0%)
	S13: 50 (17%, 51)
	S15: 95 (8%)
	S16: 94 (28%, 110 20%, Other 10%)
	S28: 207 (100%, 206 100%, Other 0%)
	S32: 219 (96%, 247 96%, Other 0%)
	S35: 221 (9%, 253 2%, Other 7%)
	S29: 214 (7%)
	S30: 210 (5%, 232 5%, 233 0%, Other 0%)
	S31: 204 (76%)
	S33: 247 (30%, 247 0%, Other 0%)
	S36: 222 (72%)
	S38: 225 (17%, 273)
KSK19/QP	S1: 10 (24%, 25)
	S3: 14 (21%, 27 14%, Other 7%)
	S4: 15 (23%, 28 23%, Other 0%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (10%, 30 10%, Other 0%)
	S7: 18 (59%, 31 41%, Other 18%)
	S9: 20 (25%, 33 10%, Other 15%)
	S10: 12 (27%, 34 26%, Other 1%)
	S11: 13 (3%, 35 0%, 36 3%, Other 0%)
KSK19/QP/F87V	S3: 14 (44%, 27 21%, Other 23%)
	S4: 15 (95%, 28 92%, Other 3%)
	S5: 16 (25%, 29 22%, Other 3%)
	S6: 17 (14%, 30 14%, Other 0%)
	S7: 18 (22%, 31 21%, Other 1%)
	S9: 20 (84%, 33 69%, Other 15%)
	S10: 12 (39%, 34 37%, Other 2%)
	S11: 13 (11%, 35 1%, 36 5%, Other 5%)
	S18: 96 (33%, 112 33%, Other 0%)
	S25: 192 (70%)
KT2	S3: 14 (26%, 27 23%, Other 3%)
	S4: 15 (2%, 28 2%, Other 0%)
	S5: 16 (1%, 29 1%, Other 0%)

Enzyme	Substrate (conv., product ratio)
KT2	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (8%, 31 7%, Other 1%)
	S9: 20 (7%, 33 7%, Other 0%)
	S10: 12 (2%, 34 0%, Other 2%)
	S11: 13 (2%, 35 2%, 36 0%, Other 27%)
KT2/A82G	S3: 14 (28%, 27 20%, Other 8%)
	S4: 15 (23%, 28 23%, Other 0%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (2%, 35 0%, 36 2%, Other 0%)
KT2/E267F	S3: 14 (34%, 27 26%, Other 8%)
	S4: 15 (33%, 28 33%, Other 0%)
	S5: 16 (11%, 29 11%, Other 0%)
	S6: 17 (14%, 30 14%, Other 0%)
	S7: 18 (26%, 31 25%, Other 1%)
	S10: 12 (10%, 34 9%, Other 1%)
	S11: 13 (20%, 35 2%, 36 18%, Other 0%)
KT2/E267L	S3: 14 (33%, 27 19%, Other 14%)
	S4: 15 (49%, 28 49%, Other 0%)
	S5: 16 (45%, 29 31%, Other 14%)
	S6: 17 (25%, 30 25%, Other 0%)
	S7: 18 (29%, 31 27%, Other 2%)
	S10: 12 (38%, 34 37%, Other 1%)
KT2/F87I	S3: 14 (14%, 27 14%, Other 0%)
	S4: 15 (18%, 28 18%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S10: 12 (1%, 34 0%, Other 1%)
QP	S3: 14 (8%, 27 8%, Other 0%)
	S4: 15 (1%, 28 1%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (3%, 31 2%, Other 1%)
	S9: 20 (5%, 33 5%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
QP/A82M	S3: 14 (39%, 27 31%, Other 8%)
	S4: 15 (41%, 28 40%, Other 1%)
	S5: 16 (6%, 29 6%, Other 0%)
	S6: 17 (13%, 30 13%, Other 0%)
	S7: 18 (16%, 31 96%, Other 4%)
	S9: 20 (14%, 33 14%, Other 0%)
	S10: 12 (7%, 34 6%, Other 1%)
	S11: 13 (5%, 35 0%, 36 5%, Other 0%)
R19	S3: 14 (39%, 27 36%, Other 3%)
	S4: 15 (21%, 28 20%, Other 1%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (15%, 31 14%, Other 1%)
	S8: 19 (12%, 32)
	S9: 20 (5%, 33 5%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (2%, 35 0.5%, 36 1%, Other 0.5%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
R19	S16: 94 (26%, 110 15%, Other 11%)
	S37: 223 (87%)
RLYF	S3: 14 (2%, 27 2%, Other 0%)
	S7: 18 (1%, 31 0.5%, Other 0.5%)
	S10: 12 (3%, 34 0%, Other 3%)
	S11: 13 (1%, 35 0.5%, 36 0.5%, Other 0%)
	S25: 192 (9%)
RLYF/KSK19	S3: 14 (35%, 27 28%, Other 7%)
	S7: 18 (71%, 31 56%, Other 15%)
	S9: 20 (28%, 33 8%, Other 20%)
	S10: 12 (7%, 34 6%, Other 1%)
	S18: 96 (5%, 112 5%, Other 0%)
	S25: 192 (93%)
RLYF/KSK19/AI	S1: 10 (1%, 25)
	S2: 11 (2%, 26)
