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Development of new heterocyclic leads against malaria

by

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Doctor of Philosophy

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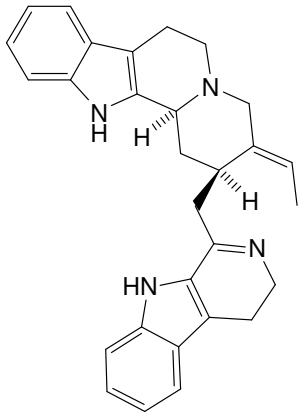
Abstract

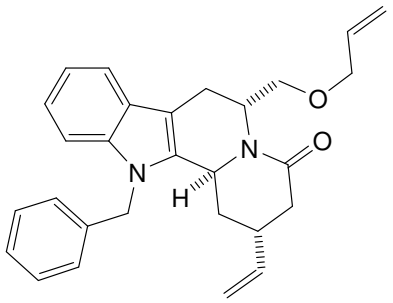
Malaria continues to pose a significant global health and socio-economic burden on those regions where it is endemic. Despite substantial investment in the delivery of artemisinin-based combination therapies, causing a fall in malaria mortality, recent data suggest that this parasitic disease still imposes a significant impact. A major problem is the narrow drug discovery pipeline, made worse by reports of artemisinin resistance.

In recent years, high-throughput screening of natural products derived from plants and marine organisms has led to the discovery of potent anti-malarial indole alkaloids (such as dihydrousambarensine **(A)**), many of which contain an indoloisoquinoline core.



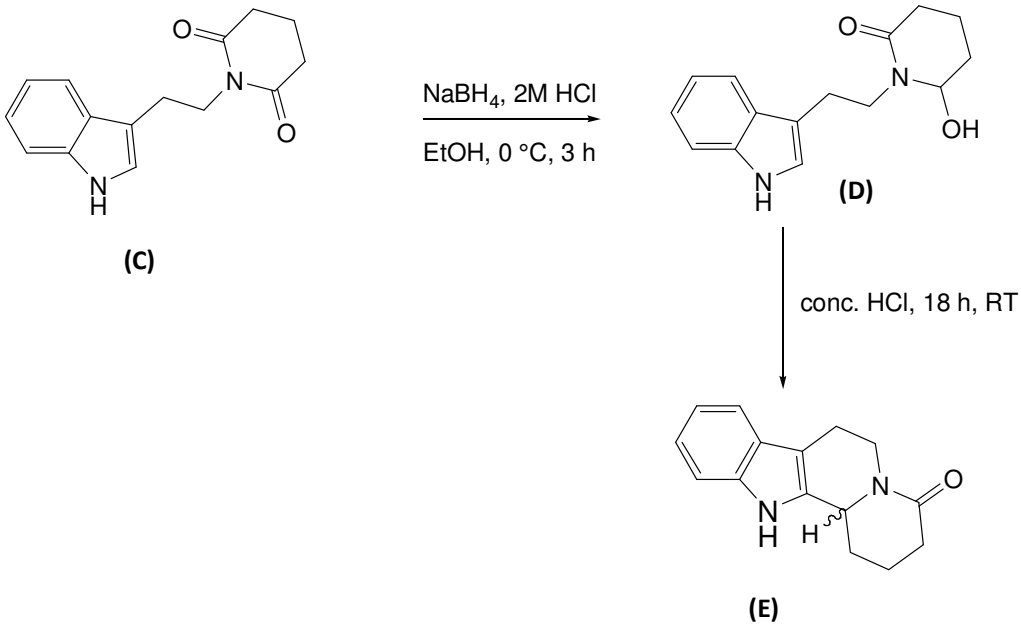
(A)

Building on previously discovered methodology in our group, we have developed a series of novel, enantiomerically pure, synthetic indoloisoquinoline and their potential as anti-malarial leads was assessed. The structure-activity relationship of these compounds was investigated in several areas and a lead compound **(B)** was generated with an activity close to that of a known anti-malarial natural product dihydrousambarensine **(A)**.



(B)

We have also developed a synthetic route to these indoloisoquinolines in racemic form, derived from compound **(E),** that give anti-malarial activity comparable to their enantiomerically pure analogues. This provides quicker and cheaper access to these anti-malarial compounds.



