

The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

Semisynthesis of Jerantinine Alkaloids

Paul Stanley, MChem.

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

March 2017

Abstract

This thesis details the synthetic studies undertaken on natural products with anti-cancer activity. The general aim was to explore the synthesis and find an efficient and effective method to generate compounds, which would be used to further examine novel biological action.

The first half of this thesis details the synthetic studies on the jerantinine family of natural products. The approach utilised the complex alkaloid (-)-tabersonine as a starting material, which was isolated from an abundant and sustainable source. Ultimately the first synthesis of (-)-jerantinine A and first enantioselective synthesis of (-)-jerantinine E were achieved. The biological activity of the synthetic material proved to be identical to that isolated from natural sources.

The final part of this work describes the synthesis of a series of compounds derived from piperlongumine, which is of the *Piperaceae* family of natural products. The synthesis was accomplished using a novel methodology, which enabled the generation of a focussed library of derivatives. The compounds were tested by collaborators and their anti-cancer activity assessed in a variety of cell lines, revealing features of the structure activity relationship.

Acknowledgements

Firstly I would like to thank my supervisor, Dr John Moses, for giving me the opportunity to work in his group and for his support, ideas and enthusiasm. Thanks also go to all members of the Moses group, past and present, for their friendship and encouragement and providing such a supportive working atmosphere.

In addition I would like to acknowledge Dr Tracey Bradshaw, Mohannad Qazzaz and Paul Wood for their most generous biological testing and for insightful scientific discussions. I would also like to thank Professor Chee-Onn Leong and Dr Chun Wai Mai for their exhaustive high throughput screening and their polite correspondence and enthusiasm toward the project.

Many thanks go to the departmental technical and analytical staff for their kind and invaluable assistance.

I would like to thank my friends for the constant reminder of what is really important. Finally my heartfelt gratitude is owed to my partner and family, for their patience and understanding over the years.

Contents

Part One – Semisynthesis of Jerantinine Alkaloids 13

1 Introduction 14

1.1 Jerantinines A-H (1-8) 14

1.2 Biological Activity 16

1.3 Biosynthesis of Monoterpenoid Indole Alkaloids 17

1.4 Jerantinine Alkaloids (1-8): Previous Literature 21

1.4.1 Waser's Synthesis of (±)-Jerantinine E (5) 21

1.5 Semisynthesis 24

1.5.1 Potier's Biomimetic Semisynthesis of Vinblastine (10) 25

1.5.2 Baran's Semisynthesis of (+)-Cortistatin A (48) 26

1.6 Aromatic Hydroxylation in Semisynthesis 28

1.6.1 Lavie's Semisynthesis of Brucine (50) 28

1.6.2 Palmisano's Semisynthesis of Vindoline (9) 30

1.7 Aims 32

2 Results and Discussion 34

2.1 Retrosynthetic Analysis of Jerantinine A (1) 34

2.2 Extraction of Tabersonine (12) 35

2.3 Preparation of Phenol 61 37

2.3.1 Attempted Aromatic Hydroxylation of Tabersonine (12) 37

2.3.1.1 Elbs Persulfate Oxidation 38

2.3.1.2 Teuber Oxidation 38

2.3.1.3 Phthaloyl Peroxide (64) Mediated Arene Hydroxylation 40

2.3.2 Synthesis of *tert*-Butyl 10-Iodotabersonine-1-carboxylate (69) 42

2.3.3 Attempted Hydroxylation of *tert*-Butyl 10-Iodotabersonine-2-carboxylate (69) 44

2.3.4 Synthesis of *tert*-Butyl Tabersonine-1-carboxylate-10-boronic Acid Pinacol Ester (77) 46

2.3.5 Synthesis of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61) 48

2.4 Preparation of Catechol Dimethyl Ether 60 51

2.4.1 Attempted Aromatic Hydroxylation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61) 51

2.4.1.1 Teuber Oxidation 51

2.4.1.2 2-Iodoxybenzoic Acid (80) Mediated Hydroxylation of Phenols 52

2.4.2 Attempted Aromatic Halogenation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61) 55

2.4.3 Attempted *Ortho* Functionalisation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61) 57

2.4.3.1 Directed *Ortho* Lithiation 57

2.4.3.2 Directed *Ortho* Borylation 60

2.4.3.3 Directed Ortho Hydroxylation 64

2.4.4 *Ortho* Formylation 68

2.4.4.1 Vilsmeier-Haack Reaction 68

2.4.4.2 Rieche Formylation 69

2.4.4.3 Reimer-Tiemann Formylation 70

2.4.4.4 Magnesium Mediated *Ortho* Formylation 71

2.4.5 Oxidation of *tert*-Butyl 10-Hydroxytabersonine-carbaldehyde-1-carboxylate (109) 73

2.4.6 Synthesis of *tert*-Butyl 10-*O*-Methyljerantinine A-1-carboxylate (60) 76

2.5 Preparation of Jerantinine A (1) and Jerantinine E (5) 77

2.5.1 Synthesis of 10-*O*-Methyljerantinine A (117) 77

2.5.2 Synthesis of Jerantinine A (1) 78

2.5.3 Synthesis of Jerantinine A Acetate (119) 81

2.5.4 Synthesis of Jerantinine E (5) 82

2.6 Biological Evaluation of Jerantinine A (1) 85

2.6.1 MTT Assay 85

2.6.2 Growth Inhibitory Effects of Jerantinine A (1) in Colorectal (HCT 116) and Breast (MCF-7) Carcinoma Cell Lines 86

2.7 Conclusions 90

3 Experimental 92

3.1 Experimental Techniques 92

3.2 Analysis 92

3.3 Cell Culture 93

3.4 MTT Assay 94

3.5 Experimental Procedures and Characterisation Data 95

Part 2 – Horner-Wadsworth-Emmons Approach to Piperlongumine and Structural Analogues 109

4 Introduction 110

4.1 Piperlongumine (123) 110

4.2 Biological Activity 111

4.3 Biosynthesis of *Piperaceae* Alkamides 112

4.4 Piperlongumine (123): Previous Literature 113

4.4.1 Simonsen's Synthesis of Piperlongumine (123) 113

4.4.2 Schreiber's Synthesis of Piperlongumine (123) 114

4.4.3 Jun's Synthesis of Piperlongumine (123) 116

4.5 Pericyclic *Syn*-Elimination 117

4.5.1 Sulfoxide Pyrolysis 118

4.5.2 Selenoxide Pyrolysis 119

4.6 Phosphorus Mediated Olefination 120

4.6.1 Wittig Reaction 120

4.6.2 Horner-Wadsworth-Emmons Reaction 123

4.7 Aims 126

5 Results and Discussion 127

5.1 First Generation Retrosynthesis – Amide Coupling Route 127

5.2 Synthesis of 5,6-dihydropyridin-2(1H)-one 130 128

5.3 Synthesis of Piperlongumine (123) 130

5.4 Second Generation Synthesis – Horner-Wadsworth-Emmons Route 132

5.5 Synthesis of a Model Phosphonoacetamide 173 133

5.6 Synthesis of Phosphonoacetamide 169 136

5.7 Synthesis of Piperlongumine Analogues 176-186 140

5.8 Biological Evaluation of Piperlongumine (123) and Analogues 176-186 141

5.8.1 Growth Inhibitory Effects of Piperlongumine (123) and Analogues 176-186 in Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Lines 142

5.8.2 Clonogenic-Survival Assay 144

5.8.3 Effects of Piperlongumine (123) and Analogues 176-186 on Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Colony Formation 144

5.8.4 ROS-Glo Hydrogen Peroxide Assay 146

5.8.5 The Effects of Piperlongumine (123) and Analogues 180-184 on Reactive Oxygen Species Levels in Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Lines 146

5.8.5.1 HCT 116 Cells 147

5.8.5.2 IGROV-1 Cells 150

5.9 Conclusion 153

6 Experimental 155

6.1 Experimental Techniques 155

6.2 Analysis 155

6.3 Cell Culture 156

6.4 MTT Assay 157

6.4.1 HCT 116 Cells 157

6.4.2 IGROV-1 Cells 159

6.5 Clonogenic Assay 160

6.6 Determination of Reactive Oxygen Species 164

6.7 Experimental Procedures and Characterisation Data 164

7 References 184

Abbreviations

7-DLGT	7-deoxyloganetic acid glucosyl transferase
8-HGO	8-hydroxygeraniol oxidoreductase
Å	angstrom
Ac	Acetyl
aq	aqueous
Boc	*tert*-butyloxycarbonyl
BTEA-ICl_2	benzyltriethylammonium dichloroiodate
Bu	butyl
CAN	ceric ammonium nitrate
Cbz	carbobenzoxy
CoA	coenzyme A
cod	cyclooctadienyl
Cy	cyclohexyl
Δ	reflux
dba	dibenzylideneacetone
DCM	dichloromethane
DLH	deoxyloganic acid 7-hydroxylase
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMG	directed metalation group
DMSO	dimethylsulfoxide
DMTMM	dimethoxytriazin-*N*-methylmorpholinium chloride

dtbpy	4,4’-di-*tert*-butyl-2,2’-dipyridyl
E^+	electrophile
Et	ethyl
FBS	foetal bovine serum
formazan	(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
G8O	geraniol 8-oxidase
GI_{50}	growth inhibition of 50%
GSH	reduced glutathione
GSSG	oxidised glutathione
GSTP1	glutathione S-transferase pi 1
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HWE	Horner-Wadsworth-Emmons
i	iso
IBX	2-iodoxybenzoic acid
IC_{50}	inhibitory concentration for growth inhibition of 50%
IO	iridoid oxidase
IS	iridoid synthase
LAMT	loganic acid methyltransferase
LDA	lithium diisopropylamide
L_n	ligand
*m*CPBA	3-chloroperbenzoic acid
Me	methyl
µW	microwave
MOM	methoxymethyl

Ms	methanesulfonate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBS	*N*-bromosuccinimide
n	normal
NIS	*N*-iodosuccinimide
NMM	4-methylmorpholine
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
Ph	phenyl
phen	1,10-phenanthroline
pin	pinacolato
ppm	parts per million
Pr	propyl
ROS	reactive oxygen species
r.t.	room temperature
SD	standard deviation
SEM	standard error of the mean
SLS	secologanin synthase
STR	strictosidine synthase
t	tertiary
TDC	tryptophan decarboxylase
THF	tetrahydrofuran

Tf	trifluoromethanesulfonate
TFA	trifluoroacetic acid
TFE	trifluoroethanol
TFAA	trifluoroacetic anhydride
TMEDA	*N*,*N*,*N*',*N*'-tetramethylethylenediamine
triflate	trifluoromethanesulfonate
UV	ultraviolet

Part One – Semisynthesis of Jerantinine Alkaloids

1 Introduction

1.1 Jerantinines A-H (1-8)

The isolation and characterisation of jerantinines A-G (**1**-**7**) was reported by Lim *et al*. in 2008 (Figure 1.1).[1] The novel, indole alkaloid natural products feature a pentacyclic carbon skeleton, each isolated as a single enantiomer.

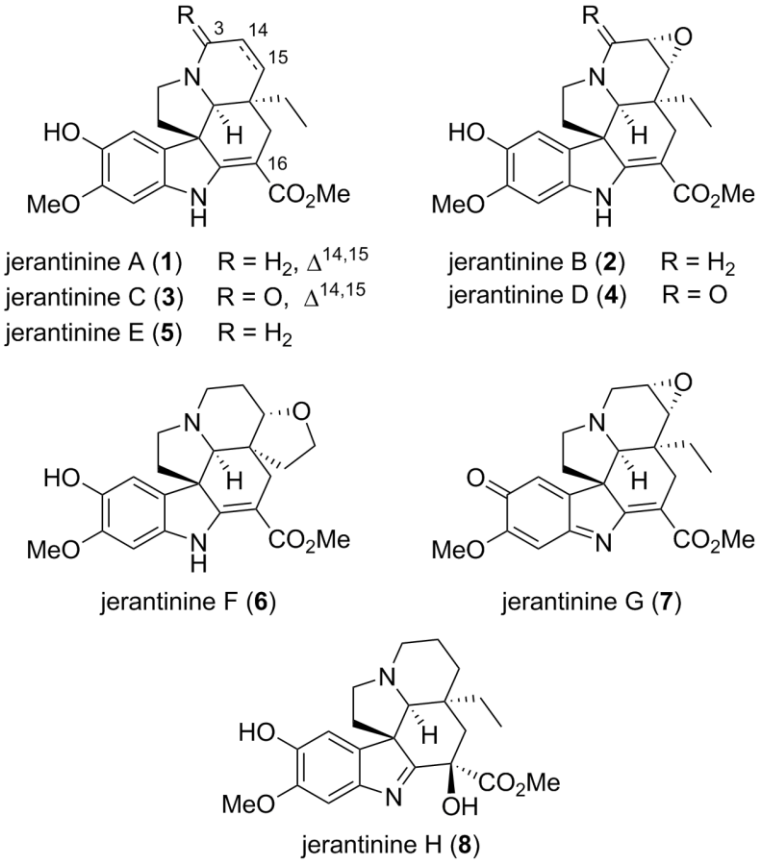


Figure 1.1: Jerantinines A-H (**1**-**8**)

As part of Lim's systematic investigation into the bioactive natural products from Malaysian plants of the genus *Tabernaemontana*, the ethanolic extract from ground leaf material of *T*. *corymbosa* was partitioned with dilute acid.[1] The resulting mixture of alkaloids was first purified by silica gel column chromatography, yielding a number of fractions. Subsequent centrifugal thin-

layer chromatography of the appropriate biologically active fractions gave each of the jerantinines A-G (**1**-**7**).

Structurally, jerantinines A-G (**1**-**7**) each possess the same *Aspidosperma* type pentacyclic core (Figure 1.1).[2] Jerantinines A-E (**1**-**5**) arise from variations in the oxidation state at carbons 3, 14 and 15. The π-bond between C14 and C15 present in jerantinine A (**1**) and C (**3**) is epoxidised to form jerantinines B (**2**) and D (**4**) respectively and reduced in jerantinine E (**5**). Additional oxidation also further distinguishes jerantinines C (**3**) and D (**4**), which have a C3 carbonyl moiety. Jerantinine F (**6**) is the only hexacyclic member of the family, where apparent oxidation at the remote, pendant ethyl group has led to an intramolecular etherification. The reportedly unstable imino quinone jerantinine G (**7**) was also isolated and observed as a degredation product upon leaving a dichloromethane solution of jerantinine B (**2**) open to air.

In the following year the same group published a second report, detailing the isolation and characterisation of another member of the jerantinine series of natural products.[3] Jerantinine H (**8**) has an indolenine moiety and concomitant C16 chiral centre, which are unique features among the family.

1.2 Biological Activity

The *Aspidosperma* type carbon skeleton is prevalent in nature. Furthermore, several pharmacologically significant compounds derive from the same pentacyclic framework, which display potent and desirable biological effects.[4] The pertinent example vindoline (**9**) makes up part of the more elaborate, clinically approved frontline tubulin inhibitors; *Vinca* alkaloids, vinblastine (**10**) and vincristine (**11**) (Figure 1.2).[5]

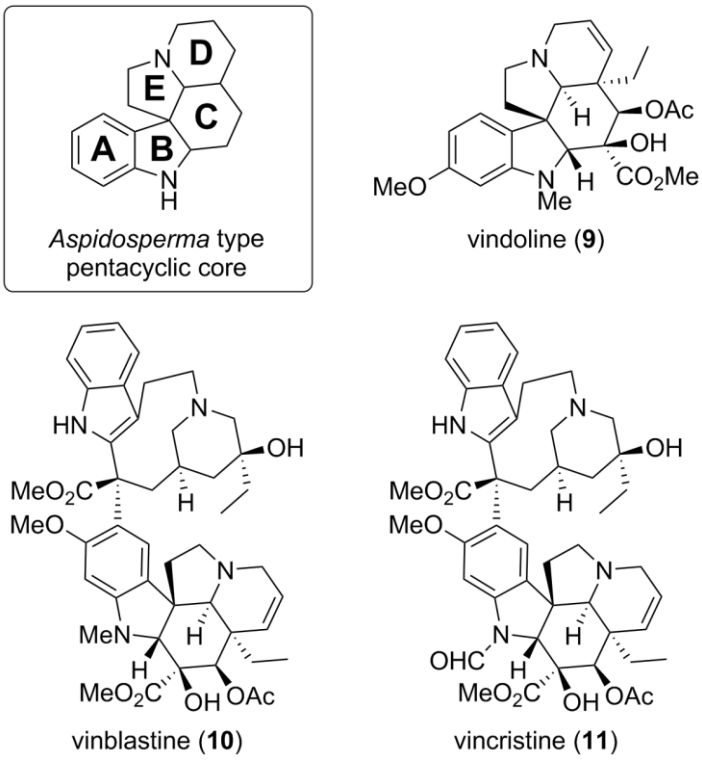


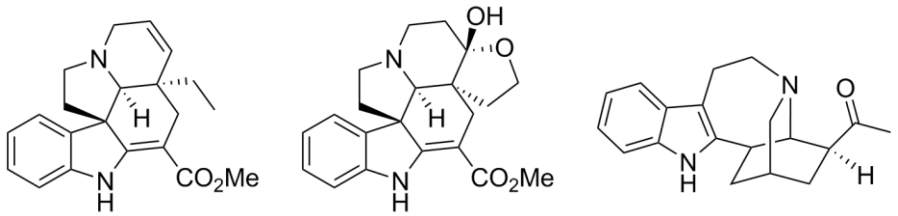
Figure 1.2: *Aspidosperma* alkaloid vindoline (**9**) and *Vinca* alkaloids vinblastine (**10**) and vincristine (**11**)

Interest in the biological activity of the jerantinine alkaloids stems from their pronounced *in vitro* cytoxicity toward vincristine resistant human oral epidermoid carcinoma cell lines. Recent investigations by Bradshaw *et al*. further explored the mode of action, revealing tubulin polymerisation as a

major mechanism potently inhibited by jerantinine A (**1**).[6] The *Vinca* alkaloids **10** and **11** are also tubulin inhibitors, which bind to the *Vinca* domain of the β-tubulin subunit. However, the cytotoxicity of the jerantinine alkaloids against vincristine (**11**) resistant cancer cells could be indicative of a different mode of action and is an appealing biological characteristic, making them desirable synthetic targets.

1.3 Biosynthesis of Monoterpenoid Indole Alkaloids

Although the biosynthetic pathways that lead to the formation of various monoterpenoid indole alkaloids have been well investigated,[7] there has been little speculation on the biogenic origin of the jerantinine alkaloids (**1**-**8**). Recently, the entire 7 gene pathway for the biogenesis of vindoline (**9**) from the simplest *Aspidosperma* alkaloid tabersonine (**12**) was published by De Luca *et al*., alongside its assembly in yeast.[8] While as yet there are no reported instances of the isolation of tabersonine (**12**) from *T*. *corymbosa*, many closely related *Aspidosperma* and *Iboga* type indole alkaloids have been observed (Figure 1.3).[9]

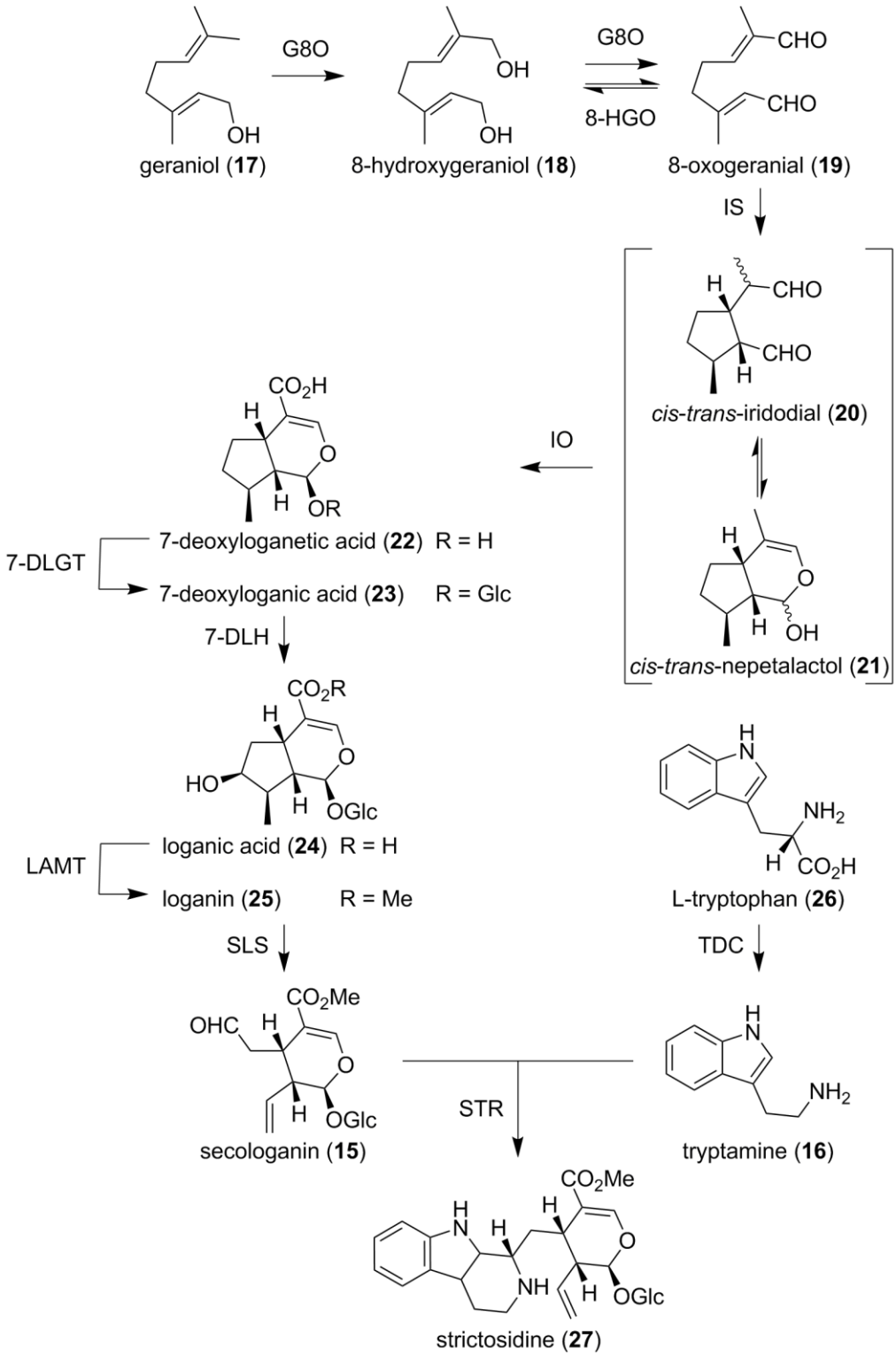


tabersonine (**12**) apocidine A (**13**) conodusine A (**14**)

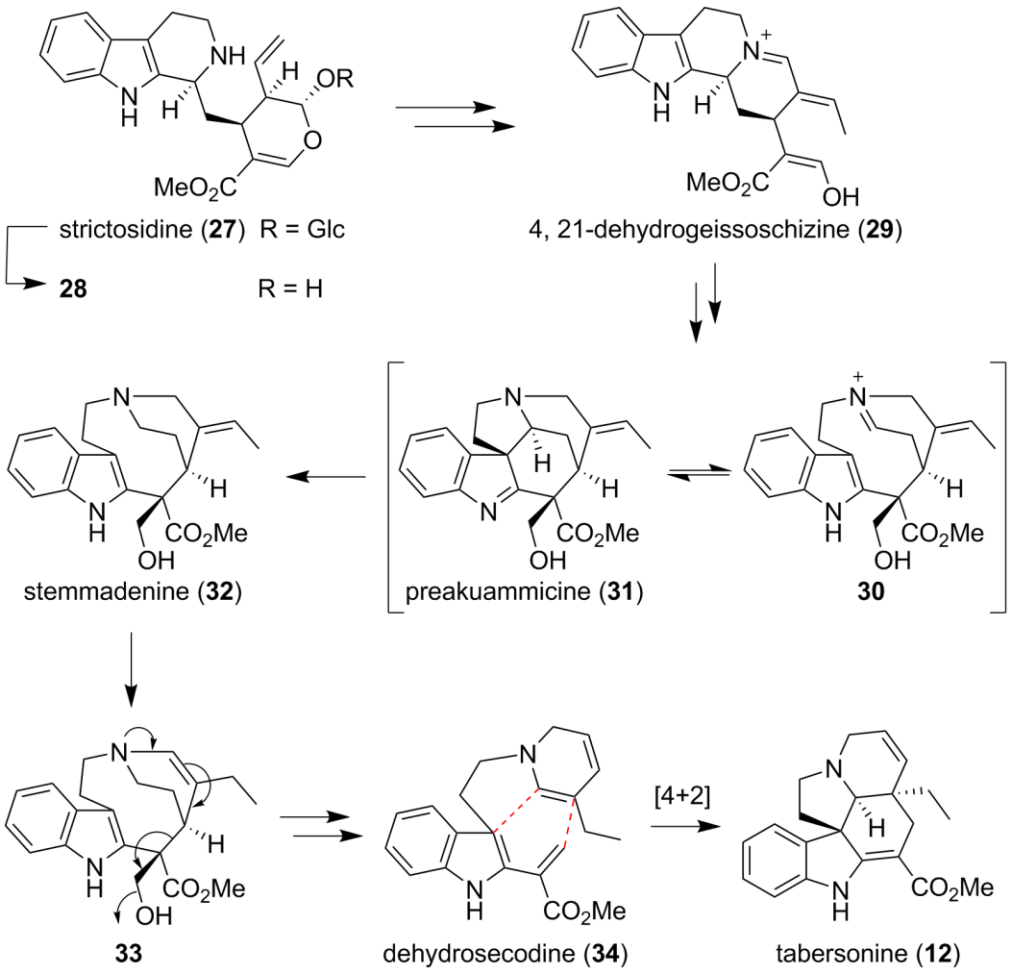
Figure 1.3: Tabersonine (**12**) and representitive alkaloids isolated from *T. corymbosa*, apocidine A (**13**) and conodusine A (**14**)

Most monoterpenoid indole alkaloids include a C_9 or C_{10} fragment originating from secologanin (**15**) and an indole moiety derived from tryptamine (**16**) (Scheme 1.1).[10] Recent efforts have successfully elucidated the entire biosynthesis of secologanin (**15**) in *C. roseus*, which derives from geraniol (**17**) by an 8 gene pathway.[11] Initial oxidation mediated by geraniol 8-oxidase (G8O) gives 8-hydroxygeraniol (**18**).[12] Further partially overlapping redox processes are then catalysed by 8-hydroxygeraniol oxidoreductase (8-HGO) and G8O, resulting in the production of 8-oxogeranial (**19**). Next, iridoid structures are formed by iridoid synthase (IS) through reductive cyclisation and hetero Diels-Alder reaction giving iridodial (**20**) and nepetalactol (**21**) respectively.[13] Both are active substrates for the enzyme iridoid oxidase (IO), which converts **20** and **21** into 7-deoxyloganetic acid (**22**). Glycosylation by 7-deoxyloganetic acid glucosyl transferase (7-DLGT) precedes further deoxyloganic acid 7-hydroxylase (DLH) mediated oxygenation. Methylation of the loganic acid (**24**) by loganic acid methyltransferase (LAMT) produces the penultimate species loganin (**25**).[14] Finally secologanin (**15**) is generated from a secologanin synthase (SLS) catalysed oxidative ring-opening.[15]

Incorporation of the heterocyclic moiety begins with decarboxylation of the amino acid L-tryptophan (**26**) by the enzyme tryptophan decarboxylase (TDC). Strictosidine synthase (STR) catalysed, Pictet-Spengler type condensation with secologanin (**15**) gives the intermediate strictosidine (**27**) and after numerous rearrangements, **27** is converted into the core structures of the *Corynanthe*, *Iboga* and *Aspidosperma* alkaloids.[16]



Scheme 1.1: Biosynthesis of strictosidine (**27**). G8O, geraniol 8-oxidase; 8-HGO, 8-hydroxygeraniol oxidoreductase; IS, iridoid synthase; IO, iridoid oxidase; 7-DLGT, 7-deoxyloganetic acid glucosyl transferase; 7-DLH, 7-deoxyloganic acid hydroxylase; LAMT, loganic acid O-methyltransferase; SLS, secologanin synthase; STR, strictosidine synthase; TDC, tryptophan decarboxylase



Scheme 1.2: Proposed biosynthesis of tabersonine (**12**) from strictosidine (**27**)

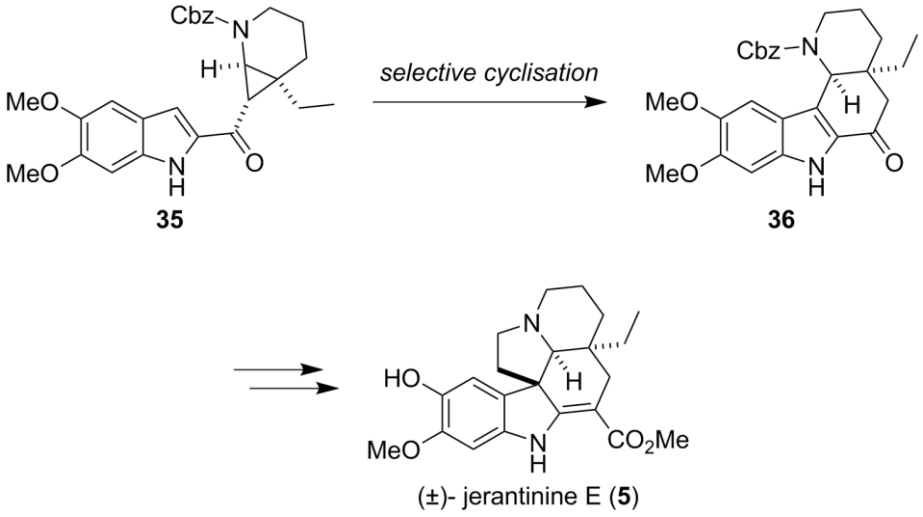
Although the precise biosynthetic pathway connecting strictosidine (**27**) and the *Aspidosperma* type pentacyclic alkaloids has yet to be fully elucidated, several intermediates have been suggested and from these some conclusions can be inferred. First, it has been proposed that cleavage of the glucose moiety leads to the unstable, highly reactive aglucon **28**, which is thought to be converted to 4,21-dehydrogeissoschizine (**29**) (Scheme 1.3.1).[17] Subsequently, a series of as yet undefined transformations generates preakuammicine (**31**), which upon reduction gives stemmadenine (**32**). Double bond migration then facilitates carbon-carbon bond cleavage and concomitant dehydration,

ultimately producing dehydrosecodine (**34**). Tabersonine (**12**) is then presumed to arise by an intramolecular [4+2] cycloaddition.

1.4 Jerantinine Alkaloids (1-8): Previous Literature

1.4.1 Waser's Synthesis of (±)-Jerantinine E (5)

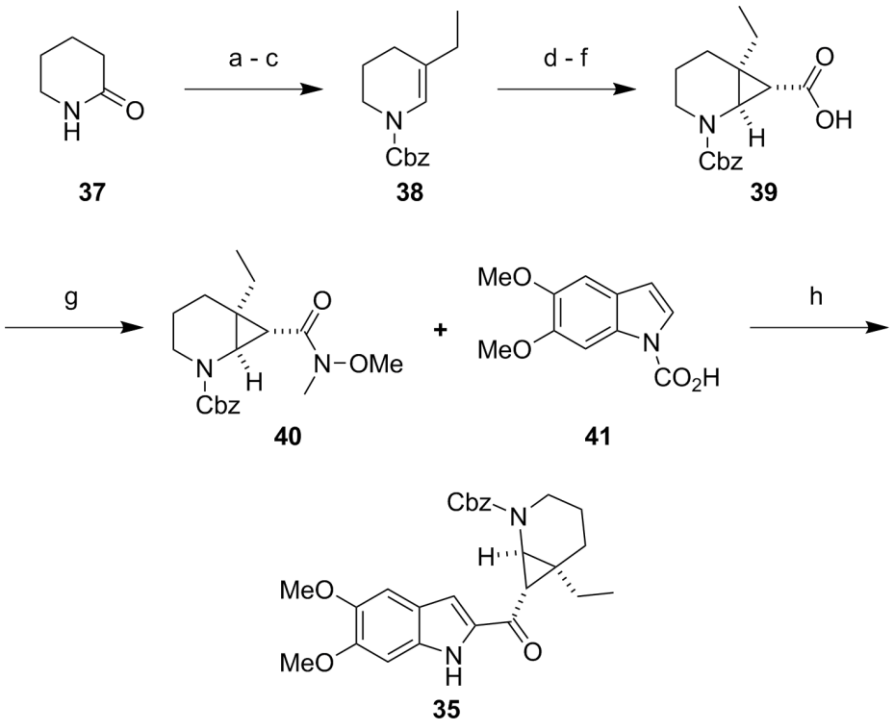
Since their isolation in in 2008, there has been only one reported total synthesis of a member of the jerantinine family of natural products. (±)-Jerantinine E (**5**) was synthesised in 2013 by Waser *et al*. using a 17 step sequence (Scheme 1.3).[18] Central to the approach was a copper catalysed selective cyclisation of the aminocyclopropane **35**.



Scheme 1.3: Synthesis of (±)-jerantinine E (**5**) accomplished by Waser *et al*. – key step

Starting from δ-valerolactam (**37**) the key cyclopropane **35** was synthesised in 8 linear steps using methodology previously published by the group (Scheme 1.4).[19] Initial one-pot protection and alkylation was followed by reduction and sulfuric acid mediated dehydration to give dehydropiperidine **38**.

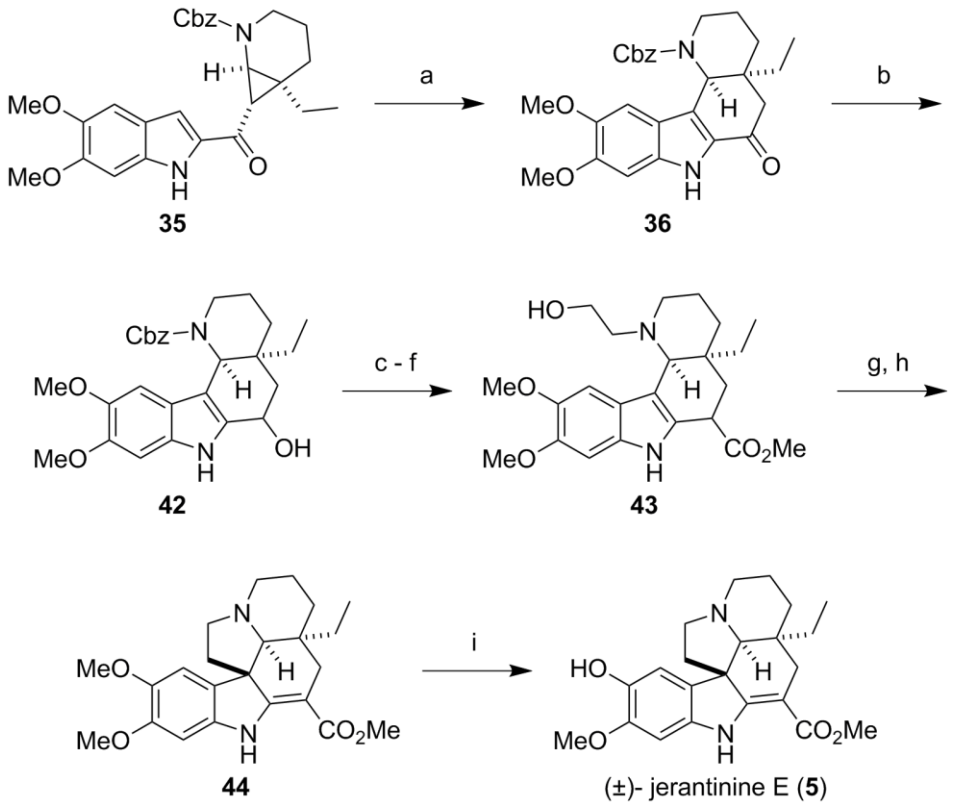
Cyclopropanation with ethyl diazoacetate catalysed by copper(I) triflate toluene complex resulted in a mixture of diastereomers. However, upon treatment with boron trifluoride diethyl etherate, equilibration to the *exo*-isomer was effected. Subsequent hydrolysis of the ethyl ester gave carboxylic acid **39**, which was converted into the corresponding Weinreb amide **40**. Finally, introduction of an indole moiety was achieved by ketone formation. Directed lithiation of the *N*-carboxy indole **41** and addition to the Weinreb amide **40** gave the cyclisation precursor, cyclopropane **35**.



Scheme 1.4: Synthesis of aminocyclopropane **35** from δ-valerolactam (**37**). Reagents and conditions: (a) ^{n}BuLi, CbzCl, EtI, THF, 0 °C, 67%; (b) $NaBH_4$, MeOH, 0 °C; (c) H_2SO_4, THF, r.t., 93% over 2 steps; (d) $(CuOTf)_2{\cdot}C_7H_8$, N_2CHCO_2Et, CH_2Cl_2, r.t., 76% (d.r. 1:1); (e) $BF_3{\cdot}Et_2O$, CH_2Cl_2, -20 → 0 °C; (f) NaOH, H_2O/THF/EtOH (1:1:3), 60 °C, 91% over 2 steps; (g) DMTMM, NMM, THF, MeNHOMe·HCl, r.t., 93%; (h) ^{t}BuLi, LiCl, THF, -78 → -20 °C, 72%

Treatment of the cyclopropane **35** with copper(II) triflate induced the key selective cyclisation, giving the fused tetracyclic core **36** as a single

diastereoisomer (Scheme 1.5). Next, reduction of the ketone gave alcohol **42**, which was converted into the ester **43** by cyanation, Pinner methanolysis, deprotection and *N*-alkylation. Ring closure occurred through mesylation and deprotonation gave pentacycle **44** after migration of the resulting double bond. Finally, selective demethylation proved to be possible by a ceric ammonium nitrate mediated oxidation, hydrolysis and subsequent *in situ* reduction, revealing the natural product (±)-jerantinine E (**5**) in an overall yield of 16% and 17 linear steps from δ-valerolactam (**37**).



Scheme 1.5: Synthesis of (±)-jerantinine E (**5**). Reagents and conditions: (a) $Cu(OTf)_2$, MeCN, r.t., 85%; (b) $LiAlH_4$, THF, 0 °C, 96%; (c) $BF_3{\cdot}OEt_2$, Me_3SiCN, CH_2Cl_2, -78 °C; (d) HCl, MeOH, 0 °C; (e) Pd/C, H_2, EtOH, r.t.; (f) Na_2CO_3, $BrCH_2CH_2OH$, MeOH, r.t., 78% over 4 steps; (g) MsCl, Et_3N, CH_2Cl_2, -20 °C; (h) KO^tBu, THF, r.t., 85% over 2 steps; (i) $(NH_4)_2Ce(NO_3)_6$, MeCN/H_2O (1:1), 0 °C, 79%

Following their completion of the first total synthesis of (±)-jerantinine E (**5**), biological properties were examined. Interestingly once separated, only the naturally occurring enantiomer (-)-jerantinine E (**5**) showed any significant activity in cellular assays. Similar cytotoxic effects were observed in both breast (MDA-MB-231 and MCF-7) and lung (A549 and HTB-178) cancer model cell lines. Furthermore, investigations into the mode of action suggested that (-)-jerantinine E (**5**) disrupts the microtubule network, indicated by the inhibition of tubulin polymerisation.

While an impressive achievement, the presented approach also highlights some of the limitations of total synthesis that can hinder the development of potential therapeutic candidates. However, the complimentary method of semisynthesis can sometimes be used to reduce the number of transformations required, improving the overall economy.

1.5 Semisynthesis

Since antiquity natural products have been at the forefront of drug discovery. Through undertaking the total synthesis of structurally intricate natural molecules, scientific developments are fuelled and access to near unobtainable material can be enabled. Unfortunately, the costs of such significant synthetic undertakings render some target molecules unfeasible drug candidates. Semisynthesis uses advanced natural material as a starting point for chemical synthesis, which can offer an attractive alternative. Again, the *Vinca* alkaloid vinblastine (**10**) provides a topical example.

1.5.1 Potier's Biomimetic Semisynthesis of Vinblastine (10)

Since it's serendipitous discovery in 1958 by Noble *et al*. vinblastine (**10**) has been recognised as one of the most significant developments in the field of anticancer medicine.[20] Isolated from *Catharanthus roseus* alongside two putative biogenic precursors catharanthine (**45**) and vindoline (**9**), the tragically low yields of vinblastine (**10**) prompted much research into chemical synthesis (Figure 1.4).

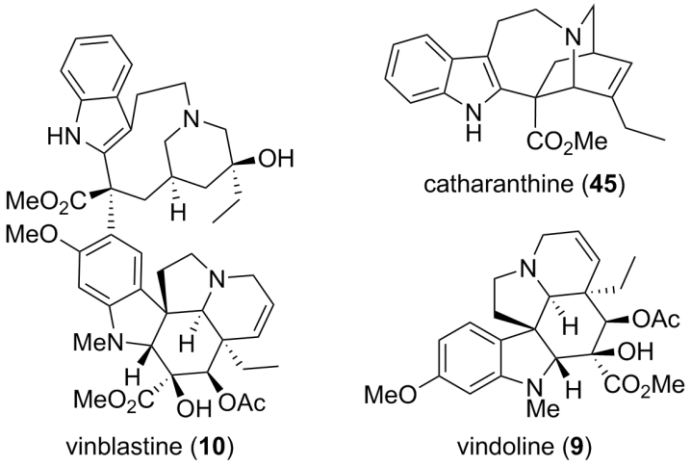
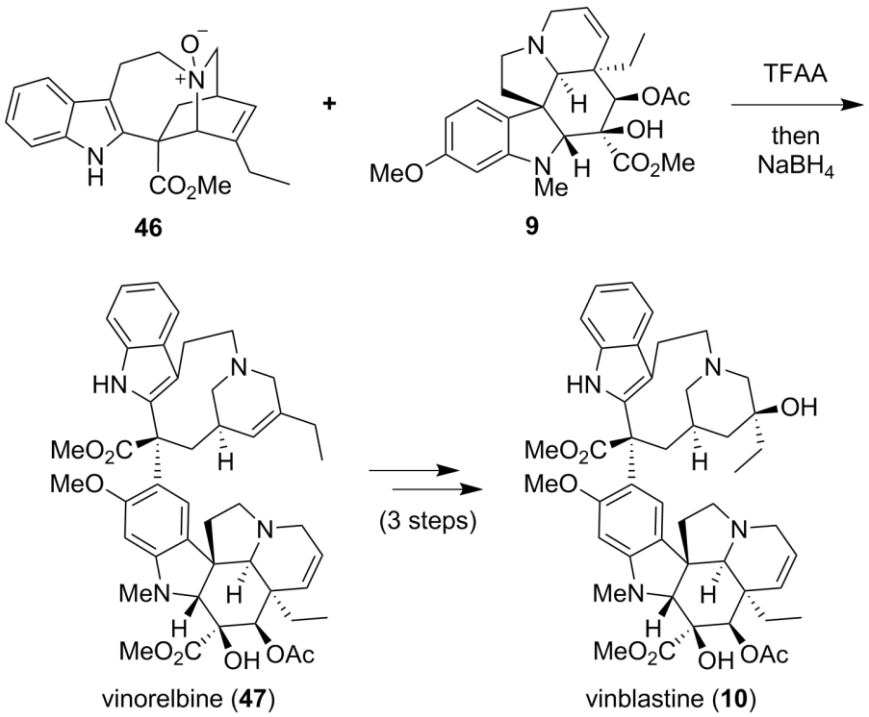


Figure 1.4: Vinblastine (**10**) and biogenic precursors vindoline (**9**) and catharanthine (**35**)

The first synthesis of vinblastine (**10**) was reported in 1979 by Potier *et al*. accomplished using a biomimetic, semisynthetic strategy (Scheme 1.6).[21] Starting from an *N*-oxide **46** derived from catharanthine (**45**), treatment with trifluoroacetic anhydride initiated a Polonovski type reaction with vindoline (**9**), which after reduction of the resulting iminium ion delivered the intermediate vinorelbine (**47**). Conversion to vinblastine (**10**) was ultimately achieved using a 3 step oxidation sequence, giving the natural product in 30% overall yield.



Scheme 1.6: Biomimetic semisynthesis of vinblastine (**10**) accomplished by Potier *et al*.

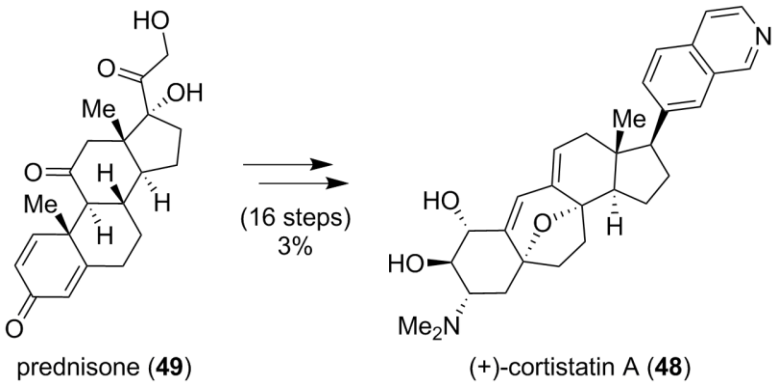
Subsequent research published by Potier *et al*. highlighted the cytotoxicity of the synthetic intermediate vinorelbine (**47**).[22] Today, both vinblastine (**10**) and vinorelbine (**47**) are used clinically in the treatment of several invasive cancers and appear on the World Health Organisation's list of essential medications.

1.5.2 Baran's Semisynthesis of (+)-Cortistatin A (48)

Semisyntheses are not necessarily restricted to biomimetic endevours, there are many examples of complex yet economically viable natural material being taken through multistep synthesis, generating entirely new products with augmented carbon architectures.[23]

In 2008 Baran *et al*. published an approach to the marine natural product (+)-cortistatin A (**48**) starting from the readily available steroid prednisone (**49**)

(Scheme 1.7).[24] Over a 15 step sequence of chemical manipulations the novel antiangiogenic compound **48** was prepared in 3% overall yield, which compares favourably with other contemporaneous total syntheses.[25]



Scheme 1.7: Semisynthesis of marine steroid (+)-cortistatin A (**48**) accomplished by Baran *et al*.

Whilst the importance of semisynthesis is best exemplified through the expedience and commercial viability of the synthetic endeavours, there remains utility for accelerating academic research. Semisynthesis is particularly effective where target compounds are closely related to well defined architectures, requiring few chemical manipulations for conversion.

Given the intricate three-dimensional pentacyclic core and the difficulty associated with enantioselective construction of contiguous chiral centres, *Aspidosperma* type natural products represent ideal candidates for the semisynthetic approach. The highly functionalised aromatic moiety of the jerantinine alkaloids (**1**-**8**) distinguishes the family and is a synthetically challenging feature.

1.6 Aromatic Hydroxylation in Semisynthesis

Derivatisation of natural products using an aromatic hydroxylation strategy has led to the semisynthesis of several targets.[26] Among the examples presented in the literature, regioselective oxidation of indole alkaloids has also been successfully applied, providing credible precedent for the semisynthetic approach envisaged for preparation of the jerantinine family (**1**-**8**).

1.6.1 Lavie's Semisynthesis of Brucine (50)

The toxic effects from plants of the genus *Strychnos* have been known since antiquity.[27] Since its identification as the bioactive component and subsequent structural characterisation, strychnine (**51**) has been well studied (Figure 1.5).[28] In nature the C2, C3 hydroxylated analogue brucine (**50**) can be isolated from the same sources, but it has a lower toxicity.[29] Interestingly, in 1904 brucine (**50**) was the organocatalyst used for the first asymmetric synthesis.[30] However, it was not until 1968 that Lavie *et al*. accomplished conversion of strychnine (**51**) into brucine (**50**) by direct aromatic hydroxylation.[31]

The semisynthesis began with amide cleavage on treatment of strychnine (**51**) with sodium ethoxide, giving strychnic acid (**52**) (Scheme 1.8). Next, Teuber oxidation installed the first hydroxyl group. Conveniently under the reaction conditions, cyclisation of the lactam was also observed and the authors were able to isolate the intermediate 2-hydroxystrychnine (**53**) in good yield. Furthermore, the newly installed phenol served to present an abstractable hydrogen atom, facilitating the second application of potassium

nitrosodisulfonate. Finally methylation of the resulting crude product gave the target brucine (**50**), confirmed by comparison with an authentic sample.

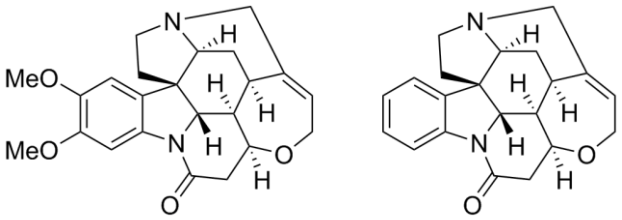
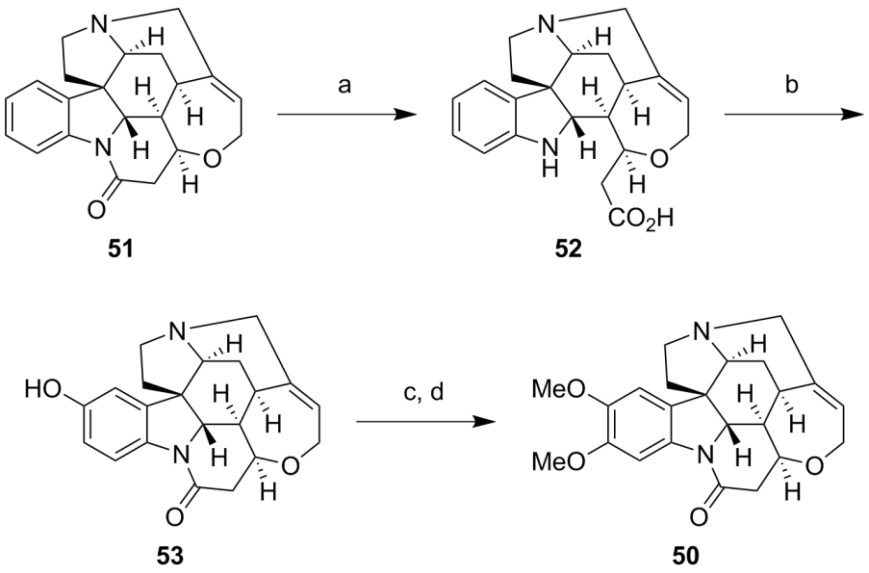


Figure 1.5: *Strychnos* alkaloids strychnine (**50**) and strychnine (**51**)



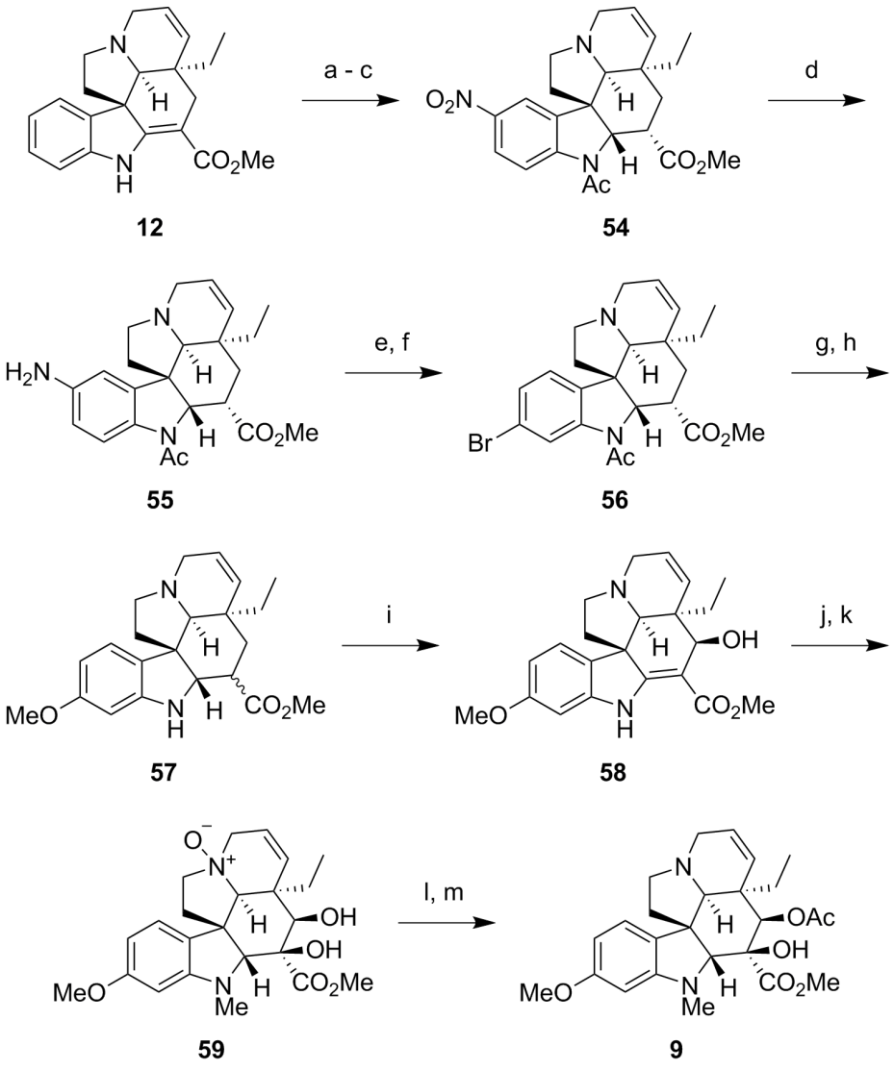
Scheme 1.8: Semisynthesis of brucine (**50**) accomplished by Lavie *et al*. Reagents and conditions: (a) NaOEt, EtOH, Δ, 90 min; (b) $(KSO_3)_2NO$, KH_2PO_4, Me_2CO/H_2O, r.t., 48 h; (c) $(KSO_3)_2NO$, $AcOH/H_2O$, r.t., 48 h; (d) CH_2N_2, $CHCl_3$/MeOH, 5 °C, 48 h

Overall, the authors accomplished conversion of strychnine (**51**) into brucine (**50**) in an effective 4 step sequence. Given that both strychnine (**51**) and the synthetic product shared the same stereochemistry, the result further served to confirm the stereochemical assignment of brucine (**50**).