	S3: 14 (5%, 27 5%, Other 0%)
	S4: 15 (4%, 28 4%, Other 0%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (11%, 31 11%, Other 0%)
	S8: 19 (8%, 32)
	S9: 20 (7%, 33 7%, Other 0%)
	S10: 12 (2%, 34 1%, Other 1%)
	S13: 50 (9%, 51)
	S15: 95 (2%)
	S16: 94 (90%, 110 90%, Other 0%)
	S17: 92 (48%, 111 36%, Other 12%)
	S19: 97 (3%, 113 3%, Other 0%)
	S21: 100 (2%, 115 0%, Other 2%)
	S22: 102 (2%, 116 2%, Other 0%)
	S23: 99 (45%, 117 25%, 118 20%, Other 0%)
	S26: 143 (61%)
	S28: 207 (100%, 206 100%, Other 0%)
RP	S2: 11 (8%, 26)
	S3: 14 (65%, 27 42%, Other 23%)
	S4: 15 (49%, 28 49%, Other 0%)
	S5: 16 (12%, 29 12%, Other 0%)
	S6: 17 (19%, 30 19%, Other 0%)
	S7: 18 (25%, 31 25%, Other 0%)
	S8: 19 (13%, 32)
	S9: 20 (7%, 33 7%, Other 0%)
	S10: 12 (6%, 34 4%, Other 2%)
	S11: 13 (19%, 35 19%, 36 0%, Other 53%)
	S12: 45 (1%, 46 1%, Other 0%)
	S13: 50 (79%, 51)
	S14: 93 (1%, 108 0%, Other 1%)
	S15: 95 (61%)
	S16: 94 (23%, 110 23%, Other 0%)
	S17: 92 (43%, 111 43%, Other 0%)
	S18: 96 (20%, 112 20%, Other 0%)
	S19: 97 (62%, 113 36%, Other 26%)
	S21: 100 (10%, 115 0%, Other 10%)
	S23: 99 (43%, 117 13%, 118 30%, Other 0%)

Enzyme	Substrate (conv., product ratio)
RP	S26: 143 (28%)
	S28: 207 (100%, 206 64%, Other 36%)
	S30: 210 (100%, 232 76%, 233 24%)
	S31: 204 (85%)
	S32: 219 (14%, 247 4%, Other 10%)
	S33: 247 (44%, 247 0%, Other 0%)
	S35: 221 (90%, 253 12%, Other 78%)
	S36: 222 (71%)
	S38: 225 (10%, 273)
RP/AM/IA	S3: 14 (37%, 27 33%, Other 4%)
	S4: 15 (22%, 28 22%, Other 0%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (6%, 30 6%, Other 0%)
	S7: 18 (17%, 31 16%, Other 1%)
	S9: 20 (11%, 33 11%, Other 0%)
	S10: 12 (6%, 34 4%, Other 2%)
	S11: 13 (2%, 35 2%, 36 0%, Other 0%)
	S18: 96 (10%, 112 10%, Other 0%)
	S25: 192 (42%)
RP/AP	S1: 10 (1%, 25)
	S2: 11 (22%, 26)
	S3: 14 (10%, 27 6%, Other 4%)
	S4: 15 (4%, 28 2%, Other 2%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (4%, 30 4%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S8: 19 (16%, 32)
	S9: 20 (6%, 33 3%, Other 3%)
	S11: 13 (2%, 35 0.5%, 36 1.5%, Other 0%)
	S12: 45 (2%, 46 2%, Other 0%)
	S13: 50 (20%, 51)
	S15: 95 (77%)
	S16: 94 (43%, 110 32%, Other 10%)
	S17: 92 (23%, 111 23%, Other 0%)
	S18: 96 (10%, 112 10%, Other 0%)
	S19: 97 (34%, 113 34%, Other 0%)
	S21: 100 (10%, 115 0%, Other 10%)
	S22: 102 (3%, 116 3%, Other 0%)
	S23: 99 (3%, 117 3%, 118 0%, Other 0%)
	S26: 143 (5%)
	S28: 207 (100%, 206 16%, Other 84%)
	S30: 210 (58%, 232 34%, 233 24%, Other 0%)
	S32: 219 (14%, 247 4%, Other 10%)
	S34: 220 (15%, 248 0%, Other 15%)
	S35: 221 (98%, 253 14%, Other 84%)
RP/AP/AM	S2: 11 (1%, 26)
	S3: 14 (12%, 27 12%, Other 0%)
	S4: 15 (4%, 28 3%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (4%, 30 4%, Other 0%)
	S7: 18 (6%, 31 6%, Other 0%)
	S8: 19 (15%, 32)
	S9: 20 (12%, 33 12%, Other 0%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RP/AP/AM	S10: 12 (3.4%, 34 1.5%, Other 1.5%)
	S16: 94 (42%, 110 11%, Other 31%)
	S30: 210 (77%, 232 45%, 233 32%, Other 0%)
	S32: 219 (13%, 247 5%, Other 8%)
	S33: 247 (8%, 247 0%, Other 0%)
	S35: 221 (16%, 253 6%, Other 10%)
RP/AP/PV	S3: 14 (3%, 27 3%, Other 0%)
	S4: 15 (1%, 28 1%, Other 0%)
	S7: 18 (1%, 31 0.5%, Other 0.5%)
	S9: 20 (3%, 33 3%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