(C) **(D)** **(E)**

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Chapter 1 – Introduction

Abbreviations

AIBN	=	azobis*iso*butyronitrile
br	=	broad
Bn	=	benzyl
Boc	=	*tert*-butoxycarbonyl
d	=	doublet
DBN	=	1,5-diazabicyclo[4.3.0]non-ene
DCM	=	dichloromethane
DDT	=	dichlorodiphenyltrichloroethane
DIBAL	=	di*iso*butylaluminium hydride
DMAP	=	*N,N*-dimethylaminopyridine
DMF	=	dimethylformamide
DMSO	=	dimethyl sulfoxide
E	=	electrophile
ee	=	enantiomeric excess
EI	=	electron ionisation
CI	=	chemical ionisation
EtOH	=	ethanol
g	=	grams
h	=	hours
IBX	=	*o*-iodoxybenzoic acid
IR	=	infrared
J	=	coupling constant
MeOH	=	methanol
LDA	=	lithium di*iso*propylamide
µM	=	micromolar
m	=	multiplet
min	=	minute
ml	=	millitres
mmol	=	millimoles
Mp	=	melting point
MS	=	mass spectrum
nM	=	nanomolar
NMR	=	nuclear magnetic resonance
PCC	=	pyridinium chlorochromate
PDC	=	pyridinium dichromate
q	=	quartet
RT	=	room temperature
s	=	singlet
se	=	septet
si	=	sextet
t	=	triplet
TBAF	=	tetra-*n*-butylammonium fluoride
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran

TLC	=	thin layer chromatography
TMSCl	=	trimethylsilyl chloride
UV	=	ultraviolet

Acknowledgments

Firstly, I would like to thank my supervisor Professor Steve Allin for his guidance and the opportunity to undertake this project, and Dr Liam Duffy for his supervision and help throughout the first couple of years. I would like to thank Dr Paul Horrocks and his research team in Life Sciences for their provision of the anti-malarial data and Irene Hallyburton for the toxicity data. I would like to thank Professor Pat Bailey, Dr Tess Philips and the Bailey group for help and support. I would also like to thank all the academic staff, technical staff and my fellow researchers at Keele University.

I would like thank my family and friends for their support.

I would also like to thank Keele University for the funding.

Chapter 1

Introduction

1.1 Introduction to Malaria, Causes and Treatments.

1.1.1. Malaria

Malaria is a parasitic disease, endemic in 105 countries throughout the tropical and temperate regions. In 2010 alone malaria caused an estimated 655,000 deaths and a further 216 million clinical cases. [1] Although everyone is susceptible, the majority of fatal cases occur among African children, where malaria accounts for approximately 22% of childhood deaths. Malaria can also cause high rates of miscarriage or low birth weight. An estimated 200,000 infants die per year as a result of the disease during pregnancy, making young children and pregnant women the most vulnerable against malaria.

Malaria is caused by a protozoan parasite of the genus *Plasmodium*. There are four main species that can cause infection in humans; *P. vivax, P. ovale, P. malariae* and *P. falciparum*. *P. vivax* and *P. falciparum* are the most common, with the latter being the most deadly. [1] Recently, there have been reported cases of *P. knowlesi* in humans, [2, 3] creating a 5th species that is a danger. Until now, *P. knowlesi* had only been found in monkeys in South-East Asia. The *Plasmodium* parasites are transmitted by the female *Anopheles* mosquito of which 50-60 species can spread the infection.

The clinical symptoms of malaria vary between species. It most commonly presents with flu-like symptoms of a periodic fever, joint pains, vomiting and headaches. If left untreated the disease can cause more serious symptoms of renal failure, hypoglycaemia, and anaemia. In severe cases the parasite enters the brain, a condition known as cerebral malaria, and can cause a coma, leading to death. [1] Due to the non-specificity of the early, less severe symptoms, malaria is often over-diagnosed in endemic areas, and therefore treatment is over-prescribed, which can lead to resistance against anti-malarials.

The life cycle of the parasite also varies between species. Figure 1 shows the life cycle of *P. falciparum*.

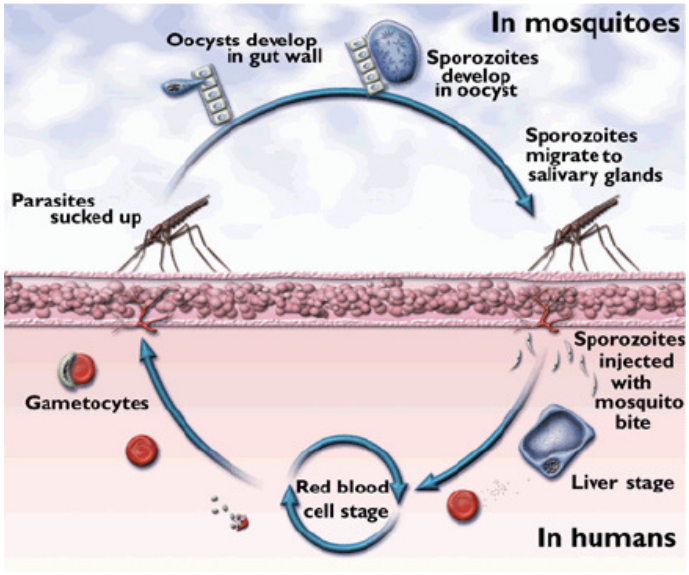
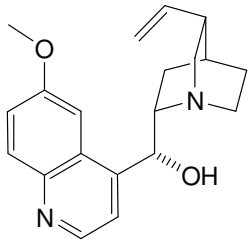