1.6.2 Palmisano's Semisynthesis of Vindoline (9)

As previously described, vindoline (**9**) is a major alkaloid of *Catharanthus roseus* and both a biosynthetic and synthetic precursor to the important antitumor agent vinblastine (**10**).[32] Given the widespread clinical use, it is unsurprising that there has been much interest in the chemical synthesis of vinblastine (**10**) and its predecessors vindoline (**9**) and catharanthine (**45**).[33] Although several total syntheses of *Aspidosperma* alkaloids have been reported, the semisynthetic strategy reported by Palmisano *et al*. presents an inherently concise alternative (Scheme 1.9).[34] The authors elected to use the more readily available natural product, tabersonine (**12**) as the starting material, providing the complete carbocyclic skeleton of vindoline (**9**).

Starting with tabersonine (**12**), reduction of the π-bond between C2 and C16 using sodium cyanoborohydride and protection of the indoline enabled facile aromatic nitration. The resulting nitro compound **54** was then chemoselectively reduced using powdered zinc in acetic acid giving an aniline **55**, which served to direct subsequent functionalisation to the desired C11 position. After electrophilic bromination on treatment with *N*-bromosuccinimide, diazotisation and protodeamination left the required aromatic substitution pattern. Unfortunately the forcing conditions required for methoxylation of bromide **56** cleaved both the acetamido and ester functionality and epimerised the C16 chiral centre.



Scheme 1.9: Semisynthesis of vindoline (**9**) accomplished by Palmisano *et al*. Reagents and conditions: (a) $NaBH_3CN$, AcOH, r.t., 90 min, 95%; (b) NaOAc, Ac_2O, r.t., 8 h, 99%; (c) HNO_3, TFA, 0 °C → r.t., 30 min, 86%; (d) Zn, AcOH, r.t., 2 h, 89%; (e) NBS, DMF, r.t., 15 min, 81%; (f) $Me_2CH(CH_2)_2ONO$, THF, Δ, 1 h, 84%; (g) NaOMe, CuI, 2,4,6-collidine, 120 °C, 6 h; (h) HCl, MeOH, Δ, 2 h, 66% (over 2 steps); (i) $(PhSeO)_2O$, C_6H_6, 40 °C, 10 min; (j) *m*CPBA, K_2CO_3, CH_2Cl_2/H_2O, 0 °C, 20 min; (k) $NaBH_3CN$, CH_2O, NaOAc/AcOH, r.t., 10 min; (l) Raney Ni, MeOH, r.t., 10 min; (m) Ac_2O, NaOAc, r.t., 24 h, 55% (over 5 steps)

Next, oxidation of both epimers of **57** using benzeneseleninic anhydride saw coalescence to the β-anilinoacrylate **58**, which was stereospecifically hydroxylated upon treatment with 3-chloroperbenzoic acid, generating a tertiary amine oxide in the process. Direct reductive methylation was required to prevent degradation of the resulting unstable intermediate, giving the diol

59. Amine oxide cleavage with Raney nickel and finally acetylation gave vindoline (**9**) as a single enantiomer. Overall the semisynthesis of vindoline (**9**) was achieved in 13 linear steps and 18% yield.

The use of pre-functionalisation to both direct and facilitate aromatic hydroxylation was clearly key to the semisynthetic approach. Incorporation of both an aniline directing group and halide leaving group ensured the correct selectivity for the required substitution.

1.7 Aims

This body of research was undertaken in order to supply the novel, anti-cancer alkaloid jerantinine A (**1**) for further development as a therapeutic candidate. It was proposed that this could be accomplished by using a semisynthetic strategy, simplifying the overall transformation and therefore reducing the number of steps required.

The complex *Aspidosperma* alkaloid tabersonine (**12**) was selected as the starting material for synthesis, which contains the complete carbon skeleton of jerantinine A (**1**). Initial experimentation was aimed towards the selective manipulation of the natural framework and accordingly potential methods were evaluated.

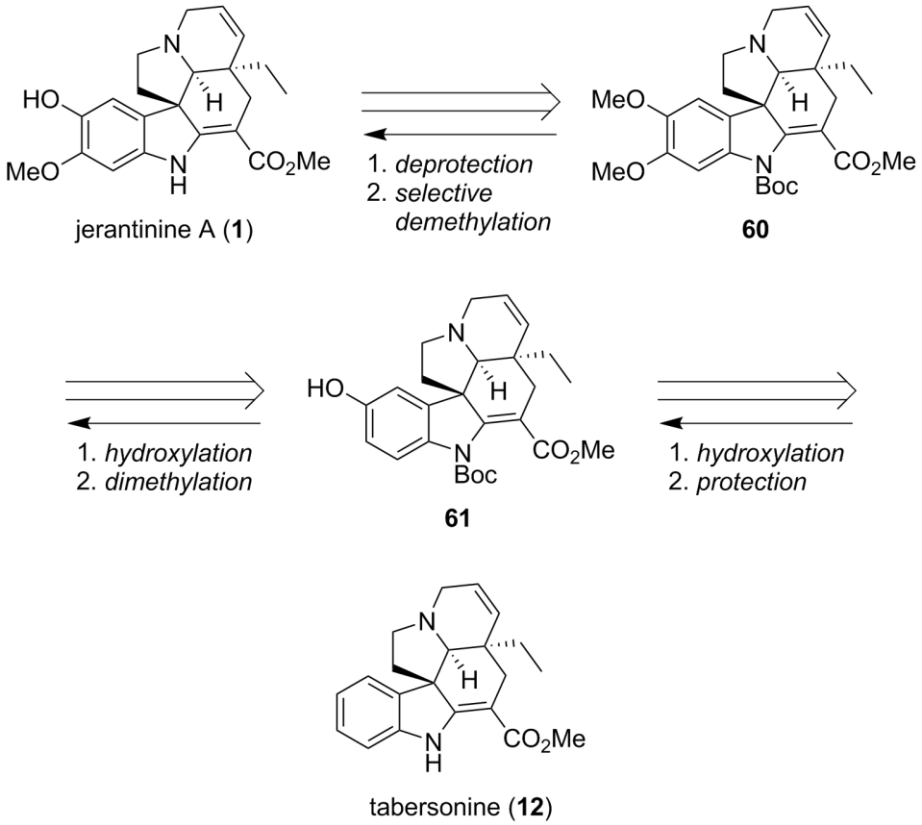
Finally the synthetic jerantinine A (**1**) was supplied for biological evaluation, which involved analysis of the cytotoxic activity against two different

carcinoma cell lines. The data obtained was then compared to that from a sample of natural origin.

2 Results and Discussion

2.1 Retrosynthetic Analysis of Jerantinine A (1)

Retrosynthetically, through application of the selective demethylation logic employed by Waser *et al*., jerantinine A (**1**) can be envisaged to derive from the catechol dimethyl ether **60** (Scheme 2.1).[18] The ether moieties present a retron for dimethylation. Subsequent disconnection of the C11 hydroxyl group can then be rationalised, considering the substituent directing effects in the corresponding phenol **61**. The proposed final transform reveals the known alkaloid tabersonine (**12**) as the ideal starting material.



Scheme 2.1: Retrosynthetic analysis of jerantinine A (**1**)

In the forward direction, synthesis would commence with directed aromatic hydroxylation of tabersonine (**12**). Protection of the indole nitrogen with an electron-withdrawing group is proposed, giving the phenol **61**, which would promote the required regioselectivity in the subsequent hydroxylation. Dimethylation of the resulting catechol gives the corresponding dimethyl ether **60**. Protecting group cleavage and finally precedented selective demethylation would yield the synthetic target jerantinine A (**1**).

2.2 Extraction of Tabersonine (12)

The natural product tabersonine (**12**) is a monoterpenoid indole alkaloid of the *Aspidosperma* class (Figure 2.1).[35] It is well known in the literature for participation in the biogenesis of vindoline (**9**) and as such in the biosynthesis of vinblastine (**10**).[36] Structurally, tabersonine (**12**) comprises the complete pentacyclic carbon skeleton present in the jerantinine family of alkaloids (**1**-**8**). Unfortunately from commercial sources, complex organic material such as natural products generally warrant high prices, limiting the overall economy of a semisynthetic strategy. Instead, the approach presented was to extract the valuable starting material (**12**) from naturally abundant and commercially readily available sources.

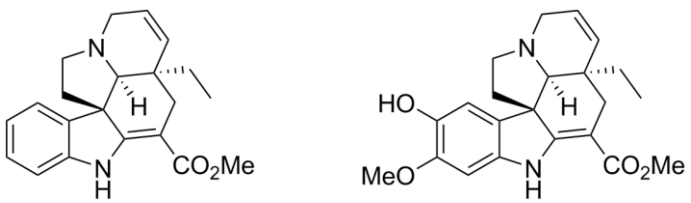


Figure 2.1: The natural product tabersonine (**12**) and target jerantinine A (**1**)

Tabersonine (**12**) has been isolated from numerous natural sources.[37] Among these, the seeds of the African tree *Voacanga africana* have been reported as particularly abundant, giving yields of tabersonine (**12**) up to 3% of the mass.[38] Furthermore, the *Voacanga* genus is closely related to *Tabernaemontana*; the producing genus of the jerantinine alkaloids (**1**-**8**). The seeds are commercially available in large quantities, significantly increasing the overall economy and viability of the semisynthetic strategy.

Adopting a modified literature procedure, the extraction of tabersonine (**12**) commenced by first grinding the *Voacanga africana* seeds into a fine powder.[39] The resulting solid was then suspended in an aqueous sulfuric acid solution and stirred vigorously overnight. In so doing the tabersonine (**12**) present in the seeds was protonated, enhancing the aqueous solubility. Removal of the extracted seed by filtration and further washing of the residue with additional sulfuric acid solution gave a cloudy aqueous solution containing the tabersonine (**12**) salt.

Next, sodium chloride was added to the solution and the mixture stirred overnight. Salt exchange with the tabersonine (**12**) present in the aqueous mixture gave the corresponding hydrochloride salt, which has relatively improved solubility in organic solvent. Chloroform was then added and the resulting biphasic mixture vigorously stirred for 3 hours. Upon standing, the solution separated into organic and aqueous fractions and the upper aqueous emulsion could be removed. The remaining organic layer was then filtered through a large plug of diatomaceous earth, clarifying the solution into distinct

phases, which could be readily separated. Removal of the organic solvent gave a crude tabersonine (**12**) hydrochloride salt product, which was finally converted to the free base on treatment with ammonia solution. The resulting crude material proved by ^{1}H NMR spectroscopy to be mainly comprised of the desired product (**12**).

Purification of the combined crude product from several batches of extractions was accomplished by flash chromatography. Using a petroleum ether and ethyl acetate solvent system, pure material could be consistently obtained, even on relatively large scales (>10 g). The pure tabersonine (**12**) proved to be spectroscopically identical to that presented in the literature. In addition, comparison of the specific rotation with those literature examples confirmed the absolute stereochemical configuration to be consistent with (-)-tabersonine (**12**). With the presented method, *Voacanga africana* seed were extracted in 200 g batches, giving isolated yields of pure (-)-tabersonine (**12**) averaging 1% of the mass of material used.

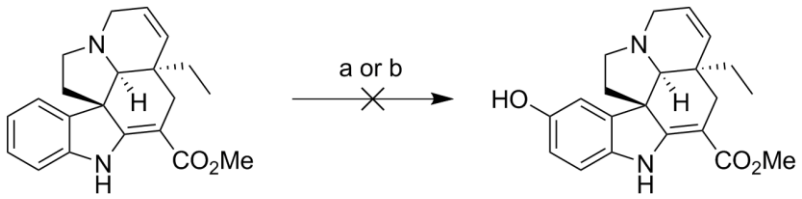
2.3 Preparation of Phenol 61

2.3.1 Attempted Aromatic Hydroxylation of Tabersonine (12)

The direct oxidation of remote aromatic carbon-hydrogen bonds is a challenging synthetic task. Several methods have been long reported in the literature, which normally utilise forcing conditions in combination with strong oxidising agents.[40]

2.3.1.1 Elbs Persulfate Oxidation

Elbs conditions for persulfate mediated oxidation of arenes were first investigated (Scheme 2.2).[41] Although the Elbs reaction has been known since 1893, it was not until 1953 that a modification was published by Boyland *et al*., extending the substrate scope to include aromatic amines.[42] Unfortunately, substitution in these cases is normally observed to occur *ortho* to the directing amino moiety. However, despite the preferred regioselectivity, treatment of tabersonine (**12**) with either potassium persulfate or potassium peroxymonosulfate failed to yield any detectable quantity of hydroxylated product **62**. Instead, inseparable mixtures of several unidentified degradation products were observed in both cases, presumably resulting from non-chemoselective oxidation.



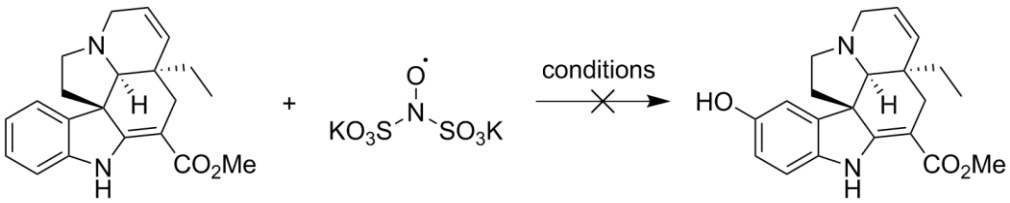
12 **62**

Scheme 2.2: Attempted Elbs persulfate oxidation of tabersonine (**12**). Reagents and conditions: (a) $K_2S_2O_8$, KOH, MeOH, 0 °C → r.t., 1 h; (b) $KHSO_5$, MeOH, 0 °C → r.t., 1 h

2.3.1.2 Teuber Oxidation

Another strong oxidant proven capable of direct arene hydroxylation is the persistent radical potassium nitrosodisulfonate (**63**) (Frémy's salt).[43] Known as the Teuber oxidation, Frémy's salt (**63**) first generates an aryl radical from the substrate, which is then quenched by a second equivalent of the reagent.[44]

Upon hydrolysis of the resulting intermediate, phenol products are obtained. Conditions were adopted from published procedures for similar transformations (Table 2.1).[45] Usually, due to the instability of Frémy's salt (**63**) to acidic reaction media, oxidations are performed in buffered aqueous solutions.



12 Frémy's salt (**63**) **62**

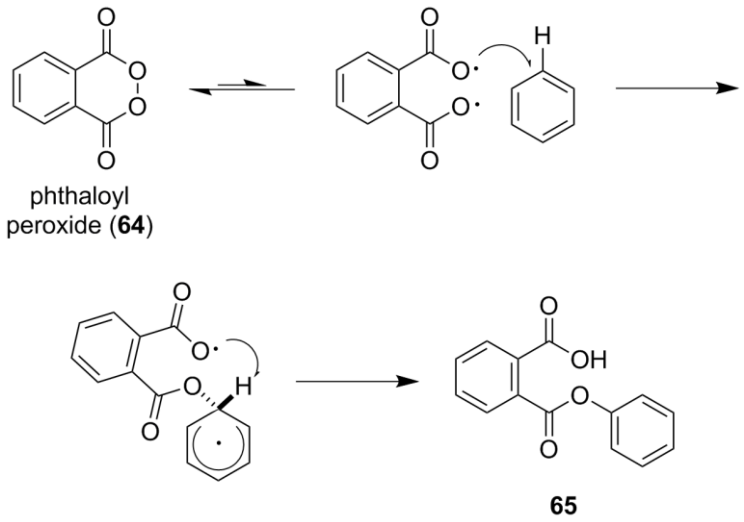
Entry	Conditions	Product
1	KH_2PO_3, H_2O/Me_2CO, r.t.	starting material
2	H_2O/heptane, r.t.	starting material
3	AcOH, H_2O, r.t.	degradation
4	NaOAc/AcOH, H_2O, r.t.	degradation

Table 2.1: Conditions attempted for the Teuber oxidation of tabersonine (**12**)

Despite screening several methods the desired oxidation was not observed. Experiments at neutral pH saw no conversion of the starting material (Table 2.1, entries 1 and 2). Generally in acidic media, coloured mixtures of several unidentifiable and unstable products were obtained, along with large quantities of the starting material (Table 2.1, entries 3 and 4). The lack of any detectable aromatic functionalisation in the ^{1}H NMR spectrum of the crude mixtures served to further discourage the application of this methodology.

2.3.1.3 Phthaloyl Peroxide (64) Mediated Arene Hydroxylation

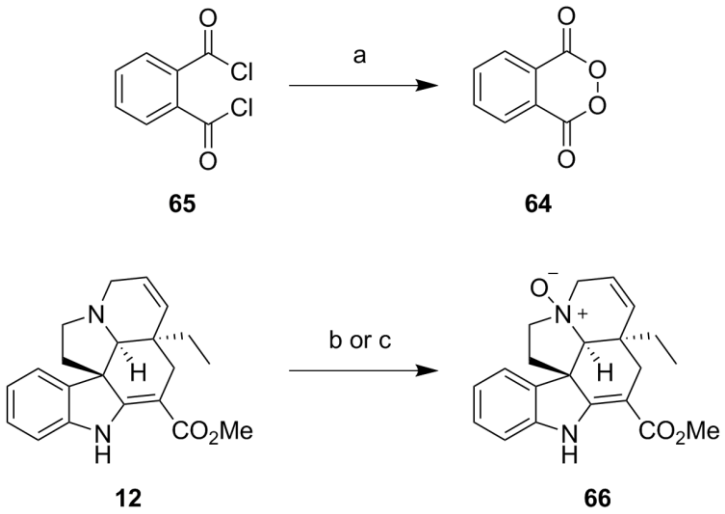
More recently, arene hydroxylation has also been accomplished using phthaloyl peroxide (**64**).[46] The diradical intermediate generated through homolysis of the peroxide bond is able to first add into arene substrates (Scheme 2.3). Concomitant intramolecular hydrogen atom abstraction gives an ester **65**, which can be readily hydrolysed under mild basic conditions.



Scheme 2.3: Proposed mechanism of phthaloyl peroxide (**64**) arene oxidation[46]

Intrigued by the expedient hydroxylation protocol, the non-commercial oxidant was prepared from phthaloyl chloride following literature precedent (Scheme 2.4).[47] The recently published procedure employs sodium percarbonate, circumventing the concentrated hydrogen peroxide solutions normally required.[48] The crude material obtained by reaction of phthaloyl chloride (**65**) and sodium percarbonate is further purified by precipitation from benzene, giving high purity crystalline phthaloyl peroxide (**64**). Unfortunately, treatment of tabersonine (**12**) with the oxidant **64** under the prescribed literature reaction conditions failed to give any aromatic hydroxylation.

Instead, consumption of the starting material was accompanied by formation of a single major compound, which was spectroscopically determined to be an unwanted amine oxide (**66**). Given the initially reported scope of the reaction had not been extended to substrates with amine functionality, it is likely that the competing reaction pathways are favoured over aromatic hydroxylation.



65 **64** **12** **66**

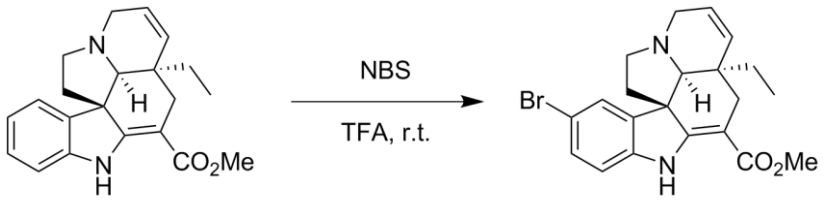
Scheme 2.4: Preparation of phthaloyl peroxide (**64**) and the attempted aromatic hydroxylation of tabersonine (**12**). Reagents and conditions: (a) $Na_2CO_3 \cdot 1.5H_2O_2$, CH_2Cl_2, r.t., 3 h, 65%; (b) phthaloyl peroxide (**64**), TFE, r.t., 1 h; (c) phthaloyl peroxide (**64**), HFIP, r.t., 1 h

The unwanted selectivity demonstrated by the strong oxidising agents examined for the direct hydroxylation of tabersonine (**12**) prompted investigation into suitable alternative manipulations. Another method for aromatic hydroxylation involves pre-functionalisation of the substrates with a suitable group, which requires less forcing conditions for conversion.

2.3.2 Synthesis of *tert*-Butyl 10-Iodotabersonine-1-carboxylate (69)

The oxidation of aryl bromides has been reported to occur under copper or palladium catalysis.[49] However, such procedures often require bespoke catalyst preparations and elevated reaction temperatures.[50] Alternative methods include lithium-halogen exchange and trapping of the resulting organolithium with an appropriate electrophile.[51]

Given the weaker carbon-halogen bond strength, aryl iodides generally undergo more facile hydroxylation than the corresponding bromides using the same conditions. Therefore, literature precedent for the preparation of the brominated tabersonine derivative **67** prompted evaluation of a selective iodination procedure using the same rationale. (Scheme 2.5).[52]

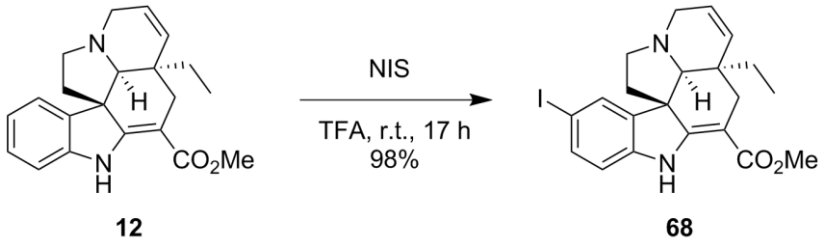


12 **67**

Scheme 2.5: Preparation of the brominated tabersonine derivative **67** accomplished by Lewin *et al*.[52]

Gratifyingly, treatment of a trifluoroacetic acid solution of tabersonine (**12**) with *N*-iodosuccinimide delivered the desired iodinated derivative **68** in near quantitative yield (Scheme 2.6). Furthermore, upon experimentation it was determined that purification could be expediently achieved by filtration of the impurities from a pentane solution of the crude product. The reaction also

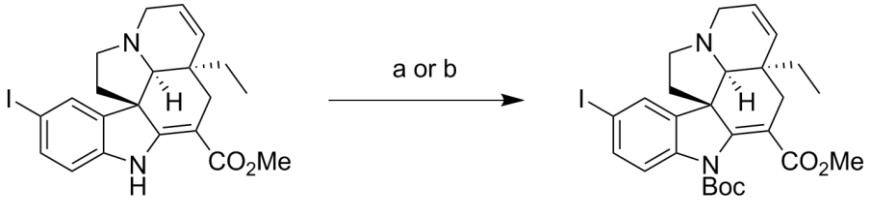
proved to be scalable; multi-gram preparations were accomplished with no discernible reduction in yield.



12 68

Scheme 2.6: Synthesis of the iodinated tabersonine derivative **68**

A protected derivative **69** was also prepared; treatment of the iodinated tabersonine **68** with di-*tert*-butyl dicarbonate and substoichiometric 4-(dimethylamino)pyridine in acetonitrile gave the product **69** in moderate yield (Scheme 2.7). Purification of the protected derivative **69** from the reaction mixture was severely impaired by the residual starting material, which even upon extended heating was never fully consumed. However, changing the solvent to tetrahydrofuran saw complete conversion of the starting material, significantly increasing the overall yield for the protection.

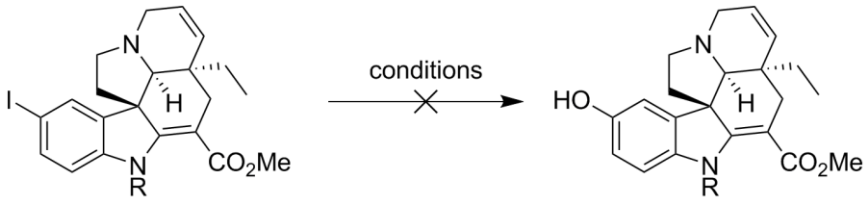


68 69

Scheme 2.7: Synthesis of the protected tabersonine derivative **69**. Reagents and conditions: (a) Boc_2O, DMAP, MeCN, 65 °C, 19 h, 62 %; (b) Boc_2O, DMAP, THF, 65 °C, 4 h, 92%

2.3.3 Attempted Hydroxylation of *tert*-Butyl 10-Iodotabersonine-1-carboxylate (69)

Next, the iodinated derivatives **68** and **69** were systematically evaluated using a series of literature procedures in an effort to effect selective hydroxylation (Table 2.2).[53] Both copper and palladium catalysis were examined under a variety of conditions.



R = H **68**
R = Boc **69**

R = H **70**
R = Boc **61**

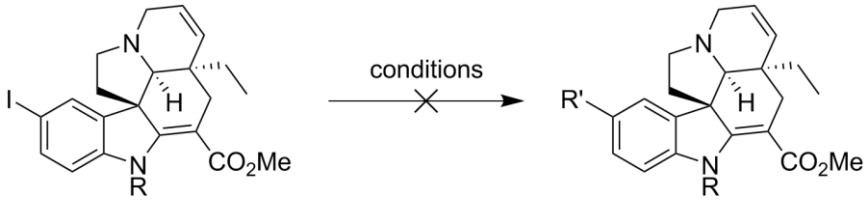
Entry	R	Conditions	Product
1	H	CuI, KOH, H_2O, 100 °C	degradation
2	H	Pd_2dba_3 (2 mol%), KOH, dioxane, 80 °C	starting material
3	Boc	CuI, KOH, H_2O, 100 °C	degradation
4	Boc	CuI, KOH, H_2O, 80 °C	degradation
5	Boc	CuI, phen, NaOH, DMSO, 120 °C	degradation
6	Boc	CuI, phen, NaOH, DMSO, 80 °C	degradation
7	Boc	Pd_2dba_3 (2 mol%), KOH, dioxane, 80 °C	starting material

Table 2.2: Conditions attempted for the hydroxylation of the iodinated tabersonine derivatives **68** and **69**

Neither copper nor palladium metal catalysis proved able to invoke the desired transformation. Under the described conditions complex mixtures were obtained from all experiments using copper catalysis, despite the decreased

temperatures also examined (Table 2.2, entries 4 and 6). Contrastingly the attempted palladium conditions returned the starting material unchanged in both cases (Table 2.2, entries 2 and 7).

In addition to the reactions described above, lithium-halogen exchange was also investigated (Table 2.3). Davis reagent (2-(phenylsulfonyl)-3-phenyl-oxaziridine) was selected as a trapping reagent, which is an electrophilic oxygen source.[54] The more conventional boron reagents, trimethyl borate and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane were also examined. While more circuitous, the oxidation of boronic esters is known to occur readily, on treatment with a variety of oxidants.[55]



R = H **68**
R = Boc **69**

R' = OH, $B(OMe)_2$ or Bpin

Entry	R	Conditions	Product
1	H	^{n}BuLi (1 equiv.), THF, -78 °C	starting material[a]
2	H	^{n}BuLi (1 equiv.), TMEDA, THF, -40 °C	starting material[a]
3	H	^{n}BuLi (2 equiv.), THF, -78 °C	degradation
4	Boc	^{n}BuLi (1 equiv.), THF, -78 °C	degradation
5	Boc	^{n}BuLi (1 equiv.), TMEDA, THF, -78 °C	degradation
6	Boc	^{t}BuLi (1 equiv.), THF, -78 °C	degradation

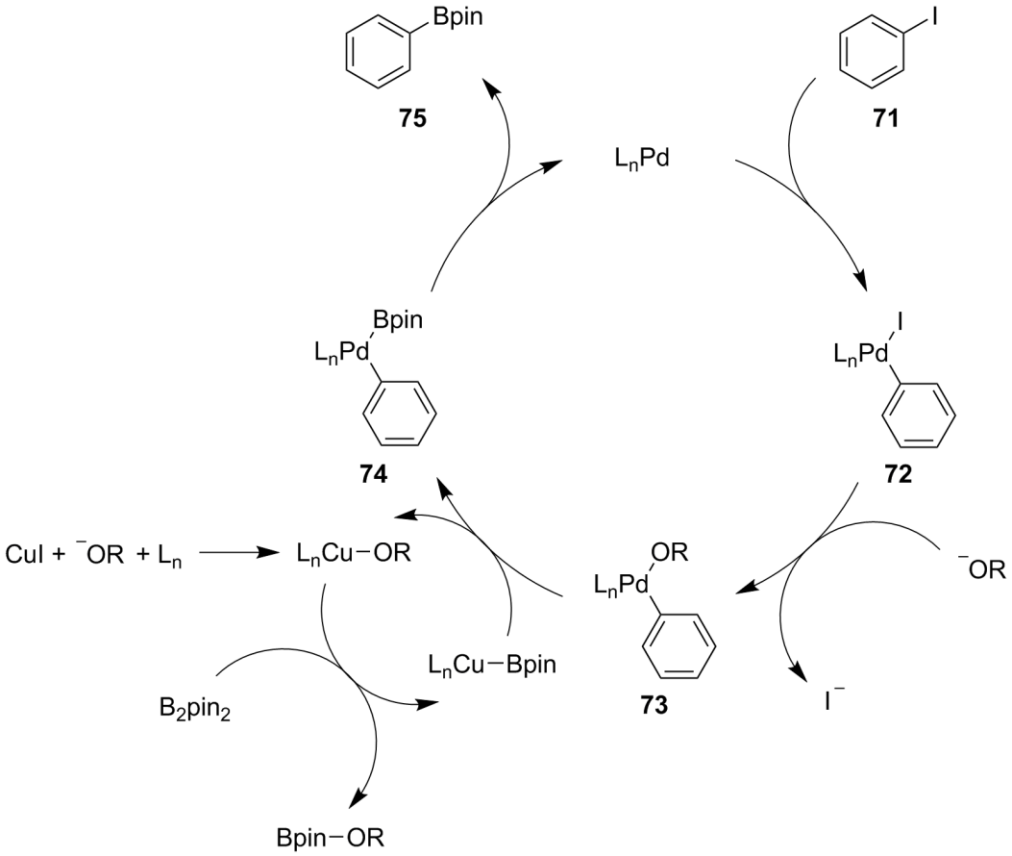
Table 2.3: Conditions attempted for lithium-halogen exchange of the iodinated derivatives **68** and **69**. [a]Reaction quenched with Davis reagent, $B(OMe)_3$ and pinBOMe

Despite screening multiple lithium sources the anticipated products were not observed. Initial experiments on the iodinated derivative **68** consistently returned the starting material, regardless of the chosen electrophile (Table 2.3, entries 1 and 2). However, increasing the molar equivalents of organolithium reagent served to generate a complex mixture of degradation products, even at the lowest temperature (Table 2.3, entry 3). Experiments using the protected derivative **69** were uniformly unsuccessful (Table 2.3, entries 4-6). Furthermore, analysis of the reaction mixtures revealed cleavage of the protecting group, evidenced by loss of the signal from the ^{1}H NMR spectrum.

2.3.4 Synthesis of *tert*-Butyl Tabersonine-1-carboxylate-10-boronic Acid Pinacol Ester (77)

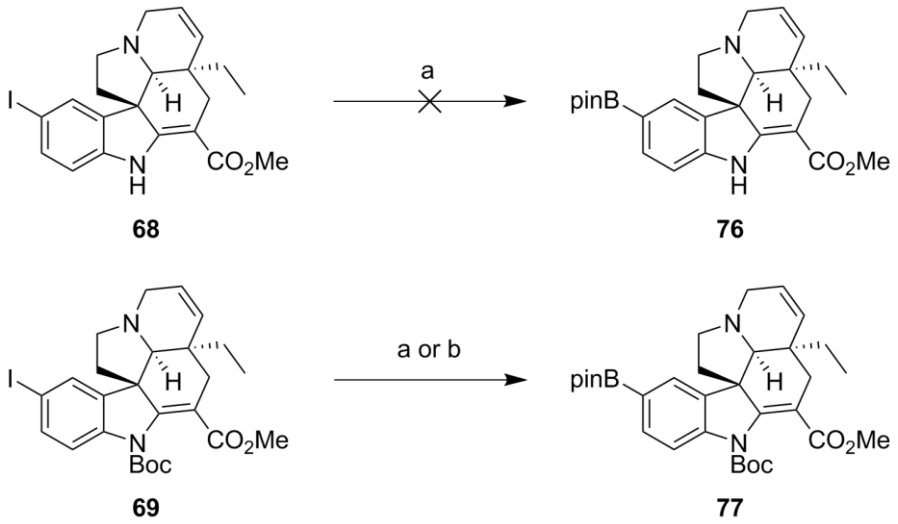
Finally, conditions were examined for the palladium catalysed borylation of the iodinated tabersonine derivatives **68** and **69**. The cross-coupling of vinyl or aryl halides with bis(pinacolato)diboron, the Miyaura borylation reaction, enables the preparation of synthetically important alkenyl and aryl boron compounds.[56] First reported in 1995 by Miyaura *et al*., the procedure finds great utility, given the widespread use of boronates in Suzuki cross-coupling reactions.[57] Among the many procedures that have been reported in the literature, the conditions described by Ratniyom *et al*. for the borylation of aryl iodides **71** stand out as particularly convenient (Scheme 2.8).[58] The authors utilise a copper(I) co-catalyst, facilitating the transmetalation of the bis(pinacolato)diboron and the intermediate palladium(II) species **73** (Scheme 2.8). The cooperative catalysis reduces the temperatures usually required,

giving high yields of the boronic ester product **75** at room temperature, without the requirement of anhydrous reaction media.



Scheme 2.8: Proposed mechanism of the Pd/Cu-catalysed Miyaura borylation reaction[58]

Borylation of the iodinated tabersonine derivatives **68** and **69** was attempted according to the literature methodology (Scheme 2.9).[58] Whilst the free amine derivative **68** remained unchanged by the reaction, significant conversion of the protected derivative **69** to the desired boronic ester **77** was observed. Additionally, increasing the catalytic loading of the palladium source and triphenylphosphine ligand employed saw full conversion of the starting material **69**.



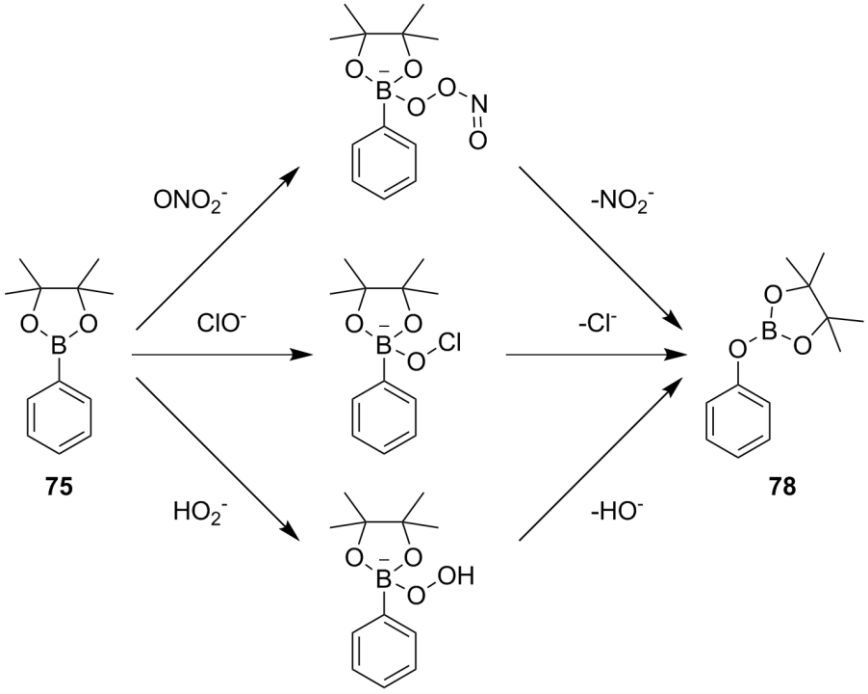
Scheme 2.9: Synthesis of the boronic ester tabersonine derivative **77**. Reagents and conditions: (a) $Pd(OAc)_2$ (2 mol%), PPh_3 (2 mol%), CuI (20 mol%), B_2pin_2, Cs_2CO_3, MeCN, r.t., 18 h, 34%; (b) $Pd(OAc)_2$ (10 mol%), PPh_3 (10 mol%), CuI (20 mol%), B_2pin_2, Cs_2CO_3, MeCN, r.t., 18 h, 74%

Purification by column chromatography delivered the boronic ester **77** in moderate yield. However, degradation of the product could be seen to occur while adsorbed onto the silica stationary phase and so once sufficient characterisation was obtained, flash chromatography was omitted from subsequent preparations. Instead, the reaction product obtained after washing with water and brine was used directly in further manipulations.

2.3.5 Synthesis of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61)

The chemistry of boronic esters is predominated by the nucleophilic character of the bonded carbon atom, imparted by the dipole of the boron-carbon bond.[59] Boronates alone are not generally nucleophilic enough to transfer an alkyl group to an electrophilic centre. However, after attack by an appropriate nucleophile, the resulting borate anion has significantly enhanced nucleophilicity.[60] Furthermore, 1,2-migration of a group on the boron can be

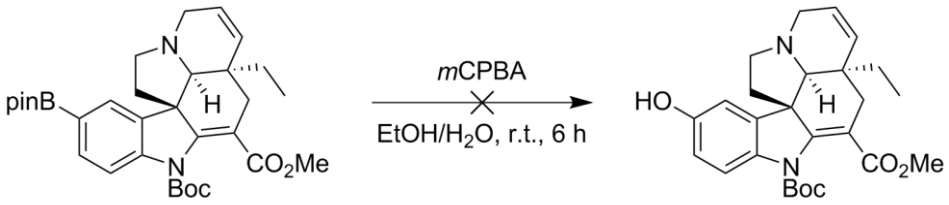
encouraged in substrates that incorporate a leaving group in the destination α-position. Therefore phenol synthesis can be accomplished by migration to an oxygen nucleophile and hydrolysis of the resulting borate ester **78** (Scheme 2.10).[61] Suitable oxidants have been shown to include peracids, peroxides and hypochlorites.[62]



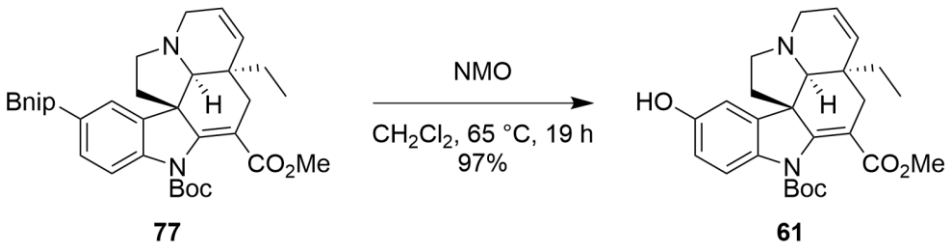
Scheme 2.10: Proposed mechanism of boronic ester oxidation[61]

In accordance with the literature precedents the oxidation of the boronic ester **77** was first attempted using the convenient, commercially available oxidant 3-chloroperbenzoic acid (Scheme 2.11).[62a] Whilst the desired product could be detected in the mass spectrum from the reaction mixture, low regioselectivity was observed, giving a complex mixture of products and preventing isolation of the phenol **61**. Discouraged from the further application of peroxides, recent literature reported by Zhu *et al*. revealed amine oxides as an attractive

alternative.[63] Both mild and rapid, the reaction is also tolerant of a variety of functional groups, offering a broad substrate scope. Gratifyingly, commercially available 4-methylmorpholine *N*-oxide (NMO) was able to effect the desired conversion (Scheme 2.12). The resulting phenol **61** was readily isolated by flash chromatography, which gave the pure material in good yield.



Scheme 2.11: Attempted oxidation of the boronic ester **77**



Scheme 2.12: Synthesis of phenol **61**

Confirmation of the desired hydroxylation was gained by examination of the spectroscopic data from the synthetic material. In combination with ^{1}H and ^{13}C NMR spectroscopy, changes in both the mass and infrared spectrum were used to confidently determine the identity of the product.

2.4 Preparation of Catechol Dimethyl Ether 60

2.4.1 Attempted Aromatic Hydroxylation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61)

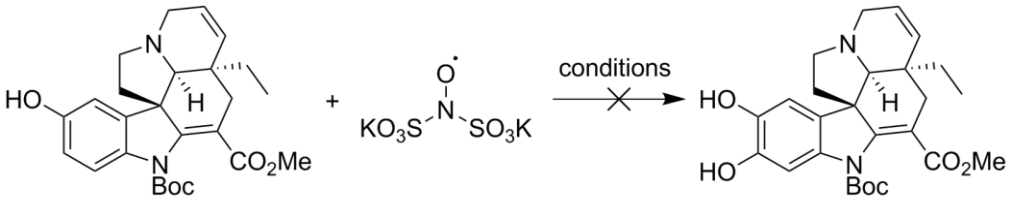
The preparation of catechols from their corresponding phenols is a relatively infrequently reported transformation. Such manipulations generally require strong oxidising agents or transition metal catalysis.[64] Accordingly several methods established in the literature were investigated for the aromatic hydroxylation of phenol **61**.

2.4.1.1 Teuber Oxidation

Through installation of the first aromatic hydroxyl group, introduction of a new, labile hydrogen atom was realised, prompting re-examination of the radical oxidant potassium nitrosodisulfonate (Frémy's salt) (Table 2.4). While the regioselectivity of Teuber oxidation normally favours generation of *para*-substituted products, in substrates where the *para*-position is blocked, *ortho*-substitution predominates.[44]

Teuber oxidation of phenol **61** was first attempted in neutral aqueous media. However, even on prolonged stirring the bulk of the starting material remained unchanged (Table 2.4, entries 1 and 2). Furthermore, gradual cleavage of the protecting group was observed to occur over the course of the reaction, likely due to the formation of unstable imino-quinone products, leading to subsequent hydrolysis of the carbamate moiety. Additionally switching to

acidic reaction media also failed to promote any significant conversion of the starting phenol **61** (Table 2.4, entries 3 and 4).



61 Frémy's salt (**63**) **79**

Entry	Conditions	Product
1	KH_2PO_3, H_2O/Me_2CO, r.t.	starting material
2	H_2O/heptane, r.t.	starting material
3	NaOAc/AcOH, H_2O, r.t.	starting material
4	AcOH, H_2O, r.t.	starting material

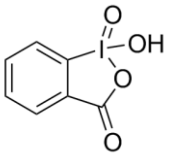
Table 2.4: Conditions attempted for the Teuber oxidation of phenol **61**

Discouraged from further application of Frémy's salt, *ortho*-selective hydroxylation was next attempted using 2-iodoxybenzoic acid (**80**) in accordance with the literature precedent for the generation of catechols.

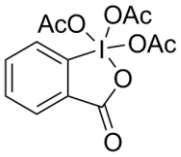
2.4.1.2 2-Iodoxybenzoic Acid (80) Mediated Hydroxylation of Phenols

The importance of hypervalent iodine reagents in organic synthesis has been extensively reviewed.[65] One of the most widespread, Dess-Martin periodinane (**81**) is a robust and convenient oxidant, frequently utilised for the oxidation of alcohols (Figure 2.2).[66] An intermediate in the synthesis, 2-iodoxybenzoic acid (**80**) is an impact-sensitive iodine(V) reagent capable of similar transformations.[67] The reactivity originates from the strongly electrophilic

nature of the iodine atom, which makes it susceptible to attack from a suitable nucleophile. The return of the hypervalent iodide to normal valency then drives its favourable reductive elimination.



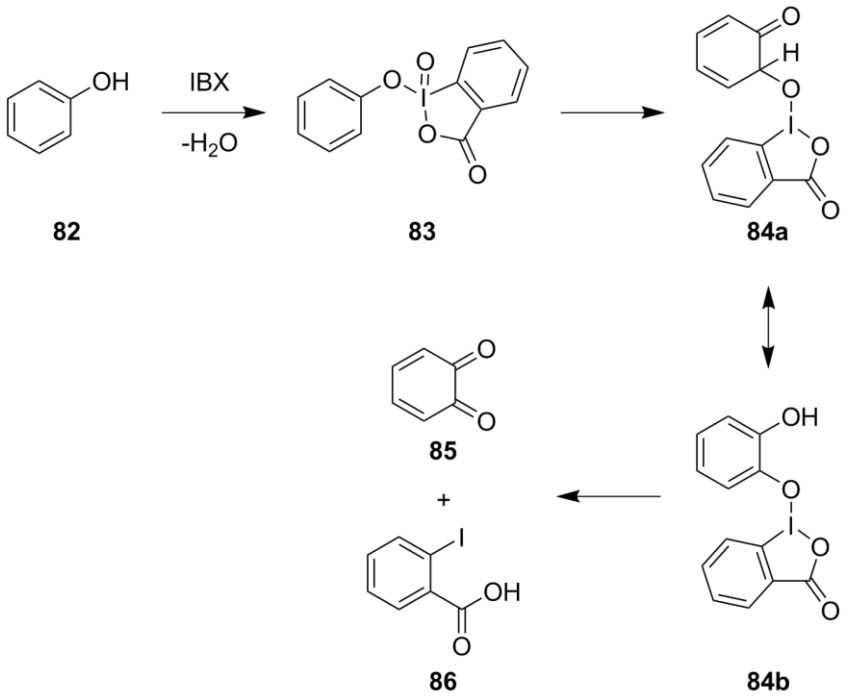
2-iodoxybenzoic acid (**80**)



Dess-Martin periodinane (**81**)

Figure 2.2: The iodine(V) reagents 2-iodoxybenzoic acid (**80**) and Dess-Martin periodinane (**81**)

Literature published in 2001 by Pettus *et al*. revealed that the regioselective generation of *ortho*-quinones from their corresponding phenols could be accomplished using 2-iodoxybenzoic acid (**80**) for an efficient double oxidation process.[68] The mechanism proposed by the authors begins with initial addition of the phenol starting material **82** to the iodine(V) centre (Scheme 2.13). Proton transfer and loss of water gives an iodine(V) intermediate **83**, which upon rearrangement makes the intramolecular delivery of the required oxygen atom to the least hindered, most nucleophilic *ortho*-position. Finally oxidative collapse of the resulting iodine(III) intermediate **84** gives an *ortho*-quinone product **85** and 2-iodobenzoic acid (**86**). Yields estimated by analysis of the ^{1}H NMR spectrum were reported and in most substrates, good conversions were observed.



Scheme 2.13: Proposed mechanism of the oxidation of phenols with 2-iodoxybenzoic acid (**80**)[68]

Accordingly application of the regioselective oxidation protocol was attempted using the hydroxylated tabersonine derivative **61** (Scheme 2.14). Unfortunately, even at reduced temperatures complex mixtures of products were obtained. Furthermore, none of the desired *ortho*-quinone product **87** could be detected upon examination of the crude reaction mixtures using ^{1}H NMR spectroscopy.



Scheme 2.14: Attempted oxidation of phenol (**61**)

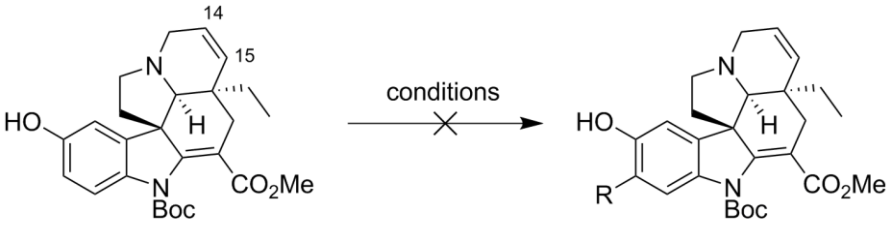
The diverse substrate scope of 2-iodoxybenzoic acid (**80**) mediated oxidation has been demonstrated in applications subsequent to its discovery.[69] However, several viable competing reaction pathways can be envisaged with the functionally rich starting material **61**.

2.4.2 Attempted Aromatic Halogenation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61)

Encouraged to simplify the required transformation through application of the oxidation sequence employed previously in the preparation of phenol **61**, conditions for aromatic halogenation were investigated (Table 2.5). Given the electron donation of the phenolic hydroxyl group, *ortho*-substitution was predicted.

The reagent utilised previously, *N*-iodosuccinimide failed to yield the desired product, despite varying the solvent (Table 2.5, entries 1 and 2). Rapid discolouration of the reaction was observed, resulting in a complex mixture of degradation products. Changing the solvent to acetonitrile (Table 2.5 entry 3) returned only the starting material, as did the mild conditions suggested by Romo *et al*. (Table 2.5, entry 4).[70] However, in both cases, exposure to air during isolation caused evolution of iodine, which could be inferred by a pronounced colour change in solution. The more exotic iodinating reagent benzyltriethylammonium dichloroiodate also proved ineffective for the desired iodination (Table 2.5, entry 5).[71] The major product, chlorination at C14 was evidenced by changes in both the mass and ^{1}H NMR spectrum. Finally,

several protocols for aromatic bromination were also unsuccessfully applied (Table 2.5 entries 6 and 7).



61 R = I or Br

Entry	R	Conditions	Product
1	I	NIS, TFA, r.t.	degradation
2	I	NIS, AcOH, r.t.	degradation
3	I	NIS, MeCN, r.t.	starting material
4	I	NIS, $In(OTf)_3$ (10 mol%), MeCN, r.t	starting material
5	I	BTEA-ICl_2, CH_2Cl_2, r.t.	C14-Cl
6	Br	NBS, AcOH, r.t.	degradation
7	Br	NBS, MeCN, r.t.	degradation

Table 2.5: Conditions for the attempted iodination of phenol **61**

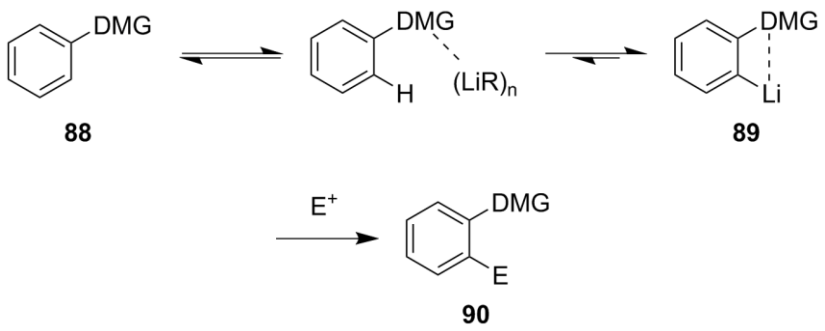
The experimental observations suggest that the nucleophilicity of phenol **61** is not directed toward aromatic halogenation with the electrophiles examined. Furthermore, reaction at the π-bond between C14 and C15 served to highlight the potential chemoselectivity issue, which prompted re-evaluation of the approach toward aromatic substitution.

2.4.3 Attempted *Ortho* Functionalisation of *tert*-Butyl 10-Hydroxytabersonine-1-carboxylate (61)

Directed functionalisation of arene carbon-hydrogen bonds, while not conceptually new, has seen significant growth as a field of research in recent years.[72] The increased demand for more benign, selective transformations with reduced waste and by-products has stimulated significant advances in directing reactivity using ancillary intramolecular features.