RP/E267i	S3: 14 (46%, 27 30%, Other 16%)
	S4: 15 (61%, 28 59%, Other 2%)
	S5: 16 (67%, 29 26%, Other 41%)
	S6: 17 (56%, 30 40%, Other 16%)
	S7: 18 (36%, 31 21%, Other 15%)
	S10: 12 (100%, 34 98%, Other 2%)
RP/E267M	S3: 14 (59%, 27 28%, Other 31%)
	S4: 15 (68%, 28 64%, Other 4%)
	S5: 16 (43%, 29 27%, Other 16%)
	S6: 17 (23%, 30 23%, Other 0%)
	S7: 18 (33%, 31 33%, Other 0%)
	S9: 20 (44%, 33 40%, Other 4%)
	S10: 12 (35%, 34 33%, Other 2%)
	S11: 13 (32%, 35 0%, 36 27%, Other 5%)
	S18: 96 (72%, 112 72%, Other 0%)
	S25: 192 (32%)
RP/EV	S2: 11 (9%, 26)
	S3: 14 (47%, 27 28%, Other 19%)
	S4: 15 (48%, 28 43%, Other 5%)
	S5: 16 (73%, 29 19%, Other 54%)
	S6: 17 (45%, 30 23%, Other 22%)
	S7: 18 (25%, 31 25%, Other 0%)
	S8: 19 (14%, 32)
	S9: 20 (25%, 33 23%, Other 2%)
	S10: 12 (86%, 34 84%, Other 2%)
	S12: 45 (15%, 46 14%, Other 1%)
	S13: 50 (100%, 51)
	S15: 95 (67%)
	S16: 94 (8%, 110 0%, Other 8%)
	S17: 92 (97%, 111 97%, Other 0%)
	S18: 96 (94%, 112 94%, Other 0%)
	S19: 97 (99%, 113 85%, Other 14%)
	S20: 98 (63%, 114 37%, Other 26%)
	S21: 100 (85%, 115 40%, Other 45%)
	S22: 102 (49%, 116 48%, Other 1%)
	S23: 99 (97%, 117 28%, 118 63%, Other 6%)
	S24: 107 (10%)
	S26: 143 (21%)
	S28: 207 (100%, 206 73%, Other 27%)
	S33: 247 (6%, 247 0%, Other 0%)
	S37: 223 (100%)
RP/F81W	S1: 10 (1%, 25)

Enzyme	Substrate (conv., product ratio)
RP/F81W	S3: 14 (57%, 27 30%, Other 27%)
	S4: 15 (15%, 28 100%, Other 0%)
	S5: 16 (10%, 29 10%, Other 0%)
	S7: 18 (18%, 31 18%, Other 0%)
	S9: 20 (31%, 33 18%, Other 13%)
	S10: 12 (8%, 34 7%, Other 1%)
	S11: 13 (25%, 35 0%, 36 24%, Other 1%)
	S18: 96 (18%, 112 18%, Other 0%)
	S25: 192 (91%)
RP/F87A/A328I	S3: 14 (20%, 27 20%, Other 0%)
	S4: 15 (4%, 28 3%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (5%, 31 5%, Other 0%)
	S9: 20 (24%, 33 15%, Other 9%)
	S10: 12 (3%, 34 2%, Other 1%)
	S11: 13 (2%, 35 2%, 36 0%, Other 0%)
RP/F87A	S3: 14 (38%, 27 29%, Other 9%)
	S4: 15 (34%, 28 34%, Other 0%)
	S5: 16 (4%, 29 4%, Other 0%)
	S6: 17 (11%, 30 11%, Other 0%)
	S7: 18 (33%, 31 24%, Other 9%)
	S10: 12 (17%, 34 16%, Other 1%)
	S11: 13 (6%, 35 0%, 36 6%, Other 0%)
	S30: 210 (61%, 232 43%, 233 16%, Other 2%)
	S32: 219 (78%, 247 78%, Other 0%)
	S34: 220 (73%, 248 0%, Other 73%)
	S35: 221 (100%, 253 38%, Other 62%)
RP/FV	S1: 10 (2%, 25)
	S2: 11 (6%, 26)
	S3: 14 (63%, 27 32%, Other 31%)
	S4: 15 (48%, 28 45%, Other 3%)
	S5: 16 (30%, 29 24%, Other 6%)
	S6: 17 (21%, 30 21%, Other 0%)
	S7: 18 (20%, 31 18%, Other 2%)
	S8: 19 (13%, 32)
	S9: 20 (27%, 33 22%, Other 5%)
	S10: 12 (23%, 34 23%, Other 0%)
	S11: 13 (15%, 35 3%, 36 12%, Other 0%)
	S13: 50 (97%, 51)
	S15: 95 (37%)
	S16: 94 (98%, 110 84%, Other 14%)
	S18: 96 (60%, 112 60%, Other 0%)
	S19: 97 (11%, 113 11%, Other 0%)
	S21: 100 (31%, 115 17%, Other 14%)
	S23: 99 (3%, 117 2%, 118 1%, Other 0%)
	S26: 143 (39%)
	S28: 207 (100%, 206 0%, Other 100%)
	S31: 204 (91%)
	S38: 225 (33%, 273)
RP/FV/EV	S1: 10 (99%, 25)
	S2: 11 (77%, 26)
	S3: 14 (87%, 27 60%, Other 27%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RP/FV/EV	S4: 15 (100%, 28 72%, Other 28%)
	S5: 16 (70%, 29 52%, Other 18%)
	S6: 17 (68%, 30 54%, Other 14%)
	S7: 18 (29%, 31 27%, Other 2%)
	S8: 19 (95%, 32)
	S9: 20 (33%, 33 26%, Other 7%)
	S10: 12 (85%, 34 84%, Other 4%)