Figure 1: Life cycle of *P. falciparum*. [4]

The parasite undergoes sexual reproduction in the mosquito's digestive tract and asexual reproduction in the body cavity. Reproduction in the mosquito's digestive tract produces sporozites which migrate into the salivary glands and are then passed into the human host when the mosquito feeds. The sporozites travel to the liver and undergo asexual reproduction. This forms a schizont, which contains approximately 40,000 merozoites. When the schizont matures, it erupts, releasing the merozoites which invade the host's red blood cells. Here, the merozoites mature through a ring stage to form a new schizont containing approximately 24 merozoites. When this is mature the merozoites are released to infect further red blood cells. The schizonts mature and erupt every 36-48 hours depending on the species of parasite. This accounts for the periodic fever. Not all merozoites invade red blood cells, however; some form gametocytes which are then taken up by another mosquito host when it feeds. [5]

1.1.2. Brief history of treatment

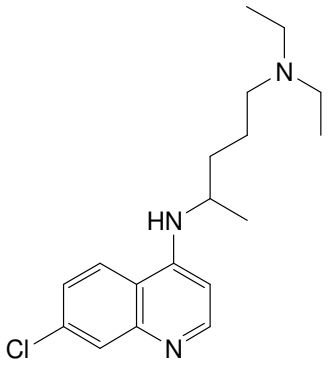
The first anti-malarial drug to be used was quinine **(1)**, an alkaloid discovered in the bark of the *Cinchona* tree (Peruvian fever tree) traditionally used to treat fevers. Resistance to quinine has been reported, but it is rare and the drug is still one of the most effective anti-malarials used today. [6]



(1)

Since the first use of quinine in the 17th century, no other drugs were used until the 20th century, when pharmacological research allowed the discovery of new compounds.

Chloroquine **(2)**, the second breakthrough in malaria treatment, was first discovered in 1934; however it was not until 1946 that it was established as a safe and effective anti-malarial. [7] It works by inhibiting the development of asexual erythrocytic forms of the parasite, thus reducing parasitaemia. [8] Resistance to chloroquine by *P. falciparum* was first documented in the 1950's in South East Asia and South Africa, and has become widespread since then. [9]

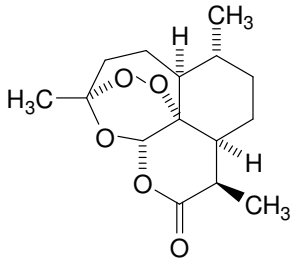


(2)

Many other drugs have been used as treatments and prophylaxis for malaria. These include mefloquine, proguanil, mepacrine, primaquine and pyrimethamine. Resistance to all these drugs has been reported. [10]

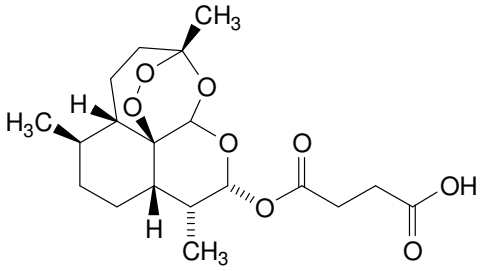
1.1.3. Current treatments

The current front-line treatments for malaria are a class of compounds known as the artemisinins. Artemisinin **(3)** resides in the leaves of the Chinese plant *Artemisia annua* which has been used in traditional Chinese medicine for over a thousand years. In 1967 the leaves were screened and found to have potent anti-malarial activity. In 1972, the active ingredient was isolated, purified and named Artemisinin. [11]



(3)

Derivatives of this compound have been synthesised; the most of important of these include artesunate **(4)**, artemether **(5)**, artemotil **(6)** and dihydroartemisinin **(7)**. The artemisinins have been widely used as anti-malarials in combination therapies since wide-spread resistance to other anti-malarials occurred. [12]



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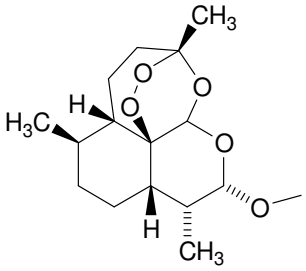

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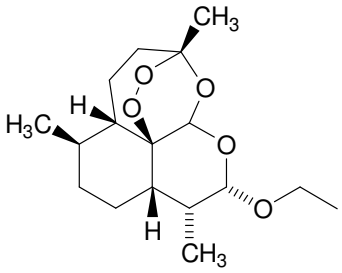

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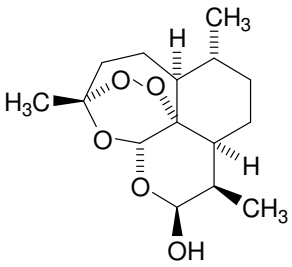


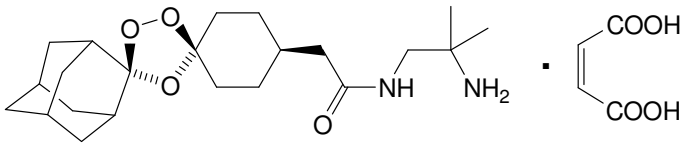
(7)

They act quickly, and are eliminated quickly, with a half-life of roughly one hour. The exact mechanism of action is not known, however studies have proven that the *endo* peroxide bridge is essential for activity. [13] This suggests that free radicals are involved in the mechanism and there is evidence to support this. There is also evidence to support that binding to the haem group in the host's blood also contributes to activity. The *endo* peroxide bridge although essential, causes problems in manufacturing and co-formulation, due to it's instability, an instability that is accelerated under tropical conditions.