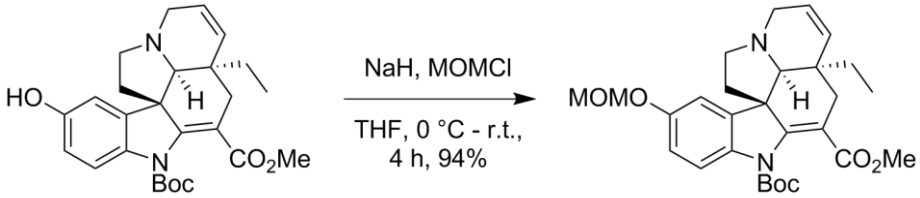
2.4.3.1 Directed *Ortho* Lithiation

Among the popularised methods of directed substitution, *ortho*-metalation has been particularly widely utilised (Scheme 2.15).[73] Substrates incorporate a directing group, normally a strongly Lewis basic moiety capable of chelating the Lewis acidic metalating species, which increases the kinetic acidity of the proton in the *ortho*-position. The resulting *ortho*-metalated intermediate **89** can be further quenched using an electrophile, giving the corresponding substituted arene product **90**.[74] Several electrophiles have been disclosed in the surrounding literature, particularly convenient is the generation of aryl boronates through the use of borate esters as quenching reagents.[75]



Scheme 2.15: The directed *ortho*-metalation of arenes

Given the prerequisite of removability, the strongly directing methoxymethyl group was selected as an appropriate chelating moiety.[76] Methoxymethyl ethers can be reliably cleaved under acidic conditions and have been successfully applied to several effective total syntheses.[77] Therefore, preparation of the required ether **91** was achieved using an adapted Williamson ether synthesis (Scheme 2.16). Generation of the alkoxide from phenol **61** upon deprotonation with sodium hydride, followed by addition of chloromethyl methyl ether gave the desired product **91** in high yield and without need of chromatographic purification.

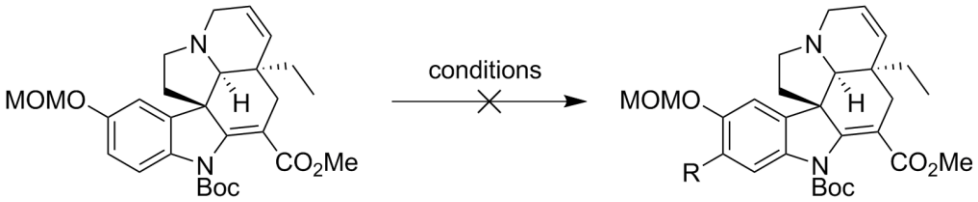


61 **91**

Scheme 2.16: Synthesis of the methoxymethyl ether **91**

Literature conditions were next examined for the lithiation of ether **91**, selected metalating reagents were added to a cooled solution of the starting material and after stirring for 30 minutes, reactions were quenched.[73] Although several electrophiles and organolithium reagents were examined, none of the desired *ortho*-functionalised products could be detected spectroscopically (Table 2.6). Where reactions used the strong alkyl lithium bases products were obtained in mixtures; in each of these cases the mass ion consistent with alkylation could be detected (Table 2.6, entries 1 and 2). While the regioselectivity of the addition could not be readily determined from the

^{1}H NMR spectrum of the crude mixtures, incompatibility of the Boc-group was evidenced by the absence of characteristic resonances. Experiments using the alternative metalating agent, lithium diisopropylamide, returned the starting material **91** unchanged (Table 2.6, entries 3 and 4). Products eluding to successful *ortho*-metalation could not be detected upon quenching with any of the chosen electrophiles. Furthermore, increasing the temperature saw no variation in reactivity (Table 2.6, entries 5 and 6).



91

R = I, $B(OMe)_3$ or Bpin

Entry	Conditions	Product
1	^{n}BuLi (1 equiv.), THF, -78 °C	degradation
2	^{t}BuLi (1 equiv.), THF, -78 °C	degradation
3	LDA (1 equiv.), THF, -78 °C → r.t.	starting material[a]
4	LDA (2 equiv.), THF, -78 °C → r.t.	starting material[a]
5	LDA (1 equiv.), THF, -40 °C → r.t.	starting material[a]
6	LDA (2 equiv.), THF, -40 °C → r.t.	starting material[a]

Table 2.6: Conditions for the attempted directed *ortho*-metalation of the methoxymethyl ether (**91**). [a]Reaction quenched with NIS, $B(OMe)_3$ and pinBOMe

Deterred from further application of organolithium reagents, alternative directed carbon-hydrogen bond functionalisation methods were sought. Ideally the directing group would be easily cleaved, facilitating expedient

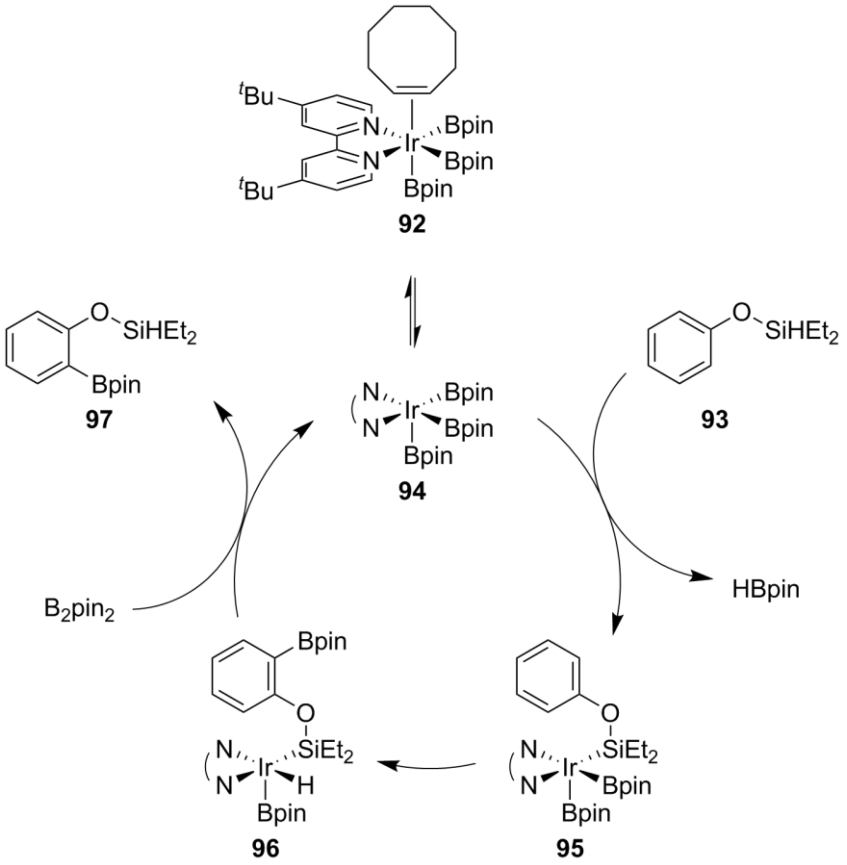
unveiling of the natural product in later steps. Mild conditions for subsequent functional group interconversion were also desirable. The previously successful application of boronic esters in the present work prompted consideration of directed borylation methodologies.

2.4.3.2 Directed *Ortho* Borylation

The borylation of aromatic carbon-hydrogen bonds is an important synthetic transformation, largely popularised since the widespread use of palladium cross-coupling.[60] Such procedures often employ precious metal catalysts and bulky, sterically hindering ligands, which assist the reductive elimination of products from the catalytic species. Iridium, rhodium and palladium have each been successfully utilised for the borylation of arenes.[78] However, systems employing palladium have not been extensively used due to the potential for transmetalation of the products. Similarly rhodium catalysed borylation finds less frequent application, generally requiring much higher temperatures than an equivalent iridium mediated process.

Iridium catalysed borylation has been comparably well studied and several examples of directed procedures have been developed.[79] Three major strategies have shown efficacy; chelate-directed, relay-directed and outer sphere hydrogen-bond directed borylations giving *ortho*-substituted products have been reported.[78] Among the systems described in the literature, elegant use of silanes as directing groups was published by Hartwig *et al*., enabling the preparation of *ortho*-borylated products in good yields.[80] Furthermore,

development of a convenient one-pot procedure using phenols as substrates was also reported (Scheme 2.17).

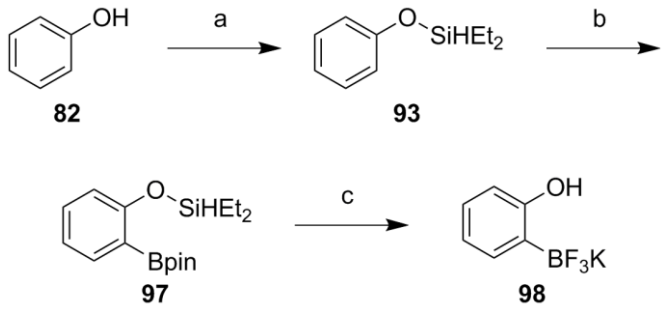


Scheme 2.17: Proposed mechanism of the silyl-directed, iridium catalysed *ortho* borylation[80]

The mechanism of the *ortho*-borylation presented by Hartwig *et al*. begins with coordination of the hydrosilyl group present in the substrate **93** to the active catalytic iridium species **94** by σ-bond metathesis and expulsion of pinacolborane (Scheme 2.17). Intramolecular oxidative addition then occurs preferentially into the carbon-hydrogen bond in the *ortho*-position. After reductive elimination of the boronate ester, reaction of the resulting intermediate **96** with bis(pinacolato)diboron liberates the *ortho*-borylated product **97** and regenerates the active catalyst **94**. Application of the same

methodology to phenols can also be accomplished, using a one-pot protocol encompassing formation of the silyl ether **93** and its cleavage upon completion of the borylation.

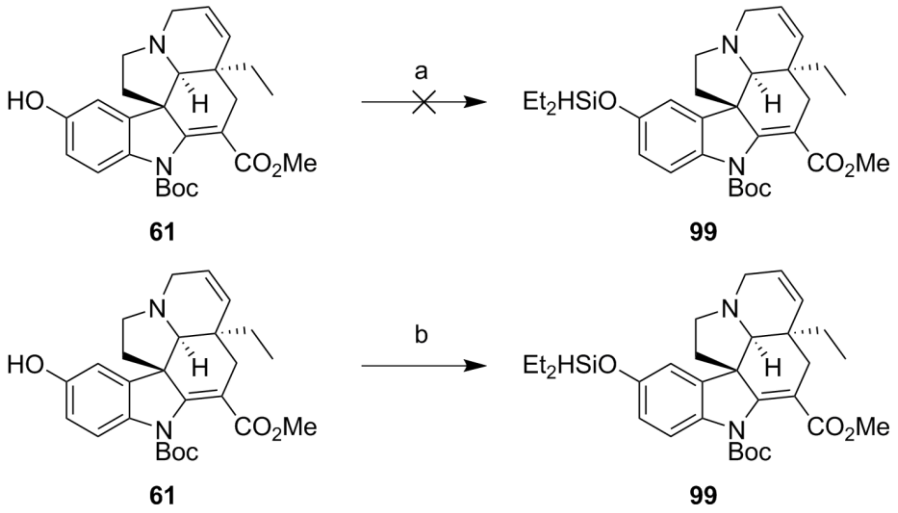
Preparation of the silyl ether **93** by dehydrogenative coupling of the phenol **82** with diethylsilane was catalysed using the same commercially available iridium complex as used for borylation, enabling the subsequent transformation to be performed in the same reaction vessel (Scheme 2.18). Finally, upon completion the addition of potassium bifluoride serves not only to cleave the silyl ether, but also to facilitate purification of the *ortho*-borylated product **97**, which is converted to the corresponding trifluoroborate salt **98**.



Scheme 2.18: Preparation of *ortho*-borylated phenols accomplished by Hartwig *et al*.[80] Reagents and conditions: (a) Et_2SiH_2, $[Ir(cod)Cl]_2$ (0.5 mol%), C_6H_6, r.t., 100 min; (b) B_2pin_2, HBpin (5 mol%), $[Ir(cod)Cl]_2$ (1 mol%), dtbpy (2 mol%), THF, 80 °C, 2 h; (c) KHF_2, THF, r.t., 16 h

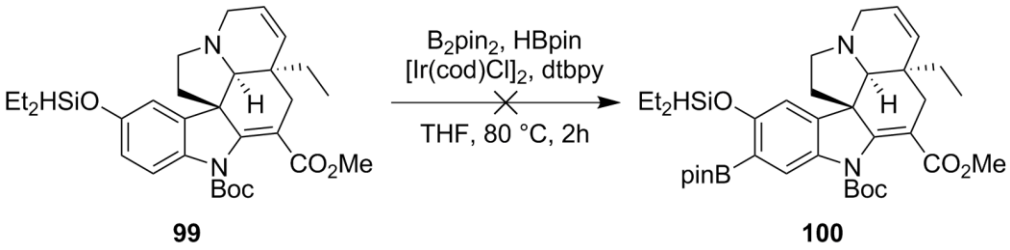
In accordance with the observations by Hartwig *et al*., the *ortho*-borylation of phenol **61** was attempted using the hydrosilane directing group. First, preparation of the required silyl ether **99** using the disclosed dehydrogenative coupling proved unsuccessful (Scheme 2.19). Fortunately upon base mediated reaction with the corresponding chlorosilane the desired silyl ether **99** could

be detected. During chromatography significant hydrolysis of the directing moiety was observed, prompting use of the substrate without further purification.



Scheme 2.19: Synthesis of the silyl ether **99**. Reagents and conditions: (a) Et_2SiH_2, $[Ir(cod)Cl]_2$ (0.5 mol%), C_6H_6, r.t., 100 min; (b) Et_2SiHCl, imidazole, THF, r.t., 17 h, 86%

Next the *ortho* borylation was examined, but no reaction was observed when using the protocol described (Scheme 2.20). The silyl ether **99** remained unchanged after treatment with bis(pinacolato)diboron under iridium catalysis and upon increasing the temperature over 80 °C the starting material was observed to gradually degrade. While the product was not determined, loss of the protective groups was inferred from the ^{1}H NMR spectrum.

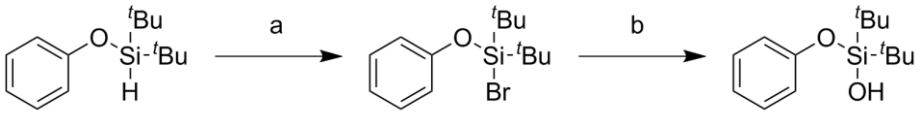


Scheme 2.20: Attempted *ortho* borylation of silyl ether **99**

The lack of discernible reaction displayed in either the preparation of silyl ether **99** or during its borylation suggest a loss of activity of the catalytic iridium species, possibly due to chelation at a different site. Potentially this could be further assessed using super-stoichiometric loadings of the catalyst. However, given the cost of bis(1,5-cyclooctadiene)diiridium(I) dichloride, the economy of semisynthesis would be substantially reduced.

2.4.3.3 Directed *Ortho* Hydroxylation

Directed aromatic functionalisation has also been reported using palladium catalysis.[81] Precedent for the synthesis of catechols from their corresponding phenols was established by Gevorgyan *et al*. using silanols as chelating groups to direct aryl metalation.[82] The preparation of silanols is also disclosed, which can be conveniently achieved from silyl ethers by bromination and subsequent aqueous hydroxylation (Scheme 2.21).

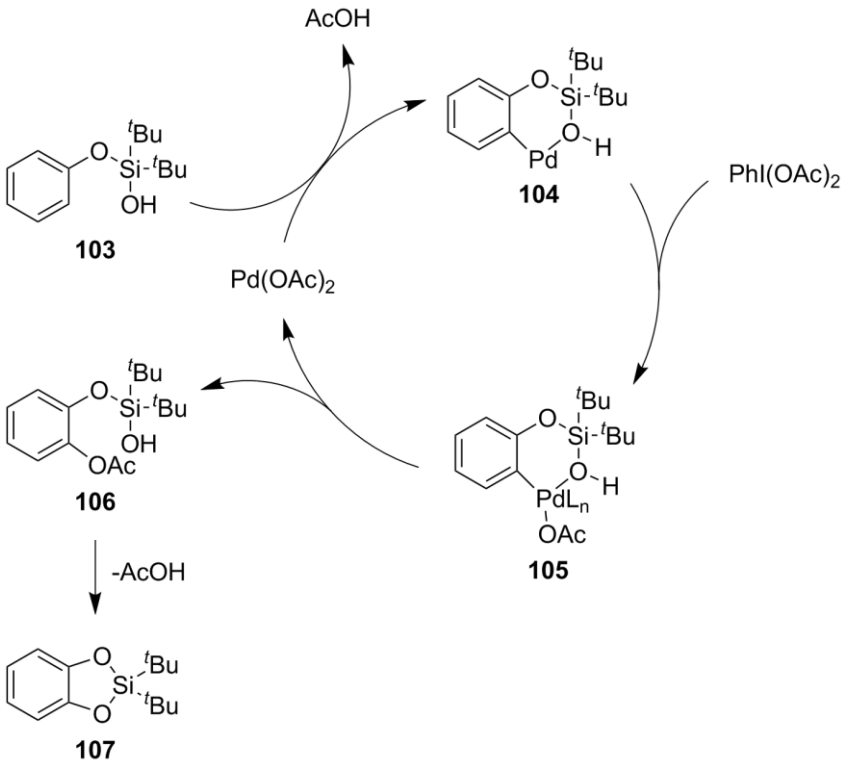


101 **102** **103**

Scheme 2.21: Preparation of silanols accomplished by Gevorgyan *et al*.[82] Reagents and conditions: (a) NBS, CH_2Cl_2, r.t.; (b) NaOH, DMF, r.t.

The mechanism of the hydroxylation begins with insertion of the palladium into the aryl carbon-hydrogen bond as directed by the silanol moiety in the starting material **103**, accompanied by loss of acetic acid (Scheme 2.22). Oxidation of the palladium(II) in the resulting palladacycle **104** by (diacetoxyiodo)benzene, gives the presumed intermediate **105**; a less

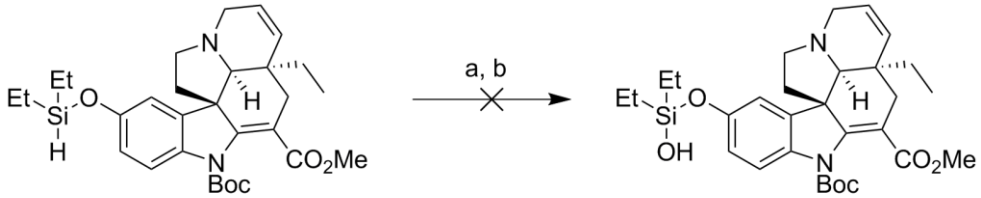
frequently encountered higher-oxidation-state palladium complex. Isotopic labelling experiments using oxygen-18 labelled silanols provide evidence against direct reductive cyclisation. Instead the authors propose reductive acetoxylation, which gives the observable aryl acetate **106**. Ultimately ring closure upon loss of acetic acid leads to the cyclic, silicon protected catechol **107**.



Scheme 2.22: Proposed mechanism of the palladium catalysed, silanol directed *ortho* hydroxylation[82]

With the silyl ether **99** conveniently in hand, preparation of the required silanol derivative **108** began with bromination of the silicon group, which was attempted according to the described conditions (Scheme 2.23). Treatment of the silyl ether **99** with *N*-bromosuccinimide resulted in a vigorous exotherm and rapid discolouration of the reaction mixture, even at reduced

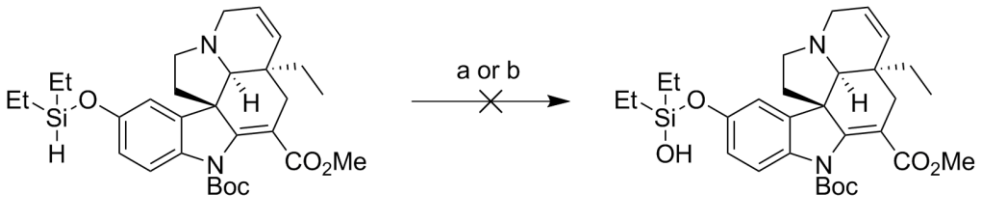
temperatures. Examination of the resulting crude material revealed a mixture of several compounds, impeding accurate determination of the reaction outcome using ^{1}H and ^{13}C NMR spectroscopy. However, encouraged to proceed by the detection of the desired ion in the mass spectrum, the crude material was utilised without further purification, pursuant to the instability of silyl bromides. Unfortunately, upon hydrolysis with aqueous sodium hydroxide the desired silanol **108** could not be detected. Furthermore the ion attributed to the starting material remained present in the mass spectrum, suggesting inadequate characterisation of the assumed intermediate.



99 **108**

Scheme 2.23: Attempted preparation of silanol **108**. Reagents and conditions: (a) NBS, CH_2Cl_2, 0 °C → r.t., 15 min; (b) NaOH, DMF, r.t., 2 h

While alternate methods for the preparation of silanols have been disclosed in the literature, protocols often exhibit poor selectivity, giving high yields of disiloxane side products.[83] More recently, the increased importance of silicon-based polymeric materials has stimulated the development of efficient, catalytic procedures for the oxidation of silanes directly to the corresponding silanols. Accordingly, both iridium and titanium catalytic systems were evaluated for the synthesis of the required silanol **108** from the silyl ether **99** (Scheme 2.24).



99 **108**

Scheme 2.23: Attempted oxidation of silanol **108**. Reagents and conditions: (a) H_2O, $[Ir(cod)Cl]_2$, MeCN, r.t., 24 h; (b) $Ti(O^iPr)_4$, ^tBuOOH, MeCN, r.t., 2 h

Treatment of the silyl ether **99** with catalytic bis(1,5-cyclooctadiene)diiridium(I) dichloride under aqueous conditions failed to induce the reported conversion.[84] Instead the starting material **99** remained unchanged, even on prolonged stirring. Conversely, the literature protocol for titanium catalysed oxidation saw complete degradation of the starting material, giving a mixture of unidentified products.[83] Uniform cleavage of the protecting group and silicon group was particularly evident on analysis of the ^1H NMR spectrum from the reaction mixture.

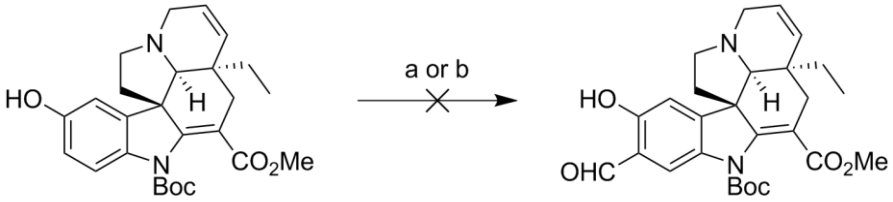
Whilst *ortho*-directed, transition metal catalysed processes have found great general utility, the problematic synthesis of the required substrate **108** prompted investigation of alternative *ortho*-selective functionalisation methods. Given the precedent for the preparation for catechols from the corresponding *ortho*-formyl phenols, conditions to effect *ortho* formylation of phenol **61** were sought.

2.4.4 *Ortho* Formylation

The formylation of an aromatic ring is an important synthetic transformation and the manipulation of the resulting carbonyl moiety permits access to a wide range of functional groups. Several robust methods for the formylation of arenes are well known in the published literature and a selection of these were examined using the phenol **61** starting material.

2.4.4.1 Vilsmeier-Haack Reaction

Perhaps the most well-known conditions for the formylation of arenes are those developed by Vilsmeier *et al*. in 1927, which use a highly electrophilic iminium cation generated from phosphorus oxychloride and dimethylformamide.[85] After attack by an electron-rich arene the product is readily hydrolysed to give the corresponding aldehyde. Unfortunately, when applied to the phenol **61**, gradual cleavage of the protecting group was evidenced from changes in the ^{1}H NMR spectrum (Scheme 2.24). Furthermore, aromatic functionalisation could not be detected. An alternative protocol was also examined, which makes use of cyanuric chloride in place of phosphorus(V) oxychloride.[86] However, the same product was observed from the reaction.



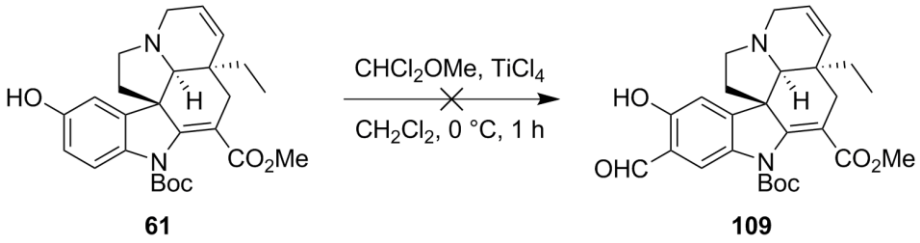
61 **109**

Scheme 2.24: Attempted Vilsmeier-Haak reaction of phenol **61**. Reagents and conditions: (a) $POCl_3$, DMF, CH_2Cl_2, r.t., 4 h; (b) cyanuric chloride, DMF, CH_2Cl_2, r.t., 4 h

The lack of discernible aromatic substitution was likely due to competing reaction with the hydroxyl group generating an unstable formate ester, which hydrolysed to return the starting material. Cleavage of the protecting group can also be rationalised by considering the stoichiometric hydrogen chloride generated over the course of the reaction.

2.4.4.2 Rieche Formylation

Carbonylation can also be accomplished using dichloromethyl methyl ether, catalysed by the strong lewis acid titanium tetrachloride in a process known as the Rieche formylation.[87] Although the mechanism has not been fully elucidated, titanium mediated carbon-carbon bond formation and subsequent hydrolysis yields aldehyde products.[88] Furthermore, research by Albericio *et al*. revealed promising *ortho*-selectivity observed with phenol substrates, warranting investigation.[89] Application of the methodology using the phenol **61** as starting material failed to yield the desired *ortho*-formylated product **109**, instead giving a mixture of compounds (Scheme 2.25).



61 **109**

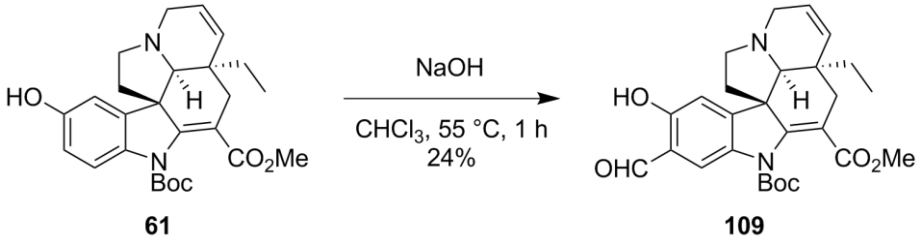
Scheme 2.25: The attempted Rieche formylation of phenol **61**

Examination of the resulting crude material using ^{1}H NMR spectroscopy revealed complete cleavage of the protecting group, enabling side reactions

with the free amino moiety. Titanium tetrachloride is known to generate hydrochloric acid upon hydrolysis, which occurs readily on contact with moisture in the air. In addition, reaction with the hydroxyl group in the substrate would also liberate hydrogen chloride. Given that *tert*-butyl carbamate cleavage is overall catalytic under acidic conditions, relatively small amounts of acid could be sufficient to effect the observed deprotection.

2.4.4.3 Reimer-Tiemann Formylation

An *ortho*-selective formylation of phenols was also investigated; the Reimer-Tiemann reaction uses a nucleophilic phenoxide anion to attack the highly reactive dichlorocarbene, generated *in situ* from chloroform by deprotonation and then spontaneous loss of chloride from the resultant carbanion.[90] The convenient procedure usually utilises chloroform in excess as the reaction solvent and aqueous potassium hydroxide as base. Typically the mixture is heated to initiate the reaction and under the prescribed conditions, basic hydrolysis of the resulting dichloromethyl substituted phenol gives the *ortho*-formylated phenol product. Gratifyingly, when applied to the phenol **61**, gradual conversion of the starting material was observed (Scheme 2.26).



61 **109**

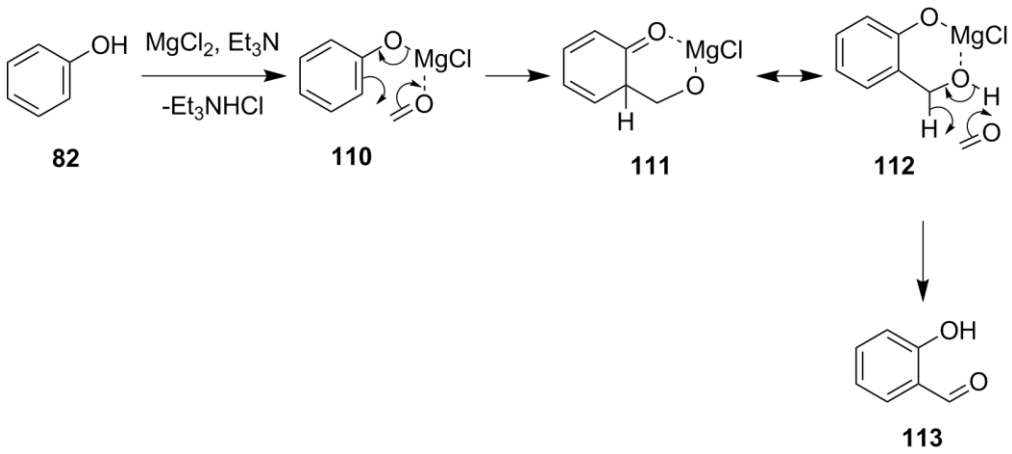
Scheme 2.26: The synthesis of *ortho*-formylated phenol **109**

Despite incomplete consumption of the starting phenol **61**, confirmation of the regiochemistry could be readily inferred from the crude ^{1}H NMR. The *ortho*-formylated product **109** was isolated from the reaction mixture by column chromatography, albeit in low yield. Increased reaction time saw no significant improvement in yield and in fact, several additional by-products were observed to appear upon prolonged heating. While the identities of these products were never ascertained, their retention times during chromatography were close enough to that of the desired product **109** to impede its isolation from the crude mixture. However, encouraged by the initial result, *ortho*-formylation procedures were further examined.

2.4.4.4 Magnesium Mediated *Ortho* Formylation

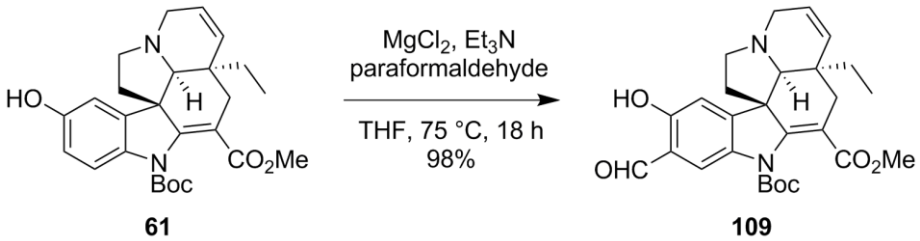
The magnesium mediated *ortho*-formylation of phenols with paraformaldehyde was first reported by Casnati *et al*. in 1978.[91] The original process used phenoxymagnesium bromides as substrates, which were prepared by reaction of the parent phenols with akyl Grignard reagents. Reaction with paraformaldehyde then gave exclusively the *ortho*-formylated phenol product. Several years later, an improved procedure was described by Levin *et al*., using magnesium salts prepared simply by reaction of the phenol with magnesium methoxide.[92] However, removal of the solvent from the phenoxides was still required prior to their reaction with paraformaldehyde, giving scope for the further enhancements made by Skattebøl *et al*.; the development of a one-pot method.[93] First, reaction of the phenol substrate **82** with magnesium chloride gives the phenoxymagnesium chloride salt **110**

(Scheme 2.27). Coordination of formaldehyde triggers a formal ene-type reaction, which gives the magnesium salt **111** and after tautomerisation benzylic alcohol **112**. Finally oxidation by a second equivalent of formaldehyde gives the desired *ortho*-formylated phenol **113**.



Scheme 2.27: Proposed mechanism of the magnesium mediated *ortho*-formylation of phenols[93]

Pleasingly, application of the magnesium mediated *ortho*-formylation developed by Skattebøl *et al*. saw full conversion of the starting phenol **61** to the desired *ortho*-formylated product **109** (Scheme 2.28). Purification was accomplished using column chromatography, which was hindered by residual paraformaldehyde until a methanol and dichloromethane solvent system was employed; the product could then be isolated in excellent yield.



Scheme 2.28: Magnesium mediated synthesis of *ortho*-formylated phenol **109**

With the *ortho*-formyl phenol **109** in hand, conversion to a catechol was envisaged through oxidation of the newly installed aldehyde. The oxidative cleavage of a carbon-carbon bond adjacent to a carbonyl, which changes an aldehyde or ketone into an ester is known as Baeyer-Villiger oxidation.[94]

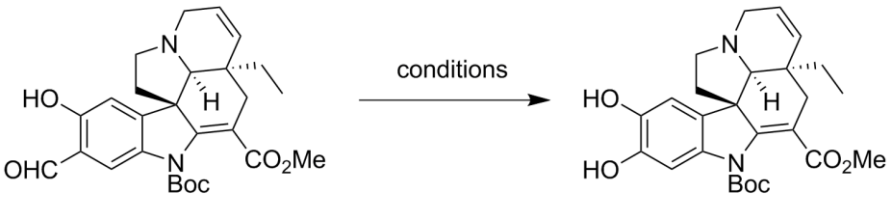
2.4.5 Oxidation of *tert*-Butyl 10-Hydroxy-11-tabersoninecarbaldehyde-1-carboxylate (109)

The Baeyer-Villiger reaction occurs by addition of a peroxidic oxidant to an aldehyde or ketone giving a Criegee intermediate. Subsequent collapse prompts migration of one of the carbonyl substituents, yielding an ester product. There have been several adapted processes reported in the published literature since the original method presented by Baeyer *et al*. in 1899, which make use of modern peroxidic oxidants and catalysis for enhanced yield and selectivity.[95]

The Dakin reaction, a modification of the Baeyer-Villiger oxidation published in 1909, allows the preparation of phenols from aryl aldehydes or ketones.[96] The procedure makes use of the nucleophilic hydroperoxide anion, generated by deprotonation of hydrogen peroxide, which on addition to the carbonyl group gives a similar tetrahedral intermediate. Collapse triggers migration of the aryl substituent yielding an ester, which under the basic reaction conditions is rapidly hydrolysed to the corresponding phenol.

While many elegant procedures have been published over the last century, a focussed selection of potential methods were first examined for the oxidation

of the *ortho*-formyl phenol **109**. The protocol originally developed by Dakin *et al*. was applied, alongside another basic oxidation using sodium percarbonate and acidic conditions employing either boric acid or 3-chloroperbenzoic acid (Table 2.7).[97] In addition, selenium dioxide catalysed oxidation, reported by Guzmán *et al*. for the mild and selective Baeyer-Villiger oxidation of benzaldehydes to aryl formates, was also examined.[98]



109 **114**

Entry	Conditions	Product
1	NaOH, H_2O_2, r.t.	degradation
2	$Na_2CO_3 \cdot 1.5H_2O_2$, THF/H_2O, r.t.	degradation
3	H_3BO_3, H_2O_2, THF, r.t.	degradation
4	*m*CPBA, AcOH, r.t.	*N*-oxide
5	SeO_2 (2 mol%), H_2O_2, ^{t}BuOH, r.t.	mixture

Table 2.7: Conditions for the attempted oxidation of *ortho*-formyl phenol **109**

The results of the basic reaction conditions screened were disappointing (Table 2.7, entries 1 and 2). Rapid discolouration of the reaction mixture was observed and upon spectroscopic examination of the resulting crude material, similar complex mixtures of unidentified degradation products were all that could be discerned. The examples of acid mediated oxidations were also unsuccessful (Table 2.7, entries 3 and 4). However, the identity of a recurrent

compound in both reactions was inferred to be an amine oxide of a proto-deformylation product, from both the mass spectrum and crude ^{1}H NMR. Interestingly, the selenium catalysed protocol gave a mixture of three major components, which upon chromatography were seen to degrade rapidly (Table 2.7, entry 5). The compounds were assigned to be a mixture of the desired catechol **114**, formyl ester **115** and an unanticipated, brightly coloured *ortho*-quinone **116** (Figure 2.3). Given the observed instability of the compounds during chromatography, the crude mixture of products was used directly, without further purification.

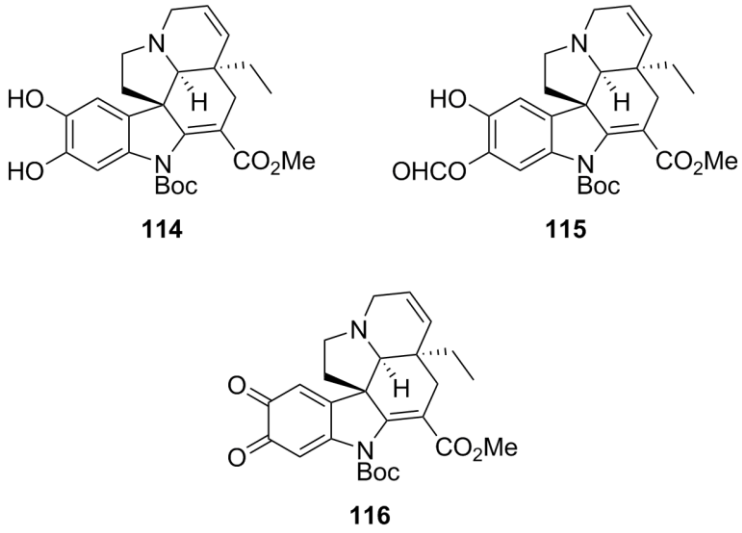
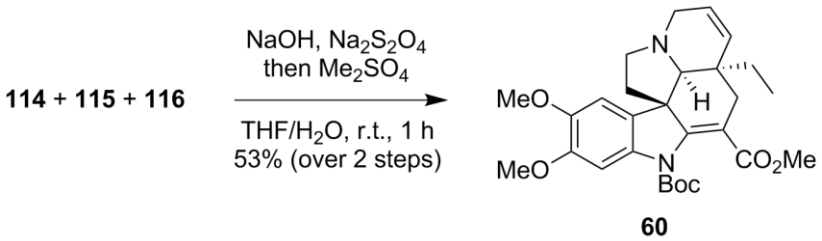


Figure 2.3: Products of the selenium dioxide catalysed Baeyer-Villiger oxidation of *ortho* formyl phenol **109**

In order to proceed with methylation, coalescence of the crude material to the catechol **114** was required. Inspired by the reduction, methylation sequence developed by Bodwell *et al*. in their preparation of pyreneyl ethers, applying a similar logic prompted development of an efficient one-pot hydrolysis, reduction and methylation sequence.[99]

2.4.6 Synthesis of *tert*-Butyl 10-*O*-Methyljerantinine A-1-carboxylate (60)

The methylation sequence began with convenient homogenous conditions for the hydrolysis of the formyl ester **115**. Using a mixture of tetrahydrofuran and water, which remained monophasic upon the addition of aqueous sodium hydroxide, cleavage of the ester moiety resulted in instantaneous discolouration of the reaction mixture at room temperature. Subsequent addition of sodium dithionite served to reduce any of the *ortho*-quinone **116**, which formed during the Baeyer-Villiger oxidation and on exposure of the catechol **114** to the air. The reduction was conveniently accompanied by the complete loss of colour from the stirring reaction, indicating rapid completion. Finally, adding dimethyl sulfate to the mixture served to methylate the catechol **114** sodium salt, giving the dimethyl ether **60** in moderate yield.



Scheme 2.29: Synthesis of the catechol dimethyl ether **60**

Isolation of the desired product was achieved by column chromatography. Complete characterisation of the compound served to confirm the identity of the dimethyl ether **60**, requiring only the appropriate deprotection steps for conversion into the target, jerantinine A (**1**).

2.5 Preparation of Jerantinine A (1) and Jerantinine E (5)

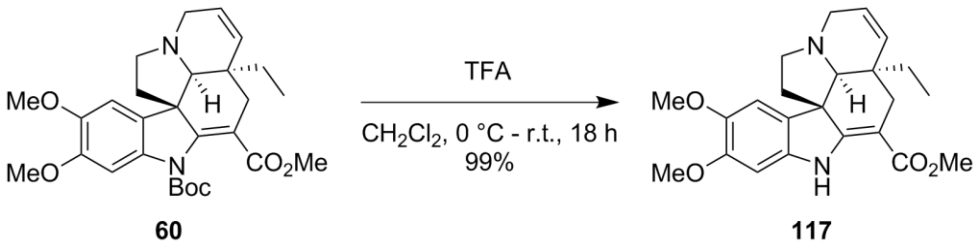
The remaining stages in the presented semisynthesis comprise cleavage of a *tert*-butyl carbamate protecting group, which precedes a final selective demethylation in accordance with that reported by Waser *et al*. in their synthesis of jerantinine E (**5**).[18]

2.5.1 Synthesis of 10-*O*-Methyljerantinine A (117)

Deprotection of *tert*-butyl carbamate masked amines is a well-documented transformation. Much of the practicality of the protecting group comes from the relative ease associated with its installation and cleavage.[100] While many procedures have been developed to encompass specific substrates, most frequently removal is accomplished using acidic conditions.[101] Protonation triggers loss of 2-methylprop-1-ene and carbon dioxide, giving the free amine. Many acids have been proven efficacious, but particularly widespread is the use of trifluoroacetic acid, normally in combination with dichloromethane as a reaction solvent.

Gratifyingly, treatment of the protected catechol dimethyl ether **60** with trifluoroacetic acid in dichloromethane solvent gave the desired product, 10-*O*-methyljerantinine A (**117**) in essentially quantitative yield (Scheme 2.30). The reaction proved slow at 0 °C prompting increase of the temperature to ambient, which saw gradual conversion of the starting material. Unfortunately, the retention time during chromatography of the protected derivative **60** proved the same as the free amine product **117** despite using a variety of

eluents and stationary phases. Consumption of the starting material **60** was therefore monitored to completion by examination of the ^{1}H NMR and mass spectra directly from the reaction mixture.



60 **117**

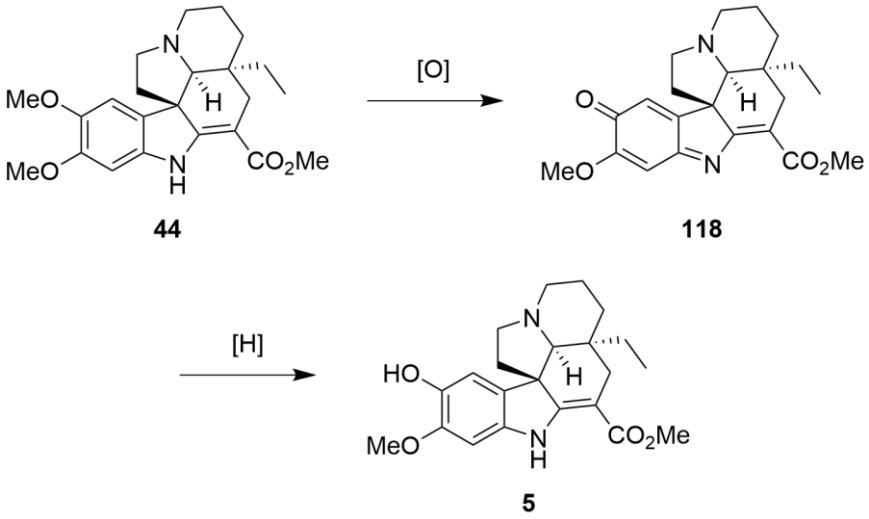
Scheme 2.30: Synthesis of 10-*O*-methyljerantinine A **117**

The 10-*O*-methyljerantinine A (**117**) proved to have spectroscopic data identical to that presented in the literature by Lim *et al*., taken from material synthesised by methylation of an authentic, natural sample of jerantinine A (**1**).[1]

2.5.2 Synthesis of Jerantinine A (1)

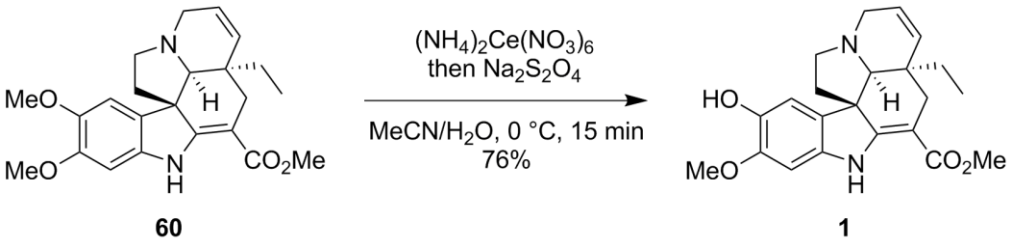
Credible precedent for the selective demethylation of 10-*O*-methyljerantinine A (**117**) can be found in the literature published by Waser *et al*., where oxidative demethylation of 10-*O*-methyljerantinine E (**44**) and subsequent reduction was shown to yield jerantinine E (**5**) (Scheme 2.31).[18] Their method used the strong, single electron oxidant ceric ammonium nitrate, which was sufficient to generate the corresponding iminoquinone **118** from the starting indoline **44**, after hydrolysis of the intermediate oxonium cation. Finally, reduction *in situ* by addition of sodium dithionite to the reaction mixture

served to complete the desired transformation. Using this sequence a 79% yield of jerantinine E (**5**) was reported from the dimethyl precursor **44**.



Scheme 2.31: Preparation of jerantinine E (**5**) from 10-*O*-methyljerantinine E (**44**) accomplished by Waser *et al*.[18] Reagents and conditions: $(NH_4)_2Ce(NO_3)_6$, MeCN/H_2O, 0 °C, 5 min then $Na_2S_2O_4$, 0 °C, 10 min, 79%

The described selective demethylation protocol was applied, using 10-*O*-methyljerantinine A (**117**) as the starting material (Scheme 2.32). Analogous conversion to the desired product jerantinine A (**1**) was demonstrated (Figure 2.4). The oxidative instability of **1**, as noted by Lim *et al*. during its first isolation, was particularly apparent during thin-layer chromatography, where the corresponding band would rapidly develop a bright yellow colour on exposure to air.[1]



Scheme 2.32: Synthesis of jerantinine A (**1**)

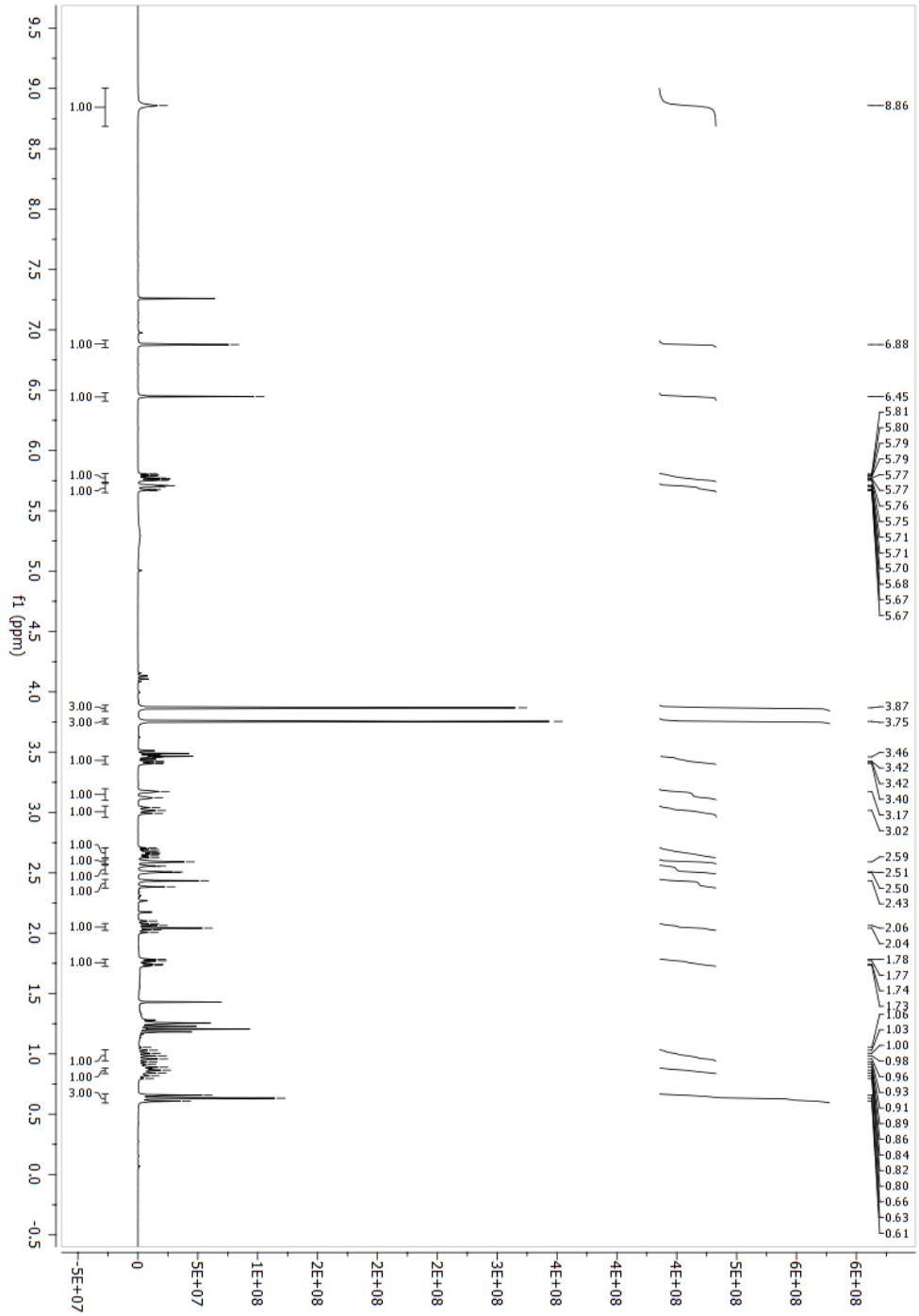


Figure 2.4: The ^{1}H NMR spectrum of jerantinine A

Fortunately, the relatively clean reaction of the 10-*O*-methyljerantinine A (**117**) simplified chromatographic purification, permitting rapid elution of the product **1**, which was isolated in 76% yield. Unfortunately significant contamination with residual solvent can be seen in the ^{1}H NMR spectrum

obtained. However, excluding signals from diethyl ether, ethyl acetate and hexane, spectroscopic data from the synthetic material corresponds with that originally reported by Lim *et al*., which further verified the product to be jerantinine A (**1**).[1] The present study represents the first synthesis of jerantinine A (**1**) and is the only example to date of an asymmetric preparation of any member of the jerantinine family of natural products (**1**-**8**), highlighting the benefit of the semisynthetic approach. Overall, the semisynthesis of jerantinine A (**1**) was accomplished in 28% yield over 9 steps, starting from tabersonine (**12**).

2.5.3 Synthesis of Jerantinine A Acetate (119)

During both the described synthesis and isolation from natural sources, jerantinine A (**1**) was observed to undergo oxidation readily in air, which is presumed to give an unstable iminoquinone.[1] Enhanced biological activity was found to result from acetylation during initial *in vitro* cytotoxicity screening, which also conveniently blocked the facile oxidation. Preparation of jerantinine A acetate (**119**) was therefore undertaken, using the conditions described by the authors.

Reaction of jerantinine A (**1**) with acetic anhydride in pyridine rapidly gave the desired product **119**, which was isolated by column chromatography in near quantitative yield (Scheme 2.33). Additionally, none of the degradation associated with the parent compound was observed during thin-layer chromatographic analysis of the jerantinine A acetate (**119**). Again the identity

of the product was further confirmed by comparison of the spectroscopic data with that available in the published literature.[1]



1 **119**

Scheme 2.33: Synthesis of jerantinine A acetate (**119**)

With an initial route to the natural product (**1**) and its potent acetate derivative **119** established, the investigation moved to the preparation of the remaining jerantinine alkaloids (**2**-**8**). Given that the existing total synthesis of jerantinine E (**5**) by Waser *et al*. was achieved in 16% yield over 17 linear steps, semisynthesis from jerantinine A (**1**) could offer significant advantages and enable direct comparison of the presented approach with the state of the art.

2.5.4 Synthesis of Jerantinine E (5)

Conversion of jerantinine A (**1**) into jerantinine E (**5**) can be envisaged through selective reduction of the π-bond between C14 and C15. Whilst the exact transformation was unknown, the analogous reduction of tabersonine (**12**) to yield vincadifformine (**120**) was reported by Andrade *et al*. during their total synthesis of several *Aspidosperma* alkaloids (Scheme 2.34).[102] Under platinum(IV) oxide catalysis, hydrogenation of tabersonine (**12**) was shown to be regioselective, giving the corresponding reduced product **120** in good yield.