	S11: 13 (92%, 35 20%, 36 63%, Other 9%)
	S12: 45 (17%, 46 16%, Other 1%)
	S13: 50 (73%, 51)
	S15: 95 (65%)
	S16: 94 (74%, 110 74%, Other 0%)
	S18: 96 (99%, 112 22%, Other 77%)
	S19: 97 (93%, 113 72%, Other 21%)
	S20: 98 (18%, 114 18%, Other 0%)
	S21: 100 (81%, 115 19%, Other 62%)
	S22: 102 (97%, 116 6%, Other 91%)
	S23: 99 (50%, 117 19%, 118 31%, Other 0%)
	S24: 107 (13%)
	S26: 143 (10%)
	S28: 207 (100%, 206 7%, Other 93%)
	S29: 214 (33%)
	S30: 210 (38%, 232 31%, 233 6%, Other 1%)
	S31: 204 (34%)
	S32: 219 (25%, 247 25%, Other 0%)
	S34: 220 (66%, 248 40%, Other 26%)
	S35: 221 (81%, 253 7%, Other 74%)
	S38: 225 (7%, 273)
RP/FV/EV/A184I	S1: 10 (25%, 25)
	S2: 11 (27%, 26)
	S8: 19 (14%, 32)
	S9: 20 (5%, 33 4%, Other 1%)
	S30: 210 (100%, 232 34%, 233 25%, Other 41%)
	S31: 204 (9%)
	S32: 219 (10%, 247 10%, Other 0%)
	S34: 220 (24%, 248 0%, Other 24%)
	S35: 221 (100%, 253 58%, Other 42%)
	S36: 222 (37%)
	S38: 225 (32%, 273)
RP/FV/EV/A330W	S5: 16 (24%, 29 21%, Other 3%)
	S6: 17 (21%, 30 21%, Other 0%)
RP/FV/EV/F81W	S3: 14 (66%, 27 53%, Other 13%)
	S4: 15 (78%, 28 70%, Other 8%)
	S5: 16 (51%, 29 44%, Other 7%)
	S6: 17 (40%, 30 40%, Other 0%)
	S7: 18 (36%, 31 33%, Other 3%)
	S9: 20 (24%, 33 19%, Other 5%)
	S10: 12 (20%, 34 19%, Other 1%)
	S11: 13 (41%, 35 3%, 36 30%, Other 8%)
RP/FV/EV/L188Q	S3: 14 (97%, 27 60%, Other 37%)
	S5: 16 (80%, 29 63%, Other 17%)
	S6: 17 (72%, 30 59%, Other 13%)
	S7: 18 (30%, 31 28%, Other 2%)

Enzyme	Substrate (conv., product ratio)
RP/FV/EV/L188Q	S9: 20 (54%, 33 41%, Other 13%)
	S10: 12 (76%, 34 75%, Other 1%)
	S11: 13 (69%, 35 10%, 36 52%, Other 7%)
	S18: 96 (99%, 112 22%, Other 77%)
	S25: 192 (18%)
RP/FV/EV/L437LV	S2: 11 (9%, 26)
	S3: 14 (44%, 27 32%, Other 12%)
	S4: 15 (44%, 28 40%, Other 4%)
	S5: 16 (66%, 29 47%, Other 19%)
	S6: 17 (39%, 30 39%, Other 0%)
	S7: 18 (24%, 31 23%, Other 1%)
	S9: 20 (11%, 33 11%, Other 0%)
	S10: 12 (15%, 34 14%, Other 1%)
	S11: 13 (22%, 35 2%, 36 4%, Other 16%)
	S12: 45 (7%, 46 6%, Other 1%)
	S13: 50 (58%, 51)
	S15: 95 (83%)
	S16: 94 (96%, 110 96%, Other 0%)
	S17: 92 (100%, 111 100%, Other 0%)
	S18: 96 (72%, 112 72%, Other 0%)
	S19: 97 (100%, 113 64%, Other 36%)
	S20: 98 (30%, 114 30%, Other 0%)
	S21: 100 (78%, 115 43%, Other 35%)
	S23: 99 (13%, 117 9%, 118 4%, Other 0%)
	S24: 107 (1%)
	S26: 143 (8%)
	S28: 207 (100%, 206 0%, Other 100%)
	S30: 210 (26%, 232 26%, 233 0%, Other 0%)
	S31: 204 (5%)
	S32: 219 (8%, 247 8%, Other 0%)
	S33: 247 (43%, 247 0%, Other 0%)
	S34: 220 (24%, 248 0%, Other 24%)
	S35: 221 (22%, 253 15%, Other 7%)
	S38: 225 (25%, 273)
RP/FV/EV/L437VL	S2: 11 (4%, 26)
	S3: 14 (58%, 27 37%, Other 21%)
	S4: 15 (60%, 28 59%, Other 1%)
	S5: 16 (52%, 29 47%, Other 5%)
	S6: 17 (40%, 30 40%, Other 0%)
	S7: 18 (30%, 31 30%, Other 0%)
	S8: 19 (90%, 32)
	S9: 20 (14.1%, 33 100%, Other 0%)
	S10: 12 (12.2%, 34 90.9%, Other 9.1%)
	S11: 13 (24%, 35 10%, 36 8%, Other 6%)
	S12: 45 (3%, 46 3%, Other 0%)
	S13: 50 (35%, 51)
	S15: 95 (84%)
	S16: 94 (45%, 110 45%, Other 0%)
	S17: 92 (77%, 111 77%, Other 0%)
	S18: 96 (27%, 112 27%, Other 0%)
	S19: 97 (11%, 113 11%, Other 0%)
	S20: 98 (52%, 114 0%, Other 52%)
	S21: 100 (15%, 115 5%, Other 10%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RP/FV/EV/L437VL	S22: 102 (7%, 116 7%, Other 0%)