Resistance to the artemisinins was slow to emerge and was not reported until 2009. [13] The reasons for this are believed to be due to:

1) The short half-life and quick elimination from the body of the drug, therefore the parasite is not exposed to it for long.
2) Activity against gametes: this is the stage at which the parasite is taken from the blood into the mosquito and transmitted to a new host. Killing the gametes reduces transmission; therefore gametes exposed to artemisinins are less likely to infect a new host.
3) Use in combination therapy, which tends to delay the onset of resistance.

The most recent anti-malarial, named Synriam, was launched by Indian drug company Ranbaxy on world malaria day, 2012. It is the first drug in recent years not to be based on artemisinin; however it still contains a peroxide bridge in its structure, thought to be causing its activity. It is given as a fixed-dose prescription as a combination of arterolane maleate **(8)** and piperaquine phosphate **(9)**. Ranbaxy report a cure rate of 95% when three doses are taken over three days, providing a very simple and effective regime. [14]



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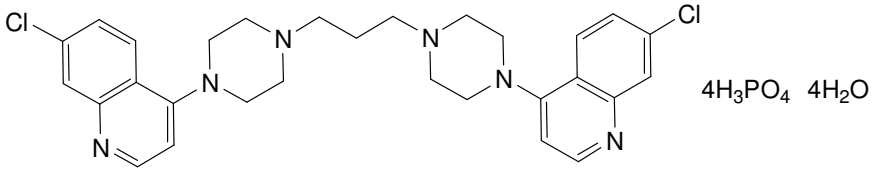
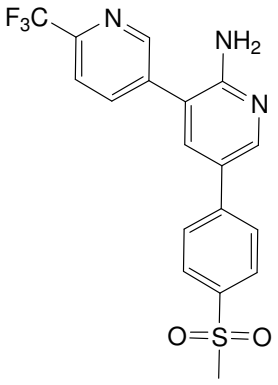


(9)

Another drug in the pipeline comes from the Medicine for Malaria Venture and is code named MMV390048 **(10)**. [15] It was identified during a high throughput screening campaign in which several millions of chemical samples were screened to identify new compounds with anti-malarial activity. It was synthesised in 2010, displaying potent activity and high stability. Animal testing in 2011 discovered that a low oral dosage could completely cure the test subject. It has been found to be active against a wide variety of drug-resistant strains and against multiple points in the parasites' life cycle. This is a very promising drug candidate which is hoped to enter clinical trials in 2013.



(10)

1.1.4 Vaccine

A lot of research today focuses on creating a vaccine for malaria. Vaccines display an advantage over drugs as they prevent people from contracting and spreading the disease and have led to the complete eradication of certain illnesses in the past (*e.g.* smallpox). RTS,S is the most clinically-advanced current vaccine candidate and the first to ever enter large-scale phase 3 clinical trials. Created in 1987 by GlaxoSmithKline, and developed since with funding from the Bill and Melinda Gates foundation, it has been shown to protect young children against *P. falciparum*. The vaccine induces the production of

antibodies and T cells that are believed to affect the parasites ability to infect, develop and survive in the human liver. [16] Studies have shown that it remains effective for at least 18 months after administration in reducing clinical malaria by 35% and severe malaria by 49%. [17, 18]

1.1.5. Other methods to combat malaria

Other methods to try to combat malaria include environmental control, biological control and vector control. The latter is concerned with the eradication of the *Anopheles* mosquito which carries the parasite. This can be done by spraying areas where the vector may rest with an insecticide or impregnating materials with one, which can then be made into mosquito nets. This method was highly successful until resistance to the insecticides used (pyrethroids and DDT) was reported. [19, 20]

Biological control involves releasing species that consume mosquito larvae, introducing bacteria that produce toxins into breeding sites and sterilisation of male mosquitoes. Environmental control is the reduction of breeding sites by draining areas of stagnant water. These methods although effective, are on their own not enough.