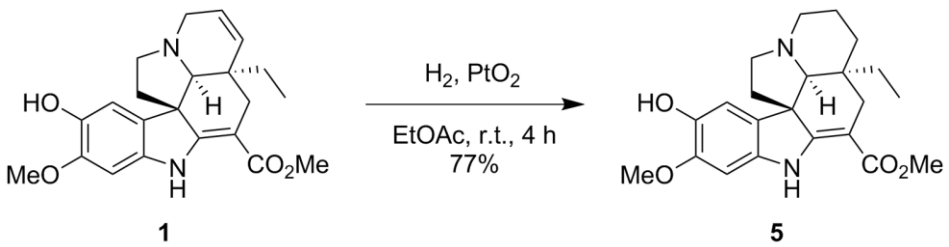


12 **120**

Scheme 2.34: Preparation of vincadifformine (**120**) from tabersonine (**12**) accomplished by Andrade *et al*.[102]

Platinum(IV) oxide, also known as Adams' catalyst, is useful for hydrogenation and hydrogenolysis in a variety of situations.[103] During the reaction, platinum black agglomerates are formed *in situ* upon exposure to a hydrogen atmosphere, which are proposed to be the true catalytic species.[104] Coordination of an olefin to the highly active platinum nanoparticles enables facile hydrogenation, usually under relatively mild experimental conditions.

Given the literature precedent for related *Aspidosperma* alkaloids, selective reduction of jerantinine A (**1**) was attempted (Scheme 2.35). Pleasingly, on exposure of the starting material **1** to an atmosphere of hydrogen, under platinum(IV) oxide catalysis, smooth conversion to the desired reduction product **5** was observed (Figure 2.5). Purification of the crude material by column chromatography gave jerantinine E (**5**) as a single enantiomer in 77% yield.



1 **5**

Scheme 2.35: Synthesis of jerantinine E (**5**)

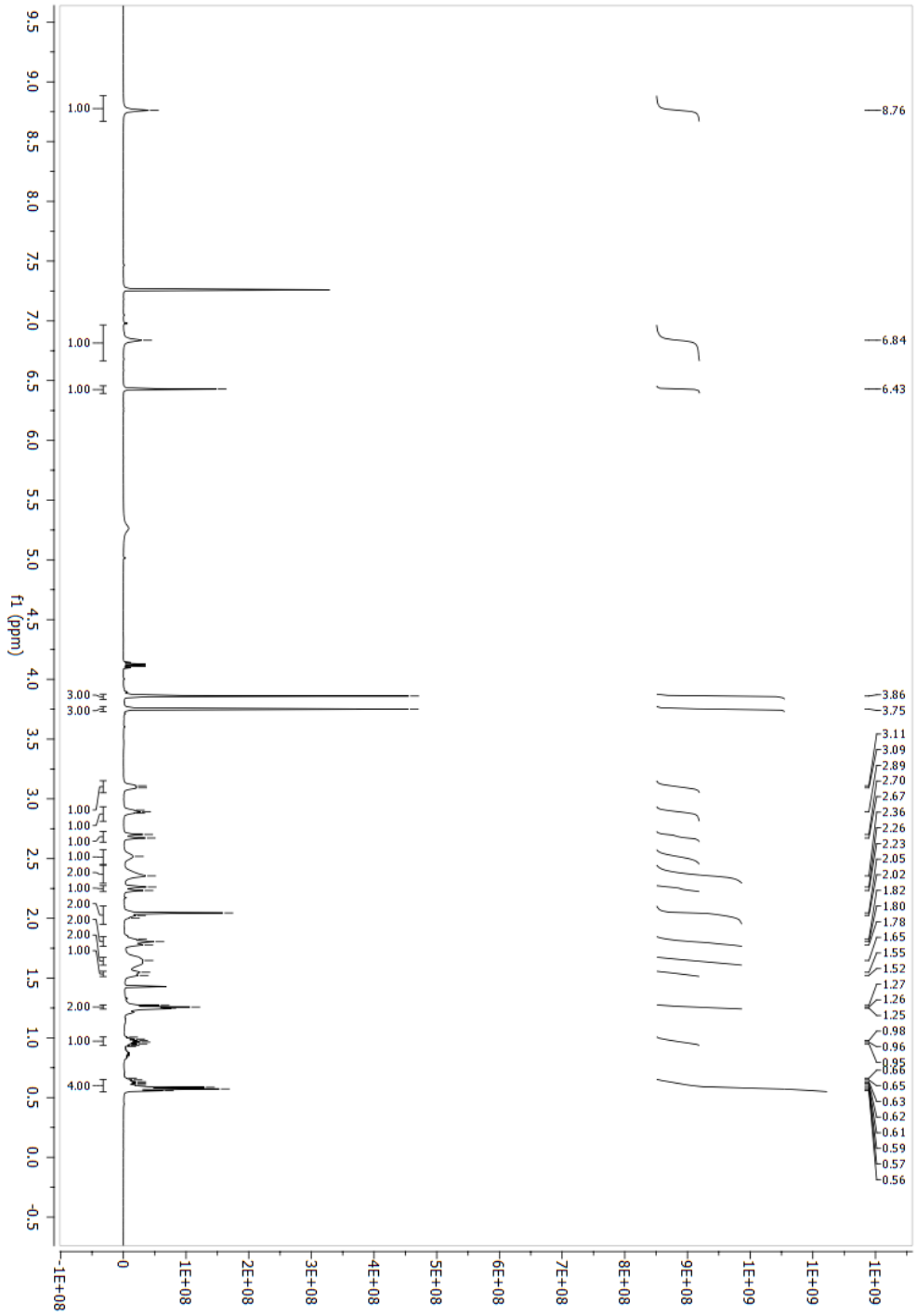


Figure 2.5: The 1H NMR spectrum of jerantinine E

Signals in the ^{1}H NMR spectrum of jerantinine E appear broad at room temperature. However, spectroscopic data obtained from the synthetic compound correlates with that reported by Lim *et al*., from material of natural origin.[1] The semisynthesis of jerantinine E (**5**) was achieved in 22% overall yield

starting from tabersonine (**12**), using a 10 step sequence. The present approach compares favourably with the total synthesis reported by Waser *et al*.; despite only 6% increase in yield the stepwise economy represents a significant improvement in terms of cost and efficiency.

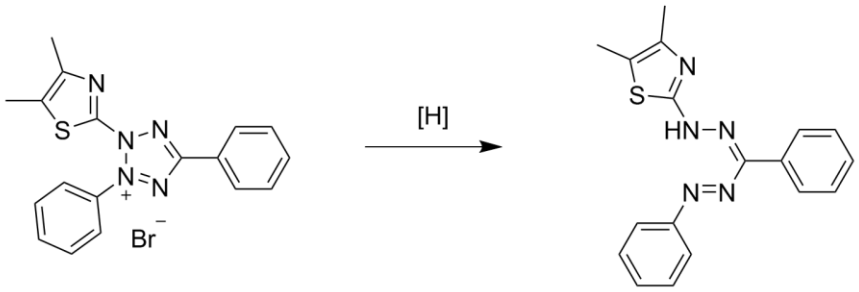
2.6 Biological Evaluation of Jerantinine A (1)

A sample of synthetic jerantinine A (**1**) was submitted to collaborating biologists in the research group of Dr Tracey Bradshaw for initial *in vitro* biological analysis and direct comparison with an authentic sample of natural origin. All experiments were generously performed and the results interpreted by Dr Mohannad Qazzaz.

2.6.1 MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**121**) (MTT) assay is used to determine the growth inhibitory properties of test compounds in carcinoma cell lines. Cells are seeded in multi-well plates in an appropriate quantity of cell culture medium. The plates are then incubated to allow cell adherence. At the time of agent administration an MTT assay is performed on an untreated plate, allowing an initial optical density to be determined. Test agents are then diluted to working concentrations in complete medium and the appropriate volume added to each well of the plate. Cells are incubated and after the required exposure MTT (**121**) is added to each well; the metabolic activity of any remaining viable cells reduce the tetrazolium dye to an insoluble formazan product (**122**) (Scheme 2.36). Plates are further incubated, allowing

the formazan **122** crystals to form. The resulting aqueous medium is aspirated and dimethyl sulfoxide added, solubilising the formazan **122** crystals. Absorbance readings of the resulting solutions are recorded using a plate reader; the concentration of formazan dye is directly proportional to the number of viable cells. Average values of repeat experiments are taken and from the data, GI_{50} values (the agent concentrations that inhibit cell growth by 50%) can be estimated.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (**121**)

(E,Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan) (**122**)

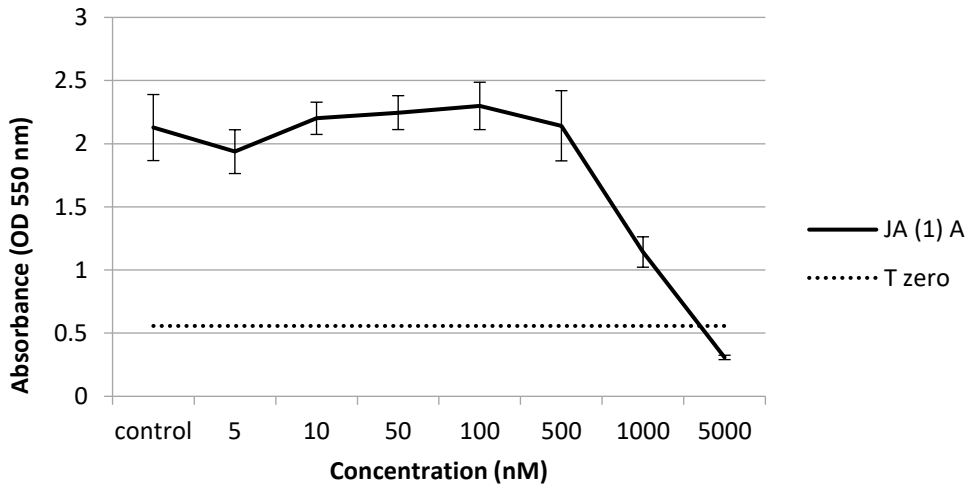
Scheme 2.36: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**121**) and (E,Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (**122**).

2.6.2 Growth Inhibitory Effects of Jerantinine A in Colorectal (HCT 116) and Breast (MCF-7) Carcinoma Cell Lines

During their previously published examination of the anti-proliferative activity of the jerantinine alkaloids (**1**-**8**), Bradshaw *et al*. demonstrated the potent growth inhibitory properties of jerantinine A (**1**) against a variety of carcinoma cell lines.[6] Furthermore, they generously offered to reproduce the assay using the material produced in the present study. Therefore, a sample of the synthetic jerantinine A (**1**) was submitted to collaborating biologists Dr

Mohannad Qazzaz and Dr Tracey Bradshaw for initial *in vitro* biological analysis and comparison with an authentic sample of natural origin.

HCT 116 is a colorectal tumour cell line, which displayed the greatest sensitivity to jerantinine A (**1**) when previously assessed.[6] MCF-7 is a breast cancer cell line that was also reported to be potently inhibited. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**121**) (MTT) assays were performed at the time of addition of the tested agent (T zero) and following 72 hour exposure of the cells to the test compound (Figures 2.6 and 2.7). Compound concentrations required to inhibit cell growth by 50% (GI_{50}) were calculated by non-linear regression analysis (Table 2.8).



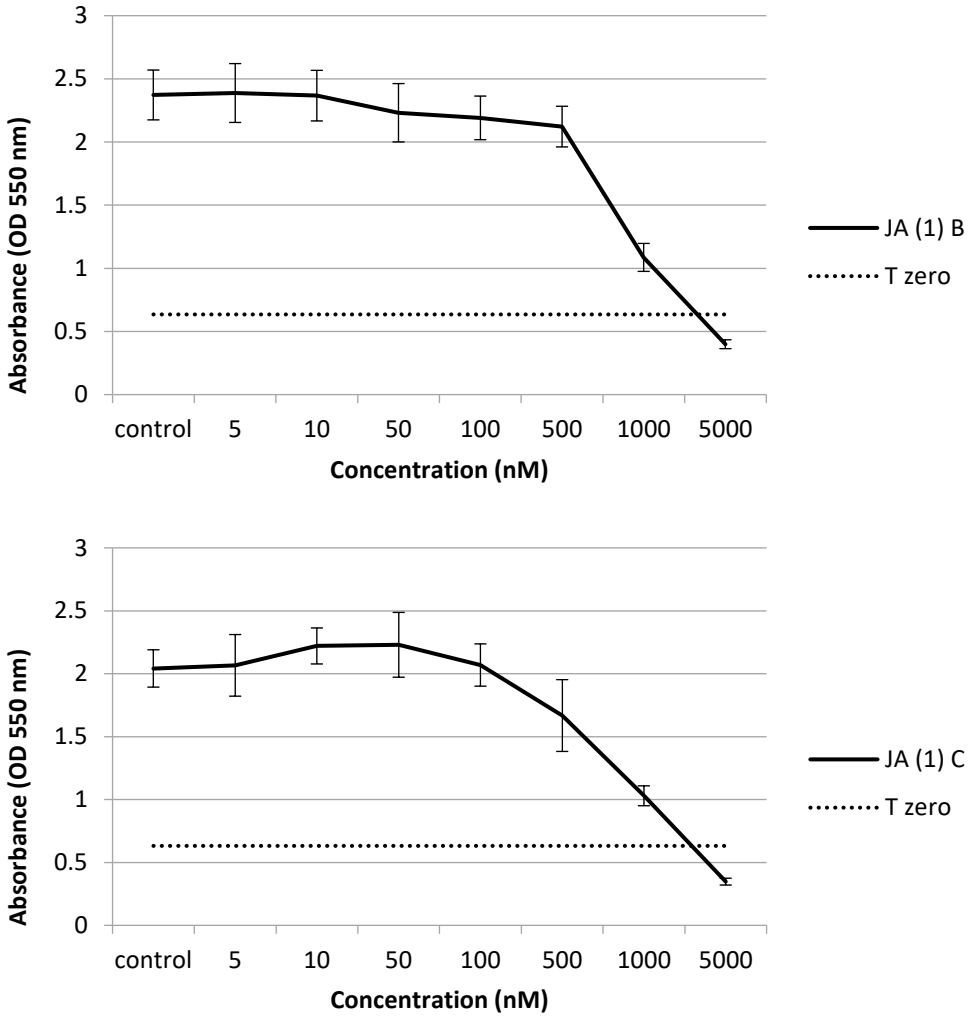
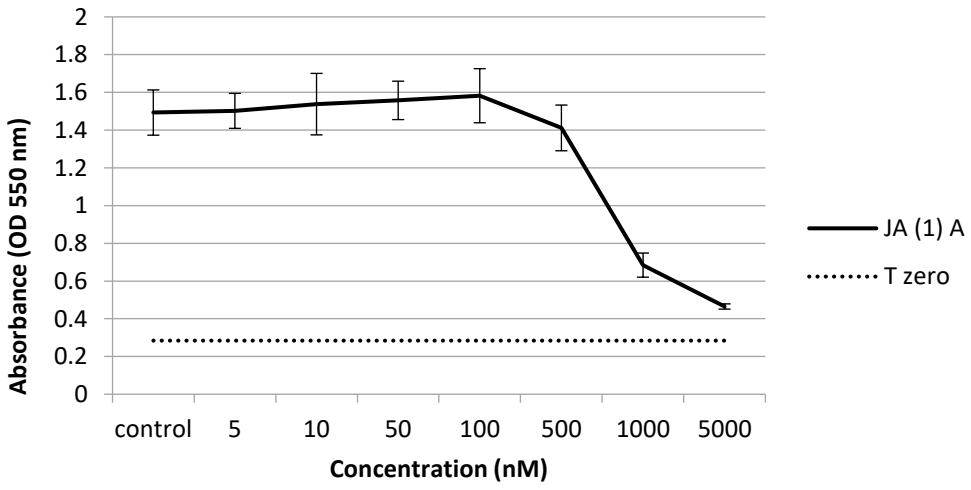


Figure 2.6: Effect of jerantinine A (**1**) on the growth of HCT 116 cells. Data shown are mean ± SD of representative MTT assays (*n* = 4 per sample)



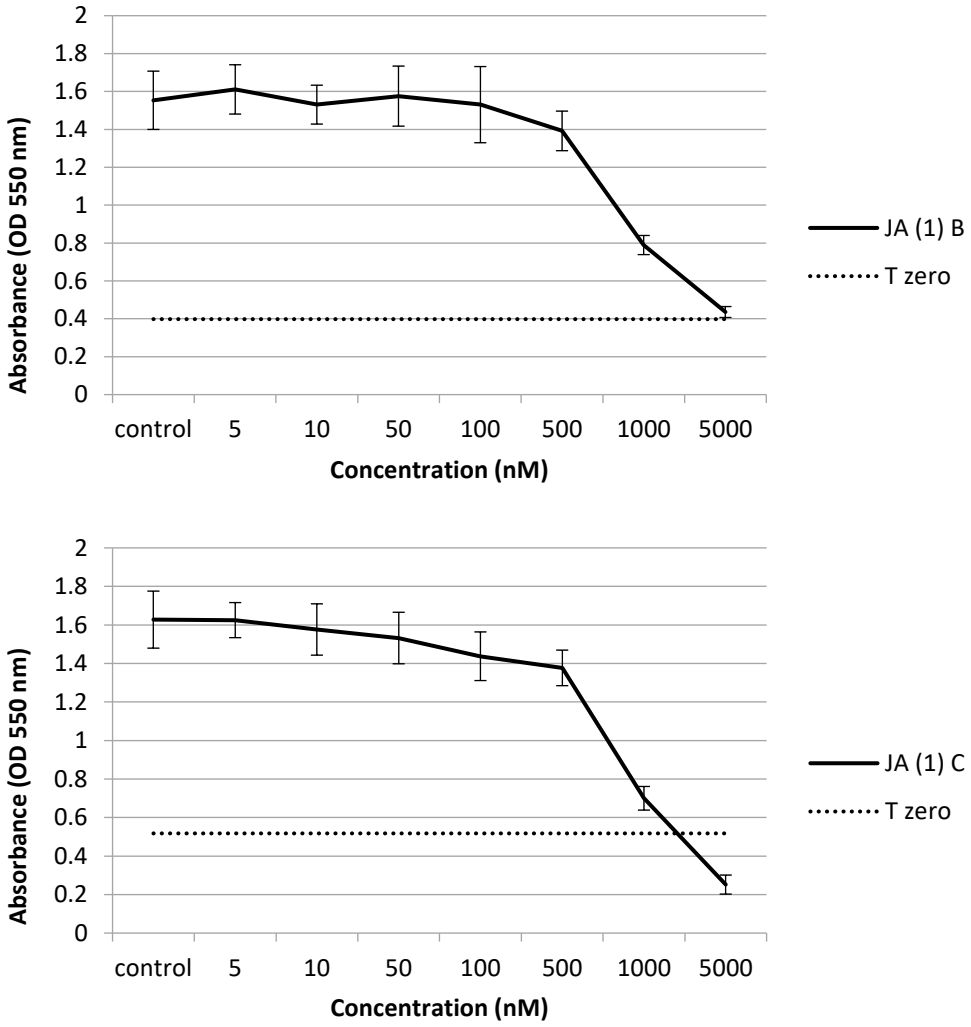


Figure 2.7: Effect of jerantinine A (**1**) on the growth of MCF-7 cells. Data shown are mean ± SD of representative MTT assays (*n* = 4 per sample)

	GI_{50} (μM)	
Compound	**HCT 116**	**MCF-7**
synthetic jerantinine A (**1**)	0.819 ± 0.07	0.809 ± 0.07
natural jerantinine A (**1**)	0.762 ± 0.13	0.853 ± 0.09

Table 2.8: Growth inhibitory activity of natural and synthetic jerantinine A (**1**) in HCT 116 and MCF-7 cell lines. Data represent mean ± SD GI_{50} (μM) values from ≥3 independent MTT assays (*n* = 4 per concentration in each trial)

Both the HCT 116 and MCF-7 cell lines showed dose-dependent sensitivity to the compounds investigated; increasing concentrations reduced the growth/proliferation. Pleasingly, the growth inhibitory effect of the synthetic jerantinine A (**1**) proved essentially identical to that displayed by the natural material.

2.7 Conclusion

The *Aspidosperma* alkaloid tabersonine (**12**) was sustainably obtained by extraction from commercially readily available *Voacanga africana* seeds, providing an economic route to a structurally intricate organic framework. The material was then used as the basis for the semisynthesis of the alkaloid jerantinine A (**1**), which is a desirable target with intriguing biological activity.

Several synthetic methods were evaluated for functionalisation of tabersonine (**12**). Ultimately a sequence comprising regioselective iodination, palladium catalysed Miyaura borylation and mild oxidation was employed for the required C10 hydroxylation. The newly generated phenol was then used to direct a magnesium mediated *ortho*-formylation, which enabled installation of the C11 hydroxyl group through the application of Baeyer-Villiger chemistry. Final stage selective demethylation in accordance with literature precedent revealed the natural product jerantinine A (**1**), which was obtained in 28% yield over 9 linear steps. Furthermore, catalytic hydrogenation of the synthetic material resulted in conversion to jerantinine E (**5**).

The present work represents the first synthesis to date of jerantinine A (**1**) and the first example of an asymmetric preparation of jerantinine E (**5**). Additionally, the viability of a semisynthetic approach to the jerantinine family of alkaloids (**1**-**8**) has been demonstrated. Both the overall yield and stepwise economy compared favourably with the total synthesis previously reported in the published literature.

Initial *in vitro* biological analysis of the synthetic jerantinine A (**1**) was generously conducted by collaborators, alongside a sample of material isolated from natural sources. The activity of both compounds was shown to be identical against the cell lines examined. The jerantinine A (**1**) that was supplied will also be further utilised in ongoing research to fully elucidate the mechanisms behind the observed cytotoxicity.

3 Experimental

3.1 Experimental Techniques

Reactions were performed in flame-dried glassware under an atmosphere of argon and stirred magnetically unless otherwise specified. For use as reaction solvents CH_2Cl_2 was freshly distilled from CaH_2; THF was freshly distilled from sodium and benzophenone; MeCN was distilled from CaH_2 and stored under an atmosphere of argon over 4Å molecular sieves. For use as a reagent Et_3N, was distilled from CaH_2 and stored under an atmosphere of argon over 4Å molecular sieves. All other solvent and reagents were obtained commercially and used without further purification. Flash column chromatography was performed using Merck silica gel 60 as the stationary phase. Where not otherwise stated flash column chromatography was performed using petrol-EtOAc mixtures of increasing polarity (appropriate to the R_f value of the compound to be isolated) as the mobile phase. Petrol refers to petroleum spirit (b.p. 40-60 °C).

3.2 Analysis

Compounds previously described in the literature were characterised to consistent levels with that reported. Thin layer chromatography was carried out on aluminium-backed plates pre-coated with silica (0.2 mm, 60 F_{254}, Merck). The plates were developed using UV light and basic potassium permanganate solution. Melting points were recorded using a STUART SMP3 apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Bruker Tensor 27

FT-IR spectrophotometer with a neat film on an NaCl disc or as a solution in $CHCl_3$. IR peaks are quoted as vmax in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at ambient temperature using Bruker DPX400 (400 MHz), AV400 (400 MHz) and AV(III)400 (400 MHz) spectrometers. Data is expressed as chemical shifts in parts per million (ppm) relative to residual solvent signals of $CDCl_3$ (^{1}H NMR 7.27; ^{13}C NMR 77.0) as the internal standard on the δ scale. The following abbreviations are used to designate the multiplicity of each signal; s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; t, triplet; tt, triplet of triplets; tq, triplet of quartets; q, quartet; m, multiplet; br, broad. Coupling constants *J* are given in Hz. High-resolution mass spectra were obtained using a Bruker MicroTOF mass spectrometer operating in electrospray ionisation (ESI) mode.

3.3 Cell Culture

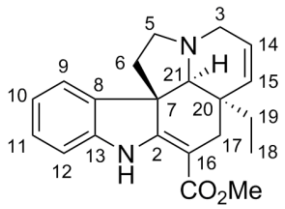
All carcinoma cell lines were sourced from the American Type Tissue Cell Culture Collection (ATCC) and cultured in RPMI 1640 nutrient medium supplemented with 10 % foetal bovine serum (FBS). Cells were passaged twice weekly to maintain logarithmic growth and incubated at 37 °C in an atmosphere constituting 5 % CO_2. Cell stocks were cryopreserved (−155 °C in liquid nitrogen) and upon re-introduction to culture were used in experiments ≤30 passages to minimise phenotypic/genotypic drift. Compound stocks were stored at −20 °C in aliquots of 10 mM in DMSO and thawed immediately prior to use.

3.4 MTT Assay

Compounds were prepared as 10 mM top stocks in DMSO. HCT 116 and MCF-7, human-derived colorectal and breast adenocarcinoma cells respectively, were cultivated at 37 °C in RPMI 1640 medium supplemented with 10% FBS. Cells were seeded at a density of 3×10^3 per well into 96-well microtiter plates and allowed to adhere for 24 h before test agent introduction (final concentrations 5 nM–5000 nM, $n = 4$). Dilutions were prepared in medium prior to each assay. At the time of agent addition and following 72 h exposure, MTT was added to each well (final concentration 400 μg mL^{-1}). Incubation at 37 °C for 2.5 h allowed reduction of MTT by viable cells (mitochondrial dehydrogenases) to insoluble formazan crystals. Well supernatants were removed, and formazan solubilised by addition of DMSO (150 μL). Absorbance was read at 550 nm using a Perkin Elmer plate reader. Non-linear regression analysis was used to calculate compound concentrations required to inhibit cell growth by 50% (GI_{50}).

3.5 Experimental Procedures and Characterisation Data

Tabersonine (12)

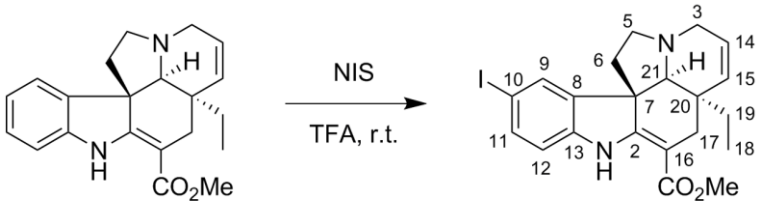


12

Voacanga africana seeds (200 g) were ground into a fine powder and suspended in an aqueous solution of 1% H_2SO_4 (2.0 L). The resulting mixture was then stirred overnight. The acidic extract was filtered and NaCl (200 g) added to the filtrate, which was stirred overnight. $CHCl_3$ (1.5 L) was then added and the biphasic solution vigorously stirred for 3 hours. The aqueous layer was removed and discarded and the remaining organic emulsion filtered through Celite®. The resulting solution was then separated and the organic fraction dried over anhydrous $MgSO_4$ and filtered. Solvent was then removed under reduced pressure and the resulting material dissolved in $CHCl_3$ (50 mL) before adding 30% aqueous NH_3 (30 mL). The organic layer was separated and the aqueous phase extracted with $CHCl_3$ (3 x 50 mL). The combined organic fractions were then dried over anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude material obtained was then purified by column chromatography (SiO_2, 0-10% EtOAc in petroleum ether 40-60 °C) to give the desired product **12** (2.8 g) as a white solid. m.p.: 78 °C; $[\alpha]_D$ -250.12 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1} 3367, 3023, 2960, 1670, 1604, 1131; δ_H(400 MHz, $CDCl_3$) 0.64 (3H, t, *J* 7.4, 18-H_3), 0.86 (1H, dq, *J* 14.3, 7.3, 19-*H*H),

1.00 (1H, dq, *J* 14.5, 7.5, 19-H*H*), 1.79 (1H, dd, *J* 11.6, 3.9, 6-*H*H), 2.07 (1H, td, *J* 11.3, 6.5, 6-H*H*), 2.44 (1H, d, *J* 15.1, 17-*H*H), 2.56 (1H, dd, *J* 15.1, 1.8, 17-H*H*), 2.76 – 2.65 (2H, m, 5-*H*H and 21-H), 3.04 (1H, ddd, *J* 7.9, 6.4, 0.8, 5-H*H*), 3.19 (1H, d, *J* 15.9, 3-*H*H), 3.46 (1H, ddd, *J* 15.8, 4.7, 1.5, 3-H*H*), 3.77 (3H, s, CO_2CH_3), 5.71 (1H, ddd, *J* 9.9, 2.3, 1.6, 15-H), 5.79 (1H, ddd, *J* 9.9, 4.7, 1.4, 14-H), 6.81 (1H, d, *J* 7.8, 12-H), 6.87 (1H, t, *J* 7.4, 10-H), 7.14 (1H, td, *J* 7.7, 1.2 Hz, 11-H), 7.23 (d, *J* 7.3 Hz, 9-H), 9.00 (1H, s, NH); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 27.0 (C-19), 28.6 (C-17), 41.4 (C-20), 44.6 (C-6), 50.7 (C-5), 51.1 (C-3), 51.1 (CO_2CH_3), 55.2 (C-7), 70.2 (C-21), 92.3 (C-16), 109.4 (C-12), 120.7 (C-10), 121.6 (C-9), 125.0 (C-14), 127.8 (C-11), 133.2 (C-15), 138.2 (C-8), 143.3 (C-13), 166.9 (C-2), 169.1 (CO_2CH_3); Found (ESI): 337.1834 $[MH]^+$, (required $C_{21}H_{25}N_2O_2$ 337.1911). Spectroscopic data in accordance with the literature.[35]

10-Iodotabersonine (68)

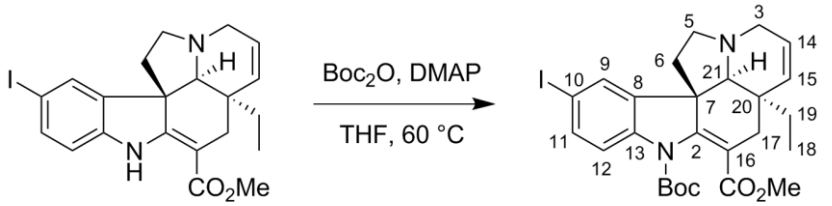


12 **68**

To a solution of tabersonine (**12**) (2.93 g, 8.71 mmol, 1.0 equiv.) in TFA (120 mL) was added NIS (1.96 g, 8.71 mmol, 1.0 equiv.) in 4 portions over 30 minutes and the reaction mixture stirred overnight under an argon atmosphere. The solution was then cooled to 0 °C and poured into an aqueous solution of NaOH (2M, 1 L) at 0 °C before extracting with DCM (4 x 50 mL). The combined organic fraction was dried over anhydrous $MgSO_4$, filtered and the solvent evaporated

under reduced pressure. The resulting solid was then dissolved in hot pentane (250 mL) and allowed to cool to room temperature before filtering. The solvent was then removed under reduced pressure to give the desired product **68** (3.79 g, 94%) as a white solid. m.p.: 85 °C; $[\alpha]_D$ -119 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1}: 3359, 3022, 2959, 1671, 1131; δ_H(400 MHz, $CDCl_3$) 0.64 (3H, t, *J* 7.4, 18-H_3), 0.85 (1H, dq, *J* 14.2, 7.3, 19-*H*H), 0.97 (1H, dq, *J* 14.9, 7.5, 19-H*H*), 1.81 (1H, ddd, *J* 11.7, 4.9, 1.4, 6-*H*H), 2.05 (1H, td, *J* 11.2, 6.5, 6-H*H*), 2.40 (1H, d, *J* 15.1, 17-*H*H), 2.54 (1H, dd, *J* 15.1, 1.8, 17-H*H*), 2.62 (1H, d, *J* 1.7, 21-H), 2.68 (1H, ddd, *J* 10.8, 8.5, 4.9, 5-*H*H), 3.04 (1H, ddd, *J* 7.9, 6.3, 0.9, 5-H*H*), 3.20 (1H, dt, *J* 15.9, 1.9, 3-*H*H), 3.44 (1H, ddd, *J* 15.9, 4.7, 1.4, 3-H*H*), 3.76 (3H, s, CO_2CH_3), 5.69 (1H, ddd, *J* 9.9, 2.2, 1.7, 15-H), 5.78 (1H, ddd, *J* 10.0, 4.7, 1.5, 14-H), 6.60 (1H, d, *J* 8.1, 12-H), 7.43 (1H, dd, *J* 8.2, 1.7, 11-H), 7.49 (1H, d, *J* 1.7, 9-H), 8.98 (1H, s, NH); δ_C(101 MHz, $CDCl_3$) 7.5 (C-18), 27.0 (C-19), 28.5 (C-17), 41.0 (C-20), 44.5 (C-6), 50.4 (C-5), 50.9 (C-3), 51.1 (CO_2CH_3), 55.1 (C-7), 70.0 (C-21), 82.4 (C-10), 92.8 (C-16), 111.3 (C-12), 124.8 (C-14), 130.3 (C-8), 132.7 (C-15), 136.4 (C-11), 140.6 (C-9), 143.0 (C-13), 165.5 (C-2), 168.9 (CO_2CH_3); Found (ESI): 463.0823 $[MH]^+$, (required $C_{21}H_{23}IN_2O_2$: 462.0804). Spectroscopic data in accordance with the literature.[105]

***tert*-Butyl 10-iodotabersonine-1-carboxylate (69)**

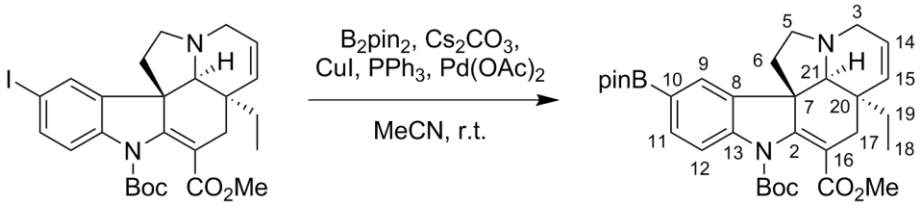


68 **69**

To a stirred solution of 10-iodotabersonine (**68**) (11.9g, 25.7 mmol, 1.0 equiv.) in anhydrous THF (250 mL) was added Boc_2O (8.2 mL, 77.1 mmol, 3.0 equiv.) and DMAP (0.313 g, 2.57 mmol, 0.1 equiv.) and the resulting mixture heated at 60 °C for 18 hours. The solvent was then evaporated under reduced pressure and the residue dissolved in DCM (200 mL) and washed with H_2O (3 x 50 mL). The organic fraction was dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude material obtained was then purified by column chromatography (SiO_2, 10% EtOAc in petroleum ether 40-60 °C) to give the desired product **69** (13.1 g, 90 %) as a white solid. m.p.: 83 °C; $[\alpha]_D$ -44.32 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1}: 2933, 2777, 1714, 1147; δ_H(400 MHz, $CDCl_3$) 0.66 (3H, t, *J* 7.5, 18-H_3), 0.98 (1H, dq, *J* 14.2, 7.3, 19-*H*H), 1.12 (1H, dq *J* 14.5, 7.5, 19-H*H*), 1.56 (9H, s, $C(CH_3)_3$), 1.81 (1H, dd, *J* 11.9, 5.0, 6-*H*H), 2.17 (1H, td, *J* 11.8, 6.7, 6-H*H*), 2.32 (1H, dd, *J* 15.1, 1.9, 17-*H*H), 2.52 (1H, td, *J* 10.4, 8.8, 5.2, 5-*H*H), 2.59 (1H, s, 21-H), 2.71 (1H, d, J 15.2, 17-H*H*), 3.06 (1H, t, *J* 7.7, 5-H*H*), 3.12 (1H, d, *J* 15.9, 3-*H*H), 3.51 (1H, dd, *J* 15.8, 4.8, 3-H*H*), 3.78 (3H, s, CO_2CH_3), 5.70 (1H, dt, *J* 9.9, 2.1, 15-H), 5.85 (1H, ddd, *J* 9.8, 3.4, 1.4, 14-H), 7.46 (1H, d, *J* 8.4, 12-H), 7.49 (1H, d, *J* 1.8, 9-H), 7.56 (1H, dd, *J* 8.4, 1.8, 11-H); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 26.4 (C-19), 28.2 ($C(CH_3)_3$), 31.1 (C-17), 41.3 (C-20), 43.0 (C-

6), 50.9 (C-5), 51.3 (C-3), 51.7 (CO_2CH_3), 52.5 (C-7), 68.1 (C-21), 82.7 ($C(CH_3)_3$), 86.6 (C-10), 112.0 (C-12), 118.0 (C-16), 125.09 (C-14), 129.8 (C-8), 132.41 (C-15), 136.4 (C-11), 140.5 (C-9), 141.0 (C-13), 150.8 ($NCO_2C(CH_3)_3$), 165.2 (C-2), 167.8 (CO_2CH_3); Found (ESI): 563.1322 $[MH]^+$, (required $C_{26}H_{32}IN_2O_4$ 563.1401).

tert-Butyl tabersonine-1-carboxylate-10-boronic acid pinacol ester (77)

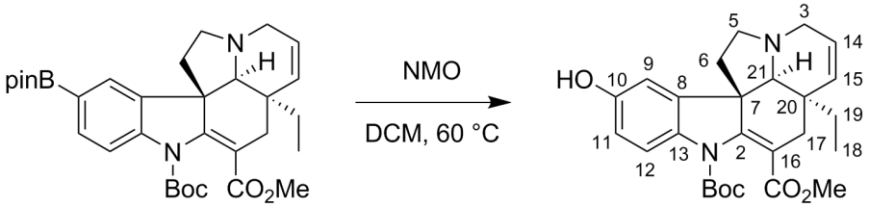


68 **77**

To a solution of *tert*-butyl 10-iodotabersonine-1-carboxylate (**69**) (0.5 g, 0.88 mmol, 1.0 equiv.) in anhydrous MeCN (25 mL) was added $CsCO_3$ (0.43 g, 1.32 mmol, 1.5 equiv.), B_2Pin_2 (0.33 g, 1.32 mmol, 1.5 equiv.), CuI (34 mg, 0.18 mmol, 0.2 equiv.), PPh_3 (21 mg, 0.09 mmol, 0.1 equiv.) and $Pd(OAc)_2$ (20 mg, 0.09 mmol, 0.1 equiv.) and the resulting mixture stirred at room temperatue overnight. The reaction was then filtered through Celite® and the solvent evaporated under reduced pressure to yield material, which was purified by column chromatography (SiO_2, 20% EtOAc in petroleum ether 40-60°C) to give the desired product **77** (0.37 g, 75%) as a green solid. m.p.: 94 °C; $[\alpha]_D$ -16.62 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1} 2974, 2932, 1720, 1140; δ_H(400 MHz, $CDCl_3$) 0.60 (3H, t, *J* 7.5, 18-H_3), 0.93 (1H, dq, *J* 14.3, 7.3, 19-*H*H), 1.08 (1H, dq, *J* 14.8, 7.4, 19-H*H*), 1.34 (12H, s, 2 x $C(CH_3)_2$), 1.53 (9H, s, $C(CH_3)_3$), 1.75 (1H, dd, *J* 11.7, 4.9, 6-*H*H), 2.13 (1H, td, *J* 11.7, 6.7, 6-H*H*), 2.29 (1H, dd, *J* 15.1, 1.9, 17-*H*H), 2.58 (1H,

ddd, *J* 11.8, 8.5, 5.1, 5-*H*H), 2.65-2.76 (2H, m, 17-H*H* and 21-H), 3.00 (1H, dd, *J* 8.5, 6.7, 5-H*H*), 3.16 (1H, dt, *J* 15.8, 1.9, 3-*H*H), 3.48 (1H, ddd, *J* 15.9, 4.8, 1.3, 3-H*H*), 3.74 (3H, s, CO_2CH_3), 5.65 (1H, dt, *J* 9.8, 1.9, 15-H), 5.78 (1H, ddd, *J* 9.9, 4.8, 1.5, 14-H), 7.55 (1H, s, 9-H), 7.65 (1H, d, *J* 7.9, 12-H), 7.72 (1H, d, *J* 8.1, 11-H); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 24.9 (C(*C*H_3)$_3$), 26.3 (C-19), 28.2 (C(*C*H_3)$_2$), 30.9 (C-17), 41.5 (C-20), 43.0 (C-6), 50.8 (C-5), 51.4 (C-3), 51.6 (CO_2CH_3), 52.5 (C-7), 67.8 (C-21), 82.4 (*C*$(CH_3)_3$), 83.7 (2 x *C*$(CH_3)_2$), 111.5 (C-12), 115.2 (C-10), 125.1 (C-14), 126.7 (C-11), 132.6 (C-15), 135.1 (C-9), 137.8 (C-8), 143.3 (C-13), 150.2 (N*C*O_2C$(CH_3)_3$), 150.9 (C-2), 168.0 (*C*O_2CH_3); δ_B(96 MHz, $CDCl_3$) 29.1; Found (ESI): 563.3222 $[MH]^+$, (required $C_{32}H_{44}BN_2O_6$ 563.3287).

tert-Butyl 10-hydroxytabersonine-1-carboxylate (61)

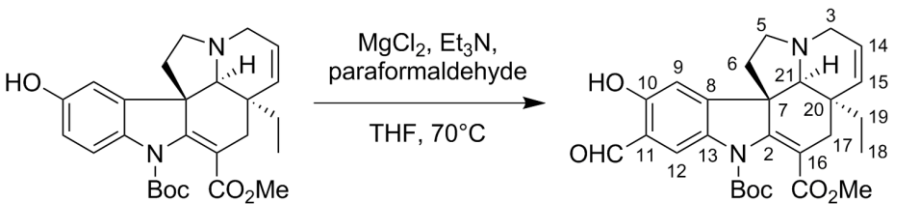


77 **61**

To a solution of *tert*-butyl tabersonine-1-carboxylate-10-boronic acid pinacol ester (**77**) (50 mg, 0.09 mmol, 1.0 equiv.) in anhydrous DCM (0.5 mL) was added NMO (18 mg, 0.15 mmol, 1.7 equiv.) and the mixture heated at 60 °C and stirred for 19 hours. After removal of the solvent under reduced pressure, the resulting material was purified by column chromatography (SiO_2, 20-30% EtOAc in petroleum ether 40-60 °C) to give the desired product **61** (40 mg, 97%) as a white solid. m.p.: 128 °C; $[\alpha]_D$ -30.33 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1}: 3347, 3025,

2767, 1720, 1683, 1112; δ_H(400 MHz, $CDCl_3$) 0.65 (3H, t, *J* 7.4, 18-H_3), 1.02 (1H, dq, *J* 14.5, 7.3, 19-*H*H), 1.12 (1H, dq, *J* 14.7, 7.5, 19-H*H*), 1.56 (9H, s, C(CH_3)$_3$), 1.81 (1H, dd, *J* 11.8, 5.0, 6-*H*H), 2.17 (1H, td, *J* 10.4, 8.4, 5.0, 6-H*H*), 2.30 (1H, dd, *J* 15.1, 1.8, 17-*H*H), 2.50 (1H, td, *J* 10.4, 8.4, 5.0, 5-*H*H), 2.59 (1H, s, 21-H), 2.72 (1H, d, *J* 15.1, 17-H*H*), 2.99-3.14 (2H, m, 3-*H*H and 5-H*H*), 3.50 (1H, ddd, *J* 15.6, 5.2, 1.5, 3-H*H*), 3.78 (3H, s, CO_2CH_3), 5.09 (1H, br s, OH), 5.65 (1H, dt, *J* 9.9, 2.0, 15-H), 5.80 (ddd, *J* 9.9, 4.8, 1.5, 14-H), 6.68 (1H, dd, *J* 8.6, 2.6, 11-H), 6.76 (1H, d, *J* 2.5, 9-H), 7.51 (1H, d, *J* 8.6, 12-H); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 26.4 (C-19), 28.2 (C(*C*H_3)$_3$), 31.2 (C-17), 41.4 (C-20), 42.9 (C-6), 51.0 (C-5), 51.4 (C-3), 51.6 (CO_2*C*H_3), 52.6 (C-7), 68.1 (C-21), 82.1 (*C*(CH_3)$_3$), 108.9 (C-9), 111.7 (C-16), 113.5 (C-11), 116.9 (C-12), 125.1 (C-14), 132.7 (C-15), 134.1 (C-13), 140.0 (C-8), 150.0 (N*C*O_2C(CH_3)$_3$), 151.2 (C-2), 152.1 (C-10), 168.3 (*C*O_2CH_3); Found (ESI): 453.2319 $[MH]^+$, (required $C_{26}H_{34}N_2O_5$ 453.2384).

tert-Butyl 10-hydroxy-11-tabersoninecarbaldehyde-1-carboxylate (109)

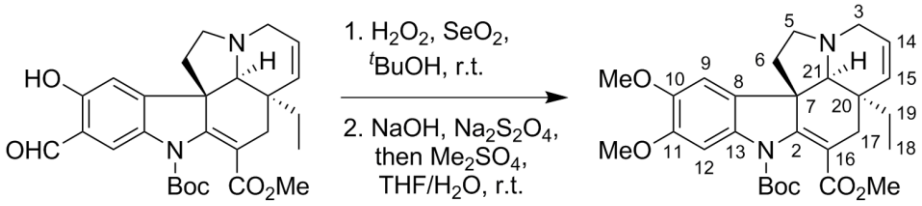


61 **109**

To a stirred solution of *tert*-butyl 10-hydroxytabersonine-1-carboxylate (**61**) (100 mg, 0.22 mmol, 1.0 equiv.) in anhydrous THF (1 mL) was added $MgCl_2$ (23 mg, 0.25 mmol, 1.1 equiv.), paraformaldehyde (30 mg, 1 mmol, 4.5 equiv.) and Et_3N (114 µL, 0.85 mmol, 3.9 equiv.) and the resulting mixture heated at 70 °C for 17 hours. After cooling to room temperature the reaction was poured into

aqueous HCl (1M, 20 mL) and then neutralised with a saturated solution of $NaHCO_3$ before extracting with DCM (3 x 10 mL). The combined organic fraction was then dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification of the residue by column chromatography (SiO_2, 0-1% MeOH in DCM) gave the desired product **109** (102 mg, 98%) as a yellow solid. m.p.: 190 °C; $[\alpha]_D$ -48.48 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1}: 2963, 2930, 1711, 1651, 1298, 1144; δ_H(400 MHz, $CDCl_3$) 0.63 (3H, t, *J* 7.4, 18-H_3), 0.80-1.12 (2H, m, 19-H_2), 1.54 (9H, s, $C(CH_3)_3$), 1.80 (1H, dd, *J* 12.0, 5.0, 6-*H*H), 2.10-2.22 (1H, m, 6-H*H*), 2.30 (1H, dd, *J* 15.2, 1.8, 17-*H*H), 2.43-2.54 (1H, m, 5-*H*H), 2.63 (1H, s, 21-H), 2.71 (1H, d, *J* 15.2, 17-H*H*), 2.96-3.21 (2H, m, 3-*H*H and 5-H*H*), 3.50 (1H, d, *J* 14.1, 3-H*H*), 3.77 (3H, s, CO_2CH_3), 5.64 (1H, d, *J* 9.6, 15-H), 5.81 (1H, dd, *J* 10.5, 4.5, 14-H), 6.87 (1H, s, 9-H), 7.85 (1H, s, 12-H), 9.84 (1H, s, CHO), 11.27 (1H, s, OH); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 26.4 (C-19), 28.1 ($C(CH_3)_3$), 31.2 (C-17), 41.3 (C-20), 43.0 (C-6), 51.0 (C-5), 51.4 (C-3), 51.7 (CO_2CH_3), 53.1 (C-7), 67.9 (C-21), 82.7 ($C(CH_3)_3$), 110.6 (C-9), 112.5 (C-16), 118.7 (C-11), 119.3 (C-12), 125.2 (C-14), 132.3 (C-15), 133.5 (C-8), 148.6 (C-13), 148.6 (C-2), 151.0 ($NCO_2C(CH_3)_3$), 159.5 (C-10) 167.7 (CO_2CH_3), 196.0 (CHO); Found (ESI): 481.2263 $[MH]^+$, (required $C_{27}H_{33}N_2O_6$ 481.2333).

tert-Butyl tabersonine-1-carboxylate-10,11-diol dimethyl ether (60)

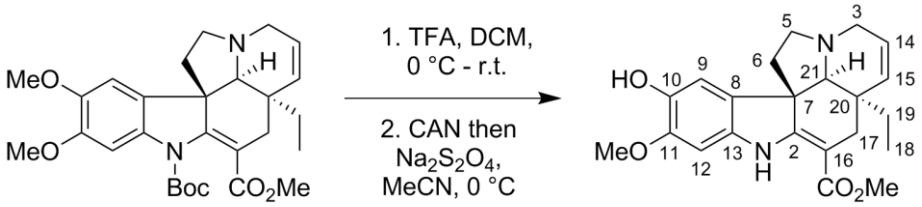


109 **60**

To a solution of *tert*-butyl 10-hydroxy-11-tabersoninecarbaldehyde-1-carboxylate (**109**) (50 mg, 0.1 mmol, 1.0 equiv.) in ^{t}BuOH (1 mL) was added SeO_2 (0.2 mg, 0.002 mmol, 0.02 equiv.) and aqueous 30% H_2O_2 (18 µL, 0.18 mmol, 1.8 equiv.) and the mixture stirred at room temperature. After 30 minutes the reaction was poured into saturated aqueous NaCl (5 mL) and extracted with DCM (3 x 5 mL). The combined organic fraction was then dried over anyhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The resulting material was then dissolved in a mixture of THF and H_2O (1:1, 1 mL) and an aqueous solution of NaOH (2.6 M, 0.5 mL) was added at room temperature. The reaction was stirred for 5 minutes before adding $Na_2S_2O_4$ (105 mg, 0.6 mmol, 6.0 equiv.). After a further 5 minutes, Me_2SO_4 (9.5 µL, 0.1 mmol, 1.0 equiv.) was added and the mixture stirred at room temperature for an hour. The aqueous phase was then extracted with DCM (3 x 5 mL) and the resulting combined organic fraction dried over anhydrous $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, purification by column chromatography (SiO_2, 10-20% EtOAc in petroleum ether 40-60 °C) gave the desired product **60** (26 mg, 53%) as a white solid. m.p.: 85 °C; $[\alpha]_D$ -45.57 (*c* 0.10, $CHCl_3$); ν_{max}/cm^{-1}: 2963, 2874, 1721, 1651, 1157, 1063; δ_H(400 MHz, $CDCl_3$) 0.63 (3H, t, *J* 7.5, 18-H_3), 1.01 (1H, dq, *J* 14.2, 7.3, 19-*H*H),

1.11 (1H, dq, *J* 14.2, 7.3, 19-H*H*), 1.55 (9H, s, C(CH_3)$_3$), 1.77 (1H, dd, *J* 11.8, 5.0, 6-*H*H), 2.10-2.20 (1H, m, 6-H*H*), 2.29 (1H, dd, *J* 15.1, 1.9, 17-*H*H), 2.50 (1H, ddd, *J* 11.5, 8.6, 5.1, 5-*H*H), 2.61 (1H, d, *J* 1.7, 21-H), 2.72 (1H, d, *J* 15.1, 17-H*H*), 3.02 (1H, dd, *J* 8.5, 6.7, 5-H*H*), 3.08 (1H, dt, *J* 15.8, 2.0, 3-*H*H), 3.51 (1H, ddd, *J* 15.8, 4.8, 1.6, 3-H*H*), 3.77 (3H, s, CO_2CH_3), 3.92 (3H, s, 10-OCH_3), 3.94 (3H, s, 11-OCH_3), 5.64 (1H, dt, *J* 9.9, 2.1, 15-H), 5.80 (1H, ddd, *J* 9.9, 4.8, 1.5, 14-H), 6.75 (1H, s, 9-H), 7.44 (1H, s, 12-H); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 26.4 (C-19), 28.2 (C(*C*H_3)$_3$), 31.2 (C-17), 41.5 (C-20), 42.8 (C-6), 51.1 (C-5), 51.5 (C-3), 51.5 (CO_2*C*H_3), 52.6 (C-7), 56.1 (10-OCH_3), 56.8 (11-OCH_3), 68.0 (C-21), 82.0 (*C*(CH_3)$_3$), 101.8 (C-9), 105.6 (C-12), 111.8 (C-16), 125.1 (C-14), 129.5 (C-8), 132.8 (C-15), 134.2 (C-13), 145.4 (C-11), 148.4 (C-2), 150.2 (C-10), 151.2 (N*C*O_2C(CH_3)$_3$), 168.1 (CO_2CH_3); Found (ESI): 497.2656 $[MH]^+$, (required $C_{28}H_{38}N_2O_6$ 496.2646).