	S23: 99 (3%, 117 3%, 118 0%, Other 0%)
	S24: 107 (21%)
	S26: 143 (82%)
	S28: 207 (100%, 206 12%, Other 88%)
RP/FV/EV/V78F	S3: 14 (99%, 27 44%, Other 55%)
	S4: 15 (89%, 28 82%, Other 7%)
	S5: 16 (93%, 29 75%, Other 18%)
	S6: 17 (68%, 30 57%, Other 11%)
	S7: 18 (39%, 31 37%, Other 2%)
	S9: 20 (42%, 33 33%, Other 9%)
	S10: 12 (38%, 34 37%, Other 1%)
	S11: 13 (42%, 35 2%, 36 15%, Other 25%)
	S18: 96 (57%, 112 54%, Other 3%)
	S25: 192 (25%)
RP/H171L	S3: 14 (71%, 27 38%, Other 33%)
	S4: 15 (70%, 28 59%, Other 11%)
	S5: 16 (52%, 29 42%, Other 10%)
	S6: 17 (61%, 30 53%, Other 8%)
	S7: 18 (41%, 31 40%, Other 1%)
	S10: 12 (11%, 34 10%, Other 1%)
	S11: 13 (97%, 35 83%, 36 10%, Other 4%)
	S18: 96 (36%, 112 36%, Other 0%)
	S25: 192 (63%)
RP/H171L/I263G	S18: 96 (96%, 112 96%, Other 0%)
	S25: 192 (95%)
RP/IA	S3: 14 (32%, 27 18%, Other 14%)
	S4: 15 (6%, 28 5%, Other 1%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (20%, 31 20%, Other 0%)
	S9: 20 (13%, 33 7%, Other 6%)
	S10: 12 (3%, 34 1.5%, Other 1.5%)
	S11: 13 (5%, 35 5%, 36 0%, Other 0%)
RP/IA/EV	S1: 10 (12%, 25)
	S2: 11 (24%, 26)
	S3: 14 (66%, 27 32%, Other 34%)
	S4: 15 (64%, 28 59%, Other 5%)
	S5: 16 (81%, 29 18%, Other 63%)
	S7: 18 (35%, 31 27%, Other 8%)
	S8: 19 (29%, 32)
	S9: 20 (10%, 33 5%, Other 5%)
	S10: 12 (69%, 34 67%, Other 2%)
	S11: 13 (94%, 35 13%, 36 72%, Other 9%)
	S12: 45 (18%, 46 17%, Other 1%)
	S13: 50 (99%, 51)
	S14: 93 (2%, 108 2%, Other 0%)
	S15: 95 (2%)
	S17: 92 (100%, 111 100%, Other 0%)
	S18: 96 (100%, 112 100%, Other 0%)
	S19: 97 (97%, 113 65%, Other 32%)
	S20: 98 (79%, 114 77%, Other 2%)
	S21: 100 (100%, 115 44%, Other 56%)

Enzyme	Substrate (conv., product ratio)
RP/IA/EV	S22: 102 (81%, 116 80%, Other 1%)
	S23: 99 (100%, 117 85%, 118 9%)
	S26: 143 (30%)
	S28: 207 (100%, 206 72%, Other 28%)
	S30: 210 (93%, 232 61%, 233 32%, Other 0%)
	S31: 204 (100%)
	S32: 219 (40%, 247 37%, Other 3%)
	S34: 220 (86%, 248 14%, Other 72%)
	S35: 221 (93%, 253 50%, Other 43%)
RP/IA/EV/H171L	S5: 16 (14%, 29 10%, Other 4%)
	S6: 17 (23%, 30 23%, Other 0%)
	S7: 18 (19%, 31 19%, Other 0%)
RT2	S1: 10 (1%, 25)
	S2: 11 (3%, 26)
	S3: 14 (39%, 27 31%, Other 7%)
	S4: 15 (19%, 28 18%, Other 1%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (11%, 31 11%, Other 0%)
	S8: 19 (19%, 32)
	S9: 20 (5%, 33 5%, Other 0%)
	S10: 12 (2%, 34 1%, Other 1%)
	S11: 13 (5%, 35 5%, 36 0%, Other 30%)
	S12: 45 (1%, 46 1%, Other 0%)
	S13: 50 (53%, 51)
	S14: 93 (1%, 108 0%, Other 1%)
	S15: 95 (38%)
	S16: 94 (37%, 110 17%, Other 19%)
	S18: 96 (41%, 112 41%, Other 0%)
	S21: 100 (12%, 115 5%, Other 7%)
	S22: 102 (9%, 116 9%, Other 0%)
	S23: 99 (80%, 117 3%, 118 71%, Other 6%)
	S26: 143 (7%)
	S28: 207 (100%, 206 64%, Other 36%)
RT2/A330W	S1: 10 (1%, 25)
	S3: 14 (7%, 27 6%, Other 1%)
	S4: 15 (1%, 28 1%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (7%, 31 7%, Other 0%)
	S9: 20 (14%, 33 8%, Other 6%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (5%, 35 0%, 36 5%, Other 0%)
	S25: 192 (13%)
	S31: 204 (100%)
	S38: 225 (6%, 273)
RT2/A330W/A82M	S3: 14 (9%, 27 6%, Other 3%)
	S4: 15 (4%, 28 3%, Other 1%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (11%, 30 11%, Other 0%)
	S7: 18 (10%, 31 9%, Other 1%)