As mentioned above, resistance has been documented against the artemisinins. The discovery of new lead compounds with high anti-malarial activity that can be developed into effective drug candidates is highly desirable, but resistance to these is likely to appear after a few years. The development of a promising vaccine is also helpful, but it is not 100% effective. All established treatments for malaria have been met with resistance from the parasite, and all species of the parasite are showing resistance, therefore there is still an urgent need for new anti-malarial compounds. [21]

1.2 Natural Compounds with Anti-Malarial Properties

1.2.1 Traditional medicines

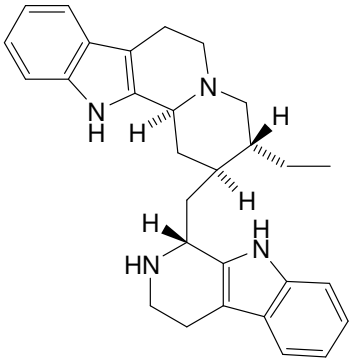
Plants have been used as medicines for thousands of years and indeed, some of the major breakthroughs in modern medicine have been derived from natural sources. The discovery of penicillin from a fungus; aspirin derived from willow bark, and the previously mentioned quinine and artemisinin are just a few examples. In 1996, it was reported that 30% of the current top prescribed drugs were derived from natural compounds and according to the World Health Organisation only 4 out of the current 15 anti-malarial drugs are totally synthetic. [22]

Traditional medicines for malaria have often come from locally sourced plants or trees in areas where the disease is endemic. The bark of the *Cinchona* tree, native to South America, was used long before quinine was isolated and identified. Also from this region, the bark of *Geissospermum vellosii* [23] is used by the native population. In traditional Congolese medicine *Anisopappus chinensis, Entandrophragma palustre, Melia azedarach, Physalis angulata* and *Strychnos icaja* are all used to treat malaria. [24] *Strychnos icaja* is also native to central Africa and is used as a medicine along with the related species *Strychnos spinosa* and *Stychnos henningsii*. [25]

1.2.2 Indole alkaloids

Many of plants mentioned in 1.2.1 have been screened for activity and the active compounds have been isolated, purified and individually tested against *P. falciparum*. These compounds, in general, are indole alkaloids. The most notable of these are ochrolifuanine A **(11)** and isotrychnopentamine A **(12)** which both have an IC_{50} <500 nM (0.5 µM) against all *Plasmodium* lines tested, Another notable compound is dihydrousambarensine **(13)** which has an IC_{50} value of < 2 µM against all strains tested

and has a reported IC_{50} of 39 nM (0.039 µM) against a particular chloroquine-resistant strain. [26]



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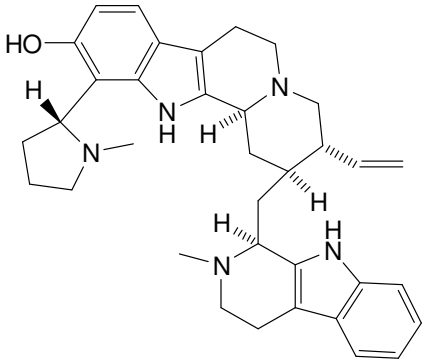

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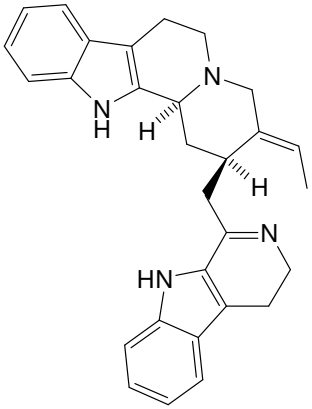
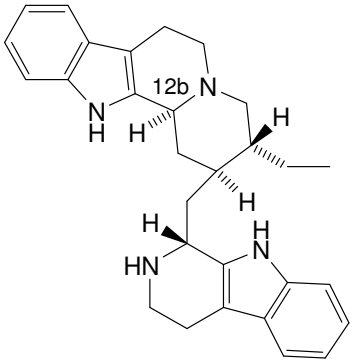


(13)

These structures are fairly similar, and the screening of numerous compounds has shown some patterns that could be the cause of high anti-malarial activity. It has been reported that the indole moiety is important for activity, [27, 28] and many report that bis-indole compounds are far more active than mono-indoles. [23, 25, 26, 27, 29] Frederich *et al.* reported that of all structures screened, the highest activity for a mono-indole compound was an IC_{50} of 10 µM, whereas all the bis-indoles showed values of less than 2 µM. [26] This is supported by Giradot *et al.,* who found bis-indoles from *Muntafara sessilifolia* (a plant

native to Madagascar whose bark is traditionally used to treat fevers) to be more active than the mono-indoles. [27]

Stereochemistry also plays a key role. This is shown clearly with ochrolifuanine A **(11)** and its enantiomer ochrolifuanine E **(14)**, which has an IC_{50} value of <2 μM compared to ochrolifuanine A's activity of <500 nM (<0.5 μM). Many of the naturally occurring compounds with high potency, including dihydrousambarensine, share the same stereochemistry at position 12b as ochrolifuanine A.