Jerantinine A (1)



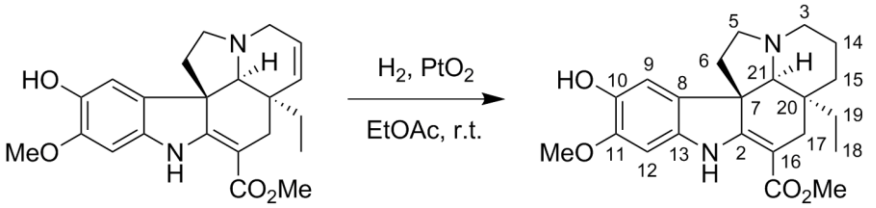
60 **1**

tert-Butyl tabersonine-1-carboxylate-10,11-diol dimethyl ether (**60**) (50 mg, 0.10 mmol, 1.0 equiv.) was dissolved in a mixture of TFA and DCM (1:1, 0.5 mL) at 0 °C and stirred. After 30 minutes the solution was warmed to room temperature and stirred for 3 hours. The reaction mixture was then poured into an aqueous solution of NaOH (2M, 5 mL) at 0 °C before extracting with

DCM (3 x 5 mL). The combined organic fraction was dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The resulting material was then dissolved in MeCN (3 mL) and cooled to 0 °C. A solution of CAN (0.12 g, 0.22 mmol, 2.2 equiv.) in H_2O (1 mL) at 0 °C was added and the resulting mixture stirred for 5 minutes. The reaction was then poured into an aqueous solution of $Na_2S_2O_4$ (1M, 30 mL) at 0 °C and stirred for 10 minutes, before diluting with DCM (10 mL). The aqueous phase was extracted with DCM (3 x 5 mL) and the combined organic fraction dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification of the residue by column chromatography (SiO_2, 10-20% EtOAc in petroleum ether 40-60 °C) and recombination of the appropriate fractions using Et_2O gave, after evaporation of the solvent, the desired product **1** (26 mg, 72%) as a white solid. m.p.: 63.5 °C; $[\alpha]_D$ -231 (*c* 0.10, $CHCl_3$); IR ν_{max}/cm^{-1}: 3548, 3379, 3035, 2965, 1669, 1605, 1110; δ_H(400 MHz, $CDCl_3$) 0.65 (3H, t, *J* 7.4, 18-H_3), 0.85 (1H, dq, *J* 14.2, 7.3, 19-*H*H), 1.00 (1H, dq, *J* 14.3, 7.4, 19-H*H*), 1.75 (1H, ddd, *J* 11.5, 4.7, 1.1, 6-*H*H), 2.01-2.11 (1H, m, 6-H*H*), 2.42 (1H, d, *J* 15.1, 17-*H*H), 2.53 (1H, dd, *J* 15.1, 1.5, 17-H*H*), 2.60 (1H, d, *J* 1.8, 21-H), 2.68 (1H, ddd, *J* 10.5, 8.2, 4.5, 5-*H*H), 3.03-3.10 (1H, m, 5-H*H*), 3.18 (1H, br d, J 15.9, 3-*H*H), 3.45 (1H, ddd, *J* 16, 4.5, 1.2, 3-H*H*), 3.77 (3H, s, CO_2CH_3), 3.88 (3H, s, OCH_3), 5.34 (1H, br s, OH), 5.69 (1H, ddd, *J* 9.9, 2.3, 1.5, 15-H), 5.79 (1H, ddd, *J* 9.9, 4.6, 1.4, 14-H), 6.46 (1H, s, 12-H), 6.89 (1H, s, 9-H), 8.87 (1H, br s, NH); δ_C(100 MHz, $CDCl_3$) 7.5 (C-18), 26.9 (C-19), 28.4 (C-17), 41.5 (C-20), 44.4 (C-6), 50.6 (C-3), 50.8 (C-5), 50.9 (CO_2CH_3), 55.3 (C-7), 56.3 (OCH_3), 70.2 (C-21), 91.8 (C-16), 94.5 (C-12), 108.8 (C-9), 124.9 (C-14), 130.1 (C-8), 133.1 (C-15), 136.1 (C-13), 139.9 (C-10), 145.9

(C-11), 167.8 (C-2), 169.1 (CO_2CH_3); Found (ESI): 383.1977 $[MH]^+$, (required $C_{22}H_{27}N_2O_4$ 383.1965). Residual solvent signals from hexane, EtOAc and Et_2O are evident in the ^1H and ^{13}C NMR spectrum, spectroscopic data is otherwise in accordance with the literature.[1]

Jerantinine E (5)

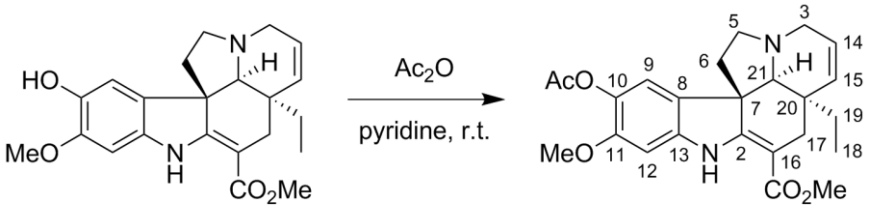


1 **5**

To a solution of jerantinine A (**1**) (6 mg, 0.016 mmol, 1.0 equiv.) in EtOAc (1 mL) was added PtO_2 (0.7 mg, 0.003 mmol, 0.2 equiv.) and the resulting mixture stirred under an atmosphere of H_2 for 4 hours. The reaction was then filtered through Celite® before removal of the solvent under reduced pressure. Purification of the residue by column chromatography (SiO_2, 10% EtOAc in petroleum ether 40-60 °C) gave the desired product **5** (4.6 mg, 77%) as a colourless oil. $[\alpha]_D$ -351 (*c* 0.10, $CHCl_3$); IR ν_{max}/cm^{-1}: 3535, 3365, 1668, 1602; δ_H(400 MHz, $CDCl_3$) 0.57 (3H, t, *J* 7.0, 18-H_3), 0.63 (1H, dq, *J* 14.0, 7.0, 19-*H*H), 0.97 (1H, dq, *J* 13.1, 6.8, 19-H*H*), 1.22-1.28 (1H, m, 15-*H*H), 1.50-1.57 (1H, m, 14-*H*H), 1.65-1.70 (1H, m, 6-*H*H), 1.76-1.88 (2H, m, 14-H*H* and 15-H*H*), 2.00-2.07 (1H, m, 6-H*H*), 2.25 (1H, d, *J* 14.9, 17-*H*H), 2.32-2.42 (2H, m, 3-*H*H and 21-H), 2.47-2.58 (1H, m, 5-*H*H), 2.69 (1H, d, *J* 15.0, 17-H*H*), 2.89 (1H, t, *J* 7.4, 5-H*H*), 3.10 (1H, d, *J* 9.6, 3-H*H*), 3.75 (3H, s, CO_2CH_3), 3.86 (3H, s, OCH_3), 5.26 (1H, br

s, OH), 6.43 (1H, s, 12-H), 6.84 (1H, s, 9-H), 8.76 (1H, br s, NH); δ_C(100 MHz, $CDCl_3$) 7.3 (C-18), 22.4 (C-14), 25.6 (C-17), 29.9 (C-19), 33.0 (C-15), 38.4 (C-20), 45.2 (C-6), 50.9 (C-3), 51.1 ($CO_2\mathit{C}H_3$), 51.8 (C-5), 55.7 (C-7), 56.4 (OCH_3), 72.9 (C-21), 92.4 (C-16), 94.7 (C-12), 108.9 (C-9), 130.1 (C-8), 136.3 (C-13), 140.0 (C-10), 145.8 (C-11), 169.0 (C-2), 169.42 (CO_2CH_3); Found (ESI): 385.2132 $[MH]^+$, (required $C_{22}H_{27}N_2O_4$ 385.2108). Residual solvent signals from EtOAc are evident in the ^1H and ^{13}C NMR spectrum, spectroscopic data is otherwise in accordance with the literature.[1]

Jerantinine A Acetate (119)



1 **119**

Jerantinine A (**1**) (10 mg, 0.026 mmol) was dissolved in an anhydrous mixture of Ac_2O and pyridine (1:1, 0.6 mL) and stirred at room temperature for 20 minutes. The reaction was then diluted with H_2O (3 mL) and basified to pH 9 using an aqueous 10% Na_2CO_3 solution. The mixture was then extracted with DCM (3 x 5 mL) and the combined organic fraction dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification of the residue by column chromatography (SiO_2, 10% EtOAc in petroleum ether 40-60 °C) and recombination of the appropriate fractions using Et_2O gave, after evaporation of the solvent, the desired product **119** (10.9 mg, 99%) as a

colourless oil. $[\alpha]_D$ -222 (*c* 0.10, $CHCl_3$); δ_H(400 MHz, $CDCl_3$) 0.64 (3H, t, *J* 7.4, 18-H_3), 0.87 (1H, dq, *J* 14.3, 7.2, 19-*H*H), 1.00 (1H, dq, *J* 14.9, 7.6, 19-H*H*), 1.84 (1H, ddd, *J* 11.6, 4.9, 1.3, 6-*H*H), 2.05 (1H, td, *J* 11.1, 6.6, 6-H*H*), 2.29 (3H, s, $COCH_3$), 2.39 (1H, d, *J* 15.1, 17-*H*H), 2.54 (1H, dd, *J* 15.2, 1.8, 17-H*H*), 2.61 (1H, d, *J* 1.3, 21-H), 2.66 (1H, ddd, *J* 10.5, 8.5, 4.9), 3.02 (1H, t, *J* 7.5, 5-H*H*), 3.16 (1H, d, *J* 15.9, 3-*H*H), 3.39-3.46 (1H, m, 3-H*H*), 3.76 (3H, s, CO_2CH_3), 3.81 (3H, s, OCH_3), 5.69 (1H, d, *J* 10.0, 15-H), 5.77 (1H, ddd, *J* 10.0, 4.8, 1.4, 14-H), 6.50 (1H, s, 12-H), 6.93 (1H, s, 9-H), 8.97 (1H, br s, NH); δ_C(100 MHz, $CDCl_3$) 7.6 (C-18), 20.8 (CO*C*H_3), 27.3 (C-19), 28.9 (C-17), 41.2 (C-20), 44.5 (C-6), 50.5 (C-3), 51.2 (C-5), 51.2 (CO_2*C*H_3), 55.2 (C-7), 56.4 (OCH_3), 70.29 (C-21), 92.9 (C-16), 95.2 (C-12), 116.5 (C-9), 124.9 (C-14), 126.6 (C-8), 133.1 (C-15), 133.6 (C-13), 141.8 (C-11), 151.0 (C-10), 167.1 (C-2), 169.1 (*C*O_2CH_3), 169.6 (*C*OCH_3); Found (ESI): 425.2075 $[MH]^+$, (required $C_{24}H_{29}N_2O_5$ 425.2071). Residual solvent signals from Et_2O are evident in the ^1H and ^{13}C NMR spectrum, spectroscopic data is otherwise in accordance with the literature.[1]

Part 2 – Horner-Wadsworth-Emmons Approach to Piperlongumine and Structural Analogues

4 Introduction

4.1 Piperlongumine (123)

The alkaloid piperlongumine (**123**) belongs to the *Piperaceae* family of natural products and is found in the fruit of the long pepper (*Piper longum* L.), which has long been used in traditional Ayurvedic medicine (Figure 4.1).[106] The structure of **123** was first correctly assigned in 1968, suggesting contrary to previous reports[107] a 5,6-dihydropyridin-2(1*H*)-one, which is connected to a 3,4,5-trimethoxyphenyl group *via* an *E*-acryloyl group.[108] Further corroboration of the structure of **123** through total synthesis and X-ray crystal structure determination was later published by Simonsen *et al*. in 1984.[109]

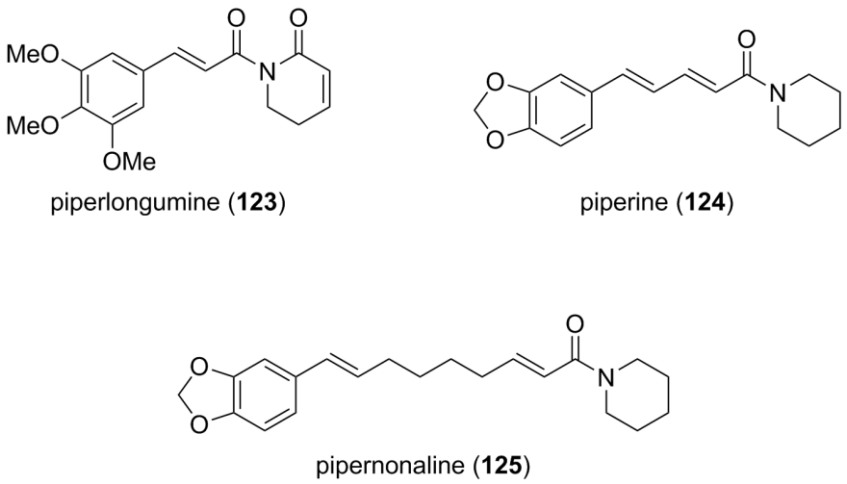


Figure 4.1: Representative members of the *Piperaceae* family of natural products

Recently interest in piperlongumine (**123**) has once again burgeoned after high impact research published by Schreiber *et al*. revealed selective cytotoxicity towards neoplastic cells.[110]

The overwhelming majority of clinical anti-cancer agents target DNA synthesis and cell division. However, while these treatments are indeed effective they often lack selectivity for cancerous cells, leading to severe side effects.[111] One of the greatest challenges in anti-cancer chemotherapy is the selective killing of cancer cells on the basis of cancer-specific features, rather than generic properties that are shared with normal cells.

Although piperlongumine (**123**) had been previously shown to exhibit cytotoxic activity,[112] the study by Schreiber *et al*. revealed that **123** had little inhibitory effect on either rapidly or slowly dividing normal cells, demonstrating impressive selectivity.[110] Subsequent research has further demonstrated piperlongumine (**123**) as a promising anti-cancer drug lead.[113]

4.2 Biological Activity

Piperlongumine (**123**) has been shown to exhibit anti-tumour, anti-metastatic and antiangiogenic activities[114] and has also been proposed to kill cancer cells through generation of reactive oxygen species (ROS).[110] ROS play a crucial role in signal transduction and clearance of pathogens during innate immune responses. Increase of ROS levels may cause significant damage to cell structures and tumour progression.[115] Piperlongumine (**123**) causes an increase in intracellular ROS with high cancer cell selectivity in an antioxidant pathway-independent and oncogene-independent manner, initiating cancer cell death with an average GI_{50} value <7 μM.[110] Screening the binding effect with various proteins has also established glutathione S-transferase pi 1

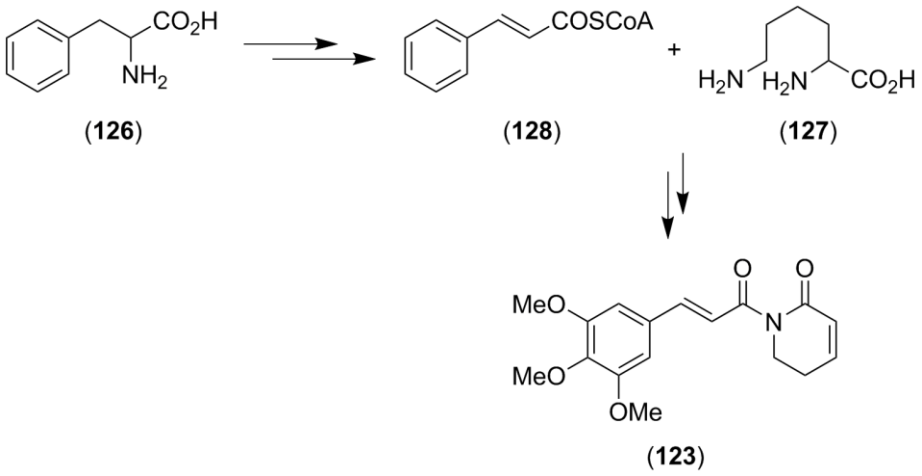
(GSTP1) as the probable binding target upon administration to cell lines.[110] Piperlongumine (**123**) is proposed to interact directly with GSTP1 causing protein glutathionylation, which leads to a decrease in reduced glutathione (GSH) and an increase in oxidised glutathione (GSSG). Lower levels of GSH can be catastrophic due to its importance in reducing intracellular ROS. The observed selectivity towards malignant cells, high efficacy and low systemic toxicity warrant further investigation of piperlongumine (**123**) as a potential therapeutic candidate.

4.3 Biosynthesis of *Piperaceae* Alkamides

Many aspects of the biogenesis of the *Piperaceae* family of natural products have not yet been fully elucidated. However, several intermediates in the piperlongumine (**123**) biosynthesis were suggested by Prabhu *et al*. based on the results of radioisotopic labelling (Scheme 4.1).[116] Feeding experiments demonstrated L-[U-^{14}C]phenylalanine and L-[U-^{14}C]lysine incorporation and subsequent degradation of the resulting labelled piperlongumine (**123**) revealed the positions of the radioisotopes. From these results it is inferred that in nature, piperlongumine (**123**) originates from the condensation of precursors derived from L-phenylalanine (**126**) and L-lysine (**127**).[117]

Therefore, it is probable that after deamination catalysed by phenylalanine ammonia lyase, acid-thiol ligation of the resultant cinnamic acid would yield cinnamoyl-CoA (**128**), an intermediate in phenylpropanoid metabolism.[118] The location of L-[U-^{14}C]lysine incorporation infers the origin of the piperidone,

which suggests biosynthesis through eventual condensation of these putative biogenic fragments.

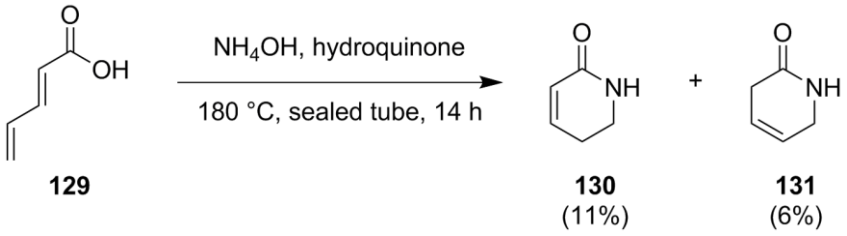


Scheme 4.1: Intermediates in the piperlongumine (**123**) biosynthesis

4.4 Piperlongumine (123): Previous Literature

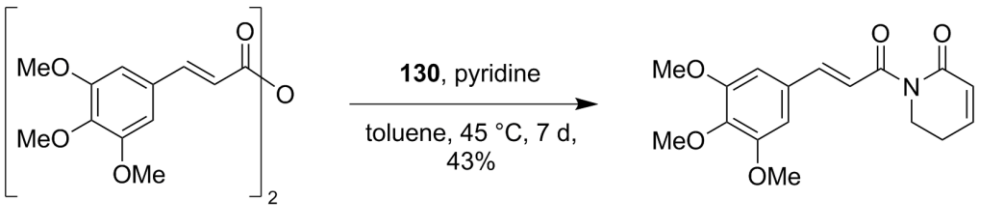
4.4.1 Simonsen's Synthesis of Piperlongumine (123)

The first conclusive total synthesis of piperlongumine (**123**) was published by Simonsen *et al*. in 1984.[109] Their synthetic route began from vinylacrylic acid (**129**), which was heated under pressure of ammonia to deliver a mixture of isomeric lactams **130** and **131** (Scheme 4.2). The desired 5,6-dihydropyridin-2(1*H*)-one **130** was isolated by fractional crystallisation in 11% yield.



Scheme 4.2: Preparation of the 5,6-dihydropyridin-2(1*H*)-one **130** accomplished by Simonsen *et al*.

The synthesis concluded with the pyridine mediated coupling of lactam **130** with (*E*)-3,4,5-trimethoxycinnamic anhydride (**132**), which was accomplished in 43% yield over 7 days giving the natural product piperlongumine (**123**) in 2 steps and overall yield of 5% (Scheme 4.3).



132 **123**

Scheme 4.3: Synthesis of piperlongumine (**123**) accomplished by Simonsen *et al*.

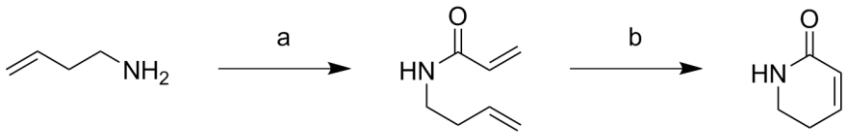
The completed synthesis was published alongside full structural characterisation, including crystallographic data, providing unequivocal corroborative evidence for the structure.

4.4.2 Schreiber's Synthesis of Piperlongumine (123)

Following their initial research highlighting the impressive selectivity of piperlongumine (**123**) for cancer cells, Schreiber *et al*. published a further study in 2012 examining the mode of biological action.[119] During the course of the investigation a library of 28 analogues was synthesised. Starting from the homoallylamine **133**, the amide **134** was prepared by reaction with acryloyl chloride (Scheme 4.4). Subsequent ring closing metathesis gave the key lactam **130** in 2 steps. However, no yields were reported for either transformation.

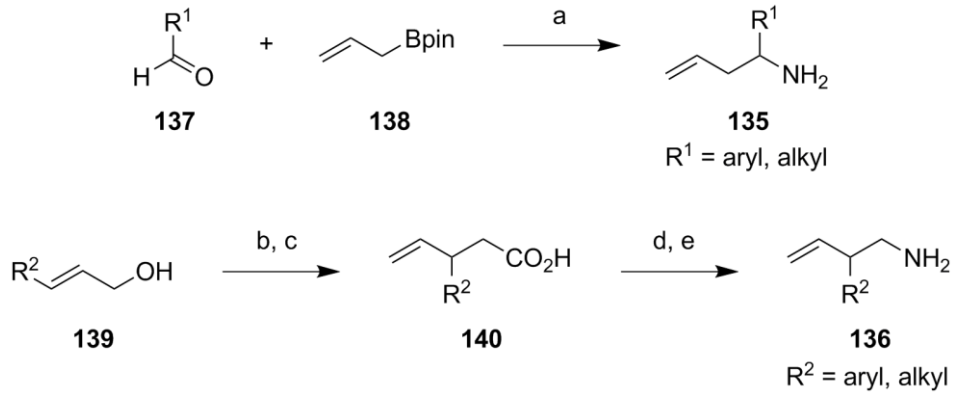
Furthermore by preparation of bespoke homoallylic amines **135** and **136**, the group were able to produce derivatives with varied substitution patterns

(Scheme 4.5). The first method utilised a Petasis reaction, whereby the R substituent on the aldehyde component **137** could be varied. The second method used a Johnson-Claisen rearrangement followed by hydrolysis and Curtius rearrangement, enabling variation of the R substituent in the allylic alcohol **139** starting material.



133 **134** **130**

Scheme 4.4: Preparation of the 5,6-dihydropyridin-2(1*H*)-one **130** accomplished by Schreiber *et al*. Reagents and conditions: (a) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C; (b) Grubbs II, CH_2Cl_2, Δ

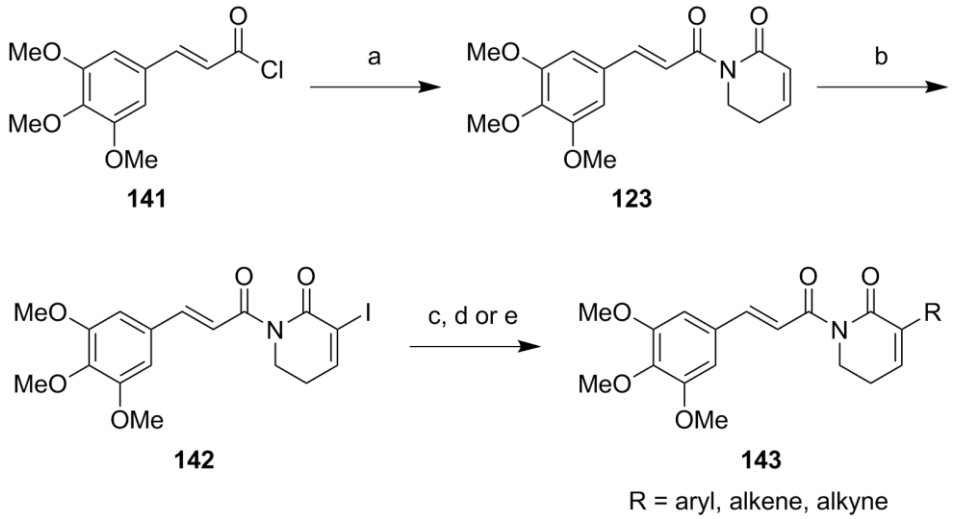


137 **138** **135**
R^1 = aryl, alkyl

139 **140** **136**
R^2 = aryl, alkyl

Scheme 4.5: Synthesis of substituted homoallylic amines **135** and **136** accomplished by Schreiber *et al*. Reagents and conditions: (a) NH_3(aq), dodecylbenzenesulfonic acid, r.t.; (b) $(MeO)_3CCH_3$, Δ; (c) LiOH, THF/H_2O (1:1), r.t.; (d) diphenylphosphoryl azide, Et_3N, C_6H_6, Δ then ^{t}BuOH, Δ; (e) TFA, CH_2Cl_2, r.t.

Finally, deprotonation of the lactam **130** and its addition into the cinnamic acid chloride **141** gave the natural product piperlongumine (**123**) (Scheme 4.6). Several more analogues were also prepared from **123** by regioselective iodination, followed by palladium catalysed cross-coupling using the Suzuki, Sonogashira or Negishi procedures.



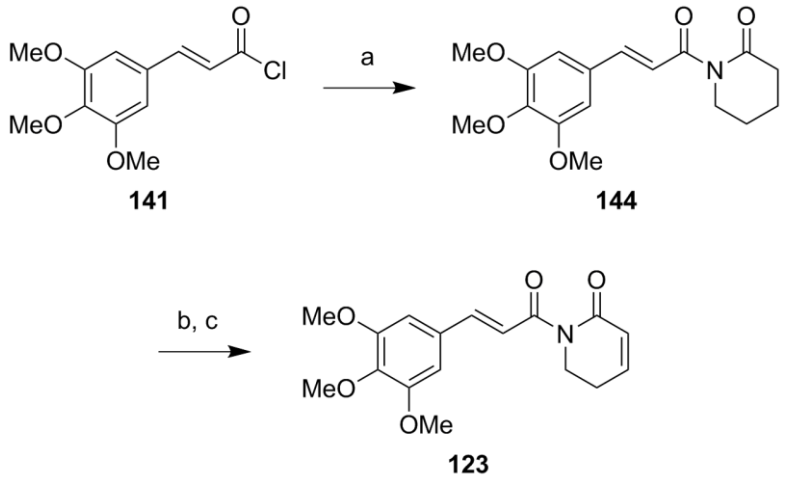
Scheme 4.6: Synthesis of piperlongumine (**123**) and analogues accomplished by Schreiber *et al*. Reagents and conditions: (a) **130**, ^{n}BuLi, THF, -78 °C; (b) I_2, pyridine, CCl_4, r.t.; (c) $RB(OH)_2$, $Pd(PCy_3)_2Cl_2$, Cs_2CO_3, 1,4-dioxane/H_2O (9:1), 100 °C; (d) RCΞCH, $Pd(Ph_3P)_2Cl_2$, CuI, DIPEA, 1,4-dioxane, r.t.; (e) RZnCl, $Pd_2(dba)_3$, RuPhos, DMA, r.t.

4.4.3 Jun's Synthesis of Piperlongumine (123)

More recently, Jun *et al*. published the synthesis of piperlongumine (**123**) and several analogues, which were made as part of an effort to find new anti-inflammatory drug candidates (Scheme 4.7).[120] Utilising the precursor (*E*)-3,4,5-trimethoxycinnamoyl chloride (**141**) as the starting material, coupling with δ-valerolactam (**37**) was followed by an α-selenation, oxidation, elimination sequence giving the natural product **123** in 3 steps and 18% overall yield.

The group were also able to produce a series of analogues, by varying the amine component of the initial coupling. Both acyclic and saturated amines were also utilised, but the yields obtained were generally inconsistent. However, the novel application of selenoxide pyrolysis for installation of the

heterocyclic double bond provides precedent for the application of pericyclic *syn*-elimination.



141 **144** **123**

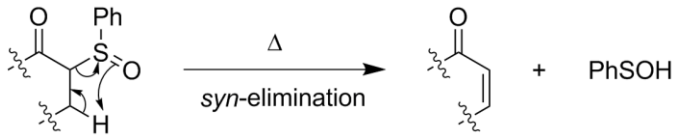
Scheme 4.7: Jun's synthesis of piperlongumine (**123**). Reagents and conditions: (a) **37**, toluene, Δ, 18 h, 95%; (b) PhSeCl, LDA, THF, -78 → -50 °C, 5.5 h, 27%; (c) H_2O_2, THF, 0 °C → r.t., 45 min, 71%

4.5 Pericyclic *Syn*-Elimination

The elimination of substituents on an alkane framework is a robust method for the generation of alkenes. When the substituents are vicinal, *syn*-elimination occurs through a cyclic transition state, which is known as the E_i mechanism. There are several functional groups that have been popularised for their participation in such pericyclic *syn*-elimination processes, giving rise to several frequently encountered named reactions.[121]

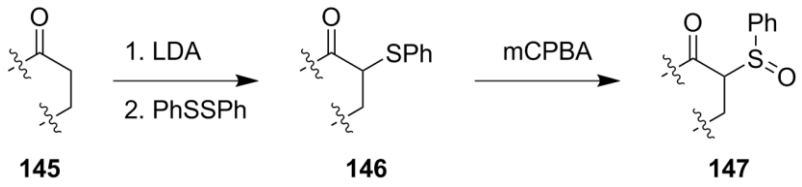
4.5.1 Sulfoxide Pyrolysis

Phenylsulfoxides are known to undergo thermal elimination through a 5-membered cyclic transition state (Scheme 4.8).[122] When the participating sulfoxide substituent is situated in the C2 position relative to a carbonyl group, the reaction product is an α,β-unsaturated derivative, alongside the unstable phenylsulfenic acid, which decomposes into volatile compounds.[123]



Scheme 4.8: Pericyclic *syn*-elimination of sulfoxides

Incorporation of sulfur into the substrates is usually achieved using electrophilic sulfenylating reagents, such as diphenyl disulfide (Scheme 4.9). Base mediated nucleophilic addition of an enolate to diphenyl disulphide generates an intermediate phenylsulfide **146**, which is finally oxidised to obtain a *syn*-elimination precursor **147**.



Scheme 4.9: Preparation of phenylsulfoxides **147**

Oxidation can be readily effected by treatment with a single equivalent of either peroxides or peracids.[124] Using excess oxidant will lead to a further oxidation, giving unwanted sulfone products. Thermal elimination of phenylsulfoxides is driven partly by the formation of a conjugated system but

also by the irreversible loss of phenylsulfenic acid, which is removed from the reaction mixture as volatile degradation products.

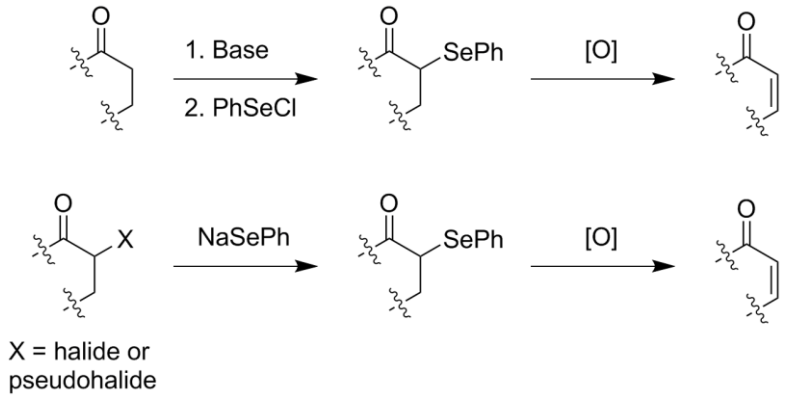
4.5.2 Selenoxide Pyrolysis

After the development of sulfoxide elimination as a method for the generation of alkenes, it was discovered by Jones *et al*. that the analogous selenium species undergo a similar process (Scheme 4.10).[125] However, selenoxide elimination occurs much more rapidly; most selenoxides decompose to the corresponding alkenes below room temperature.

In a series of contemporaneous publications, Sharpless *et al*. fully defined the selenoxide elimination and disclosed several methods for the preparation of phenylselenides using both nucleophilic and electrophilic organoselenium reagents (Scheme 4.11).[126] Among the methods presented, treatment of enolates with commercially available phenylselenyl chloride is very convenient.[127] However, nucleophilic substitution of a halide or pseudohalide is also possible, accomplished by using sodium phenylselenide, prepared by reduction of diphenyl diselenide.



Scheme 4.10: Pericyclic *syn*-elimination of phenylselenoxides



Scheme 4.11: Preparation of phenylselenides

Suitable oxidants for the reaction include peroxides and peracids, which result in rapid elimination of phenylselenenic acid and generation of the corresponding alkene. Whilst selenoxide pyrolysis is an experimentally convenient procedure further precautions are necessary when using organoselenium compounds, which are known for their extreme toxicity.

4.6 Phosphorus Mediated Olefination

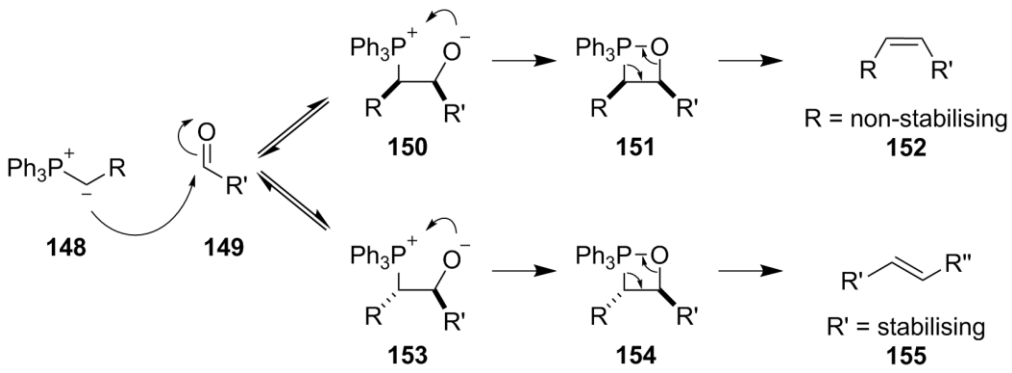
Other popular methods for the synthesis of carbon-carbon π-bonds can involve the use of phosphorus, forming alkenes from carbonyl compounds and generating a phosphine oxide by-product.

4.6.1 Wittig Reaction

The reaction of an aldehyde or ketone with a phosphonium ylide to generate alkenes and phosphine oxide waste was first described by Wittig *et al*. in 1954.[128] For the discovery Georg Wittig was awarded the Nobel Prize in Chemistry in 1979 and today the Wittig reaction remains one of the most robust and widely used methods for the preparation of unsaturated carbon

compounds.[129] Generally the stereochemistry observed in the products is dependent on the nature of the phosphonium ylide **148**; those stabilised by electron withdrawing groups tend to deliver (*E*)-alkenes. Using a non-stabilised ylide will often give (*Z*)-stereoselectivity.

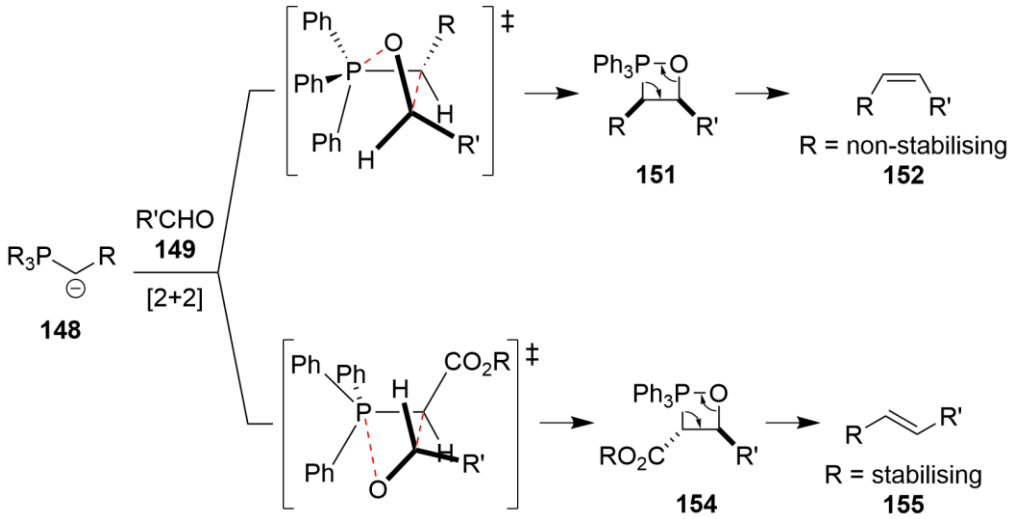
The classically accepted mechanism proceeds by nucleophilic attack of the phosphonium ylide onto the aldehyde **149** carbonyl moiety to generate a charged intermediate known as a betaine (Scheme 4.12).[130] The steric bulk of the ylide **148** governs the stereochemical preference of the initial nucleophilic attack, rapidly giving the *erythro* betaine **150**. In non-stabilised ylides the rate determining step is then decomposition by ring closure and fragmentation to produce (*Z*)-alkenes **152**. However, stabilised ylides decrease the rate of nucleophilic addition, which leads to an increase in the proportion of *threo* betaine **153** and concomitantly (*E*)-alkene product **155**.



Scheme 4.12: Classical mechanism of the Wittig reaction with stabilised and non-stabilised phosphonium ylides[130]

More recently the previously accepted mechanism has come under scrutiny and an alternative theory centred upon a concerted cycloaddition has been suggested for lithium salt free systems (Scheme 4.13).[131] The (*Z*)-selectivity

observed in non-stabilised systems as rationalised by Vedejs *et al*., is attributed to the lowest energy transition state for the process.[132] Model system calculations for alkylidenetriphenylphosphoranes corroborate a puckered transition state, where the carbonyl R' substituent occupies a *pseudo*-equatorial position. This arrangement minimises steric interactions between the carbonyl R' substituent and the *P*-phenyl groups (1–3 interactions), which are in a *pseudo*-tetrahedral arrangement around the phosphorus. It also results in the minimisation of steric interactions between the carbonyl R' substituent and the ylide R substituent (1–2 interactions). Decomposition of the cycloaddition product, oxaphosphetane **151**, gives the (*Z*)-alkene product **152**.



Scheme 4.13: Favoured transition states for the lithium salt-free Wittig reaction of stabilised and non-stabilised ylides

In systems with stabilised phosphonium ylides the (*E*)-stereoselectivity is also proposed to arise from a puckered transition state (Scheme 4.13). Calculations by Aggarwal and Harvey *et al*. based on the reaction of

(methoxycarbonylmethylidene)triphenyl-phosphorane with benzaldehyde revealed an electrostatically favourable antiparallel orientation of the carbonyl C-O and ylide C-C(O) bond dipoles.[133] The large aldehyde substituent is then positioned *pseudo*-equatorial to minimise both 1-2 and 1-3 steric interactions. After expulsion of triphenylphosphine oxide from the resulting oxaphosphetane **154**, (*E*)-alkene products **155** are observed.

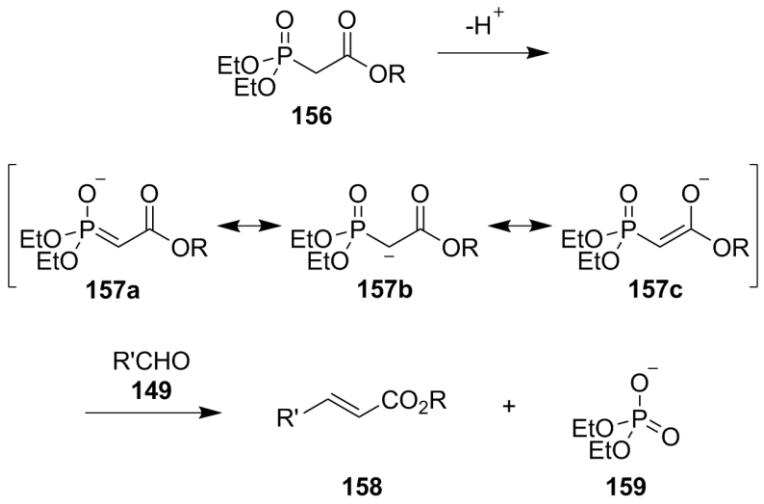
Wittig reagents are usually prepared by deprotonation of alkylphosphonium salts, which in turn can be prepared by the quaternisation of triphenylphosphine with an alkyl halide. Whilst tolerant to a variety of other functionality, slower rates of reaction, reduced selectivity and decreased yields can result from using more hindered ketone substrates or more substituted phosphoranes.

4.6.2 Horner-Wadsworth-Emmons Reaction

The Horner-Wadsworth-Emmons (HWE) olefination is another powerful synthetic method for the generation of an alkene by the reaction of an aldehyde **149** with a stabilised phosphorous ylide (phosphonate carbanion **157**) (Scheme 4.14).[134] The resulting alkene **158** is usually formed with predominantly (*E*)-stereoselectivity alongside a stoichiometric dialkylphosphate by-product.

Since its discovery in 1958 by Horner *et al*., there are a number of features of the HWE reaction that have helped to make its use widespread.[135] Reaction conditions are usually mild, even when compared to the other extremely

useful phosphorus based olefination, the Wittig reaction. It shares the same regioselectivity; the alkene is invariably formed from the ylide α-carbon to the carbonyl carbon. Additionally, phosphonate carbanions are tolerant of a number of other functional groups, giving scope for application to the synthesis of complex molecules.

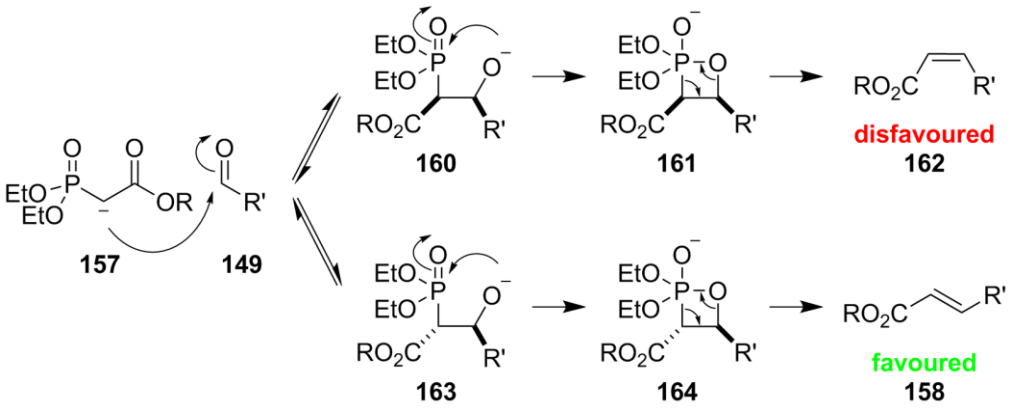


Scheme 4.14: The Horner-Wadsworth-Emmons reaction

Several mechanisms have been proposed for the HWE reaction but the most prevalent involves the nucleophilic addition of the phosphonate carbanion **157** into the electophilic carbonyl **149** (Scheme 4.15).[136] Ring closure of the intermediate **163** yields oxaphosphetane **164**, which upon collapse gives the (*E*)-alkene product **158** and dialkylphosphate **159** waste.

The origin of the stereospecificity was attributed to the stability of the phosphonate carbanion **157**. Therefore the reversibility of the initial addition allows the slower formation of the *threo* anionic intermediate **163**. Upon ring closure and rapid, irreversible collapse the more thermodynamically stable (*E*)-

alkene **158** is generated.[136] Recently this mechanism has been further examined and evidence to support an asynchronous concerted [2+2] cycloaddition was presented.[137] However, calculations of the transitions state geometries for these types of phosphorous ylides have not yet been published.



Scheme 4.15: Mechanism of the Horner-Wadsworth-Emmons reaction

There have been a wide variety of modern developments to the HWE reaction, offering benefits such as enhanced substrate scope.[138] The modification published by Still *et al*. enabling the stereoselective synthesis of (*Z*)-alkenes has found particularly wide application.[139] Substituting more electron-withdrawing 2,2,2-trifluoroethoxy groups on the phosphonate component reverses the stereoselectivity normally observed for HWE reactions, producing predominantly (*Z*)-alkenes. The use of a non-coordinating counterion was also found to be critical for high levels of (*Z*)-selectivity; typically accomplished by using a base with a potassium counterion in the presence of 18-crown-6.

4.7 Aims

The principal aim of this body of research was to investigate the synthesis of piperlongumine (**123**) and its analogues. Throughout the course of research it was envisaged that further insight into the novel selective cytotoxicity could encourage more rapid development through collaboration.

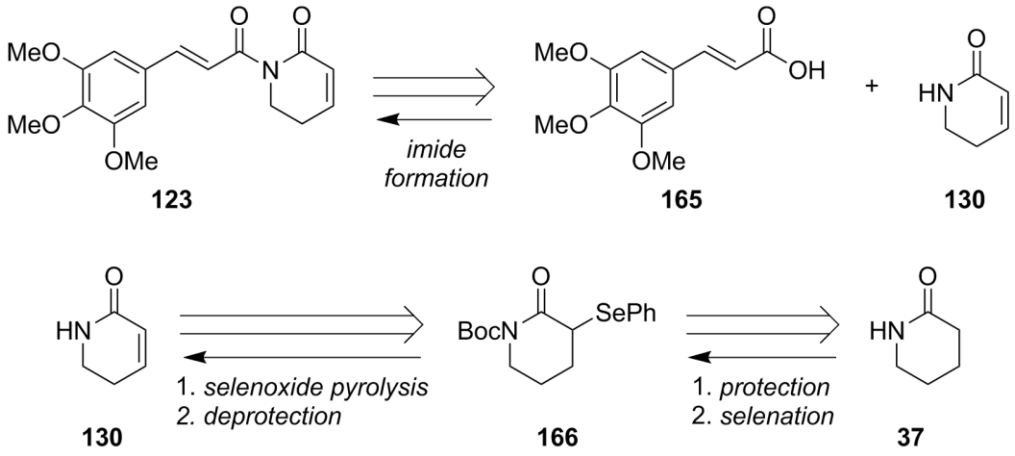
Initially the synthesis was planned using previously established methodologies; coupling of cinnamic acid derivatives with an unsaturated lactam. However, during the course of research several reactions proved capricious, prompting development of a new procedure. It was postulated that a Wittig reaction could be used for the final coupling step. Synthesis was subsequently directed towards the appropriate precursor and a method for its utility.

Finally the compounds generated were to be submitted for evaluation of their cytotoxicity profile against a variety of both normal and transformed cell types and the information generated used to develop an understanding of the structure activity relationship.

5 Results and Discussion

5.1 First Generation Retrosynthesis – Amide Coupling Route

At the commencement of the research endeavour, several effective methods for the synthesis of piperlongumine (**123**) had already been published, each of which utilised a similar coupling step.[109, 119-120] Therefore, using the same imide formation logic as applied in the literature, retrosynthetic analysis gave the key 5,6-dihydropyridin-2(1*H*)-one **130** along with the commercially available (*E*)-3,4,5-trimethoxycinnamic acid (**165**) (Scheme 5.1). Furthermore the synthesis of **130** was envisaged from the protected organoselenide **166** by oxidation and concomitant pericyclic *syn*-elimination of phenylselenenic acid. The α-phenylselenocarbonyl **166** would finally derive from the commercially available δ-valerolactam (**37**).

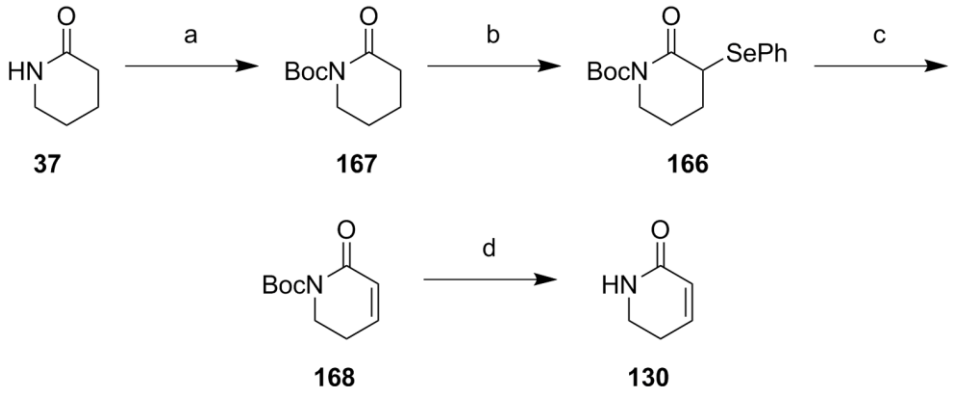


Scheme 5.1: Retrosynthesis of piperlongumine (**123**)

In the forward direction, starting with **37**, protection would precede selenation, which can be achieved by treatment with an electrophilic organoselenide such as phenylselenyl chloride.[126a] Peroxide or peracid oxidants have been shown

to be suitable to effect the elimination of phenylselenenic acid. Deprotection and finally coupling with (*E*)-3,4,5-trimethoxycinnamic acid (**165**) in accordance with literature precedent would complete the synthesis.

5.2 Synthesis of 5,6-dihydropyridin-2(1*H*)-one 130



Scheme 5.2: Synthesis of 5,6-dihydropyridin-2(1*H*)-one **130**. Reagents and conditions: (a) ^{n}BuLi, Boc_2O, THF, -78 °C → r.t., 1 h, 99%; (b) LDA, PhSeCl, THF, -78 °C → r.t., 1 h, 44%; (c) H_2O_2 (30% aq), THF, 0 °C, 1 h, 40%; (d) TFA, CH_2Cl_2, 0 °C → r.t., 1 h, 27%

The synthesis commenced from the commercially available precursor δ-valerolactam (**37**), which was protected by lithiation and subsequent addition to di-*tert*-butyl dicarbonate (Scheme 5.2). Full conversion of the starting material could be observed after 1 hour in the crude ^{1}H NMR and near quantitative yields of the protected derivative **167** were obtained after purification by column chromatography.

The α-phenylselenocarbonyl compound **166** was prepared from **167** by metalation with freshly prepared lithium diisopropylamide and treatment of the resulting lithium enolate with phenylselenyl chloride. Unfortunately, despite careful control of stoichiometry and the order/rate of additions a

separable mixture of single and double addition products was consistently observed. However, upon chromatographic purification the desired organoselenide product **166** was obtained in moderate yield.

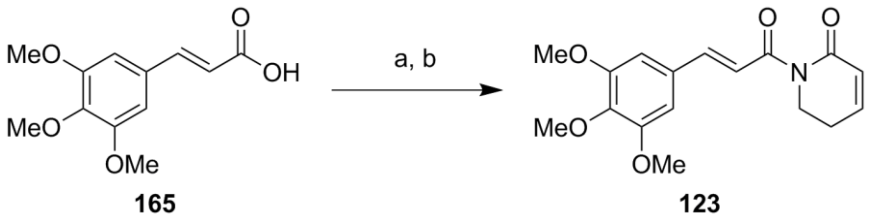
Oxidation of the phenylselenide **166** was experimentally easy to perform. Addition of aqueous hydrogen peroxide to a cooled solution of **166** saw rapid consumption of the starting material and generation of the unsaturated product **168**. The crude product obtained contained significant amounts of insoluble polymeric material, which may have arisen from cleavage of the protecting group during the course of the reaction and subsequent intermolecular condensations. Fortunately the desired product **168** could be readily isolated by chromatography, albeit in moderate yield.

Finally cleavage of the protecting group was achieved upon treatment of a cooled, dichloromethane solution of **168** with trifluoroacetic acid. Analysis of the ^{1}H NMR from the reaction confirmed the complete consumption of the starting material. However, after extraction from the reaction mixture the yield of 5,6-dihydropyridin-2(1*H*)-one **130** was apparently poor, even though no side products were evident. Examination of the aqueous extracts from the reaction revealed a significant quantity of the desired product, but unfortunately none of the material could be recovered.

Overall the preparation of 5,6-dihydropyridin-2(1*H*)-one **130** was achieved in 4 linear steps from the commercially available δ-valerolactam (**37**) in 5% total

yield. Despite the need for improvement, the sequence delivered a sufficient quantity of material to examine the final stage of the synthesis.