	S9: 20 (23%, 33 23%, Other 0%)
	S10: 12 (7%, 34 4%, Other 3%)
	S11: 13 (2%, 35 0%, 36 1%, Other 1%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RT2/A330W/I263A	S3: 14 (1%, 27 1%, Other 0%)
	S7: 18 (1%, 31 0.5%, Other 0.5%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (1%, 35 0.5%, 36 0.5%, Other 0%)
	S31: 204 (71%)
	S38: 225 (11%, 273)
RT2/AI/L437LV	S3: 14 (4%, 27 4%, Other 0%)
	S4: 15 (2%, 28 2%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (3%, 31 2%, Other 1%)
	S9: 20 (10%, 33 10%, Other 0%)
	S10: 12 (2%, 34 0%, Other 2%)
RT2/AP	S1: 10 (2%, 25)
	S2: 11 (2%, 26)
	S3: 14 (11%, 27 7%, Other 4%)
	S4: 15 (4%, 28 3%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S8: 19 (42%, 32)
	S9: 20 (12%, 33 11%, Other 1%)
	S10: 12 (2%, 34 1%, Other 1%)
	S11: 13 (2%, 35 2%, 36 0%, Other 0%)
	S13: 50 (36%, 51)
	S15: 95 (16%)
	S18: 96 (7%, 112 7%, Other 0%)
	S19: 97 (37%, 113 22%, Other 15%)
	S21: 100 (6%, 115 2%, Other 4%)
	S23: 99 (3%, 117 3%, 118 0%, Other 0%)
	S26: 143 (22%)
	S28: 207 (100%, 206 42%, Other 58%)
	S31: 204 (100%)
	S38: 225 (6%, 273)
RT2/AP/A184I	S1: 10 (4%, 25)
	S2: 11 (94%, 26)
	S3: 14 (11%, 27 4%, Other 7%)
	S4: 15 (5%, 28 4%, Other 1%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (12%, 30 12%, Other 0%)
	S7: 18 (14%, 31 13%, Other 1%)
	S8: 19 (25%, 32)
	S9: 20 (16%, 33 16%, Other 0%)
	S10: 12 (5%, 34 4%, Other 1%)
	S11: 13 (12%, 35 12%, 36 0%, Other 63%)
	S12: 45 (2%, 46 2%, Other 0%)
	S13: 50 (33%, 51)
	S14: 93 (2%, 108 0%, Other 2%)
	S15: 95 (72%)
	S16: 94 (84%, 110 74%, Other 10%)
	S17: 92 (69%, 111 69%, Other 0%)
	S18: 96 (24%, 112 24%, Other 0%)
	S19: 97 (58%, 113 52%, Other 6%)
	S21: 100 (5%, 115 1%, Other 4%)

Enzyme	Substrate (conv., product ratio)
RT2/AP/A184I	S23: 99 (26%, 117 26%, 118 0%, Other 0%)
	S26: 143 (34%)
	S28: 207 (100%, 206 10%, Other 90%)
	S31: 204 (90%)
	S33: 247 (24%, 247 0%, Other 0%)
	S38: 225 (49%, 273)
RT2/AP/A184I/F81W	S3: 14 (10%, 27 6%, Other 4%)
	S4: 15 (5%, 28 4%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (6%, 31 5%, Other 1%)
	S9: 20 (21%, 33 21%, Other 0%)
	S10: 12 (3%, 34 2%, Other 1%)
	S11: 13 (8%, 35 1%, 36 6%, Other 1%)
RT2/AP/A82F	S3: 14 (26%, 27 4%, Other 22%)
	S4: 15 (6%, 28 5%, Other 1%)
	S5: 16 (6%, 29 6%, Other 0%)
	S6: 17 (15%, 30 15%, Other 0%)
	S7: 18 (12%, 31 11%, Other 1%)
	S9: 20 (49%, 33 44%, Other 5%)
	S10: 12 (28%, 34 17%, Other 11%)
	S11: 13 (23%, 35 1%, 36 4%, Other 18%)
RT2/AP/A82L	S3: 14 (16%, 27 16%, Other 0%)
	S4: 15 (28%, 28 25%, Other 3%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (13%, 30 13%, Other 0%)
	S7: 18 (16%, 31 15%, Other 1%)
	S8: 19 (16%, 32)
	S9: 20 (22%, 33 22%, Other 0%)
	S10: 12 (7%, 34 3%, Other 4%)
	S11: 13 (16%, 35 0%, 36 2%, Other 14%)
	S16: 94 (70%, 110 64%, Other 6%)
RT2/AP/AI/S72A	S3: 14 (3%, 27 3%, Other 0%)
	S4: 15 (3%, 28 3%, Other 0%)
	S6: 17 (1%, 30 1%, Other 0%)
	S7: 18 (2%, 31 2%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S25: 192 (20%)
RT2/AP/AM	S2: 11 (3%, 26)
	S3: 14 (10%, 27 7%, Other 3%)
	S4: 15 (5%, 28 4%, Other 1%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (10%, 30 10%, Other 0%)
	S7: 18 (11%, 31 11%, Other 0%)
	S8: 19 (14%, 32)
	S9: 20 (23%, 33 23%, Other 0%)
	S10: 12 (5%, 34 4%, Other 1%)
	S11: 13 (7%, 35 1%, 36 1%, Other 6%)