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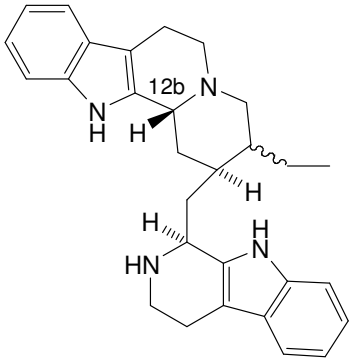
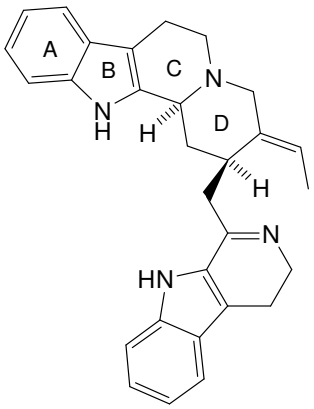


(14)

An ethyl or ethylidene side chain (as shown on compounds (**11)**-(**13)**) on the D-ring and a fully or partially aromatised second ring system have also been found to increase activity. [26, 30]

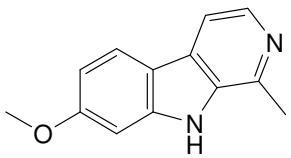


(13)

1.2.3 Mechanism of action

It is known that the basicity of chloroquine contributes to its activity, as this causes it to accumulate in the acidic food vacuole of the parasite. [31, 32] However, it has been shown by Schalkwyk *et al.* [33] that the acidity of a compound can be also be a cause of activity. The *Plasmodium* parasite is known to regulate the pH of its cytosol by pumping protons out across the parasite's plasma membrane. This generates a pH gradient which is critical to the transport of Vitamin B_5, an essential nutrient. Disruption of this pH gradient would result in inhibiting parasitic growth. Schalkwyk *et al.* showed that there was a strong, positive correlation between anti-malarial activity and the ability of the compounds tested to disrupt the cytosolic pH of the parasite.

Another possible mechanism is the interference of heat shock protein 90 (HSP 90). Heat shock proteins (named for their increase in production after periods of heat or stress) are a class of compounds that are involved with the folding and unfolding of other proteins. HSP 90 has been found to be essential for the growth of *P. falciparum* during the erythrocytic stage. [34] This mechanism was proposed by Shahinas *et al.* during their research into harmine **(15)**, an indole alkaloid from the shrub *Guiera senegalensis*, found in the savannah regions of Central and West Africa that is used as a traditional remedy for malaria. [35] Harmine has a very potent activity of 0.0501 µM against the *P. falciparum* strain 3D7. Shahinas *et al.* found that harmine inhibits *P. falciparum* HSP 90 by competing for the ATP binding site and that it also synergises with known anti-malarials chloroquine and artemisinin. [35, 36]

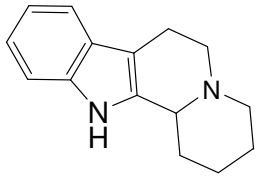


(15)

The structure of harmine is similar to the tetracyclic indolizino[2,3-a]quinolizidine ring system **(16)**. It is possible that other indole alkaloids with this basic structure inhibit *P. falciparum* the same way as harmine does.

Although the exact mechanism is unknown, it appears that the indole moiety is highly important for anti-malarial activity. All the most active compounds have a free indole NH and compounds with two indole moieties are more active still. [33, 37]

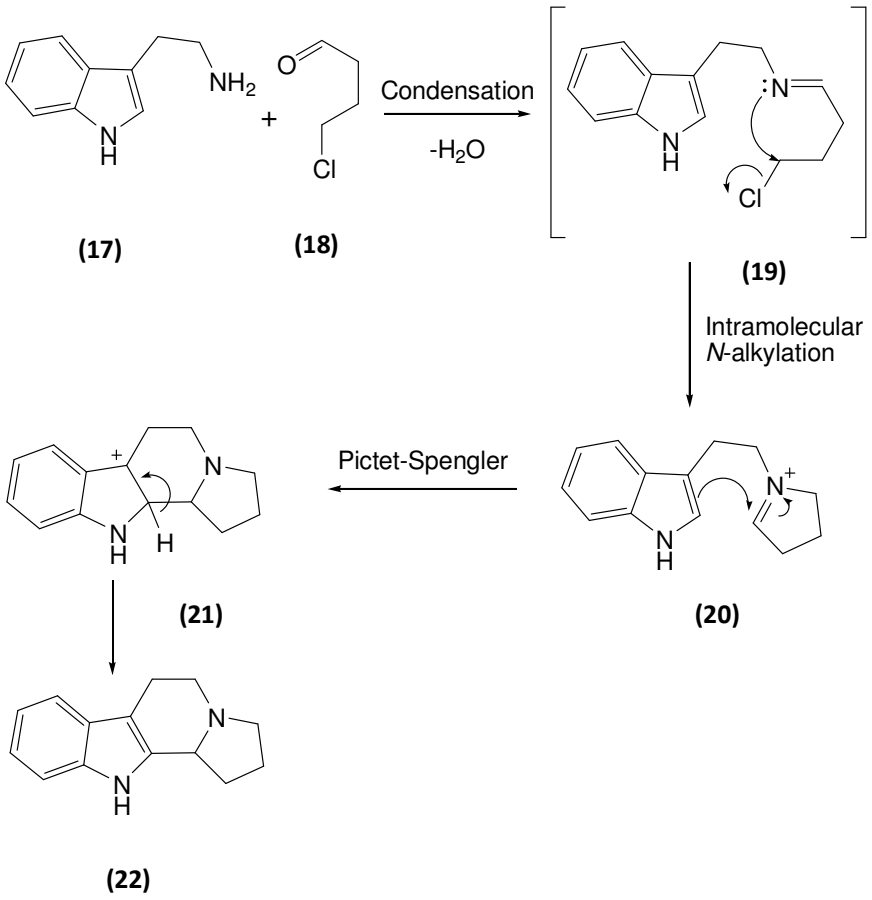
The aim of this project is to investigate the structure-activity relationships of the indolizino[2,3-a]quinolizidine ring system **(16)** and to build upon the findings to synthesise a compound with high anti-malarial activity.