5.3 Synthesis of Piperlongumine (123)

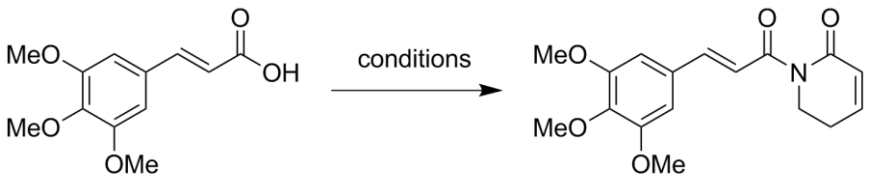


Scheme 5.3: Synthesis of piperlongumine (**123**). Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, Δ, 4 h, 99%; (b) ^{n}BuLi, **130**, THF, -78 °C → r.t., 24%

Coupling of the 5,6-dihydropyridin-2(1*H*)-one **130** with (*E*)-3,4,5-trimethoxycinnamic acid (**165**) first required preparation of the acyl chloride **141**. The required functional group interconversion was performed by refluxing a solution of **165** with thionyl chloride. Evaporation of the volatiles from the reaction mixture under reduced pressure gave the desired product **141** in quantitative yield, without need for any further purification.

Finally, in accordance with literature precedent, lithiation of 5,6-dihydropyridin-2(1*H*)-one **130** and its addition to a solution of the acyl chloride **141** gave the natural product piperlongumine (**123**).[119] However, purification of the desired compound from the reaction mixture was severely hindered by the generation of several co-eluting side products, significantly reducing the yield of piperlongumine (**123**) obtained.

In order to improve the poor yield from the coupling, alternative methods for carboxylic acid activation were investigated. However, generally poor yields and selectivity were observed in all cases (Table 5.1).



165 **123**

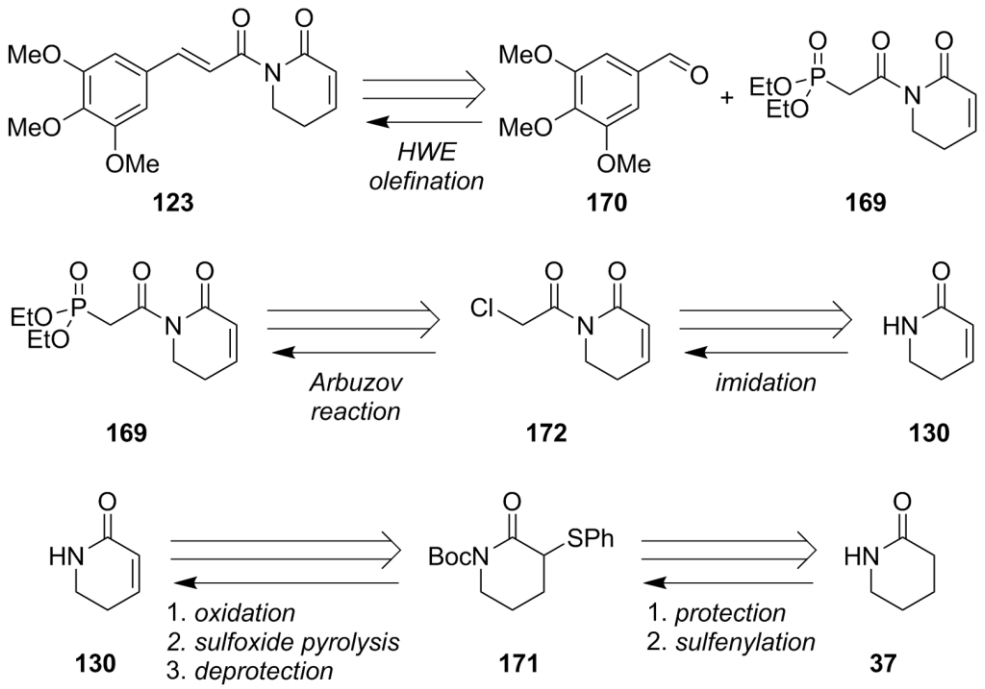
Entry	Conditions	Yield
1	pivaloyl chloride, Et_3N, THF, -10 °C, 1 h then **130**, ^{n}BuLi, THF, -78 °C, 2 h	8%
2	pivaloyl chloride, Et_3N, THF, -10 °C, 1 h then **130**, toluene, Δ, 24 h	16%
3	$SOCl_2$, CH_2Cl_2, Δ, 4 h then **130**, toluene, Δ, 24 h	16%

Table 5.1: Alternative methods for the synthesis of piperlongumine (**123**)

Forming an activated ester using pivaloyl chloride, followed by addition of lactam **130**, which was deprotonated using *n*-butyllithium, saw a significant reduction in yield (Table 5.1, entry 1). Additionally, literature conditions for thermally induced coupling also failed to improve on the initial result (Table 5.1, entries 2 and 3). The poor yields were likely observed due to competing reaction pathways, which when compounded with the poor overall yield from the preparation of 5,6-dihydropyridin-2(1*H*)-one **130**, discouraged further application of this method.

5.4 Second Generation Synthesis – Horner-Wadsworth-Emmons Route

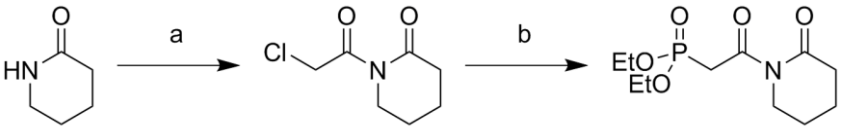
Due to the low overall yields achieved for the first generation synthesis, research was instead directed towards developing a novel method for the generation of piperlongumine analogues. It was postulated that a late stage Horner-Wadsworth-Emmons (HWE) reaction of the corresponding phosphonoacetamide **169** would enable (*E*)-stereoselective installation of the central acryloyl group directly from the aldehyde **170** (Scheme 5.4). Therefore, the approach would be amenable to the synthesis of a variety of aromatic/styryl analogues, circumventing the preparation of the bespoke cinnamates required by other synthetic methods.[119] In addition, sulfoxide pyrolysis would be used for generation of the 5,6-dihydropyridin-2(1*H*)-one **130**, obviating the use of the commercially relatively expensive and highly toxic organoselenides employed in the first generation synthesis.



Scheme 5.4: Altered retrosynthesis of piperlongumine (**123**)

In the forward direction, starting from the same, previously employed commercially available precursor δ-valerolactam (**37**), initial protection was envisaged followed by conversion into the corresponding 3-(phenylthio)piperidin-2-one **171**. Oxidation, thermal elimination and subsequent deprotection would then give 5,6-dihydropyridin-2(1*H*)-one **130**. Imidation with chloroacetyl chloride and Arbuzov reaction of the resulting α-chloro imide **172** leads to the key phosphonoacetamide **169**. Finally, HWE reaction with 3,4,5-trimethoxybenzaldehyde **170** would deliver the natural product piperlongumine (**123**).

5.5 Synthesis of a Model Phosphonoacetamide 173



37 **174** **173**

Scheme 5.5: Synthesis of the model phosphonoacetamide **173**. Reagents and conditions: (a) chloroacetyl chloride, NaH, THF, 0 °C → r.t., 1.5 h, 80%; (b) NaI, $P(OEt)_3$, DMF, µW (300 W) 110 °C, 10 min, 64%

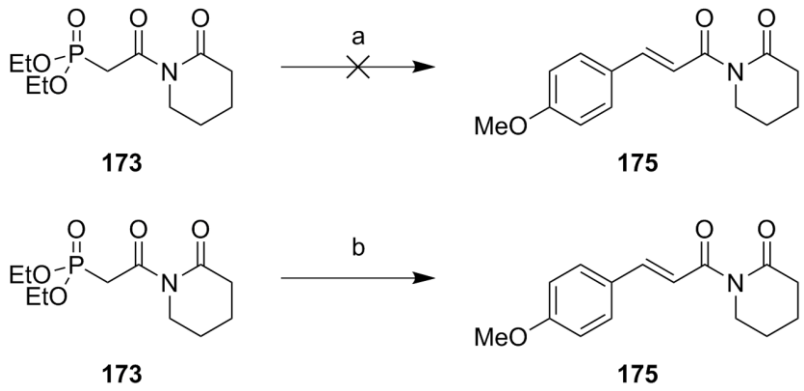
In order to test the viability of a Horner-Wadsworth-Emmons strategy for the preparation of piperlongumine analogues, the model phosphonoacetamide substrate **173** was first envisaged. The final coupling stage could then be investigated further, without the need to commit advanced synthetic material. Preparation of the model system **173** began with N-acetylation of the readily available commercial precursor δ-valerolactam (**37**) (Scheme 5.5). Initial deprotonation of **37** on treatment with sodium hydride and subsequent

gradual addition of chloroacetyl chloride gave the α-chloro imide **174**. However, alongside the desired product several unidentified trace side products could be observed in the ^{1}H NMR spectrum of the reaction mixture, which were removed upon purification by flash chromatography.

Conditions for the Arbuzov reaction were adapted from literature precedent in related systems.[140] Often purification of the desired products from such phosphonylation reactions requires reduced pressure distillation for removal of surplus triethyl phosphite, which is normally used in excess or as the reaction solvent. Instead, the adopted procedure employed an *in-situ* Finkelstein reaction with sodium iodide and efficient microwave heating to facilitate high yielding conversion in fast reaction times and with only a small excess of triethyl phosphite. Accordingly the α-chloro imide **174** was converted to phosphonoacetamide **173**. However, isolation of the product from the reaction mixture still required sequential column chromatography to ensure adequate recovery of the pure material.

With the model substrate **173** in hand, investigation of the key HWE reaction began with suitably mild conditions commonly employed for preparation of cinnamic esters from aromatic aldehydes.[141] The convenient and readily available 4-anisaldehyde was selected for the trial reaction. Consequently, deprotonation of the model phosphonoacetamide **173** was attempted using potassium *tert*-butoxide (Scheme 5.6). However, upon heating and addition of anisaldehyde no reaction was observed to take place. It was proposed that stronger base may be required for α-deprotonation of the

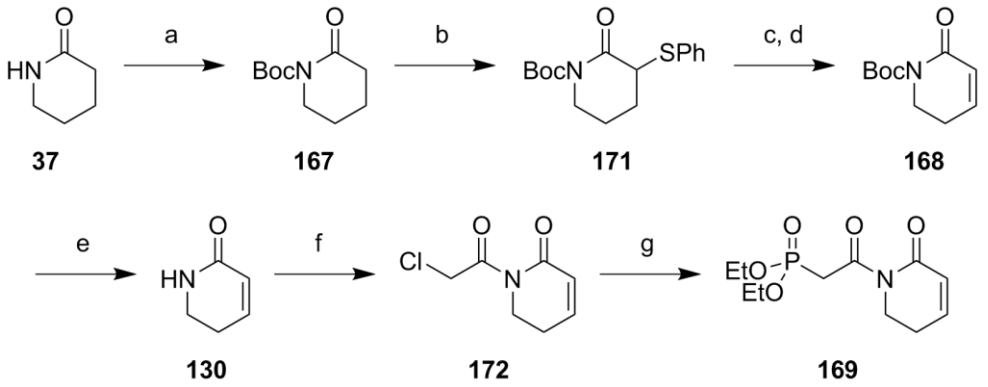
phosphonoacetamide and indeed, upon repetition using an organolithium base the desired compound **175** was detected as the major reaction product.



Scheme 5.6: Model Horner-Wadsworth-Emmons reactions. Reagents and conditions: (a) KO^{t}Bu, THF, Δ, 4 h; (b) ^{n}BuLi, THF, -78 °C → r.t., 4 h, 72%

The result served as proof of concept for the viability of an HWE strategy for the construction of piperlongumine analogues and further established a preliminary synthetic route, which could potentially be used to access the required phosphonoacetamide **169**.

5.6 Synthesis of Phosphonoacetamide 169



Scheme 5.7: Synthesis of phosphonoacetamide **169**. Reagents and conditions: (a) ^{n}BuLi, Boc_2O, THF, -78 °C → r.t., 1 h, 99%; (b) LDA, PhSSPh, THF, -78 °C → r.t., 1.5 h, 70%; (c) *m*CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C → r.t., 2 h; (d) toluene, 80 °C, 3 h, 99% over 2 steps; (e) HCl (6M in MeOH), MeOH, 0 °C, 1 h, 98%; (f) chloroacetyl chloride, NaH, THF, 0 °C → r.t., 17 h, 65%; (g) NaI, $P(OEt)_3$, DMF, µW (300 W) 110 °C, 10 min, 61%

Initially the synthesis of phosphonoacetamide **169** began with the previously applied boc-protection reaction, which consistently afforded a near quantitive yield of the protected lactam **167**. Next, generation of the lithium enolate using lithium diisopropylamide and subsequent treatment with diphenyl disulfide gave the 3-(phenylthio)piperidin-2-one **171**. An improvement in yield was observed when compared with the similar reaction previously employed, which used the electrophile phenylselenyl chloride. However, the unpleasant odor of the phenylthiol by-product still warranted careful handling of the reaction and waste produced during purification.

The required double bond was installed using an efficient two step sequence. First, oxidation with 3-chloroperbenzoic acid gave an intermediate sulfoxide, which was used directly without further purification. The crude material was dissolved in toluene and heated, initiating the desired pericyclic *syn*-

elimination. The reaction product, protected lactam **168**, was isolated by column chromatography in excellent yield.

During the previous synthetic effort a low yield was observed for deprotection of the unsaturated lactam **168**, primarily due to the aqueous solubility of the 5,6-dihydropyridin-2(1*H*)-one product **130**. Alternative methods to bring about the transformation were therefore investigated. Among these the most effective proved to be using hydrochloric acid (6M in methanol), which gave the free amide product **130** in excellent yield. With this procedure, no aqueous extraction was required and instead the crude hydrochloride salt could be isolated upon removal of the solvent. Treatment of the residue with amine base then served to facilitate purification of the free 5,6-dihydropyridin-2(1*H*)-one **130** by column chromatography.

Acetylation of the 5,6-dihydropyridin-2(1*H*)-one **130** was next accomplished using the same conditions as described for the preparation of the model substrate. Unfortunately the yield was observed to decrease slightly in comparison, due to the formation of several unidentified compounds. However, the desired α-chloro imide **172** could still be readily isolated by column chromatography as the major reaction product, so no further optimisation was undertaken.

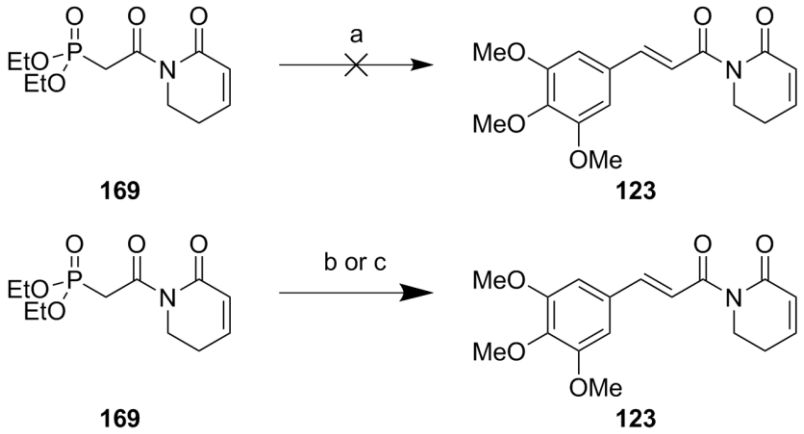
Finally, the phosphosphonoacetamide **169** was prepared using the microwave conditions previously employed. Gratifying the reaction was consistent with that observed for the model system. However, similar to the model,

purification of the phosphosphonoacetamide product **169** required sequential column chromatography to remove the residual phosphite waste, likely reducing the quantity isolated. Overall, preparation of the required phosphonoacetamide **169** was accomplished in 7 linear steps and 27% yield.

The HWE reaction with 3,4,5-trimethoxybenzaldehyde (**170**) was next examined, using the same procedure that was developed when testing the model phosphonate **173** (Scheme 5.8). However, on treatment with *n*-butyllithium and addition of the aldehyde a complex mixture of reaction products was observed. Spectroscopic analysis of the resultant crude material revealed features in both the ^{1}H NMR and mass spectrum that were indicative of alkylation. Given that the only structural alteration from the model system is the addition of a π-bond, the alkylation of this position with the organolithium reagent can be inferred. Therefore, alternative strong bases were investigated. Both lithium diisopropylamide and sodium hydride were initially selected, due to their non-nucleophilic characteristics. Pleasingly in both cases facile conversion to the desired product **123** was observed.

The synthetic piperlongumine (**123**) was readily separable from the phosphate by-product, which remained immobile during chromatography, facilitating expedient purification. Both of the isolated yields were significantly improved relative to those obtained when using amide formation methodology. However, the cleanest and most convenient conversion was observed with sodium hydride. Examination of the ^{1}H NMR confirmed an (*E*)-stereoselective olefination. Furthermore the spectroscopic data obtained for the synthetic

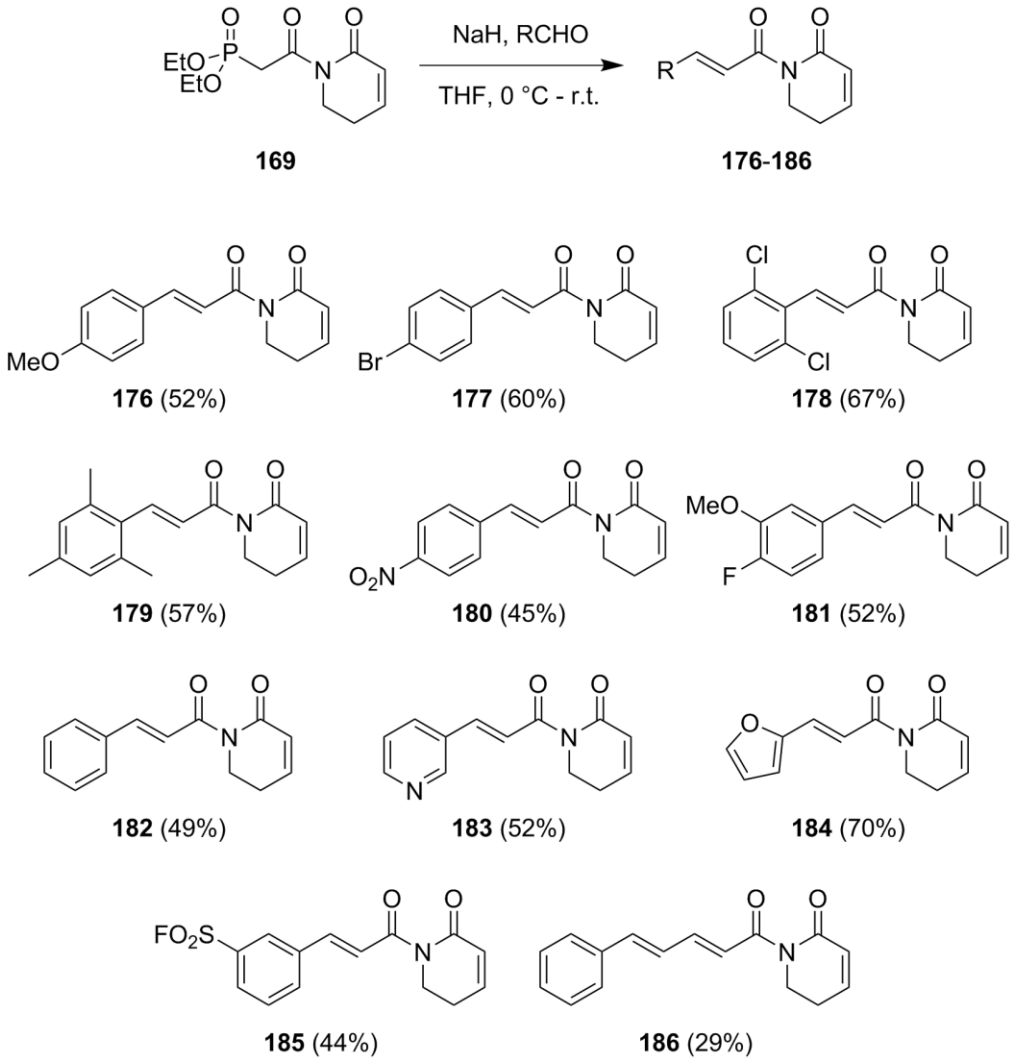
piperlongumine (**123**) proved to be identical with that reported in the literature, from material of natural origin.[109]



Scheme 5.8: Synthesis of piperlongumine (**123**) by HWE olefination. Reagents and conditions: (a) ^{n}BuLi, THF, -78 °C → r.t., 4 h; (b) LDA, THF, -78 °C → r.t., 4 h, 44%; (c) NaH, THF, 0 °C → r.t., 51%

Overall the synthesis of piperlongumine was achieved in 8 linear steps and 14% yield, starting from the commercially available precursor δ-valerolactam. The described method represents the first synthesis using an olefination strategy, providing a complimentary approach to the preparation of piperlongumine (**123**). Interested to explore the substrate scope, the investigation next moved to the generation of analogues through variation of the aromatic aldehyde.

5.7 Synthesis of Piperlongumine Analogues 176-186



Scheme 5.9: Synthesis of piperlongumine analogues **176**-**186** by HWE olefination

The developed Horner-Wadsworth-Emmons reaction conditions were next applied to a selection of aryl and heteroaryl aldehydes, giving the piperlongumine analogues **176**-**186** (Scheme 5.9). Aromatic aldehydes with electron withdrawing or electron donating substituents were well tolerated, reflected by the relatively consistent isolated yields of the respective products **176**-**182**. The lowest yield was obtained from the coupling of cinnamaldehyde.

However, with extended reaction times the desired product **186** proved isolable and therefore no further optimisation was undertaken.

The heteroaromatic aldehydes 3-pyridinecarboxaldehyde and furfural gave the resulting derivatives **183** and **184**. Furthermore, out of all the analogues prepared, **184** was obtained in the highest yield.

During the course of this investigation, contemporaneous research by Sharpless *et al*. revealed the sulfonyl fluoride moiety as a versatile and robust chemical handle.[142] Intrigued whether the newly popularised functional group would withstand the developed reaction conditions, 3-benzaldehyde sulfonyl fluoride was selected for its availability as a test substrate. Gratifyingly the corresponding sulfonyl fluoride analogue **185** was obtained in moderate yield. The presence of the fluorine atom was further confirmed using ^{19}F NMR spectroscopy.

5.8 Biological Evaluation of Piperlongumine (123) and Analogues 176-186

Samples of each of the 11 piperlongumine analogues **176**-**186**, along with the parent compound (**123**) were then submitted to collaborating biologists Mr Paul Wood and Dr Tracey Bradshaw for *in-vitro* biological analysis.

5.8.1 Growth Inhibitory Effects of Piperlongumine (123) and Analogues 176-186 in Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Lines

To determine any growth inhibitory effects of the synthetic piperlongumine (**123**) or its analogues **176**-**186**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (**121**) (MTT) assays were generously performed by collaborating biologists.

The colorectal carcinoma cell line HCT 116 shows mitochondrial-dependent reactive oxygen species (ROS) damage when treated with ROS generating agents.[143] Apoptosis can be induced by inhibition of the ROS scavenging enzyme thioredoxin reductase. The ovarian adenocarcinoma cell line IGROV-1 shows sensitivity to agents such as 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole and cisplatin, which can generate a mitochondrial-ROS damage response and pathway activation.[144] Given that the two cell lines show sensitivity to other ROS inducing agents, the postulated mechanism of action of piperlongumine (**123**) indicate that it may also induce similar effects.

The MTT assays were performed at the time of addition of the tested agent (T zero) and following 72 hour exposure of the cells to the test compound (Table 5.2). Both the HCT 116 and IGROV-1 cell lines showed dose-dependent sensitivity to each compound tested; increasing concentrations reduced growth/proliferation. While each compound assayed demonstrated promising activity, the heterocyclic derivatives **183** and **184** were among the most active agents in the ovarian carcinoma line (Table 5.2, entries 9 and 10). Compounds bearing electron withdrawing aryl substituents showed generally greater

potency than those non-substituted or with alkyl. Overall **180** and **183** proved to be the most active (Table 5.2, entries 6 and 9). To test the hypothesis that activity was cancer cell-selective, growth inhibitory properties against a non-transformed foetal lung fibroblast cell line (MRC-5) were also examined.

		GI_{50} (μM)		
Entry	**Compound**	**HCT 116**	**IGROV-1**	**MRC-5**
1	**123**	17.3 ± 1.7	4.9 ± 1.7	3.1 ± 0.04
2	**176**	23.0 ± 3.5	11.6 ± 3.5	5.5 ± 1.5
3	**177**	39.9 ± 3.3	10.8 ± 3.3	6.7 ± 0.5
4	**178**	26.1 ± 5.3	11.8 ± 5.3	5.5 ± 0.4
5	**179**	64.4 ± 2.5	36.0 ± 2.5	25.7 ± 1.2
6	**180**	7.78 ± 0.5	7.1 ± 0.5	2.8 ± 0.05
7	**181**	9.4 ± 2.8	11.4 ± 2.8	3.1 ± 0.06
8	**182**	97.0 ± 9.3	29.45 ± 9.3	6.3 ± 1.1
9	**183**	7.4 ± 0.6	4.34 ± 0.6	5.5 ± 1.7
10	**184**	17.2 ± 1.7	5.4 ± 1.7	14.1 ± 6.1
11	**185**	43.5 ± 8.0	31.0 ± 8.0	7.8 ± 0.6
12	**186**	52.8 ± 12.3	29.5 ± 12.3	17.5 ± 2.4

Table 5.2: Growth inhibitory activity of piperlongumine (**123**) and analogues **176**-**186** in HCT 116, IGROV-1 and MRC-5 cell lines. Data represent mean ± SD GI_{50} (μM) values from >3 independent MTT assays (*n* = 4 per concentration in each trial)

Contrary to previous reports, selectivity was not observed; the piperlongumine analogues appeared equi-active against MRC-5, as they were against HCT 116

and IGROV-1 cell lines. The GI_{50} results obtained for the MTT assay were then used to guide the selection of compounds to examine in further, more time consuming clonogenic analysis.

5.8.2 Clonogenic-Survival Assay

Clonogenic-survival assays are used to determine the ability of single cells to survive a brief challenge with a test agent and regain proliferative capacity to form progeny colonies. The cells are seeded in growth medium in multi-well plates. After an adherence period in an incubator, test agents are added and after a further exposure period the agent containing medium is removed and the wells washed. Fresh agent-free growth medium is then added and the plates incubated for several days. When colonies in the control wells contain >50 cells the plates are washed of growth medium, the colonies fixed by overlaying with absolute methanol and finally a chemical stain applied. Colonies can then be counted for each treatment concentration and averages of colony counts taken. Finally estimated IC_{50} values (agent concentration which inhibits colony number by 50%) are then calculated.

5.8.3 Effects of Piperlongumine (123) and Analogues 176-186 on Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Colony Formation

Clonogenic assays were performed by collaborators to determine the ability of single cells to survive a brief challenge with the piperlongumine analogues (24 hour exposure) and recover the proliferative potential to form progeny colonies. The parent compound **123** and four analogues; **180**, **181**, **183** and **184**,

which gave the lowest GI_{50} values against both cell lines, were selected for inclusion in the clonogenic assays. The non-substituted phenyl compound **182**, which yielded a high GI_{50} value against both cell lines and demonstrated the poorest performance in the MTT assay, was also included as a negative control. Compounds were tested in duplicate on three separate occasions. Dose-dependent inhibition of colony formation by piperlongumine (**123**) and analogues **180**-**184** was observed and IC_{50} values calculated (Table 5.3).

		IC50 (μM)	
Entry	**Compound**	**HCT 116**	**IGROV-1**
1	**123**	3.7 ± 0.1	3.55 ± 0.3
2	**180**	3.9 ± 0.5	3.75 ± 0.1
3	**181**	9.75 ± 0.3	6.85 ± 0.7
4	**182**	5.05 ± 0.4	7.2 ± 0.7
5	**183**	7.9 ± 0.3	7.8 ± 1.7
6	**184**	7.6 ± 0.2	7.35 ± 1.9

Table 5.3: Inhibition of colony formation by piperlongumine (**123**) and analogues **180**-**184** in HCT 116 and IGROV-1 cell lines. Data represent mean ± SD IC_{50} (μM) values (*n* = 6)

Piperlongumine (**123**) was shown to most significantly inhibit colony formation, with IC_{50} values <4 μM in both the colorectal and ovarian carcinoma cell lines. However, very similar levels of activity were also observed for analogue **180** (Table 5.3, entries 1 and 2). For both compounds **123** and **180** clonogenic IC_{50} values correlated with MTT GI_{50} values. Intriguingly, although the activity

of **182** was relatively poor in the MTT assay against HCT 116 cells (GI_{50} 97 μM), clonogenic survival did not reflect this lack of activity, indicating that single cells were less able to survive challenge by **182** to form progeny colonies (IC_{50} 5.05 μM). Against IGROV-1 cells, similar inhibition of colony formation was evident following treatment of cells with analogue **182** (IC_{50} 7.2 μM). Again greater potency was observed in the clonogenic assay compared with the MTT assay (GI_{50} 29.45 μM).

5.8.4 ROS-Glo™ Hydrogen Peroxide Assay

The ROS-Glo™ hydrogen peroxide assay allows detection of ROS that are generated in cell culture. Cells are seeded at and added to multi-well opaque white plates in an appropriate quantity of growth medium before incubation for an adherence period. Next, test agents are added to the wells. For wells with a ≤6 hour exposure period the peroxide substrate solution is added at the same time as the test agents. Those wells requiring a longer exposure are treated with the peroxide substrate for the final 6 hours of the period. Finally incubation with a pre-prepared ROS-Glo™ detection solution for 20 minutes in the dark allows relative luminescence units to be recorded using a plate reader.

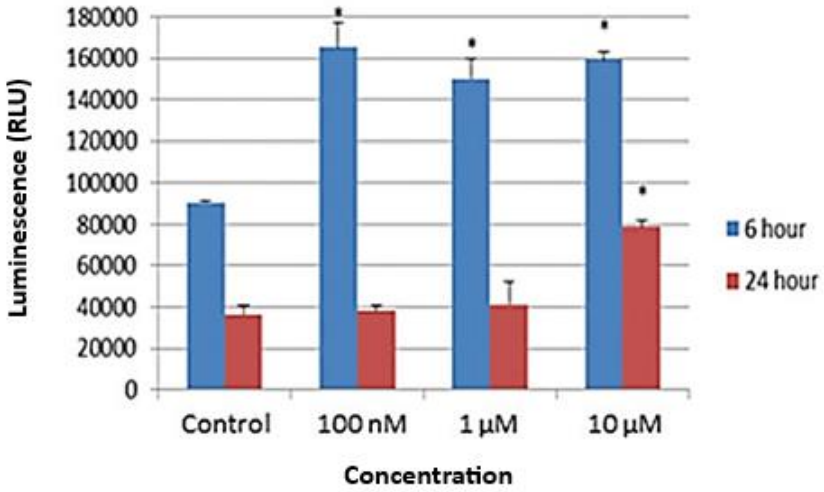
5.8.5 The Effects of Piperlongumine (123) and Analogues 180-184 on Reactive Oxygen Species Levels in Colorectal (HCT 116) and Ovarian (IGROV-1) Carcinoma Cell Lines

In an effort to further elucidate the mechanisms of action of the piperlongumine analogues, collaborators performed assays to measure cellular generation of ROS following treatment of cells with piperlongumine

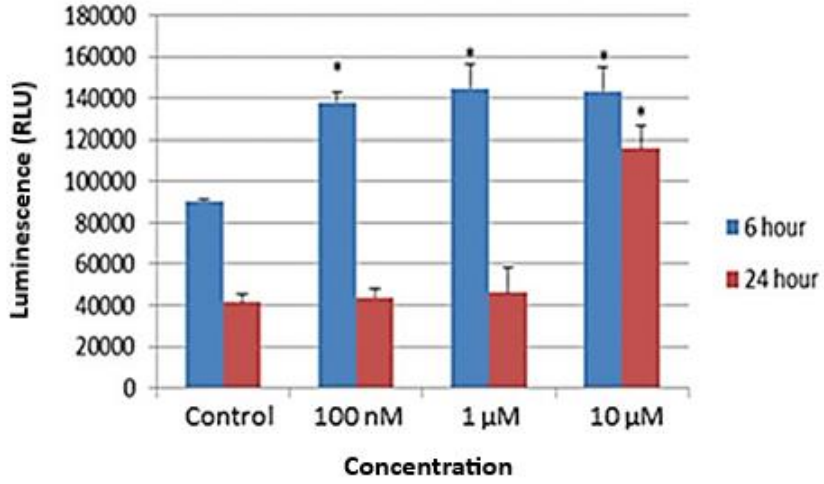
(**123**) and analogue compounds **180**-**184**. The assays were conducted following 6 hour and 24 hour exposure to the test agents.

5.8.5.1 HCT 116 Cells

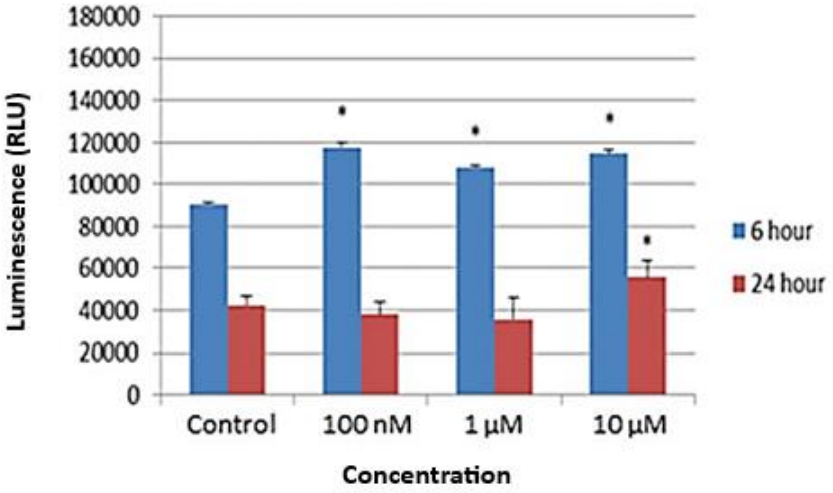
Piperlongumine (123)



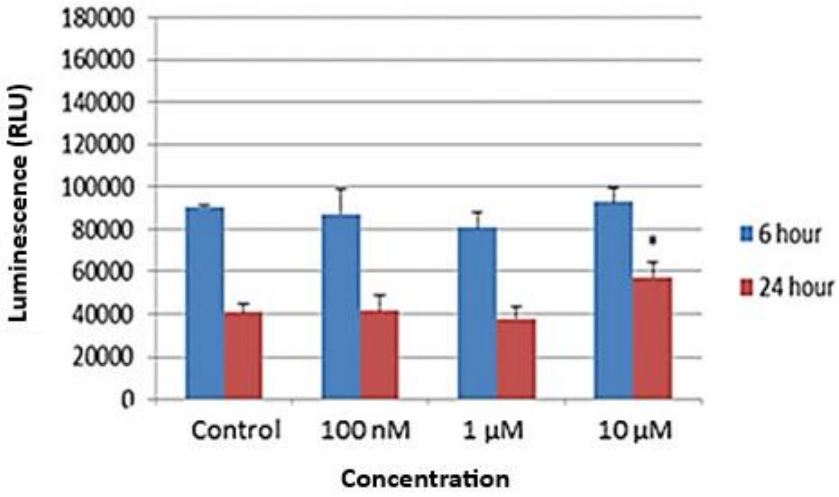
Piperlongumine Analogue 180



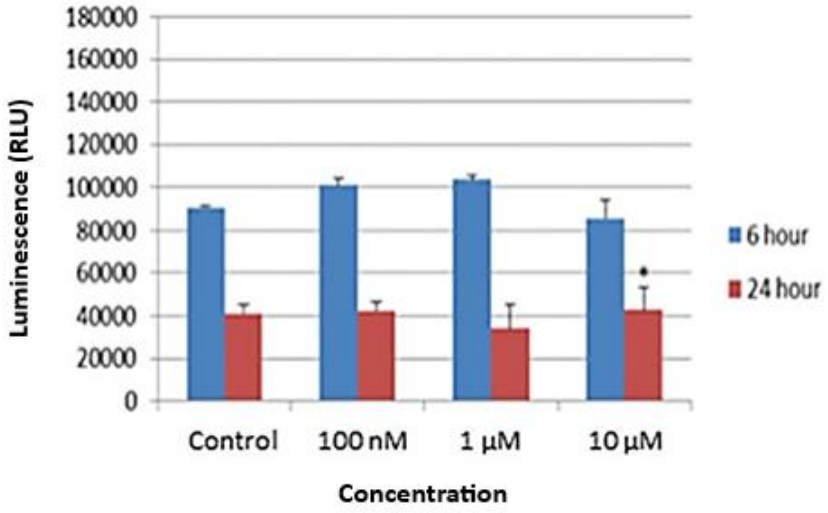
Piperlongumine Analogue 181



Piperlongumine Analogue 182



Piperlongumine Analogue 183



Piperlongumine Analogue 184

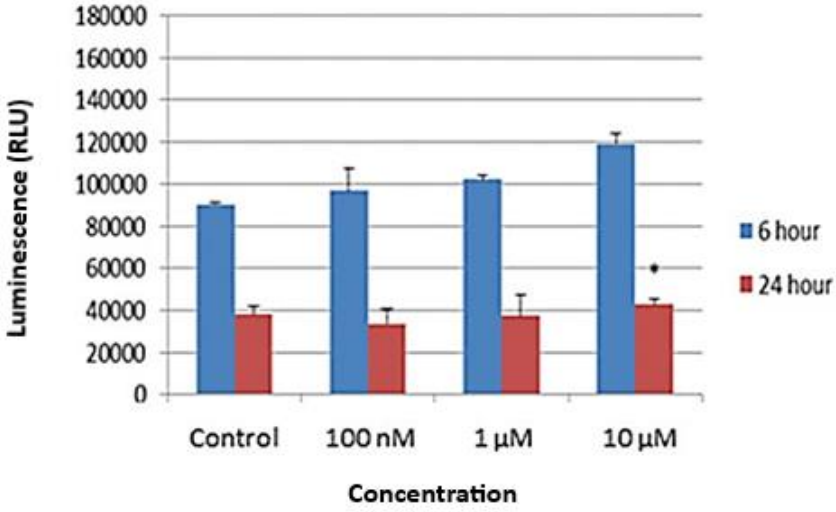


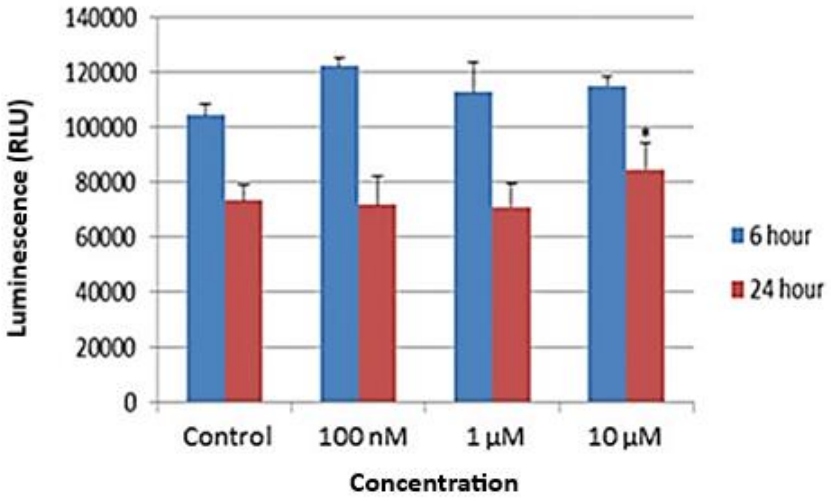
Figure 5.1: The effects of piperlongumine (**123**) and analogues **180**-**184** on ROS levels in HCT 116 cell lines following 6 and 24 hour exposure. Graphs represent mean + SEM from 2 independent repeats carried out in duplicate for both 6 hour and 24 hour data points. One way ANOVA was used to calculate significant differences with (*p < -0.05)

Following 6 hour exposure, significantly elevated ROS levels were generated in HCT 116 cells treated with piperlongumine (**123**) and analogues **180** and **181**

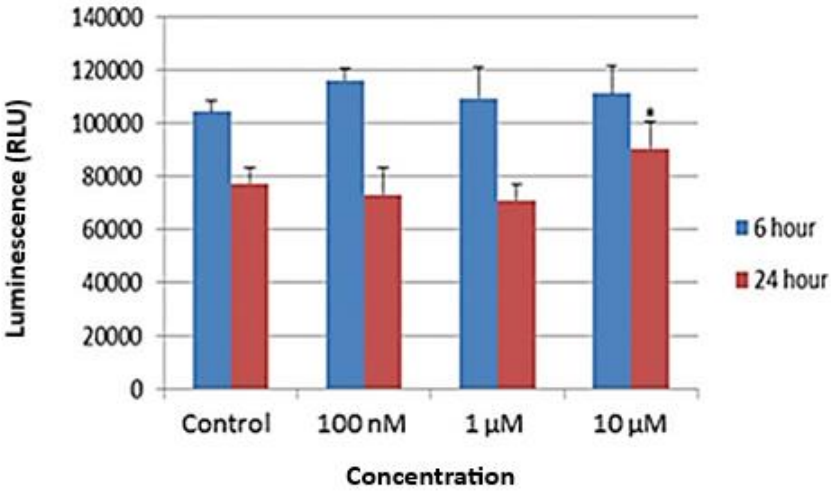
at all dilutions tested (1, 5, and 10 µM) (Figure 5.1). However, following 24 hour exposure, changes in ROS levels were reduced with significantly raised levels being detected only in cells treated with 10 µM agent concentrations.

5.8.5.2 IGROV-1 Cells

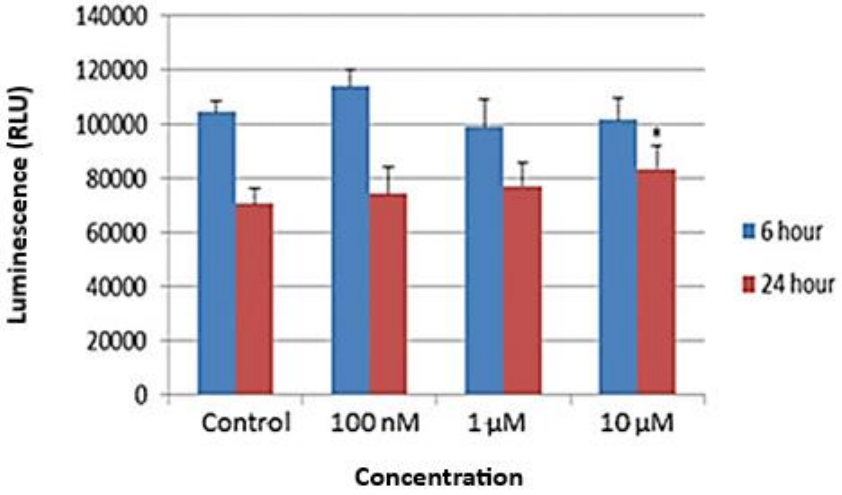
Piperlongumine (123)



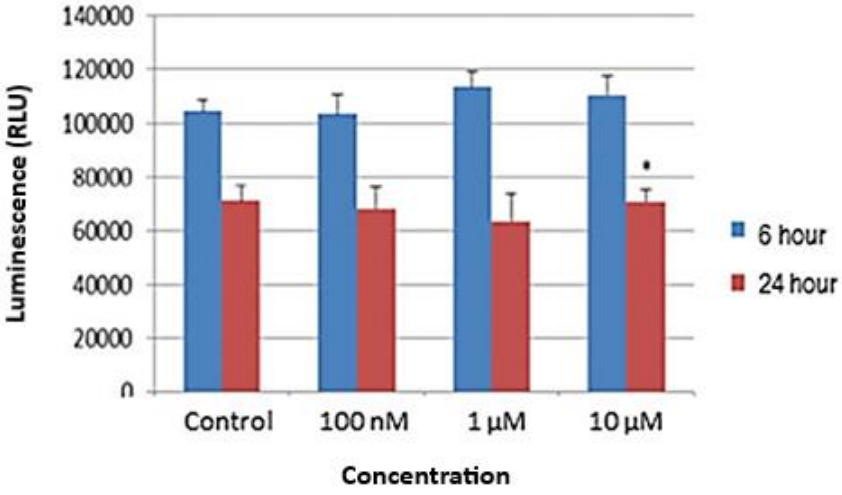
Piperlongumine Analogue 180



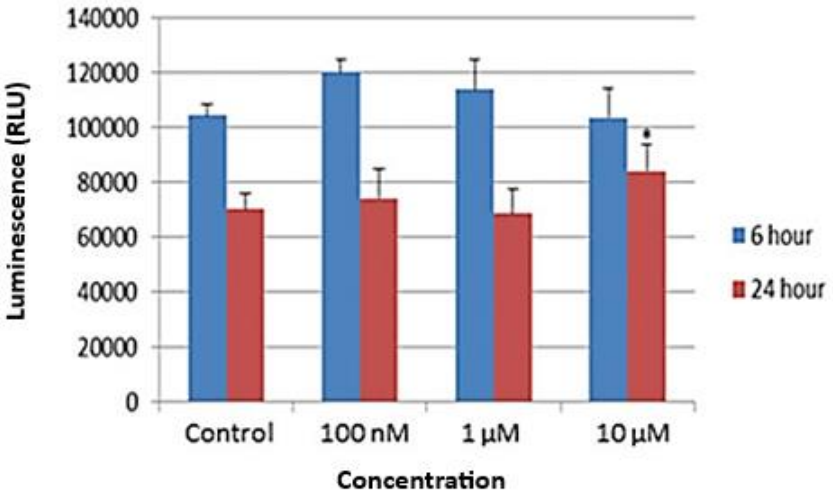
Piperlongumine Analogue 181



Piperlongumine Analogue 182



Piperlongumine Analogue 183



Piperlongumine Analogue 184

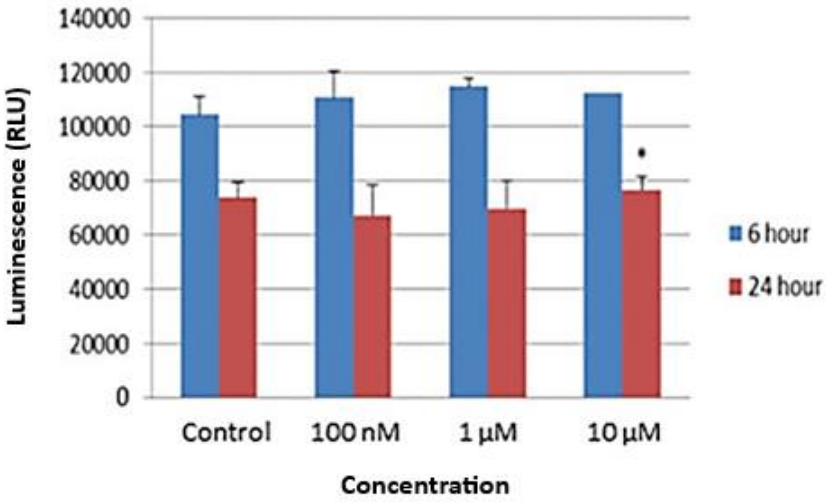


Figure 5.1: The effects of piperlongumine (**123**) and analogues **180**-**184** on ROS levels in IGROV-1 cell lines following 6 and 24 hour exposure. Graphs represent mean + SEM from 2 independent repeats carried out in duplicate for both 6 hour and 24 hour data points. One way ANOVA was used to calculate significant differences with (*p < -0.05)

In contrast with the HCT-116 cell line, following 6 hour exposure to piperlongumine (**123**) and analogues **180**-**184**, no significant variation to

cellular ROS levels was detected in treated IGROV-1 cells. After 24 hours, again increase in ROS levels was observed only in cells treated with analogue concentrations of 10 μM.

5.9 Conclusion

The alkaloid piperlongumine (**123**) is known to induce cancer specific cell death.[110] Although the mode of biological action is reported to occur through cancer cell-specific elevation of ROS levels, additional deconvolution of the mechanism is required. To further understand the intriguing activity the synthesis of piperlongumine (**123**) and analogues **176**-**186** was achieved. Central to the approach was a Horner–Wadsworth–Emmons coupling strategy, prompting the development of a novel phosphonoacetamide **169** reagent, demonstrating a new and versatile approach to piperine type alkamides.

Biological analysis of the synthetic material was undertaken by collaborators. Piperlongumine (**123**) and its analogues **176**-**186** were shown to cause growth inhibition and cytotoxicity in HCT 116 and IGROV-1 cells. In MTT assays, several compounds showed approximately equal activity to the parent piperlongumine (**123**) and of these selected analogues **180**-**184** potent anticlonogenic activity was revealed. HCT 116 and IGROV-1 cells showed similar sensitivity to piperlongumine (**123**) and its analogues **180**-**184** (IC_{50} < 10 μM). However, in the MTT assay, distinction between analogues and between cell lines was slightly more evident. Generally, IGROV-1 cells appeared more sensitive to the growth inhibitory properties of piperlongumine (**123**) and

analogues **176**-**186** than HCT 116 cells. In addition, contrary to previous reports, piperlongumine (**123**) and its analogues **176**-**186** displayed the same potent biological activity against MRC-5, as against HCT 116 and IGROV-1 cell lines.

When examined 6 hours post-treatment, the compounds **180** and **181** had generated significantly enhanced ROS levels in HCT 116 cells. The data collected strongly supported the hypothesis that ROS generation may not be a critical factor for the mechanism of action of all the piperlongumine analogues **176**-**186**, indicating a decoupling of ROS production and cell death. Nevertheless, it is noteworthy that the piperlongumine analogues possessing most potent HCT 116 growth inhibitory activity **180** and **181** also significantly elevate ROS in HCT 116 cells. Therefore, in this cell line at least, the generation of ROS may contribute to the observed growth inhibitory activity.

Further investigation of piperlongumine (**123**) and its structural analogues is warranted, including examination of activity and mechanisms of action against other cancer cell lines and additional *in vivo* studies of tolerability, stability and bioavailability. It is anticipated that piperlongumine (**123**) could be used in conjunction with ROS scavenging enzyme inhibitors to promote the damaging effects caused by ROS and induce cancer cell death.

6 Experimental

6.1 Experimental techniques

Reactions were performed in flame-dried glassware under an atmosphere of argon and stirred magnetically unless specified. For use as reaction solvents CH_2Cl_2 was freshly distilled from CaH_2; THF was freshly distilled from sodium and benzophenone; toluene was distilled from CaH_2 and stored under an atmosphere of argon over 4Å molecular sieves. For use as reagents Et_3N and diisopropylamine were distilled from CaH_2 and stored under an atmosphere of argon over 4Å molecular sieves. All other solvent and reagents were obtained commercially and used without further purification. Flash column chromatography was performed using Merck silica gel 60 as the stationary phase. Where not otherwise stated flash column chromatography was performed using petrol-EtOAc mixtures of increasing polarity (appropriate to the R_f value of the compound to be isolated) as the mobile phase. Petrol refers to petroleum spirit (b.p. 40-60 °C).

6.2 Analysis

Compounds previously described in the literature were characterised to consistent levels with that reported. Thin layer chromatography was carried out on aluminium-backed plates pre-coated with silica (0.2 mm, 60 F_{254}, Merck). The plates were developed using UV light and basic potassium permanganate solution. Melting points were recorded using a STUART SMP3 apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Bruker Tensor 27

FT-IR spectrophotometer with a neat film on an NaCl disc or as a solution in $CHCl_3$. IR peaks are quoted as vmax in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at ambient temperature using Bruker DPX400 (400 MHz), AV400 (400 MHz) and AV(III)400 (400 MHz) spectrometers. Data is expressed as chemical shifts in parts per million (ppm) relative to residual solvent signals of $CDCl_3$ (^{1}H NMR 7.27; ^{13}C NMR 77.0) as the internal standard on the δ scale. The following abbreviations are used to designate the multiplicity of each signal; s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; t, triplet; tt, triplet of triplets; tq, triplet of quartets; q, quartet; m, multiplet; br, broad. Coupling constants *J* are given in Hz. High-resolution mass spectra were obtained using a Bruker MicroTOF mass spectrometer operating in electrospray ionisation (ESI) mode.