	S12: 45 (6%, 46 6%, Other 0%)
	S13: 50 (18%, 51)
	S15: 95 (93%)
	S16: 94 (49%, 110 23%, Other 26%)
	S19: 97 (61%, 113 20%, Other 41%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RT2/AP/AM	S21: 100 (30%, 115 0%, Other 30%)
	S23: 99 (10%, 117 7%, 118 3%, Other 0%)
	S26: 143 (23%)
	S28: 207 (100%, 206 77%, Other 23%)
	S30: 210 (98%, 232 55%, 233 38%, Other 5%)
	S31: 204 (41%)
	S33: 247 (7%, 247 0%, Other 0%)
	S34: 220 (11%, 248 0%, Other 11%)
	S35: 221 (51%, 253 19%, Other 32%)
	S38: 225 (7%, 273)
RT2/AP/AM/AI	S3: 14 (13%, 27 6%, Other 7%)
	S4: 15 (14%, 28 14%, Other 0%)
	S5: 16 (7%, 29 7%, Other 0%)
	S6: 17 (21%, 30 21%, Other 0%)
	S7: 18 (19%, 31 17%, Other 2%)
	S9: 20 (18%, 33 18%, Other 0%)
	S10: 12 (11%, 34 8%, Other 3%)
	S11: 13 (1%, 35 0%, 36 1%, Other 1%)
RT2/AP/E267F	S3: 14 (8%, 27 4%, Other 4%)
	S4: 15 (2%, 28 2%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (8%, 31 7%, Other 1%)
	S9: 20 (32%, 33 30%, Other 2%)
	S10: 12 (5%, 34 2.5%, Other 2.5%)
	S11: 13 (8%, 35 1%, 36 6%, Other 1%)
RT2/AP/E267L	S3: 14 (9%, 27 3%, Other 6%)
	S4: 15 (2%, 28 2%, Other 0%)
	S5: 16 (5%, 29 3%, Other 2%)
	S6: 17 (7%, 30 7%, Other 0%)
	S7: 18 (8%, 31 7%, Other 1%)
	S9: 20 (55%, 33 49%, Other 6%)
	S10: 12 (24%, 34 23%, Other 1%)
	S11: 13 (36%, 35 9%, 36 24%, Other 3%)
RT2/AP/I401M	S3: 14 (6%, 27 6%, Other 0%)
	S4: 15 (4%, 28 3%, Other 1%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S8: 19 (13%, 32)
	S9: 20 (11%, 33 11%, Other 0%)
	S10: 12 (2%, 34 0%, Other 2%)
	S12: 45 (1%, 46 1%, Other 0%)
	S13: 50 (33%, 51)
	S15: 95 (40%)
	S18: 96 (4%, 112 4%, Other 0%)
	S19: 97 (3%, 113 3%, Other 0%)
	S21: 100 (60%, 115 0%, Other 60%)
	S22: 102 (3%, 116 3%, Other 0%)
	S23: 99 (7%, 117 7%, 118 0%, Other 0%)
	S24: 107 (35%)
	S26: 143 (25%)
	S28: 207 (100%, 206 21%, Other 79%)
RT2/AP/PV	S3: 14 (41%, 27 36%, Other 5%)

Enzyme	Substrate (conv., product ratio)
RT2/AP/PV	S4: 15 (2%, 28 2%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (3%, 30 3%, Other 0%)
	S7: 18 (18%, 31 18%, Other 0%)
	S9: 20 (6%, 33 6%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (1%, 35 0%, 36 1%, Other 0%)
	S18: 96 (14%, 112 14%, Other 0%)
	S25: 192 (46%)
RT2/AP/V78I	S1: 10 (2%, 25)
	S2: 11 (7%, 26)
	S3: 14 (3%, 27 3%, Other 0%)
	S4: 15 (2%, 28 1%, Other 1%)
	S5: 16 (1%, 29 1%, Other 0%)
	S7: 18 (1%, 31 1%, Other 0%)
	S8: 19 (10%, 32)
	S9: 20 (8%, 33 8%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (1%, 35 0%, 36 1%, Other 49%)
	S13: 50 (9%, 51)
	S15: 95 (46%)
	S16: 94 (23%, 110 15%, Other 8%)
	S21: 100 (5%, 115 0%, Other 5%)
	S24: 107 (9%)
	S26: 143 (7%)
	S28: 207 (100%, 206 21%, Other 79%)
RT2/AP/VI/AI	S3: 14 (11%, 27 6%, Other 5%)
	S4: 15 (1%, 28 1%, Other 0%)
	S6: 17 (4%, 30 4%, Other 0%)
	S7: 18 (5%, 31 4%, Other 1%)
	S9: 20 (11%, 33 11%, Other 0%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (3%, 35 0%, 36 2%, Other 1%)
	S18: 96 (14%, 112 14%, Other 0%)
	S25: 192 (24%)
RT2/F81W	S3: 14 (90%, 27 69%, Other 21%)
	S5: 16 (90%, 29 55%, Other 35%)
	S6: 17 (86%, 30 56%, Other 30%)
	S7: 18 (22%, 31 20%, Other 2%)
	S9: 20 (53%, 33 32%, Other 21%)
	S10: 12 (100%, 34 100%, Other 0%)
	S11: 13 (58%, 35 23%, 36 24%, Other 11%)
	S12: 45 (6%, 46 5%, Other 1%)
	S13: 50 (81%, 51)