(16)

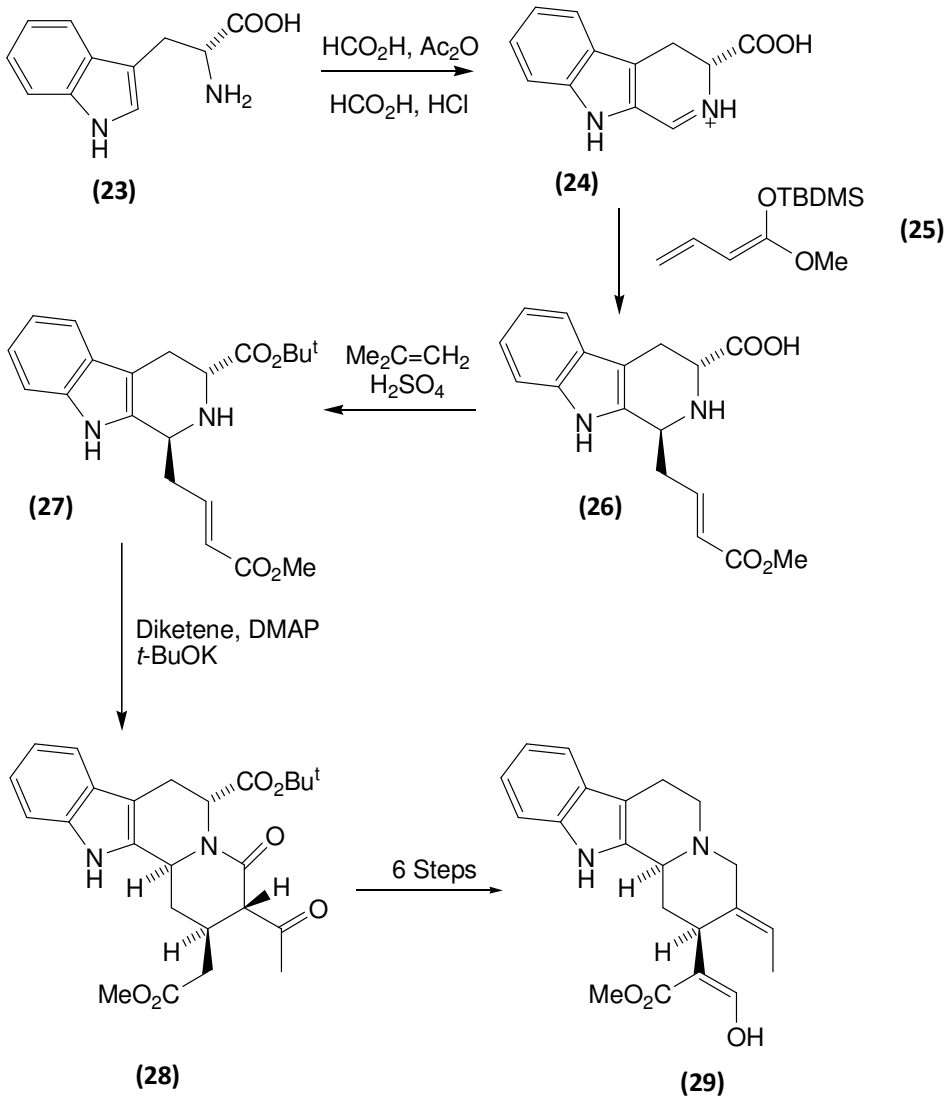
1.2.4 Synthesis of the indolizino[2,3-*a*]quinolizidine core

Many different methods have been employed for the synthesis of this synthetically important ring system. Jana *et al.* [38] utilised the condensation of various amines with haloaldehydes followed by a cyclisation and subsequent Pictet-Spengler reaction to give a one-step synthesis of many derivatives of the core (Scheme 1). They used this method to generate the *Kopsia griffithii* alkaloid harmicine **(21)** in racemic form, found to exhibit activity against the parasite that causes leishmaniasis.