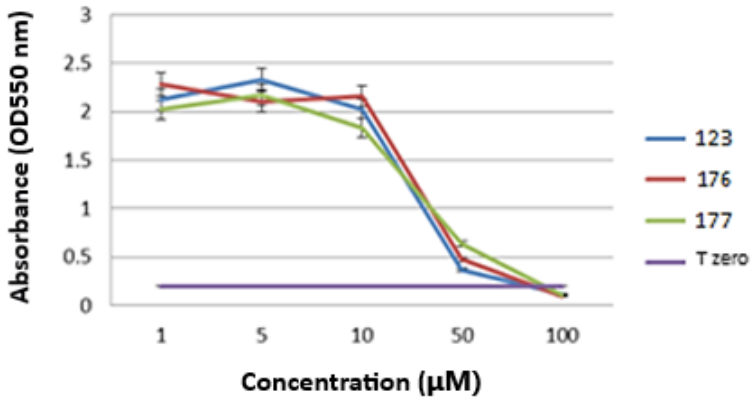
6.3 Cell Culture

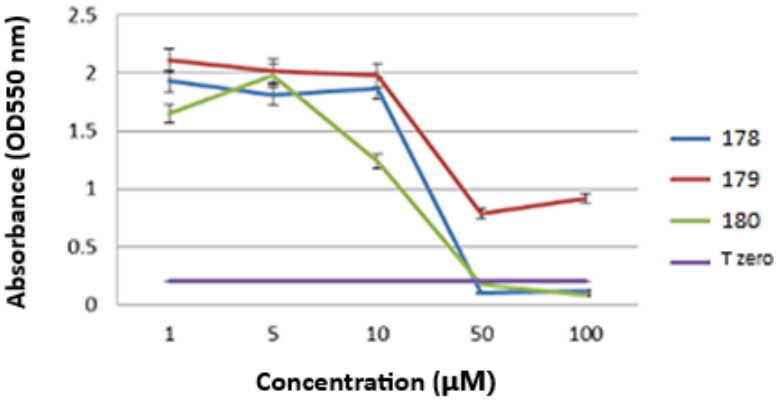
All carcinoma cell lines were sourced from the American Type Tissue Cell Culture Collection (ATCC) and cultured in RPMI 1640 nutrient medium supplemented with 10 % foetal bovine serum (FBS). Cells were passaged twice weekly to maintain logarithmic growth and incubated at 37 °C in an atmosphere constituting 5 % CO_2. Cell stocks were cryopreserved (−155 °C in liquid nitrogen) and upon re-introduction to culture were used in experiments ≤30 passages to minimise phenotypic/genotypic drift. Compound stocks were stored at −20 °C in aliquots of 10 mM in DMSO and thawed immediately prior to use.

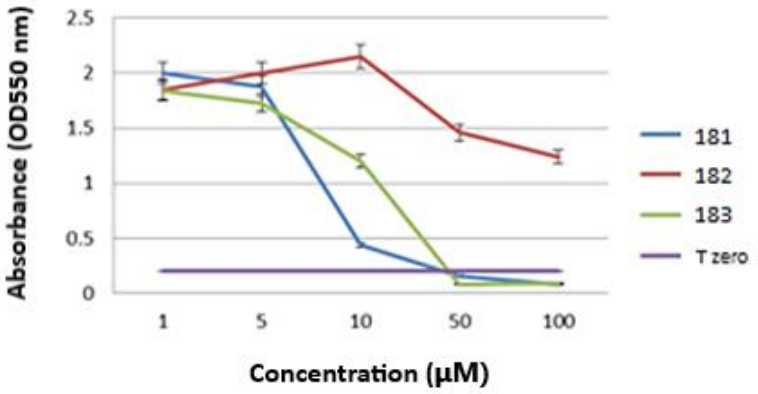
6.4 MTT Assay

Compounds were prepared as 10 mM top stocks in DMSO. HCT 116 and IGROV-1, human-derived colorectal and ovarian adenocarcinoma cells respectively, were cultivated at 37 °C in RPMI 1640 medium supplemented with 10% FBS. Cells were seeded at a density of 3×10^3 (HCT 116) or 5×10^3 (IGROV-1) per well into 96-well microtiter plates and allowed to adhere for 24 hours before test agent introduction (final concentrations 1 µM–100 µM, *n* = 4). Dilutions were prepared in medium prior to each assay. At the time of agent addition and following 72 hours exposure, MTT was added to each well (final concentration 400 µg mL^{-1}). Incubation at 37 °C for 2.5 hours allowed reduction of MTT by viable cells (mitochondrial dehydrogenases) to insoluble formazan crystals. Well supernatants were removed, and formazan solubilised by addition of DMSO (150 µL). Absorbance was read at 550 nm using a Perkin Elmer plate reader. Non-linear regression analysis was used to calculate compound concentrations required to inhibit cell growth by 50% (GI_{50}).

6.4.1 HCT 116 Cells







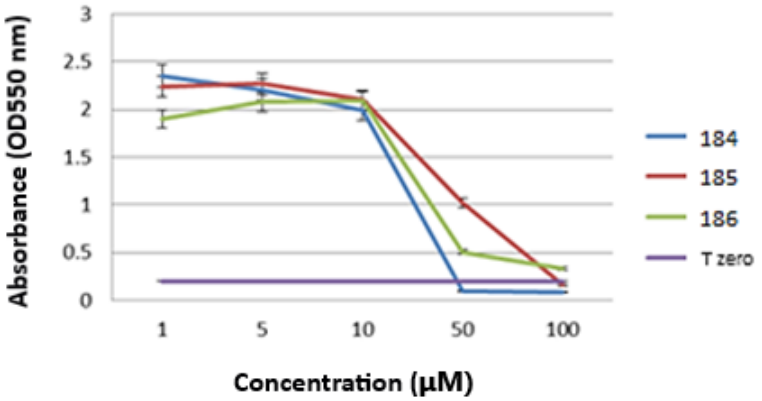
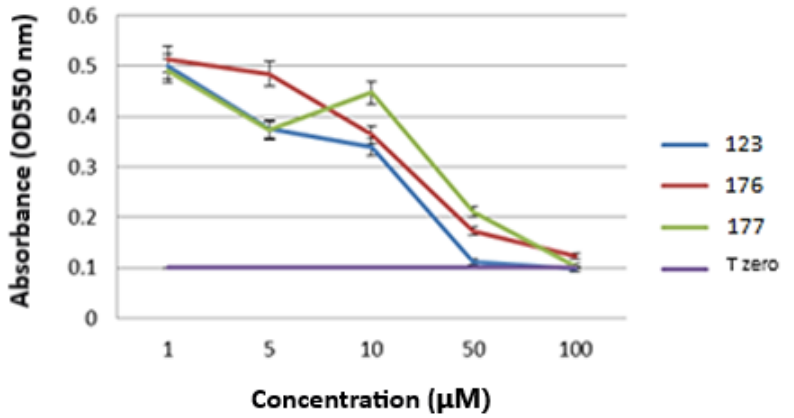
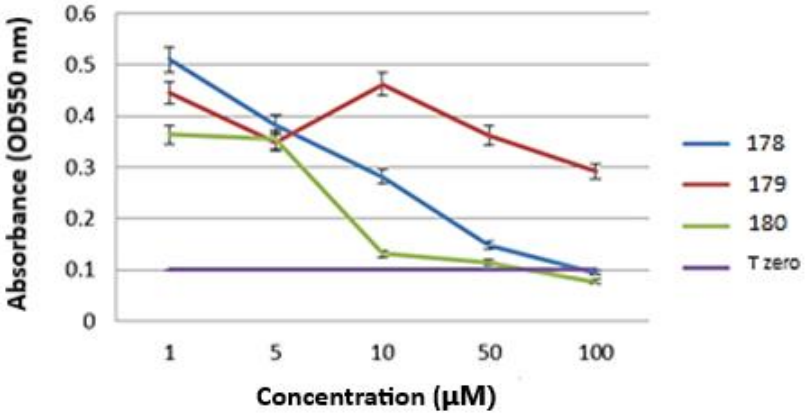
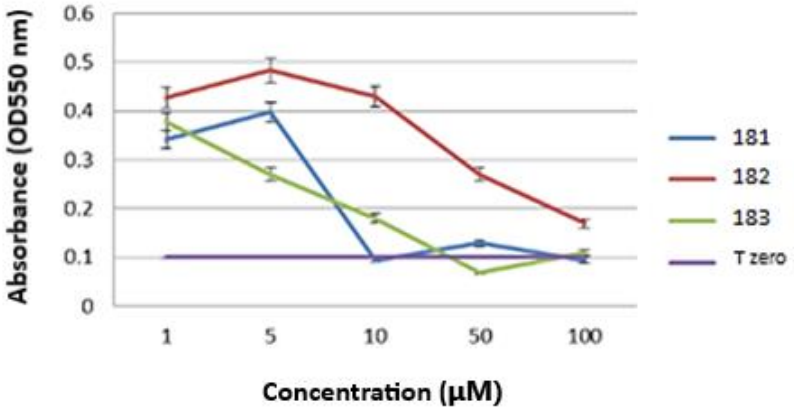


Figure 6.1: Effect of piperlongumine (**123**) and its analogues **176**-**186** on the growth of HCT 116 Cells. Data shown are the mean ± SD of representative MTT assays (*n* = 4 per sample; trials repeated ≥3 times)

6.4.2 IGROV-1 Cells







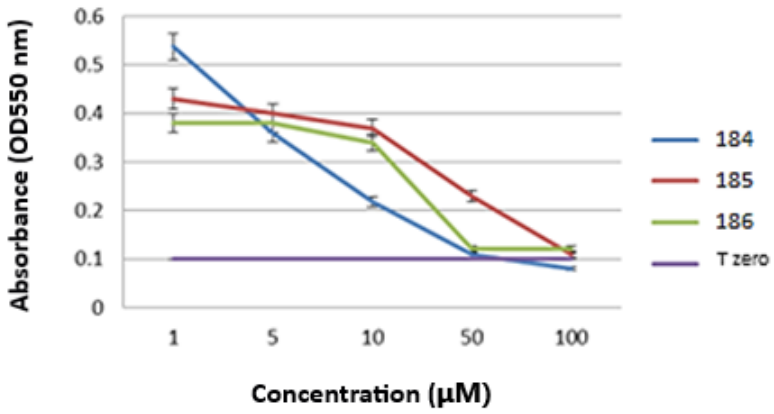
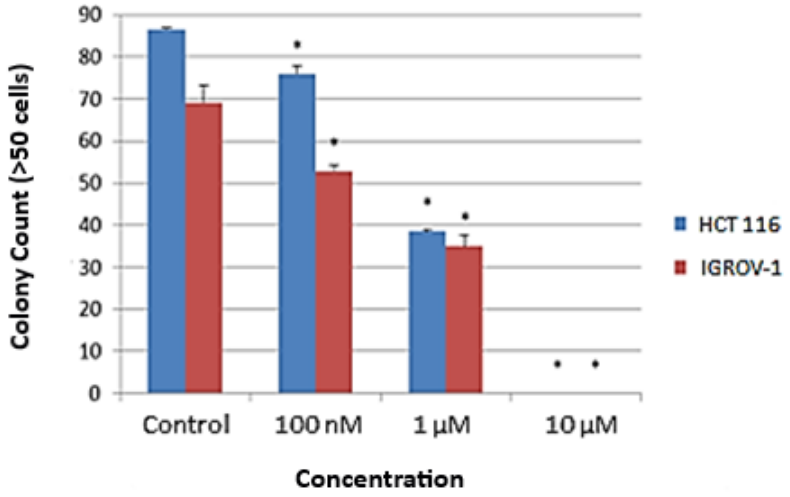


Figure 6.2: Effect of piperlongumine (**123**) and its analogues **176**-**186** on the growth of HCT 116 Cells. Data shown are the mean ± SD of representative MTT assays (*n* = 4 per sample; trials repeated ≥3 times)

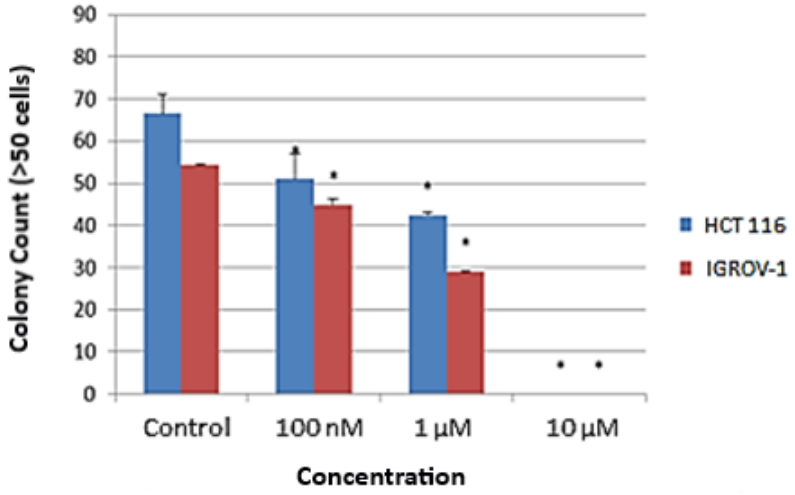
6.5 Clonogenic Assay

Clonogenic-survival assays were used to determine the ability of single cells to survive brief challenge with the compounds and retain proliferative capacity to form progeny colonies. HCT 116 and IGROV-1 cells were seeded at 250 per well in 2 mL complete medium in 6-well plates. After 24 h incubation at 37 °C, 5% CO_2, test agents were added (final concentrations 100 nM, 1 µM, 10 µM). After 24 h exposure agent containing medium was removed, cells washed (2 × 1 mL PBS) and fresh medium added to wells (3 mL). When colonies in control wells contained >50 cells (8–10 days), colonies were washed, fixed (100% methanol, 10 min), stained (0.5% methylene blue; 1 : 1 methanol : water; 10 min), washed, air dried and counted.

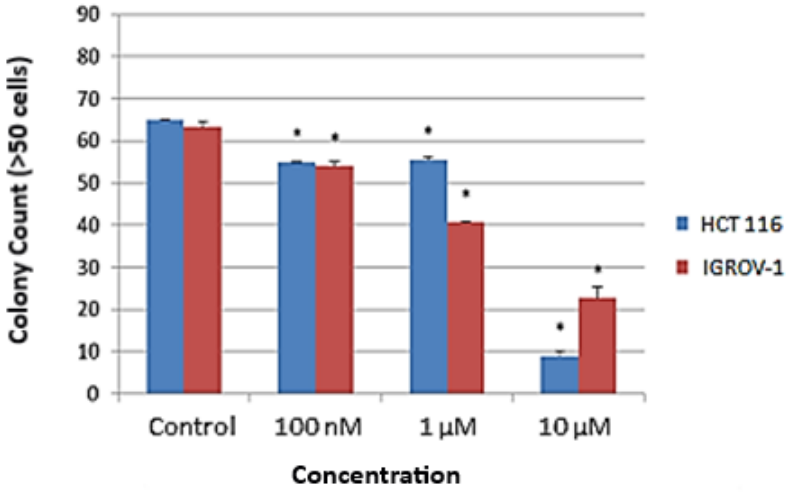
Piperlongumine (123)



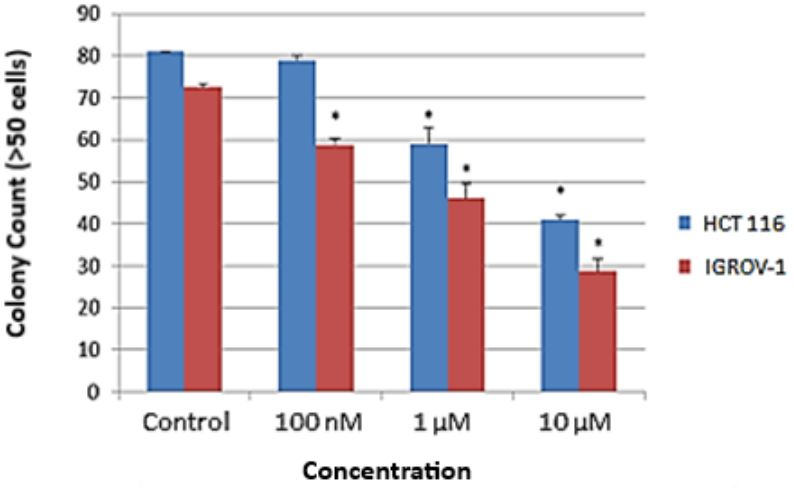
Piperlongumine Analogue 180



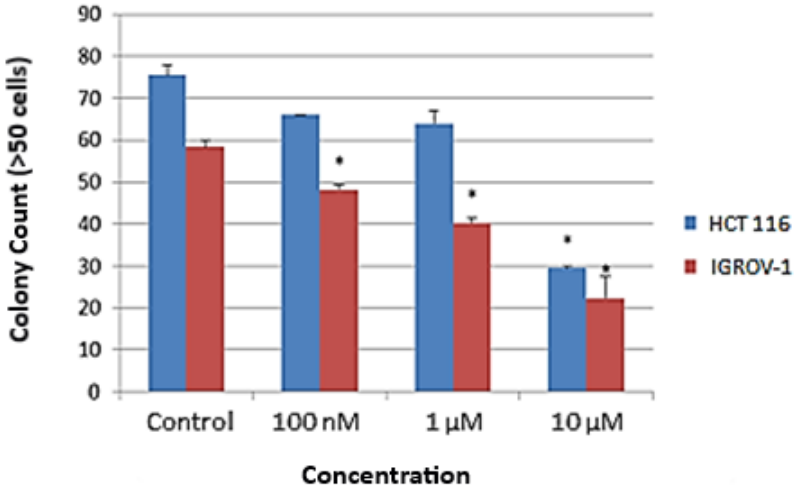
Piperlongumine Analogue 181



Piperlongumine Analogue 182



Piperlongumine Analogue 183



Piperlongumine Analogue 184

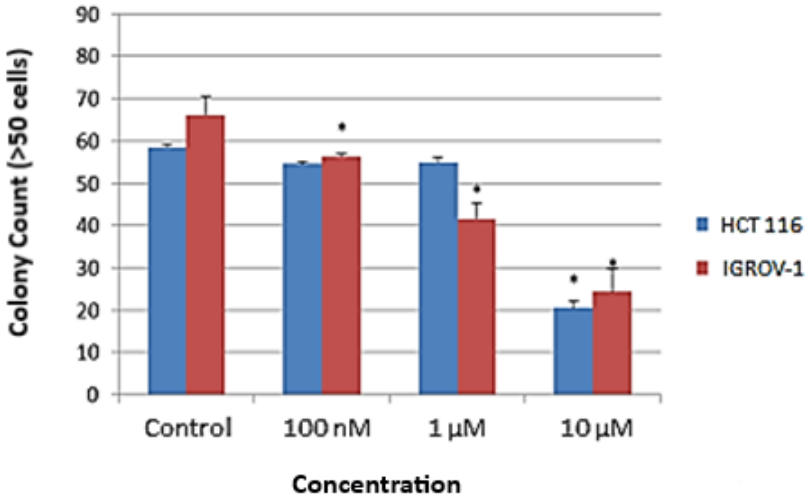


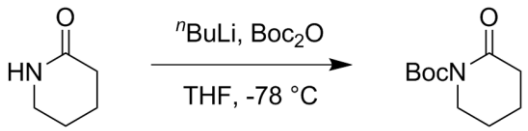
Figure 6.3: Effect of piperlongumine (**123**) and selected analogues **180**-**184** on clonogenic survival of HCT 116 and IGROV-1 cells. Data shown are mean ± SD (*n* = 6). One way ANOVA was used to calculate significant inhibition (*p < 0.05)

6.6 Determination of Reactive Oxygen Species

The ROS-Glo™ H_2O_2 (Promega) assay allows detection of ROS that are generated in cell culture. Cells were seeded at 5×10^3 in 96-well opaque white plates in 80 µL RPMI-1640 supplemented with 10% FBS. After 24 h, test agents were added to the wells (final concentrations 1 µM, 5 µM and 10 µM, *n* = 3). Immediately, or following 18 h incubation, 20 µL H_2O_2 substrate solution was added to wells. ROS-Glo™ detection solution was added to each well (100 µL) then incubated at room temperature for 20 min in the dark. Relative luminescence at wavelength 700 nm was recorded using a multilabel plate reader.

6.7 Experimental Procedures and Characterisation Data

N-(*tert*-Butyloxycarbonyl)piperidine-2-one (167)

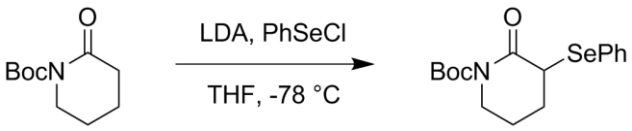


37 **167**

δ-Valerolactam (**37**) (1.00 g, 10.09 mmol, 1.0 equiv.) was dissolved in anhydrous THF (120 mL) and cooled to −78 °C. *n*-BuLi (2.43 M, 4.15 mL, 1.0 equiv.) was added dropwise and the solution was stirred for 30 min. A solution of Boc_2O (3.30 g, 15.13 mmol, 1.5 equiv.) in anhydrous THF (30 mL) was added dropwise and then the reaction was allowed to stir at room temperature for 16 h. The reaction was then quenched with saturated aqueous NH_4Cl (20 mL) and extracted with Et_2O (3 × 40 mL). The combined organic layers were dried

over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 20-30% EtOAc in petroleum ether 40-60 °C) to give the desired product **167** (1.96 g, 98%) as a colourless oil. δ_H(400 MHz, $CDCl_3$) 1.52 (9H, s, $C(CH_3)_3$), 1.81-1.83 (4H, m, 4-H_2 and 5-H_2), 2.48-2.52 (2H, m, 3-H_2), 3.63-3.66 (2H, m, 6-H_2); δ_C(100 MHz, $CDCl_3$) 20.7 and 22.9 (C-4 and C-5), 28.2 ($C(CH_3)_3$), 35.0 (C-3), 46.4 (C-6), 83.0 ($C(CH_3)_3$), 152.9 ($NCO_2C(CH_3)_3$), 171.5 (C-2). Spectroscopic data in accordance with the literature.[145]

***tert*-Butyl 2-oxo-3-(phenylselanyl)piperidine-1-carboxylate (166)**

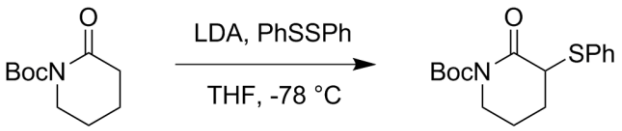


167 **166**

To a stirring solution of diisopropylamine (840 µL, 5.93 mmol, 1.2 equiv.) in anhydrous THF (8 mL) at -78 °C was added *n*-BuLi (2.198 M, 2.47 mL, 1.1 equiv.) and the resulting solution stirred for 30 min. The reaction mixture was then transferred into a stirring solution of *N*-(*tert*-butyloxycarbonyl)piperidine-2-one (**167**) (985 mg, 4.94 mmol, 1.0 equiv.) in anhydrous THF (3 mL) at -78 °C. The reaction was then stirred for 30 minutes before transferring into a stirred solution of phenylselenyl chloride (1.14 g, 5.93 mmol, 1.2 equiv) in anhydrous THF (4.5 mL) at -78 °C. The reaction mixture was allowed to warm to room temperature over 3 hours and was then quenched with H_2O (10 mL) and separated. The organic extract was washed with brine (10 mL), separated and

dried over anhydrous $MgSO_4$. After filtering the solvent was evaporated under reduced pressure and the resulting crude residue purified by column chromatography (0-10% ethyl acetate in petroleum ether) to deliver the desired product **166** (705 mg, 40%) a colourless oil. δ_H(400 MHz, $CDCl_3$) 1.53 (9H, s, $C(CH_3)_3$), 1.77 (1H, m, 5-*H*H), 2.02 (2H, m, 4-*H*H and 5-H*H*), 2.22 (1H, m, 4-H*H*), 3.60 (1H, m, 6-*H*H), 3.69 (1H, m, 6-H*H*), 3.97 (1H, t, *J* 5.7, 3-H), 7.32 (3H, m, ArH), 7.66 (2H, m, ArH). Spectroscopic data in accordance with the literature.[146]

_N_-(_tert_-Butyloxycarbonyl)-3-(phenylthio)piperidine-2-one (171)

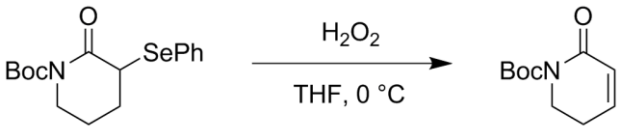


167 **171**

A solution of diisopropylamine (4.97 mL, 35.456 mmol, 1.1 equiv.) in anhydrous THF (32.3 mL) was cooled to −78 °C. *n*-BuLi (2.5 M, 12.9 mL, 32.233 mmol, 1.0 equiv.) was added dropwise and the reaction stirred at −78 °C for 30 min. A solution of *N*-(*tert*-butyloxycarbonyl)piperidine-2-one (**167**) (6.422 mmol, 32.233 mmol, 1.0 equiv.) in anhydrous THF (32.3 mL) was added dropwise and the resulting mixture stirred at −78 °C for 30 min. The reaction mixture was then added to a solution of diphenyl disulfide (7.038 g, 32.233 mmol, 1.0 equiv.) in anhydrous THF (64.5 mL) and stirred at −78 °C for another 90 min before quenching by the addition of saturated aqueous NH_4Cl (50 mL). The aqueous phase was extracted with Et_2O (3 × 100 mL) and the organic layers

combined, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 10-15% EtOAc in petroleum ether 40-60 °C) to give the desired product **171** (6.936 g, 70%) as a colourless oil. δ_H(400 MHz, $CDCl_3$) 1.52 (9H, s, $C(CH_3)_3$), 1.79 (1H, m, 5-*H*H), 1.97-2.09 (2H, m, 4-*H*H and 5-H*H*), 2.22 (1H, m, 4-H*H*), 3.83 (1H, m, 6-*H*H), 3.74 (1H, m, 6-H*H*), 3.85 (1H, t, *J* 6.0, 3-H), 7.28-7.32 (3H, m, ArH), 7.52-7.55 (2H, m, ArH); δ_C(100 MHz, $CDCl_3$) 20.8 (C-5), 28.2 ($C(CH_3)_3$), 28.3 (C-4), 45.9 (C-6), 51.6 (C-3), 83.2 ($C(CH_3)_3$), 128.3 (C-4'), 129.1 (C-2' or C-3'), 133.5 (C-1'), 133.7 (C-2' or C-3'), 153.3 ($NCO_2C(CH_3)_3$), 169.9 (C-2).

***tert*-Butyl 5,6-dihydro-2-oxopyridine-1(2*H*)-carboxylate (168) A**

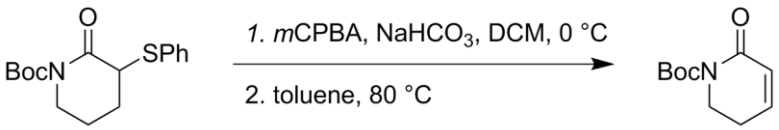


166 **168**

To a stirring solution of *N*-(*tert*-butyloxycarbonyl)-3-(phenylseleno)piperidine-2-one (**166**) (705 mg, 2.00 mmol, 1.0 equiv.) in THF (4 mL) at 0 °C was added a solution of 30% H_2O_2 (0.35 mL, 5.5 mmol, 2.75 equiv.) and the resulting mixture stirred for 1 hour. The reaction mixture was then washed with 10% aqueous NaOH solution (3 mL) and brine (3 mL) and the organic fraction dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure. The resulting crude material was purified by column chromatography (10-20% ethyl acetate in petroleum ether) to deliver the desired product **168** (156 mg,

40%) a colourless oil. ν_{max}/cm^{-1} 3010, 2984, 2093, 1759, 1709; δ_H(400 MHz, $CDCl_3$) 1.54 (9H, s, $C(CH_3)_3$), 2.40 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 3.85 (2H, t, *J* 6.4, 6-H_2), 5.95 (1H, dt, *J* 9.8, 1.9, 3-H), 6.77 (1H, dt, *J* 9.8, 4.2, 4-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 28.2 ($C(CH_3)_3$), 43.7 (C-6), 83.1 ($C(CH_3)_3$), 126.5 (C-3), 143.6 (C-4), 152.8 (NCO), 163.9 (C-2); Found (ESI): 198.1118 $[MH]^+$ (required $C_{10}H_{16}NO_3$ 198.1125); Found (ESI): 220.0946 $[MNa]^+$ (required $C_{10}H_{15}NO_3Na$ 220.0944). Spectroscopic data in accordance with the literature.[146]

tert-Butyl 5,6-dihydro-2-oxopyridine-1(2*H*)-carboxylate (168) B

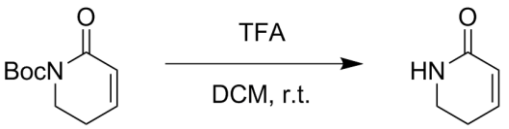


171 **168**

N-(*tert*-Butyloxycarbonyl)-3-(phenylthio)piperidine-2-one (**171**) (3.24 g, 10.54 mmol, 1.0 equiv.) was dissolved in DCM (116 mL) and saturated aqueous $NaHCO_3$ (22 mL) was added. The solution was cooled to 0 °C and *m*CPBA (1.82 g, 10.54 mmol, 1.0 equiv.) added portion wise and the reaction stirred at room temperature for 2 h. The aqueous phase was extracted with DCM (3 × 100 mL) and the combined organic fraction was dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The residue was then dissolved in toluene (34 mL) and heated to 80 °C. The reaction was stirred for 3 h before removal of the solvent under reduced pressure. The resulting crude product was purified by flash column chromatography (SiO_2, 20-40% EtOAc in

petroleum ether 40-60 °C) to give the desired product **168** (2.06 g, 99%) as a colourless oil. Spectroscopic data in accordance with the literature.[147]

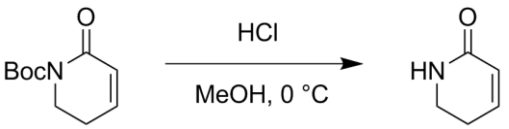
5,6-Dihydropyridin-2(1*H*)-one (130) A



168 **130**

To a stirred mixture of TFA/DCM (1 : 1, 3.8 mL) was added *tert*-butyl 5,6-dihydro-2-oxopyridine-1(2*H*)-carboxylate (**168**) (156 mg, 0.79 mmol) and the resulting mixture stirred at room temperature for 1 hour. The reaction mixture was then cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$ (3 mL) and extracted with Et_2O (5 x 10 mL). The organic fractions were combined, dried over anhydrous $MgSO_4$, filtered and the solvent evaporated to yield the desired product **130** (21 mg, 27%) a white solid. m.p. 61-62 °C; ν_{max}/cm^{-1} 3691, 3424, 3009, 1677, 1609; δ_H(400 MHz, $CDCl_3$) 2.32 (2H, tdd, *J* 7.2, 4.2, 1.9, 5-H), 3.40 (2H, td, *J* 7.2, 2.7, 6-H), 5.87 (1H, dq, *J* 9.9, 1.9, 3-H), 6.62 (1H, dt, *J* 9.9, 4.2, 4-H), 6.65 (1H, br s, NH); δ_C(100 MHz, $CDCl_3$) 23.9 (C-5), 39.6 (C-6), 124.9 (C-3), 141.6 (C-4), 166.7 (C-2). Spectroscopic data in accordance with the literature.[147]

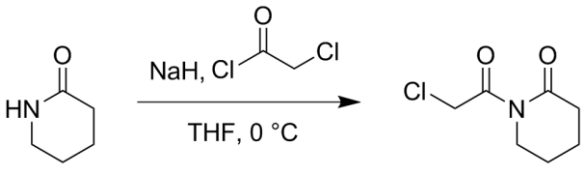
5,6-Dihydropyridin-2(1*H*)-one (130) B



168 **130**

tert-Butyl 5,6-dihydro-2-oxopyridine-1(2*H*)-carboxylate (**168**) (2.08 g, 10.54 mmol, 1.0 equiv.) was dissolved in MeOH (51.5 mL) and cooled to 0 °C. A solution of aqueous HCl (6 M, 15.4 mL, 8.8 equiv) was added dropwise and the reaction stirred at 0 °C for 1 h before concentrating under reduced pressure. The residue was treated with Et_3N (5 mL) and then concentrated under reduced pressure. The resulting crude product was purified by flash column chromatography (SiO_2, 5-10% MeOH in DCM) to give the desired product **130** (1.00g, 98%) as a white solid. Spectroscopic data in accordance with the literature.[147]

1-(2-Chloroacetyl)piperidin-2-one (174)

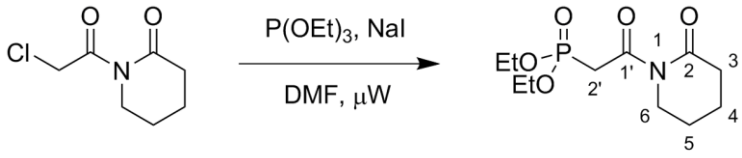


37 **174**

To a solution of δ-valerolactam (**37**) (100 mg, 1.00 mmol, 1.0 equiv.) in anhydrous THF (3 mL) at 0 °C was added a solution of NaHMDS (2M, 0.5 mL, 1.0 equiv.) and the reaction mixture warmed to room temperature and stirred for 3 hours. Chloroacetyl chloride (48 µL, 1.00 mmol, 1.0 equiv.) was then

added and the reaction stirred overnight. Solvent was then removed and the crude residue purified by column chromatography (0-20% ethyl acetate in petroleum ether) to yield the desired product **174** (117 mg, 68%) a pale yellow solid. δ_H(400 MHz, $CDCl_3$) 1.87 (4H, dt, *J* = 6.9, 3.4, 4-H_2 and 5-H_2), 2.59 (2H, m, 3-H_2), 3.79 (2H, m, 6-H_2), 4.75 (2H, s, 2'-H_2).

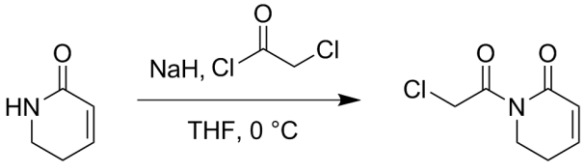
Diethyl (2-oxo-2-(2-oxopiperidin-1-yl)ethyl)phosphonate (173)



174 **173**

To a solution of 1-(2-chloroacetyl)piperidin-2-one (**174**) (120 mg, 0.68 mmol, 1.0 equiv.) was dissolved in DMF (2.7 mL), and NaI (0.020 g, 0.134 mmol, 0.2 equiv.) and $P(OEt)_3$ (0.919 mL, 5.357 mmol, 7.9 equiv.) were added. The reaction mixture was heated by microwave (300 W, 110 °C) for 10 min. The DMF was removed by reduced pressure distillation and the resulting crude product purified by flash chromatography (SiO_2, 1% MeOH in DCM) to give the desired product **173** (131 mg, 72%) a yellow oil. δ_H(400 MHz, CD_3CN) 1.26 (6H, m, 2 x OCH_2CH_3), 1.79 (4H, m, 4-H_2 and 5-H_2), 2.51 (2H, m, 3-H_2), 3.66 (2H, m, 6-H_2), 3.71 (2H, d, *J* = 21.5, 2'-H_2), 4.06 (4H, dq, *J* = 8.2, 7.1, 2 x OCH_2CH_3).

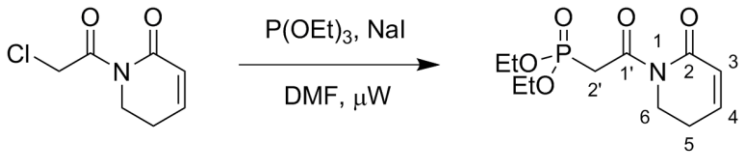
1-(2-Chloroacetyl)-5,6-dihydropyridin-2(1*H*)-one (172)



130 **172**

5,6-Dihydropyridin-2(1*H*)-one (**130**) (2.80 g, 28.83 mmol, 1.0 equiv.) was dissolved in anhydrous THF (144 mL) under an atmosphere of argon and cooled to 0 °C. NaH (60%, 1.27 g, 31.71 mmol, 1.1 equiv.) was added in portions and the reaction stirred at 0 °C for 1 h. Chloroacetyl chloride (2.52 mL, 31.71 mmol, 1.1 equiv.) was then added dropwise and the reaction stirred at room temperature overnight. Saturated aqueous NH_4Cl (200 mL) was added and the resulting mixture extracted with EtOAc (5 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product obtained was purified by flash column chromatography (SiO_2, 20-30% EtOAc in petroleum ether 40-60 °C) to give the desired product **172** (3.25 g, 65%) as a yellow oil. ν_{max}/cm^{-1} 3691, 3504, 3007, 2928, 1723, 1677, 1601; δ_H(400 MHz, $CDCl_3$) 2.48 (2H, tdd, *J* 6.6, 4.2, 1.9, 5-H_2), 4.04 (2H, t, *J* 6.6, 6-H_2), 4.77 (2H, s, 2'-H_2), 6.03 (1H, dt, *J* 9.8, 1.9, 3-H), 6.97 (1H, dt, *J* 9.8, 4.2, 4-H); δ_C(100 MHz, $CDCl_3$) 24.6 (C-5), 41.8 (C-6), 47.5 (C-2'), 125.2 (C-3), 146.4 (C-4); Found (ESI): 174.0318 [MH^+] (required $C_7H_9ClNO_2$ 174.0316); Found (ESI): 196.0143 $[MNa]^+$ (required $C_7H_8ClNaNO_2$ 196.0136).

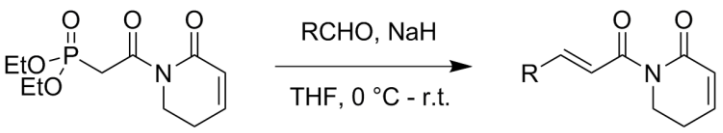
Diethyl (4-oxo-4,7-dihydropyridin-3(2*H*)-yl))phosphonacetate (169)



172 **169**

1-(2-Chloroacetyl)-5,6-dihydropyridin-2(1*H*)-one (**172**) (0.233 g, 1.339 mmol, 1.0 equiv.) was dissolved in DMF (2.7 mL), and NaI (0.020 g, 0.134 mmol, 0.1 equiv.) and $P(OEt)_3$ (0.919 mL, 5.357 mmol, 4.0 equiv.) were added. The reaction mixture was heated by microwave (300 W, 110 °C) for 10 min. The DMF was removed by reduced pressure distillation and the resulting crude product purified by flash chromatography (SiO_2, 1% MeOH in DCM) to give the desired product **169** (0.225 g, 61%) as a pale yellow oil. ν_{max}/cm^{-1} 3691, 2999, 1695; δ_H(400 MHz, $CDCl_3$) 1.33 (6H, t, *J* 7.1, 2 × OCH_2CH_3), 2.44 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 3.83 (2H, d, *J* 21.2, 2'-H_2), 4.00 (2H, t, *J* 6.4, 6-H_2), 4.16 (4H, dq, *J* 8.1, 7.1, 2 × OCH_2CH_3), 6.01 (1H, dt, *J* 9.7, 1.7, 3-H), 6.93 (1H, dt, *J* 9.7, 4.2, 4-H); δ_C(100 MHz, $CDCl_3$) 16.3 (2 × OCH_2CH_3), 24.5 (C-5), 37.6 (C-1), 41.4 (C-6), 62.4 (2 × OCH_2CH_3), 125.4 (C-3), 146.1 (C-4), 165.4 (C-1'), 167.7 (C-2); Found (ESI): 298.0799 $[MNa]^+$, (required $C_{11}H_{18}NO_5PNa$ 298.0815).

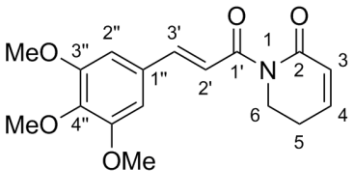
General procedure for the synthesis of piperlongumine analogues by Horner-Wadsworth-Emmons olefination



169

Diethyl (4-oxo-4,7-dihydropyridin-3(2*H*)-yl))phosphonacetate (**169**) (1 equiv.) was dissolved in anhydrous THF (0.05 M) under an atmosphere of argon and the solution cooled to 0 °C. NaH (1.1 equiv.) was added and the resulting mixture stirred until no further effervescence was observed. The aldehyde (2 equiv.) was then added and the reaction warmed to room temperature and stirred overnight. Saturated aqueous NH_4Cl was added and the aqueous phase extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography.

Piperlongumine (123)

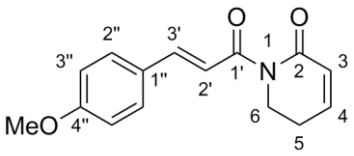


123

Piperlongumine (**123**) (0.014 g, 51%) was prepared using the general procedure as a white solid. m.p.: 120-121 °C; ν_{max}/cm^{-1} 3692, 3606, 1686, 1603, 1583; δ_H(400 MHz, $CDCl_3$) 2.47 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 3.86 (3H, s, OMe),

3.87 (6H, s, 2 × OMe), 4.02 (2H, t, *J* 6.4, 6-H_2), 6.04 (1H, dt, *J* 9.7, 1.9, 3-H), 6.79 (2H, s, ArH), 6.93 (1H, dt, *J* 9.7, 4.2, 4-H), 7.41 (1H, d, *J* 15.6, 2'-H), 7.87 (1H, d, *J* 15.6, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 56.3 (2 × 3''-OMe), 61.0 (4''-OMe), 105.6 (2 × C-2''), 121.2 (C-2'), 125.9 (C-3), 128.3 and 139.9 (C-1'' and C-4''), 130.7 (2 × C-3''), 143.9 (C-3'), 145.7 (C-4), 166.0 (C-1'), 169.0 (C-2); Found (ESI): 318.1327 $[MH]^+$, (required $C_{17}H_{20}NO_5$ 318.1336); Found (ESI): 340.1153 $[MNa]^+$, (required $C_{17}H_{19}NO_5Na$ 340.1155). Spectroscopic data in accord with the literature.[147]

(*E*)-1-(3-(4-methoxyphenyl)prop-2-enoyl)-5,6-dihydropyridin-2(1*H*)-one (176)

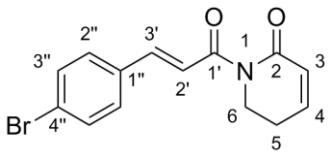


176

(*E*)-1-(3-(4-methoxyphenyl)prop-2-enoyl)-5,6-dihydropyridin-2(1*H*)-one (**176**) (0.023 g, 52%) was prepared using the general procedure as a white solid. m.p.: 93-94 °C; ν_{max}/cm^{-1} 3691, 3606, 3011, 2937, 2840, 1686, 1601, 1513; δ_H(400 MHz, $CDCl_3$) 2.46 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 3.83 (3H, s, OMe), 4.03 (2H, t, *J* 6.4, 6-H_2), 6.03 (1H, dt, *J* 9.7, 1.9, 3-H), 6.87-6.90 (2H, m, ArH), 6.92 (1H, dt, *J* 9.7, 4.2, 4-H), 7.41 (1H, d, *J* 15.6, 2'-H), 7.49-7.57 (2H, m, ArH), 7.73 (1H, d, *J* 15.6, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.8 (C-5), 41.6 (C-6), 55.4 (OMe), 114.2 (2 × C-3''), 119.4 (C-2'), 126.0 (C-3), 127.9 (C-1''), 130.0 (2 × C-2''), 143.6 (C-3'), 145.3 (C-4), 161.3 (C-4''), 165.8 (C-1'), 169.2 (C-2); Found (ESI): 258.1124 $[MH]^+$,

(required $C_{15}H_{16}NO_3$ 258.1125); Found (ESI): 280.0943 $[MNa]^+$, (required $C_{15}H_{15}NO_3Na$ 280.0944). Spectroscopic data in accord with the literature.[119]

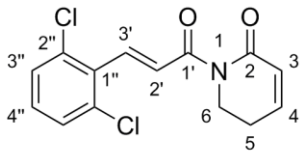
(*E*)-1-(3-(4-Bromophenyl)acryloyl)-5,6-dihydropyridin-2(1*H*)-one (177)



177

(*E*)-1-(3-(4-Bromophenyl)acryloyl)-5,6-dihydropyridin-2(1*H*)-one (**177**) (0.023 g, 60%) was prepared using the general procedure as a white solid. m.p.: 93-94 °C; ν_{max}/cm^{-1} 3692, 3607, 1686, 1618, 1602; δ_H(400 MHz, $CDCl_3$) 2.48 (2H, tdd, *J* 6.5, 4.2, 1.9, 5-H_2), 4.03 (2H, t, *J* 6.5, 6-H_2), 6.04 (1H, dt, *J* 9.7, 1.9, 3-H), 6.95 (1H, dt, *J* 9.7, 4.2, 4-H), 7.42-7.46 and 7.48-7.52 (4H, m, ArH), 7.49 (1H, d, *J* 15.6, 2'-H), 7.65 (1H, d, *J* 15.6, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 122.7 (C-2'), 124.4 (C-4''), 125.9 (C-3), 129.8-132.1 (2 × C-2'' and 2 × C-3''), 134.2 (C-1''), 142.2 (C-3'), 145.8 (C-4), 165.9 (C-1'), 169.0 (C-2); Found (ESI): 306.0123 $[MH]^+$, (required $C_{14}H_{13}BrNO_2$ 306.0124); Found (ESI): 327.9939 $[MNa]^+$, (required $C_{14}H_{12}BrNO_2Na$ 327.9944). Spectroscopic data in accord with the literature.[147]

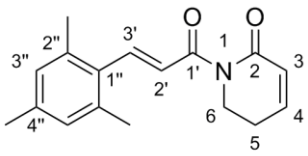
(E)-1-(3-(2,6-Dichlorophenyl)acryloyl)-5,6-dihydropyridin-2(1H)- one (178)



178

(E)-1-(3-(2,6-Dichlorophenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**178**) (0.015 g, 67%) was prepared using the general procedure as a white solid. m.p.: 126-127 °C; ν_{max}/cm^{-1} 3692, 3606, 3083, 1688, 1624, 1602; δ_H(400 MHz, $CDCl_3$) 2.49 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 4.06 (2H, t, *J* 6.4, 6-H_2), 6.03 (1H, dt, *J* 9.7, 1.9, 3-H), 6.94 (1H, dt, *J* 9.7, 4.2, 4-H), 7.17 (1H, t, *J* 8.1, 4''-H), 7.34 (2H, d, *J* 8.1, 2 × 3''-H), 7.59 (1H, d, *J* 16.0, 2'-H), 7.82 (1H, d, *J* 16.0, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 125.8 (C-3), 128.9 (2 × C-3''), 129.7 and 130.1 (C-2' and C-4''), 132.8 (C-1''), 135.3 (2 × C-2''), 136.7 (C-3'), 145.8 (C-4), 165.7 (C-1''), 168.5 (C-2); Found (ESI): 296.0240 $[MH]^+$, (required $C_{14}H_{12}Cl_2NO_2$ 296.0240); Found (ESI): 318.0057 $[MNa]^+$, (required $C_{14}H_{11}Cl_2NO_2Na$ 318.0059).

(*E*)-1-(3-Mesitylacryloyl)-5,6-dihydropyridin-2(1*H*)-one (179)

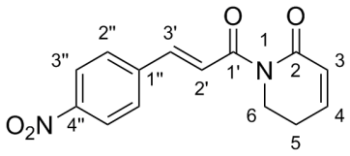


179

(*E*)-1-(3-Mesitylacryloyl)-5,6-dihydropyridin-2(1*H*)-one (**179**) (0.020 g, 57%) was prepared using the general procedure as a pale yellow solid. m.p.: 104-105 °C, ν_{max}/cm^{-1} 3691, 3067, 3011, 2926, 2857, 1687, 1608,; δ_H(400 MHz,

$CDCl_3$) 2.28 (3H, s, 4''-CH_3), 2.38 (6H, s, 2 × 2''-CH_3), 2.48 (2H, tdd, *J* 6.4, 4.2, 1.8, 5-H_2), 4.05 (2H, t, *J* 6.4, 6-H_2), 6.02 (1H, dt, *J* 9.7, 1.8, 3-H), 6.89 (2H, s, 2 × 3''-H), 6.93 (1H, dt, *J* 9.7, 4.2, 4-H), 7.09 (1H, d, *J* 16.0, 2'-H), 7.91 (1H, d, *J* 16.0, 3'-H); δ_C(100 MHz, $CDCl_3$) 21.2 (4''-CH_3), 21.3 (2 × 2''-CH_3), 24.9 (C-5), 41.8 (C-6), 126.0 (C-2'), 126.8 (C-3), 129.3 (2 × C-2''), 131.7 and 138.3 (C-1'' and C-4''), 137.4 (2 × C-3''), 142.0 (C-3'), 145.5 (C-4), 165.8 (C-1'), 169.2 (C-2); Found (ESI): 270.1494 $[MH]^+$, (required $C_{17}H_{20}NO_2$ 270.1489); Found (ESI): 292.1314 $[MNa]^+$, (required $C_{17}H_{19}NO_2Na$ 292.1308). Spectroscopic data in accord with the literature.[147]

(E)-1-(3-(4-Nitrophenyl)acryloyl)-5,6-dihydropyridin-2(1H)- one (180)

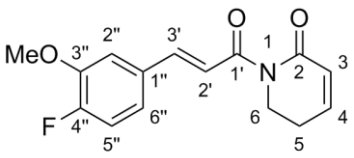


180

(E)-1-(3-(4-Nitrophenyl)acryloyl)-5,6-dihydropyridin-2(1H)- one (**180**) (0.010 g, 45%) was prepared using the general procedure as a white solid. m.p.: 178-180 °C; ν_{max}/cm^{-1} 3691, 3606, 1688, 1602, 1522; δ_H(400 MHz, $CDCl_3$) 2.51 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 4.05 (2H, t, *J* 6.4, 6-H_2), 6.06 (1H, dt, *J* 9.7, 1.9, 3-H), 6.98 (1H, dt, *J* 9.7, 4.2, 4-H), 7.58 (1H, d, *J* 15.7, 2'-H), 7.70 (1H, d, *J* 15.7, 3'-H), 7.69-7.72 and 8.21-8.24 (each 2H, each m, 2 × 2''-H and 2 × 3''-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 124.2 and 128.9 (2 × C-2'' and 2 × C-3''), 125.7 (C-3), 126.3 (C-2'), 140.0 (C-3'), 141.5 (C-1''), 146.2 (C-4), 148.4 (C-4''), 165.9 (C-

1'), 168.3 (C-2); Found (ESI): 273.0877 $[MH]^+$, (required $C_{14}H_{13}N_2O_4$ 273.0870); Found (ESI): 295.0690 $[MNa]^+$, (required $C_{14}H_{12}N_2O_4Na$ 295.0689).

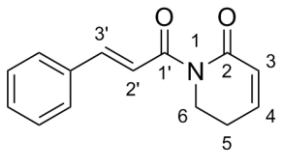
(E)-1-(3-(4-Fluoro-3 methoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (181)



181

(E)-1-(3-(4-Fluoro-3 methoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**181**) (0.013 g, 52%) was prepared using the general procedure as a white solid. m.p.: 112-114 °C; ν_{max}/cm^{-1} 3692, 3607, 3012, 2928, 2855, 1687, 1602, 1515; δ_H(400 MHz, $CDCl_3$) 2.48 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 3.92 (3H, s, OMe), 4.04 (2H, t, *J* 6.4, 6-H_2), 6.05 (1H, dt, *J* 9.7, 1.9, 3-H), 6.95 (1H, dt, *J* 9.7, 4.2, 4-H), 7.04-7.17 (3H, m, 2''-H, 5''-H and 6''-H), 7.43 (1H, dd, *J* 15.6, 0.6, 2'-H), 7.68 (1H, d, *J* 15.6, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 56.4 (OMe), 112.6 (d, *J* 2.3, C-6''), 116.5 (d, *J* 18.5, C-5''), 121.8 (d, *J* 2.3, C-2''), 122.1 (d, *J* 7.5, C-2'), 126.0 (C-3), 131.9 (d, *J* 3.9, 1''), 142.9 (C-3'), 145.7 (C-4), 148.1 (d, *J* 10.8, C-3''), 153.8 (d, *J* 251.5, C-4''), 166.0 (C-1'), 168.9 (C-2); δ_F(376 MHz, $CDCl_3$) -131.6; Found (ESI): 276.1023 $[MH]^+$, (required $C_{15}H_{15}FNO_3$ 276.1030); Found (ESI): 298.0842 $[MNa]^+$, (required $C_{15}H_{14}FNO_3Na$ 298.0850).