	S15: 95 (31%)
	S18: 96 (27%, 112 27%, Other 0%)
	S19: 97 (97%, 113 0%, Other 97%)
	S21: 100 (21%, 115 9%, Other 12%)
	S22: 102 (13%, 116 13%, Other 0%)
	S23: 99 (68%, 117 25%, 118 43%, Other 0%)
	S26: 143 (19%)
	S28: 207 (100%, 206 90%, Other 10%)
RT2/F81W/A328I	S3: 14 (20%, 27 16%, Other 4%)

Appendix B – Summary of screening data organised by $P450_{BM3}$ mutant

Enzyme	Substrate (conv., product ratio)
RT2/F81W/A328I	S4: 15 (4%, 28 4%, Other 0%)
	S6: 17 (7%, 30 7%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S9: 20 (23%, 33 23%, Other 0%)
	S10: 12 (4%, 34 2%, Other 2%)
	S25: 192 (38%)
RT2/FW	S30: 210 (91%, 232 61%, 233 19%, Other 11%)
	S31: 204 (88%)
	S32: 219 (12%, 247 12%, Other 0%)
	S34: 220 (73%, 248 55%, Other 18%)
	S36: 222 (6%)
RT2/I263W	S3: 14 (41%, 27 35%, Other 6%)
	S4: 15 (22%, 28 22%, Other 0%)
	S5: 16 (3%, 29 3%, Other 0%)
	S6: 17 (5%, 30 5%, Other 0%)
	S7: 18 (9.1%, 31 8%, Other 1%)
	S10: 12 (1%, 34 0%, Other 1%)
	S11: 13 (5%, 35 0%, 36 4%, Other 1%)
RT2/IP	S1: 10 (19%, 25)
	S2: 11 (32%, 26)
	S3: 14 (43%, 27 23%, Other 20%)
	S4: 15 (26%, 28 23%, Other 3%)
	S5: 16 (14%, 29 14%, Other 0%)
	S6: 17 (18%, 30 18%, Other 0%)
	S7: 18 (15%, 31 15%, Other 0%)
	S8: 19 (26%, 32)
	S9: 20 (10%, 33 8%, Other 2%)
	S10: 12 (11%, 34 10%, Other 1%)
	S11: 13 (45%, 35 2%, 36 24%, Other 19%)
	S12: 45 (9%, 46 9%, Other 0%)
	S13: 50 (99%, 51)
	S14: 93 (1%, 108 1%, Other 0%)
	S15: 95 (32%)
	S16: 94 (43%, 110 31%, Other 12%)
	S17: 92 (46%, 111 46%, Other 0%)
	S18: 96 (49%, 112 49%, Other 0%)
	S19: 97 (86%, 113 72%, Other 14%)
	S21: 100 (28%, 115 17%, Other 11%)
	S22: 102 (12%, 116 12%, Other 0%)
	S23: 99 (41%, 117 18%, 118 23%, Other 0%)
	S24: 107 (28%)
	S26: 143 (55%)
	S28: 207 (100%, 206 81%, Other 9%)
	S30: 210 (100%, 232 78%, 233 22%, Other 0%)
	S31: 204 (91%)
	S32: 219 (23%, 247 22%, Other 1%)
	S34: 220 (71%, 248 31%, Other 40%)
	S35: 221 (100%, 253 50%, Other 50%)
	S38: 225 (47%, 273)
	S39: 226 (7%)
RT2/IP/A184I	S3: 14 (39%, 27 6%, Other 33%)
	S4: 15 (15%, 28 13%, Other 2%)
	S5: 16 (17%, 29 11%, Other 6%)

Enzyme	Substrate (conv., product ratio)
RT2/IP/A184I	S6: 17 (23%, 30 23%, Other 0%)
	S7: 18 (15%, 31 13%, Other 2%)
	S9: 20 (40%, 33 22%, Other 18%)
	S10: 12 (66%, 34 65%, Other 1%)
RT2/IP/F87V	S3: 14 (71%, 27 23%, Other 48%)
	S4: 15 (50%, 28 48%, Other 2%)
	S5: 16 (36%, 29 29%, Other 7%)
	S6: 17 (27%, 30 27%, Other 0%)
	S7: 18 (20%, 31 15%, Other 5%)
	S10: 12 (43%, 34 41%, Other 2%)
	S11: 13 (84%, 35 36%, 36 35%, Other 14%)
RT2/IP/V78I	S30: 210 (88%, 232 52%, 233 36%, Other 0%)
	S31: 204 (14%)
	S32: 219 (10%, 247 9%, Other 1%)
	S35: 221 (64%, 253 37%, Other 27%)
	S36: 222 (34%)
RT2/V78F	S3: 14 (15%, 27 15%, Other 0%)
	S4: 15 (1%, 28 1%, Other 0%)
	S5: 16 (2%, 29 2%, Other 0%)
	S6: 17 (2%, 30 2%, Other 0%)
	S7: 18 (4%, 31 3%, Other 1%)
	S10: 12 (2%, 34 0%, Other 2%)
	S25: 192 (26%)
	S31: 204 (85%)
	S38: 225 (13%, 273)
WT	S1: 10 (3%, 25)
	S2: 11 (1%, 26)
	S3: 14 (1%, 27 1%, Other 0%)
	S4: 15 (1%, 28 0%, Other 1%)
	S7: 18 (2%, 31 2%, Other 0%)
	S9: 20 (3%, 33 2%, Other 1%)
	S10: 12 (1%, 34 0%, Other 1%)
	S13: 50 (3%, 51)
	S15: 95 (5%)
	S20: 98 (28%, 114 28%, Other 0%)
	S21: 100 (2%, 115 0%, Other 2%)
	S23: 99 (7%, 117 2%, 118 5%)
	S28: 207 (100%, 206 20%, Other 80%)