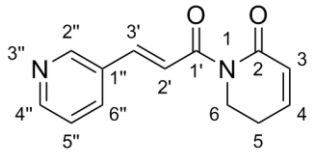
(*E*)-1-Cinnamoyl-5,6-dihydropyridin-2(1*H*)-one (182)



182

(*E*)-1-Cinnamoyl-5,6-dihydropyridin-2(1*H*)-one (**182**) (0.015 g, 49%) was prepared using the general procedure as a yellow oil. δ_H(400 MHz, $CDCl_3$) 2.42 (2H, tdd, *J* 6.4, 4.2, 1.8, 5-H_2), 4.02 (2H, t, *J* 6.4, 6-H_2), 6.01 (1H, dt, *J* 9.7, 1.8, 3-H), 6.26-7.39 (2H, m, ArH), 6.93 (1H, dt, *J* 9.7, 4.2, 4-H), 7.09 (1H, d, *J* 16.0, 2'-H), 7.44-7.61 (3H, m, ArH), 7.91 (1H, d, *J* 16.0, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 121.8 (Ar), 126.0 (C-2'), 126.8 (C-3), 128.3, 128.7 and 130.0 (Ar), 143.7 (C-3'), 145.0 (C-4), 166.2 (C-1'), 168.5 (C-2); Found (ESI): 250.0847 $[MNa]^+$, (required $C_{14}H_{13}NO_2Na$ 250.0838). Spectroscopic data in accord with the literature.[147]

(E)-1-(3-(Pyridin-3-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (183)

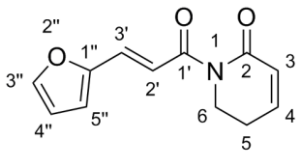


183

(E)-1-(3-(Pyridin-3-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**183**) (0.013 g, 52%) was prepared using the general procedure as a white solid. m.p.: 113-114 °C; ν_{max}/cm^{-1} 3691, 3606, 3091, 1686, 1623, 1602; δ_H(400 MHz, $CDCl_3$) 2.49 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 4.04 (2H, t, *J* 6.4, 6-H_2), 6.04 (1H, dt, *J* 9.7, 1.9, 3-

H), 6.96 (1H, dt, *J* 9.7, 4.2, 4-H), 7.31 (1H, dd, *J* 7.8, 4.6, 5''-H), 7.55 (1H, d, *J* 15.6, 2'-H), 7.69 (1H, d, *J* 15.6, 3'-H), 7.90 (1H, dt, *J* 7.7, 1.9, 6''-H), 8.58 (1H, d, *J* 4.6, 4''-H), 8.76 (1H, s, 2''-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 123.8 (C-5''), 124.1 (C-2'), 125.8 (C-3), 134.4 (C-6''), 139.6 (C-3'), 145.9 (C-4), 150.2 (C-2''), 150.8 (C-4''), 165.9 (C-1'), 168.5 (C-2); Found (ESI): 229.0966 $[MH]^+$, (required $C_{13}H_{13}N_2O_2$ 229.0972); Found (ESI): 251.0779 $[MNa]^+$, (required $C_{13}H_{12}N_2O_2Na$ 251.0791).

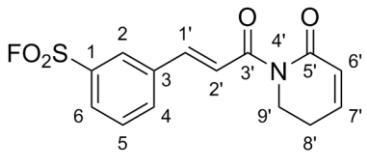
(E)-1-(3-Furan-2-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (184)



184

(E)-1-(3-Furan-2-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**184**) (0.028 g, 70%) was prepared using the general procedure as a yellow solid. m.p.: 51-53 °C; ν_{max}/cm^{-1} 3691, 3606, 3117, 3009, 2943, 2902, 1688, 1615; δ_H(400 MHz, $CDCl_3$) 2.45 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 4.01 (2H, t, *J* 6.4, 6-H_2), 6.03 (1H, dt, *J* 9.7, 1.9, 3-H), 6.45 (1H, dd, *J* 3.4, 1.8, 4''-H), 6.62 (1H, d, *J* 3.4, 5''-H), 6.91 (1H, dd, *J* 9.7, 4.2, 4-H), 7.36 (1H, d, *J* 15.4, 2'-H), 7.46 (1H, d, *J* 1.8, 3''-H), 7.51 (1H, d, *J* 15.4, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.8 (C-6), 112.4 and 114.9 (C-4'' and C-5''), 119.5 (C-2'), 126.0 (C-3), 130.3 (C-3''), 144.7 (C-3'), 145.5 (C-4), 151.8 (C-1''), 165.8 (C-1'), 168.9 (C-2); Found (ESI): 218.0813 $[MH]^+$, (required $C_{12}H_{12}NO_3$ 218.0812); Found (ESI): 240.0630 $[MNa]^+$, (required $C_{12}H_{11}NO_3Na$ 240.0631). Spectroscopic data in accord with the literature.[147]

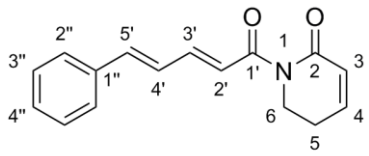
(1'E)-3-(3'-Oxo-3'-(5'-oxo-5',8'-dihydropyridin-4'(2H)-yl)prop-1'-enyl)-benzenesulfonyl fluoride (185)



185

(1'E)-3-(3'-Oxo-3'-(5'-oxo-5',8'-dihydropyridin-4'(2H)-yl)prop-1'-enyl)benzene-sulfonyl fluoride (**185**) (0.033 g, 44%) was prepared using the general procedure as a white solid. m.p. 145-146 °C; ν_{max}/cm^{-1} 3692, 3606, 3012, 2947, 2902, 1686, 1626, 1471; δ_H(400 MHz, $CDCl_3$) 2.51 (2H, tdd, *J* 6.4, 4.2, 1.9, 8'-H_2), 4.05 (2H, t, *J* 6.4, 9'-H_2), 6.06 (1H, dt, *J* 9.7, 1.9, 6'-H), 6.98 (1H, dt, *J* 9.7, 4.2, 7'-H), 7.56 (1H, d, *J* 15.7, 2'-H), 7.65 (1H, t, *J* 7.9, 5-H), 7.69 (1H, d, *J* 15.7, 1'-H), 7.94 and 7.98 (each 1H, each d, *J* 7.9, 4-H and 6-H), 8.15 (1H, s, 2-H); 24.9 (C-8'), 41.8 (C-9'), 125.6 (C-2'), 125.7 (C-6'), 127.7 (C-2), 129.1 and 134.7 (C-4 and C-6), 134.1 (d, *J* 24.6, C-1), 137.3 (C-3), 139.6 (C-1'), 146.2 (C-7'), 165.9 (C-3'), 168.2 (C-5'); δ_F(376 MHz, $CDCl_3$) 65.9; Found (EI^+): 309.0461 $[M]^+$, (required $C_{14}H_{12}NO_4FS$ 309.0471).

1-((2E,4E)-5-Phenylpenta-2,4-dienoyl)-5,6-dihydropyridin-2(1H)- one (186)



186

1-((2E,4E)-5-Phenylpenta-2,4-dienoyl)-5,6-dihydropyridin-2(1H)- one (**186**) (0.008 g, 29%) was prepared using the general procedure as a colourless oil. ν_{max}/cm^{-1} 3691, 3606, 3080, 1686, 1602; δ_H(400 MHz, $CDCl_3$) 2.46 (2H, tdd, *J* 6.4, 4.2, 1.9, 5-H_2), 4.02 (2H, t, *J* 6.4, 6-H), 6.03 (1H, dt, *J* 9.7, 1.9, 3-H), 6.88-7.03 (3H, m, ArH 4-H and 4'-H), 7.09 (1H, d, *J* 15.2, 2'-H), 7.27-7.39 (3H, m, ArH and 5'-H), 7.45-7.52 (2H, m, ArH), 7.55 (1H, dd, *J* 14.8, 9.5, 3'-H); δ_C(100 MHz, $CDCl_3$) 24.9 (C-5), 41.7 (C-6), 125.1 (C-2'), 126.0 (C-3), 127.3 and 128.9 (C-4', 2 × C-2'' and 2 × C-3''), 129.1 (C-4''), 136.4 (C-1''), 140.5 and 144.1 (C-3' and C-5'), 145.5 (C-4), 165.9 (C-1'), 169.0 (C-2); Found (ESI): 254.1182 $[MH]^+$, (required $C_{16}H_{16}NO_2$ 254.1176); Found (ESI): 276.0989 $[MNa]^+$, (required $C_{16}H_{15}NO_2Na$ 276.0995).

7 References

1. Lim, K.-H.; Hiraku, O.; Komiyama, K.; Kam, T.-S., Jerantinines A−G, Cytotoxic Aspidosperma Alkaloids from Tabernaemontana corymbosa. *Journal of Natural Products* **2008**, *71* (9), 1591-1594.

2. Cordell, G. A., The Aspidosperma Alkaloids. In *The Alkaloids: Chemistry and Physiology*, Rodrigo, R. G. A.; Manske, R. H. F., Eds. Academic Press: New York, 1979; Vol. 17, pp 199-384.

3. Lim, K. H.; Thomas, N. F.; Abdullah, Z.; Kam, T. S., Seco-tabersonine alkaloids from Tabernaemontana corymbosa. *Phytochemistry* **2009**, *70* (3), 424-429.

4. Oliveira, V. B.; Freitas, M. S. M.; Mathias, L.; Braz-Filho, R.; Vieira, I. J. C., Atividade biológica e alcalóides indólicos do gênero Aspidosperma (Apocynaceae): uma revisão. *Revista Brasileira de Plantas Medicinais* **2009**, *11* (1), 92-99.

5. Rowinsky, E. K.; Donehower, R. C., The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. *Pharmacology & Therapeutics* **1991**, *52* (1), 35-84.

6. Raja, V. J.; Lim, K.-H.; Leong, C.-O.; Kam, T.-S.; Bradshaw, T. D., Novel antitumour indole alkaloid, Jerantinine A, evokes potent G2/M cell cycle arrest targeting microtubules. *Investigational New Drugs* **2014**, *32* (5), 838-850.

7. (a) Qureshi, A. A.; Scott, A. I., Interconversion of Corynanthe, aspidosperma, and Iboga alkaloids. A model for indole alkaloid biosynthesis. *Chemical Communications (London)* **1968**, (16), 945-946; (b) O'Connor, S. E.; Maresh, J. J., Chemistry and biology of monoterpene indole alkaloid biosynthesis. *Natural Product Reports* **2006**, *23* (4), 532-547.

8. Qu, Y.; Easson, M. L. A. E.; Froese, J.; Simionescu, R.; Hudlicky, T.; De Luca, V., Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. *Proceedings of the National Academy of Sciences* **2015**, *112* (19), 6224-6229.

9. (a) Nge, C.-E.; Chong, K.-W.; Thomas, N. F.; Lim, S.-H.; Low, Y.-Y.; Kam, T.-S., Ibogan, Aspidosperman, Vincamine, and Bisindole Alkaloids from a Malayan Tabernaemontana corymbosa: Iboga Alkaloids with C-20α Substitution. *Journal of Natural Products* **2016**, *79* (5), 1388-1399; (b) Abubakar, I. B.; Loh, H.-S., A review on ethnobotany, pharmacology and phytochemistry of Tabernaemontana corymbosa. *Journal of Pharmacy and Pharmacology* **2016**, *68* (4), 423-432.

10. Zhu, X.; Zeng, X.; Sun, C.; Chen, S., Biosynthetic pathway of terpenoid indole alkaloids in Catharanthus roseus. *Frontiers of Medicine* **2014**, *8* (3), 285-293.

11. Miettinen, K.; Dong, L.; Navrot, N.; Schneider, T.; Burlat, V.; Pollier, J.; Woittiez, L.; van der Krol, S.; Lugan, R.; Ilc, T.; Verpoorte, R.; Oksman-Caldentey, K.-M.; Martinoia, E.; Bouwmeester, H.; Goossens, A.; Memelink, J.; Werck-Reichhart, D., The seco-iridoid pathway from Catharanthus roseus. *Nature Communications* **2014**, *5*.

12. Collu, G.; Unver, N.; Peltenburg-Looman, A. M. G.; van der Heijden, R.; Verpoorte, R.; Memelink, J., Geraniol 10-hydroxylase1, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis. *FEBS Letters* **2001**, *508* (2), 215-220.

13. Geu-Flores, F.; Sherden, N. H.; Courdavault, V.; Burlat, V.; Glenn, W. S.; Wu, C.; Nims, E.; Cui, Y.; O'Connor, S. E., An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. *Nature* **2012**, *492* (7427), 138-142.

14. Murata, J.; Roepke, J.; Gordon, H.; De Luca, V., The Leaf Epidermome of Catharanthus roseus Reveals Its Biochemical Specialization. *The Plant Cell Online* **2008**, *20* (3), 524-542.

15. Irmler, S.; Schröder, G.; St-Pierre, B.; Crouch, N. P.; Hotze, M.; Schmidt, J.; Strack, D.; Matern, U.; Schröder, J., Indole alkaloid biosynthesis in Catharanthus roseus: new enzyme activities and identification of cytochrome P450 CYP72A1 as secologanin synthase. *The Plant Journal* **2008**, *24* (6), 797-804.

16. Mizukami, H.; Nordlov, H.; Lee, S.-L.; Scott, A. I., Purification and properties of strictosidine synthetase (an enzyme condensing tryptamine and secologanin) from Catharanthus roseus cultured cells. *Biochemistry* **1979**, *18* (17), 3760-3763.

17. Rueffer, M.; Kan-Fan, C.; Husson, H.-P.; Stöckigt, J.; Zenk, M. H., 4,21-Dehydrogeissoschizine, an intermediate in heteroyohimbine alkaloid biosynthesis. *Journal of the Chemical Society, Chemical Communications* **1979**, (22), 1016-1018.

18. Frei, R.; Staedler, D.; Raja, A.; Franke, R.; Sasse, F.; Gerber-Lemaire, S.; Waser, J., Total synthesis and biological evaluation of jerantinine E. *Angewandte Chemie International Edition* **2013**, *52* (50), 13373-13376.

19. De Simone, F.; Gertsch, J.; Waser, J., Catalytic selective cyclizations of aminocyclopropanes: formal synthesis of aspidospermidine and total synthesis of goniomitine. *Angew Chem Int Ed Engl* **2010**, *49* (33), 5767-70.

20. Noble, R. L.; Beer, C. T.; Cutts, J. H., Role of Chance Observations in Chemotherapy: Vinca Rosea. *Annals of the New York Academy of Sciences* **1958**, *76* (3), 882-894.

21. Mangeney, P.; Zo Andriamialisoa, R.; Langlois, N.; Langlois, Y.; Potier, P., Preparation of vinblastine, vincristine, and leurosidine, antitumor alkaloids from Catharanthus species (Apocynaceae). *Journal of the American Chemical Society* **1979**, *101* (8), 2243-2245.

22. (a) Mangeney, P.; Andriamialisoa, R. Z.; Langlois, N.; Langlois, Y.; Potier, P., A new class of antitumor compounds: 5'-nor and 5',6'-seco derivatives of vinblastine-type alkaloids. *The Journal of Organic Chemistry* **1979**, *44* (22), 3765-3768; (b) Mangeney, P.; Andriamialisoa, R. Z.; Lallemand, J. Y.; Langlois, N.; Langlois, Y.; Potier, P., 5'-Nor anhydrovinblastine. *Tetrahedron* **1979**, *35* (18), 2175-2179.

23. Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T., Synthesis of biologically active taxol analogs with modified phenylisoserine side chains. *Journal of Medicinal Chemistry* **1992**, *35* (22), 4230-4237.

24. Shenvi, R. A.; Guerrero, C. A.; Shi, J.; Li, C.-C.; Baran, P. S., Synthesis of (+)-Cortistatin A. *Journal of the American Chemical Society* **2008**, *130* (23), 7241-7243.

25. (a) Lee, H. M.; Nieto-Oberhuber, C.; Shair, M. D., Enantioselective Synthesis of (+)-Cortistatin A, a Potent and Selective Inhibitor of Endothelial Cell Proliferation. *Journal of the American Chemical Society* **2008**, *130* (50), 16864-16866; (b) Nicolaou, K. C.; Sun, Y.-P.; Peng, X.-S.; Polet, D.; Chen, D. Y. K., Total Synthesis of (+)-Cortistatin A. *Angewandte Chemie International Edition* **2008**, *47* (38), 7310-7313.

26. (a) Waldvogel, S.; Malkowsky, I.; Nieger, M.; Kataeva, O., Synthesis and Properties of Optically Pure Phenols Derived from (+)-Dehydroabietylamine. *Synthesis* **2007**, *2007* (5), 773-778; (b) Saeed, M.; Zahid, M.; Rogan, E.; Cavalieri, E., Synthesis of the catechols of natural and synthetic estrogens by using 2-iodoxybenzoic acid (IBX) as the oxidizing agent. *Steroids* **2005**, *70* (3), 173-178.

27. Philippe, G.; Angenot, L.; Tits, M.; Frédérich, M., About the toxicity of some Strychnos species and their alkaloids. *Toxicon* **2004**, *44* (4), 405-416.

28. (a) Woodward, R. B.; Cava, M. P.; Ollis, W. D.; Hunger, A.; Daeniker, H. U.; Schenker, K., The Total Synthesis of Strychnine. *Journal of the American Chemical Society* **1954**, *76* (18), 4749-4751; (b) Woodward, R. B.; Cava, M. P.; Ollis, W. D.; Hunger, A.; Daeniker, H. U.; Schenker, K., The total synthesis of strychnine. *Tetrahedron* **1963**, *19* (2), 247-288.

29. (a) Morrison, R. W.; Bliss, A. R., The Strychnine-Brucine Ratio of Nux Vomica and the Relative Potency of these Alkaloids. *The Journal of the American Pharmaceutical Association (1912)* **1932**, *21* (7), 648-658; (b) Teske,

J.; Weller, J. P.; Albrecht, U. V.; Fieguth, A., Fatal Intoxication Due to Brucine. *Journal of Analytical Toxicology* **2011**, *35* (4), 248-253.

30. Marckwald, W., Ueber asymmetrische Synthese. *Berichte der deutschen chemischen Gesellschaft* **1904**, *37* (1), 349-354.

31. Tedeschi, E.; Dukler, S.; Pfeffer, P.; Lavie, D., Studies on strychnine derivatives and conversion into brucine. *Tetrahedron* **1968**, *24* (12), 4573-4580.

32. BlaskÓ, G.; Cordell, G. A., Isolation, Structure Elucidation, and Biosynthesis of the Bisindole Alkaloids of Catharanthus. In *The Alkaloids: Antitumor Bisindole Alkaloids from Catharanthus roseus (L.)*, Brossi, A.; Stuffness, M., Eds. Academic Press: New York, 1990; Vol. 37, pp 1-76.

33. (a) Kato, D.; Sasaki, Y.; Boger, D. L., Asymmetric Total Synthesis of Vindoline. *Journal of the American Chemical Society* **2010**, *132* (11), 3685-3687; (b) Ishikawa, H.; Colby, D. A.; Seto, S.; Va, P.; Tam, A.; Kakei, H.; Rayl, T. J.; Hwang, I.; Boger, D. L., Total Synthesis of Vinblastine, Vincristine, Related Natural Products, and Key Structural Analogues. *Journal of the American Chemical Society* **2009**, *131* (13), 4904-4916; (c) Yokoshima, S.; Ueda, T.; Kobayashi, S.; Sato, A.; Kuboyama, T.; Tokuyama, H.; Fukuyama, T., Stereocontrolled Total Synthesis of (+)-Vinblastine. *Journal of the American Chemical Society* **2002**, *124* (10), 2137-2139.

34. (a) Danieli, B.; Lesma, G.; Palmisano, G.; Riva, R., Aspidosperma alkaloids. Conversion of tabersonine into vindoline. *Journal of the Chemical Society, Perkin Transactions 1* **1987**, 155-161; (b) Danieli, B.; Lesma, G.; Palmisano, G.; Riva, R., Rapid access to the highly oxygenated aspidosperma alkaloids vindoline, vindorosine, and cathovaline. *Journal of the Chemical Society, Chemical Communications* **1984**, (14), 909-911.

35. Wenkert, E.; Cochran, D. W.; Hagaman, E. W.; Schell, F. M.; Neuss, N.; Katner, A. S.; Potier, P.; Kan, C.; Plat, M.; et, a., Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. XIX. Aspidosperma alkaloids. *Journal of the American Chemical Society* **1973**, *95* (15), 4990-4995.

36. Roberts, M. F., Enzymology of Alkaloid Biosynthesis. In *Alkaloids*, Roberts, M. F.; Wink, M., Eds. Plenum Publishing Corporation: New York, 1998; pp 109-146.

37. Saxton, J. E., Alkaloids of the Aspidospermine Group. In *The Alkaloids: Chemistry and Biology*, Cordell, G. A., Ed. Academic Press: New York, 1998; Vol. 51, pp 1-197.

38. *Medicinal Plants*. PROTA Foundation: Netherlands, 2008; Vol. 1, p 791.

39. Jenks, C., Extraction Studies of Tabernanthe Iboga and Voacanga Africana. *Natural Product Letters* **2002**, *16* (1), 71-76.

40. Hock, H.; Lang, S., Autoxydation von Kohlenwasserstoffen, IX. Mitteil.: Über Peroxyde von Benzol-Derivaten. *Berichte der deutschen chemischen Gesellschaft (A and B Series)* **1944**, *77* (3-4), 257-264.

41. Elbs, K., Ueber Nitrohydrochinon. *Journal für Praktische Chemie* **1893**, *48* (1), 179-185.

42. (a) Boyland, E.; Manson, D.; Sims, P., The preparation of o-aminophenyl sulphates. *Journal of the Chemical Society (Resumed)* **1953**, 3623-3628; (b) Boyland, E.; Sims, P., The oxidation of some aromatic amines with persulphate. *Journal of the Chemical Society (Resumed)* **1954**, 980-985.

43. Teuber, H. J.; Jellinek, G., Nitrosodisulfonat als Dehydrierungs- und Oxydationsmittel. *Die Naturwissenschaften* **1951**, *38* (11), 259-260.

44. Zimmer, H.; Lankin, D. C.; Horgan, S. W., Oxidations with potassium nitrosodisulfonate (Fremy's radical). The Teuber reaction. *Chemical Reviews* **1971**, *71* (2), 229-246.

45. (a) Wehrli, P. A.; Piggot, F., Oxidation with the nitrosodisulfonate radical. I. Preparation and use of disodium nitrosodisulfonate: trimethyl-p-benzoquinone *Organic Syntheses* **1972**, *52*, 83; (b) Morey, J., Undergraduate experiments with a long-lived radical (Fremy's salt): Synthesis of 1,4-benzoquinones by degradative oxidation of p-hydroxybenzyl alcohols. *Journal of Chemical Education* **1988**, *65* (7), 627-628.

46. Yuan, C.; Liang, Y.; Hernandez, T.; Berriochoa, A.; Houk, K. N.; Siegel, D., Metal-free oxidation of aromatic carbon–hydrogen bonds through a reverse-rebound mechanism. *Nature* **2013**, *499* (7457), 192-196.

47. Yuan, C.; Eliasen, A. M.; Camelio, A. M.; Siegel, D., Preparation of phenols by phthaloyl peroxide–mediated oxidation of arenes. *Nature Protocols* **2014**, *9* (11), 2624-2629.

48. Greene, F. D., Cyclic Diacyl Peroxides. I. Monomeric Phthaloyl Peroxide. *Journal of the American Chemical Society* **1956**, *78* (10), 2246-2250.

49. Alonso, D. A.; Nájera, C.; Pastor, I. M.; Yus, M., Transition-Metal-Catalyzed Synthesis of Hydroxylated Arenes. *Chemistry - A European Journal* **2010**, *16* (18), 5274-5284.

50. (a) Mann, G.; Incarvito, C.; Rheingold, A. L.; Hartwig, J. F., Palladium-Catalyzed C−O Coupling Involving Unactivated Aryl Halides. Sterically Induced Reductive Elimination To Form the C−O Bond in Diaryl Ethers. *Journal of the American Chemical Society* **1999**, *121* (13), 3224-3225; (b) Tlili, A.; Xia, N.; Monnier, F.; Taillefer, M., A Very Simple Copper-Catalyzed Synthesis of Phenols Employing Hydroxide Salts. *Angewandte Chemie* **2009**, *121* (46), 8881-8884; (c) Yang, D.; Fu, H., A Simple and Practical Copper-Catalyzed Approach to Substituted Phenols from Aryl Halides by Using Water as the Solvent. *Chemistry - A European Journal* **2010**, *16* (8), 2366-2370.

51. Sinhababu, A. K.; Borchardt, R. T., Aromatic hydroxylation. Hydroxybenzaldehydes from bromobenzaldehydes via reaction of in situ generated, lithiated α-morpholinobenzyl alkoxides with nitrobenzene. *The Journal of Organic Chemistry* **1983**, *48* (12), 1941-1944.

52. Poisson, J.; Lewin, G.; Rolland, Y., Aromatic Substitution Effects in the Aspidospermane-Eburnane Rearrangement of Indole Alkaloids. *Heterocycles* **1980**, *14* (12), 1915-1920.

53. (a) Anderson, K. W.; Ikawa, T.; Tundel, R. E.; Buchwald, S. L., The Selective Reaction of Aryl Halides with KOH: Synthesis of Phenols, Aromatic Ethers, and Benzofurans. *Journal of the American Chemical Society* **2006**, *128* (33), 10694-10695; (b) Zhao, D.; Wu, N.; Zhang, S.; Xi, P.; Su, X.; Lan, J.; You, J., Synthesis of Phenol, Aromatic Ether, and Benzofuran Derivatives by Copper-Catalyzed Hydroxylation of Aryl Halides. *Angewandte Chemie International Edition* **2009**, *48* (46), 8729-8732.

54. Davis, F. A.; Lamendola, J.; Nadir, U.; Kluger, E. W.; Sedergran, T. C.; Panunto, T. W.; Billmers, R.; Jenkins, R.; Turchi, I. J., Chemistry of oxaziridines. 1. Synthesis and structure of 2-arenesulfonyl-3-aryloxaziridines. A new class of oxaziridines. *Journal of the American Chemical Society* **1980**, *102* (6), 2000-2005.

55. (a) Bora, U.; Gogoi, A., An Iodine-Promoted, Mild and Efficient Method for the Synthesis of Phenols from Arylboronic Acids. *Synlett* **2012**, *23* (07),

1079-1081; (b) Molander, G. A.; Cavalcanti, L. N., Oxidation of Organotrifluoroborates via Oxone. *The Journal of Organic Chemistry* **2011**, *76* (2), 623-630.

56. Ishiyama, T.; Murata, M.; Miyaura, N., Palladium(0)-Catalyzed Cross-Coupling Reaction of Alkoxydiboron with Haloarenes: A Direct Procedure for Arylboronic Esters. *The Journal of Organic Chemistry* **1995**, *60* (23), 7508-7510.

57. (a) Suzuki, A., Organoboron compounds in new synthetic reactions. *Pure and Applied Chemistry* **1985**, *57* (12), 1949-1958; (b) Suzuki, A., Synthetic studies via the cross-coupling reaction of organoboron derivatives with organic halides. *Pure and Applied Chemistry* **1991**, *63* (3), 419-422.

58. Ratniyom, J.; Dechnarong, N.; Yotphan, S.; Kiatisevi, S., Convenient Synthesis of Arylboronates through a Synergistic Pd/Cu-Catalyzed Miyaura Borylation Reaction under Atmospheric Conditions. *European Journal of Organic Chemistry* **2014**, *2014* (7), 1381-1385.

59. Suzuki, A., Organoboranes in Organic Syntheses Including Suzuki Coupling Reaction. *Heterocycles* **2010**, *80* (1), 15-43.

60. Miyaura, N.; Suzuki, A., Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chemical Reviews* **1995**, *95* (7), 2457-2483.

61. Sikora, A.; Zielonka, J.; Lopez, M.; Joseph, J.; Kalyanaraman, B., Direct oxidation of boronates by peroxynitrite: Mechanism and implications in fluorescence imaging of peroxynitrite. *Free Radical Biology and Medicine* **2009**, *47* (10), 1401-1407.

62. (a) Huang, J.-M.; Chen, D.-S., A Mild and Highly Efficient Conversion of Arylboronic Acids into Phenols by Oxidation with MCPBA. *Synlett* **2013**, *24* (04), 499-501; (b) Cai, H.; Guo, S.; Lu, L., Base-Promoted, Mild and Highly Efficient Conversion of Arylboronic Acids into Phenols with tert-Butyl Hydroperoxide. *Synlett* **2013**, *24* (13), 1712-1714.

63. Zhu, C.; Wang, R.; Falck, J. R., Mild and Rapid Hydroxylation of Aryl/Heteroaryl Boronic Acids and Boronate Esters withN-Oxides. *Organic Letters* **2012**, *14* (13), 3494-3497.

64. Wu, Q.; Yan, D.; Chen, Y.; Wang, T.; Xiong, F.; Wei, W.; Lu, Y.; Sun, W.-Y.; Li, J. J.; Zhao, J., A redox-neutral catechol synthesis. *Nature Communications* **2017**, *8*.

65. Zhdankin, V. V.; Stang, P. J., Recent developments in the chemistry of polyvalent iodine compounds. *Chemical Reviews* **2002**, *102* (7), 2523-2584.

66. Dess, D. B.; Martin, J. C., Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *The Journal of Organic Chemistry* **1983**, *48* (22), 4155-4156.

67. Zhdankin, V. V., Organoiodine(V) Reagents in Organic Synthesis. *The Journal of Organic Chemistry* **2011**, *76* (5), 1185-1197.

68. Magdziak, D.; Rodriguez, A. A.; Van De Water, R. W.; Pettus, T. R. R., Regioselective Oxidation of Phenols to o-Quinones with o-Iodoxybenzoic Acid (IBX). *Organic Letters* **2002**, *4* (2), 285-288.

69. Satam, V.; Harad, A.; Rajule, R.; Pati, H., 2-Iodoxybenzoic acid (IBX): an efficient hypervalent iodine reagent. *Tetrahedron* **2010**, *66* (39), 7659-7706.

70. Zhou, C.-Y.; Li, J.; Peddibhotla, S.; Romo, D., Mild Arming and Derivatization of Natural Products via an In(OTf)3-Catalyzed Arene Iodination. *Organic Letters* **2010**, *12* (9), 2104-2107.

71. Kosynkin, D. V.; Tour, J. M., Benzyltriethylammonium Dichloroiodate/Sodium Bicarbonate Combination as an Inexpensive, Environmentally Friendly, and Mild Iodinating Reagent for Anilines. *Organic Letters* **2001**, *3* (7), 991-992.

72. Godula, K.; Sames, D., C-H Bond Functionalization in Complex Organic Synthesis. *Science* **2006**, *312* (5770), 67-72.

73. Snieckus, V., Directed ortho metalation. Tertiary amide and O-carbamate directors in synthetic strategies for polysubstituted aromatics. *Chemical Reviews* **1990**, *90* (6), 879-933.

74. Hartung, C. G.; Snieckus, V., The Directed ortho Metalation Reaction – A Point of Departure for New Synthetic Aromatic Chemistry. In *Modern Arene Chemistry*, Astruc, D., Ed. Wiley-VCH: Weinheim, 2002; pp 330-367.

75. Cederbalk, A.; Lysén, M.; Kehler, J.; Kristensen, J. L., Ortho lithiation-in situ borylation of substituted morpholine benzamides. *Tetrahedron* **2017**, *73* (12), 1576-1582.

76. Stern, R.; English, J.; Cassidy, H. G., Electron Exchange Polymers. X. A General Method for the Preparation of Phenolic Polystyrenes. *Journal of the American Chemical Society* **1957**, *79* (21), 5792-5797.

77. (a) Townsend, C. A.; Bloom, L. M., Studies of methoxymethyl-directed metalation. *Tetrahedron Letters* **1981**, *22* (40), 3923-3924; (b) Tius, M. A.; Gomez-Galeno, J.; Gu, X. Q.; Zaidi, J. H., C-Glycosylanthraquinone synthesis: total synthesis of vineomycinone B2 methyl ester. *Journal of the American Chemical Society* **1991**, *113* (15), 5775-5783.

78. Ros, A.; Fernández, R.; Lassaletta, J. M., Functional group directed C-H borylation. *Chemical Society Reviews* **2014**, *43* (10), 3229-3243.

79. Kawamorita, S.; Ohmiya, H.; Hara, K.; Fukuoka, A.; Sawamura, M., Directed Ortho Borylation of Functionalized Arenes Catalyzed by a Silica-Supported Compact Phosphine-Iridium System. *Journal of the American Chemical Society* **2009**, *131* (14), 5058-5059.

80. Boebel, T. A.; Hartwig, J. F., Silyl-Directed, Iridium-Catalyzed ortho-Borylation of Arenes. A One-Pot ortho-Borylation of Phenols, Arylamines, and Alkylarenes. *Journal of the American Chemical Society* **2008**, *130* (24), 7534-7535.

81. Neufeldt, S. R.; Sanford, M. S., Controlling Site Selectivity in Palladium-Catalyzed C–H Bond Functionalization. *Accounts of Chemical Research* **2012**, *45* (6), 936-946.

82. Huang, C.; Ghavtadze, N.; Chattopadhyay, B.; Gevorgyan, V., Synthesis of Catechols from Phenols via Pd-Catalyzed Silanol-Directed C–H Oxygenation. *Journal of the American Chemical Society* **2011**, *133* (44), 17630-17633.

83. Adam, W.; Mitchell, C. M.; Saha-Möller, C. R.; Weichold, O.; Garcia, H., The selective catalytic oxidation of silanes to silanols with H2O2 activated by the Ti-beta zeolite. *Chemical Communications* **1998**, (23), 2609-2610.

84. Lee, Y.; Seomoon, D.; Kim, S.; Han, H.; Chang, S.; Lee, P. H., Highly Efficient Iridium-Catalyzed Oxidation of Organosilanes to Silanols. *The Journal of Organic Chemistry* **2004**, *69* (5), 1741-1743.

85. Vilsmeier, A.; Haack, A., Über die Einwirkung von Halogenphosphor auf Alkyl-formanilide. Eine neue Methode zur Darstellung sekundärer und tertiärer p-Alkylamino-benzaldehyde. *Berichte der deutschen chemischen Gesellschaft (A and B Series)* **1927**, *60* (1), 119-122.

86. Oda, R.; Yamamoto, K., The Formylation of Aromatic Compounds by 1 : 2 Adduct of Dimethylformamide with Cyanuric Chloride. *Nippon kagaku zassi* **1962**, *83* (12), 1292-1294.

87. Rieche, A.; Gross, H.; Höft, E., Über α-Halogenäther, IV. Synthesen aromatischer Aldehyde mit Dichlormethyl-alkyläthern. *Chemische Berichte* **1960**, *93* (1), 88-94.

88. Heras, C.; Ramos-Tomillero, I.; Caballero, M.; Paradís-Bas, M.; Nicolás, E.; Albericio, F.; de P. R. Moreira, I.; Bofill, J. M., On the Mechanism of Phenolic Formylation Mediated by TiCl4Complexes: Existence of Diradical Intermediates Induced by Valence Tautomerism. *European Journal of Organic Chemistry* **2015**, *2015* (10), 2111-2118.

89. Ramos-Tomillero, I.; Paradís-Bas, M.; de Pinho Ribeiro Moreira, I.; Bofill, J.; Nicolás, E.; Albericio, F., Formylation of Electron-Rich Aromatic Rings Mediated by Dichloromethyl Methyl Ether and TiCl4: Scope and Limitations. *Molecules* **2015**, *20* (4), 5409-5422.

90. Reimer, K.; Tiemann, F., Ueber die Einwirkung von Chloroform auf Phenole und besonders aromatische Oxysäuren in alkalischer Lösung. *Berichte der deutschen chemischen Gesellschaft* **1876**, *9* (2), 1268-1278.

91. Casiraghi, G.; Casnati, G.; Cornia, M.; Pochini, A.; Puglia, G.; Sartori, G.; Ungaro, R., Selective reactions using metal phenoxides. Part 1. Reactions with

formaldehyde. *Journal of the Chemical Society, Perkin Transactions 1* **1978**, (4), 318-321.

92. Aldred, R.; Johnston, R.; Levin, D.; Neilan, J., Magnesium-mediated ortho-specific formylation and formaldoximation of phenols. *Journal of the Chemical Society, Perkin Transactions 1* **1994**, (13), 1823-1831.

93. Hofsløkken, N. U.; Skattebøl, L., Convenient Method for the ortho-Formylation of Phenols. *Acta Chemica Scandinavica* **1999**, *53*, 258-262.

94. Baeyer, A.; Villiger, V., Einwirkung des Caro'schen Reagens auf Ketone. *Berichte der deutschen chemischen Gesellschaft* **1899**, *32* (3), 3625-3633.

95. Strukul, G., Transition Metal Catalysis in the Baeyer-Villiger Oxidation of Ketones. *Angewandte Chemie International Edition* **1998**, *37* (9), 1198-1209.

96. Dakin, H. D., The Oxidation of Hydroxy Derivatives of Benzaldehyde, Acetophenone and Related Substances. *American Chemical Journal* **1909**, *42* (6), 477-498.

97. (a) Kabalka, G. W.; Reddy, N. K.; Narayana, C., Sodium percarbonate: A convenient reagent for the dakin reaction. *Tetrahedron Letters* **1992**, *33* (7), 865-866; (b) Roy, A.; Reddy, K. R.; Mohanta, P. K.; Ila, H.; Junjappat, H., Hydrogen Peroxide/Boric Acid: An Efficient System for Oxidation of Aromatic

Aldehydes and Ketones to Phenols. *Synthetic Communications* **1999**, *29* (21), 3781-3791.

98. Guzmán, J. A.; Mendoza, V.; García, E.; Garibay, C. F.; Olivares, L. Z.; Maldonado, L. A., Baeyer-Villiger Oxidation of β-Aryl Substituted Unsaturated Carbonyl Compounds with Hydrogen Peroxide and Catalytic Selenium Dioxide. *Synthetic Communications* **1995**, *25* (14), 2121-2133.

99. Venkataramana, G.; Dongare, P.; Dawe, L. N.; Thompson, D. W.; Zhao, Y.; Bodwell, G. J., 1,8-Pyrenylene−Ethynylene Macrocycles. *Organic Letters* **2011**, *13* (9), 2240-2243.

100. Wuts, P. G. M.; Greene, T. W., Protection for the Amino Group. In *Greene's Protective Groups in Organic Synthesis*, 4 ed.; John Wiley & Sons: Hoboken, 2006; pp 696-926.

101. (a) Srinivasan, N.; Yurek-George, A.; Ganesan, A., Rapid deprotection of N-Boc amines by TFA combined with freebase generation using basic ion-exchange resins. *Molecular Diversity* **2005**, *9* (4), 291-293; (b) Gibson, F. S.; Bergmeier, S. C.; Rapoport, H., Selective Removal of an N-BOC Protecting Group in the Presence of a tert-Butyl Ester and Other Acid-Sensitive Groups. *The Journal of Organic Chemistry* **1994**, *59* (11), 3216-3218.

102. Zhao, S.; Andrade, R. B., Domino Michael/Mannich/N-Alkylation Route to the Tetrahydrocarbazole Framework of Aspidosperma Alkaloids: Concise

Total Syntheses of (−)-Aspidospermidine, (−)-Tabersonine, and (−)-Vincadifformine. *Journal of the American Chemical Society* **2013**, *135* (36), 13334-13337.

103. Voorhees, V.; Adams, R., The Use of the Oxides of Platinum for the Catalytic Reduction of Organic Compounds. I. *Journal of the American Chemical Society* **1922**, *44* (6), 1397-1405.

104. Scheeren, C. W.; Domingos, J. B.; Machado, G.; Dupont, J., Hydrogen Reduction of Adams' Catalyst in Ionic Liquids: Formation and Stabilization of Pt(0) Nanoparticles. *The Journal of Physical Chemistry C* **2008**, *112* (42), 16463-16469.

105. Hu, L.; Lei, M.; Chen, F., Synthesis of C-10 Tabersonine Analogues by Palladium-Catalyzed Cross-Coupling Reactions. *Synthesis* **2014**, *46* (23), 3199-3206.

106. Kumar, S.; Kamboj, J.; Suman; Sharma, S., Overview for Various Aspects of the Health Benefits of Piper Longum Linn. Fruit. *Journal of Acupuncture and Meridian Studies* **2011**, *4* (2), 134-140.

107. (a) Chatterjee, A.; Dutta, C. P., Structure of piperlonguminine, an alkaloid of Piper Longum Linn. *Tetrahedron Letters* **1966**, *7* (16), 1797-1800; (b) Chatterjee, A.; Dutta, C. P., Alkaloids of Piper longum Linn—I : Structure and

synthesis of piperlongumine and piperlonguminine. *Tetrahedron* **1967**, *23* (4), 1769-1781.

108. Joshi, B. S.; Kamat, Y. N.; Saksena, A. K., On the structure of piplartine and a synthesis of dihydropiplartine. *Tetrahedron Letters* **1968**, *9* (20), 2395-2400.

109. Boll, P. M.; Hansen, J.; Simonsen, O.; Thorup, N., Synthesis and molecular structure of piplartine (=piperlongumine). *Tetrahedron* **1984**, *40* (1), 171-175.

110. Raj, L.; Ide, T.; Gurkar, A. U.; Foley, M.; Schenone, M.; Li, X.; Tolliday, N. J.; Golub, T. R.; Carr, S. A.; Shamji, A. F.; Stern, A. M.; Mandinova, A.; Schreiber, S. L.; Lee, S. W., Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature* **2011**, *475* (7355), 231-234.

111. Burgess, D. J., Anticancer Drugs: Selective oxycution? *Nature Reviews Drug Discovery* **2011**, *10* (9), 658-659.

112. Kong, E.-H.; Kim, Y.-J.; Kim, Y.-J.; Cho, H.-J.; Yu, S.-N.; Kim, K.-Y.; Chang, J.-H.; Ahn, S.-C., Piplartine induces caspase-mediated apoptosis in PC-3 human prostate cancer cells. *Oncology Reports* **2008**, *20* (4), 785-792.

113. (a) Wu, Y.; Min, X.; Zhuang, C.; Li, J.; Yu, Z.; Dong, G.; Yao, J.; Wang, S.; Liu, Y.; Wu, S.; Zhu, S.; Sheng, C.; Wei, Y.; Zhang, H.; Zhang, W.; Miao, Z., Design,

synthesis and biological activity of piperlongumine derivatives as selective anticancer agents. *European Journal of Medicinal Chemistry* **2014**, *82*, 545-551; (b) Dhillon, H.; Chikara, S.; Reindl, K. M., Piperlongumine induces pancreatic cancer cell death by enhancing reactive oxygen species and DNA damage. *Toxicology Reports* **2014**, *1*, 309-318.

114. Bezerra, D. P.; Pessoa, C.; de Moraes, M. O.; Saker-Neto, N.; Silveira, E. R.; Costa-Lotufo, L. V., Overview of the therapeutic potential of piplartine (piperlongumine). *European Journal of Pharmaceutical Sciences* **2013**, *48* (3), 453-463.

115. Liou, G.-Y.; Storz, P., Reactive oxygen species in cancer. *Free Radical Research* **2010**, *44* (5), 479-496.

116. Prabhu, B. R.; Mulchandani, N. B., Biosynthesis of piperlongumine. *Phytochemistry* **1985**, *24* (11), 2589-2591.

117. Rios, M. Y.; Olivo, H. F., Natural and Synthetic Alkamides. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Ed. Elsevier: Oxford, 2014; Vol. 43, pp 79-121.

118. Kato, M. J.; Furlan, M., Chemistry and evolution of the Piperaceae. *Pure and Applied Chemistry* **2007**, *79* (4), 529-538.

119. Adams, D. J.; Dai, M.; Pellegrino, G.; Wagner, B. K.; Stern, A. M.; Shamji, A. F.; Schreiber, S. L., Synthesis, cellular evaluation, and mechanism of action of piperlongumine analogs. *Proceedings of the National Academy of Sciences* **2012**, *109* (38), 15115-15120.

120. Seo, Y. H.; Kim, J.-K.; Jun, J.-G., Synthesis and biological evaluation of piperlongumine derivatives as potent anti-inflammatory agents. *Bioorganic & Medicinal Chemistry Letters* **2014**, *24* (24), 5727-5730.

121. (a) Cope, A. C.; Foster, T. T.; Towle, P. H., Thermal Decomposition of Amine Oxides to Olefins and Dialkylhydroxylamines. *Journal of the American Chemical Society* **1949**, *71* (12), 3929-3934; (b) Grieco, P. A.; Gilman, S.; Nishizawa, M., Organoselenium chemistry. A facile one-step synthesis of alkyl aryl selenides from alcohols. *The Journal of Organic Chemistry* **1976**, *41* (8), 1485-1486; (c) Tschugaeff, L., Ueber eine neue Methode zur Darstellung ungesättigter Kohlenwasserstoffe. *Berichte der deutschen chemischen Gesellschaft* **1899**, *32* (3), 3332-3335; (d) Burgess, E. M.; Penton, H. R.; Taylor, E. A., Synthetic applications of N-carboalkoxysulfamate esters. *Journal of the American Chemical Society* **1970**, *92* (17), 5224-5226.

122. Clayden, J.; Greeves, N.; Warren, S., *Organic Chemistry*. Oxford University Press: Oxford, 2012; p 1264.

123. Trost, B. M.; Salzmann, T. N., New synthetic reactions. Sulfenylation-dehydrosulfenylation as a method for introduction of unsaturation. *Journal of the American Chemical Society* **1973**, *95* (20), 6840-6842.

124. Drabowicz, J.; Mikołajczyk, M., Synthesis of Sulfoxides. A Review. *Organic Preparations and Procedures International* **2009**, *14* (1-2), 45-89.

125. Jones, D. N.; Mundy, D.; Whitehouse, R. D., Steroidal selenoxides diastereoisomeric at selenium; syn-elimination, absolute configuration, and optical rotatory dispersion characteristics. *Journal of the Chemical Society D: Chemical Communications* **1970**, (2), 86-87.

126. (a) Sharpless, K. B.; Lauer, R. F.; Teranishi, A. Y., Electrophilic and nucleophilic organoselenium reagents. New routes to α,β-unsaturated carbonyl compounds. *Journal of the American Chemical Society* **1973**, *95* (18), 6137-6139; (b) Sharpless, K. B.; Young, M. W.; Lauer, R. F., Reactions of selenoxides: Thermal -elimination and H218O exchange. *Tetrahedron Letters* **1973**, *14* (22), 1979-1982.

127. Sharpless, K. B.; Lauer, R. F., Mild procedure for the conversion of epoxides to allylic alcohols. First organoselenium reagent. *Journal of the American Chemical Society* **1973**, *95* (8), 2697-2699.

128. (a) Wittig, G.; Schöllkopf, U., Über Triphenyl-phosphin-methylene als olefinbildende Reagenzien (I. Mitteil.). *Chemische Berichte* **1954**, *87* (9), 1318-

1330; (b) Wittig, G.; Haag, W., Über Triphenyl-phosphinmethylene als olefinbildende Reagenzien (II. Mitteil.). *Chemische Berichte* **1955**, *88* (11), 1654-1666.

129. Hoffmann, R. W., Wittig and His Accomplishments: Still Relevant Beyond His 100th Birthday. *Angewandte Chemie International Edition* **2001**, *40* (8), 1411-1416.

130. Trippett, S., The Wittig reaction. *Quarterly Reviews, Chemical Society* **1963**, *17* (4), 406-440.

131. (a) Byrne, P. A.; Gilheany, D. G., Unequivocal Experimental Evidence for a Unified Lithium Salt-Free Wittig Reaction Mechanism for All Phosphonium Ylide Types: Reactions with β-Heteroatom-Substituted Aldehydes Are Consistently Selective for cis-Oxaphosphetane-Derived Products. *Journal of the American Chemical Society* **2012**, *134* (22), 9225-9239; (b) Byrne, P. A.; Gilheany, D. G., The modern interpretation of the Wittig reaction mechanism. *Chemical Society Reviews* **2013**, *42* (16), 6670-6696.

132. Vedejs, E.; Marth, C. F., Mechanism of the Wittig reaction: the role of substituents at phosphorus. *Journal of the American Chemical Society* **1988**, *110* (12), 3948-3958.

133. (a) Robiette, R.; Richardson, J.; Aggarwal, V. K.; Harvey, J. N., On the Origin of High E Selectivity in the Wittig Reaction of Stabilized

Ylides: Importance of Dipole−Dipole Interactions. *Journal of the American Chemical Society* **2005**, *127* (39), 13468-13469; (b) Robiette, R.; Richardson, J.; Aggarwal, V. K.; Harvey, J. N., Reactivity and Selectivity in the Wittig Reaction: A Computational Study. *Journal of the American Chemical Society* **2006**, *128* (7), 2394-2409.

134. Wadsworth, W. S.; Emmons, W. D., The Utility of Phosphonate Carbanions in Olefin Synthesis. *Journal of the American Chemical Society* **1961**, *83* (7), 1733-1738.

135. (a) Horner, L.; Hoffmann, H.; Wippel, H. G., Phosphororganische Verbindungen, XII. Phosphinoxyde als Olefinierungsreagenzien. *Chemische Berichte* **1958**, *91* (1), 61-63; (b) Horner, L.; Hoffmann, H.; Wippel, H. G.; Klahre, G., Phosphororganische Verbindungen, XX. Phosphinoxyde als Olefinierungsreagenzien. *Chemische Berichte* **1959**, *92* (10), 2499-2505.

136. Lefèbvre, G.; Seyden-Penne, J., The mechanism of the Horner–Emmons modification of the Wittig reaction. *Journal of the Chemical Society D: Chemical Communications* **1970**, *0* (20), 1308-1309.

137. Appel, R.; Loos, R.; Mayr, H., Nucleophilicity Parameters for Phosphoryl-Stabilized Carbanions and Phosphorus Ylides: Implications for Wittig and Related Olefination Reactions. *Journal of the American Chemical Society* **2009**, *131* (2), 704-714.

138. (a) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T., Horner-wadsworth-emmons reaction: Use of lithium chloride and an amine for base-sensitive compounds. *Tetrahedron Letters* **1984**, *25* (21), 2183-2186; (b) Rathke, M. W.; Nowak, M., The Horner-Wadsworth-Emmons modification of the Wittig reaction using triethylamine and lithium or magnesium salts. *The Journal of Organic Chemistry* **1985**, *50* (15), 2624-2626.

139. Still, W. C.; Gennari, C., Direct synthesis of Z-unsaturated esters. A useful modification of the Horner-Emmons olefination. *Tetrahedron Letters* **1983**, *24* (41), 4405-4408.

140. Gruber, N.; Mollo, M. C.; Zani, M.; Orelli, L. R., Microwave-Enhanced Synthesis of Phosphonoacetamides. *Synthetic Communications* **2011**, *42* (5), 738-746.

141. (a) Ando, K., Highly Selective Synthesis of Z-Unsaturated Esters by Using New Horner−Emmons Reagents, Ethyl (Diarylphosphono)acetates. *The Journal of Organic Chemistry* **1997**, *62* (7), 1934-1939; (b) Karstens, W. F. J. (Oss, NL), Conti, Paolo Giovanni Martino (Oss, NL), Timmers, Cornelis Marius (Oss, NL), Plate, Ralf (Oss, NL), Van Koppen, Christianus Johannes (Oss, NL) TSH receptor antagonizing tetrahydroquinoline compounds. 2011.

142. Dong, J.; Krasnova, L.; Finn, M. G.; Sharpless, K. B., Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry. *Angewandte Chemie International Edition* **2014**, *53* (36), 9430-9448.

143. Sreevalsan, S.; Safe, S., Reactive Oxygen Species and Colorectal Cancer. *Current Colorectal Cancer Reports* **2013**, *9* (4), 350-357.

144. (a) Callero, M. A.; Luzzani, G. A.; De Dios, D. O.; Bradshaw, T. D.; Loaiza Perez, A. I., Biomarkers of sensitivity to potent and selective antitumor 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F203) in ovarian cancer. *Journal of Cellular Biochemistry* **2013**, *114* (10), 2392-2404; (b) Le Moguen, K.; Lincet, H.; Deslandes, E.; Hubert-Roux, M.; Lange, C.; Poulain, L.; Gauduchon, P.; Baudin, B., Comparative proteomic analysis of cisplatin sensitive IGROV1 ovarian carcinoma cell line and its resistant counterpart IGROV1-R10. *Proteomics* **2006**, *6* (19), 5183-5192.

145. Garnier, E. C.; Liebeskind, L. S., Organometallic Enantiomeric Scaffolding: General Access to 2-Substituted Oxa- and Azabicyclo[3.2.1]octenes via a Brønsted Acid Catalyzed [5 + 2] Cycloaddition Reaction. *Journal of the American Chemical Society* **2008**, *130* (23), 7449-7458.

146. Winter, D. K.; Drouin, A.; Lessard, J.; Spino, C., Photochemical Rearrangement of N-Chlorolactams: A Route to N-Heterocycles through Concerted Ring Contraction. *The Journal of Organic Chemistry* **2010**, *75* (8), 2610-2618.

147. Rao, V. R.; Muthenna, P.; Shankaraiah, G.; Akileshwari, C.; Babu, K. H.; Suresh, G.; Babu, K. S.; Chandra Kumar, R. S.; Prasad, K. R.; Yadav, P. A.; Petrash, J. M.; Reddy, G. B.; Rao, J. M., Synthesis and biological evaluation of new piplartine analogues as potent aldose reductase inhibitors (ARIs). *European Journal of Medicinal Chemistry* **2012**, *57*, 344-361.