Scheme 1

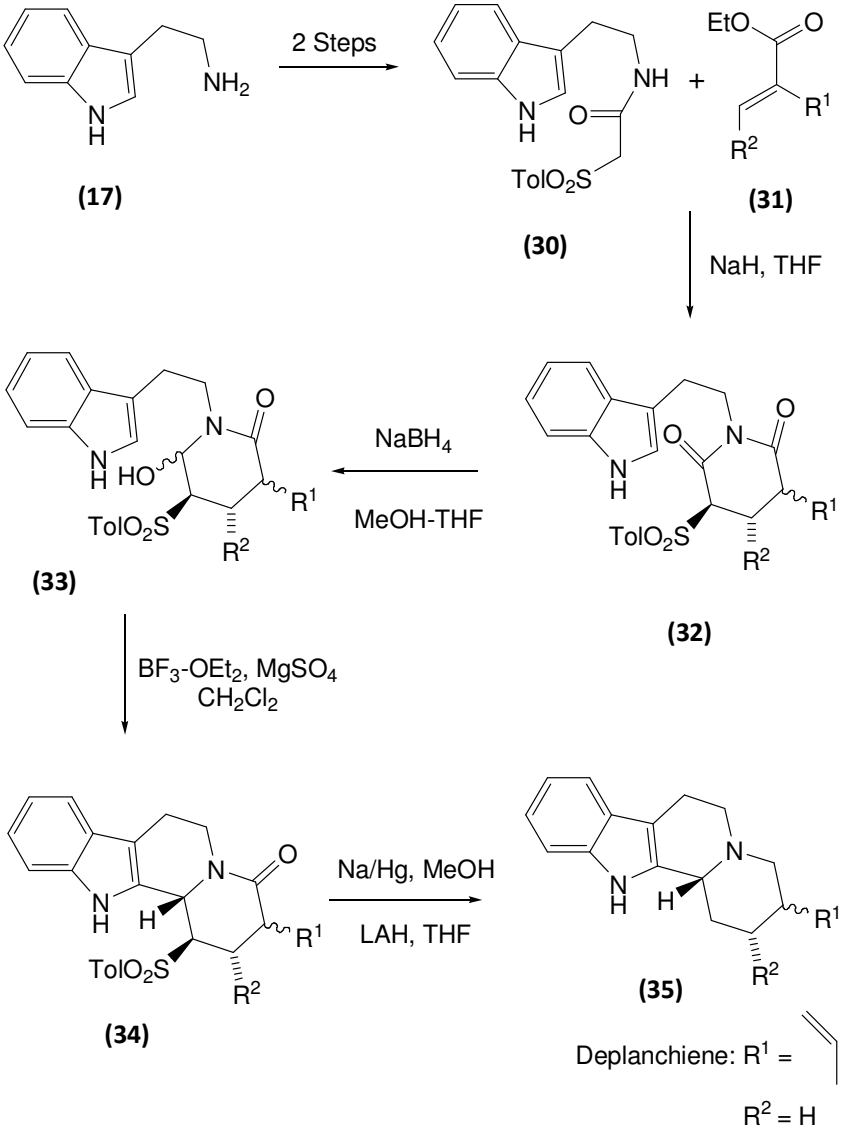
Martin *et al.* [39] utilised another method to access this template *en route* to a synthesis of geissoschizine (a pivotal intermediate in the biosynthesis of biologically-active alkaloids) (Scheme 2). It began with the conversion of D-tryptophan **(23)** into the dihydrocarboline **(24)** in a single step. This was followed by a reaction with vinyl ketene acetal **(25)** to give compound **(26)** which was then treated with isobutylene in acidic conditions to give **(27).** *N*-Acylation of **(27)** with diketene, followed by a Michael reaction gave compound **(28)** as a precursor to geissoschizine **(29)**.



Scheme 2

Chang *et al.*[40] synthesised the template *en route* to the natural product deplancheine **(35)**, a compound isolated from the New Caledonian plant *Alstonia deplanchei*, that has a simple structure containing the indolizino[2,3-*a*]quinolizidine template. Tryptamine **(17)** forms an amide with chloroacetyl chloride in the presence of triethylamine, which is then treated with *p*-toluenesulfinic acid sodium salt to give compound **(30)**. This was then reacted with various α,β-unsaturated esters **(31)** in the presence of sodium hydride, followed by a reduction with sodium borohydride and a cyclisation reaction to give the

tetracyclic template **(34)**. This was then functionalised further to give the natural product deplancheine **(35)** (Scheme 3).

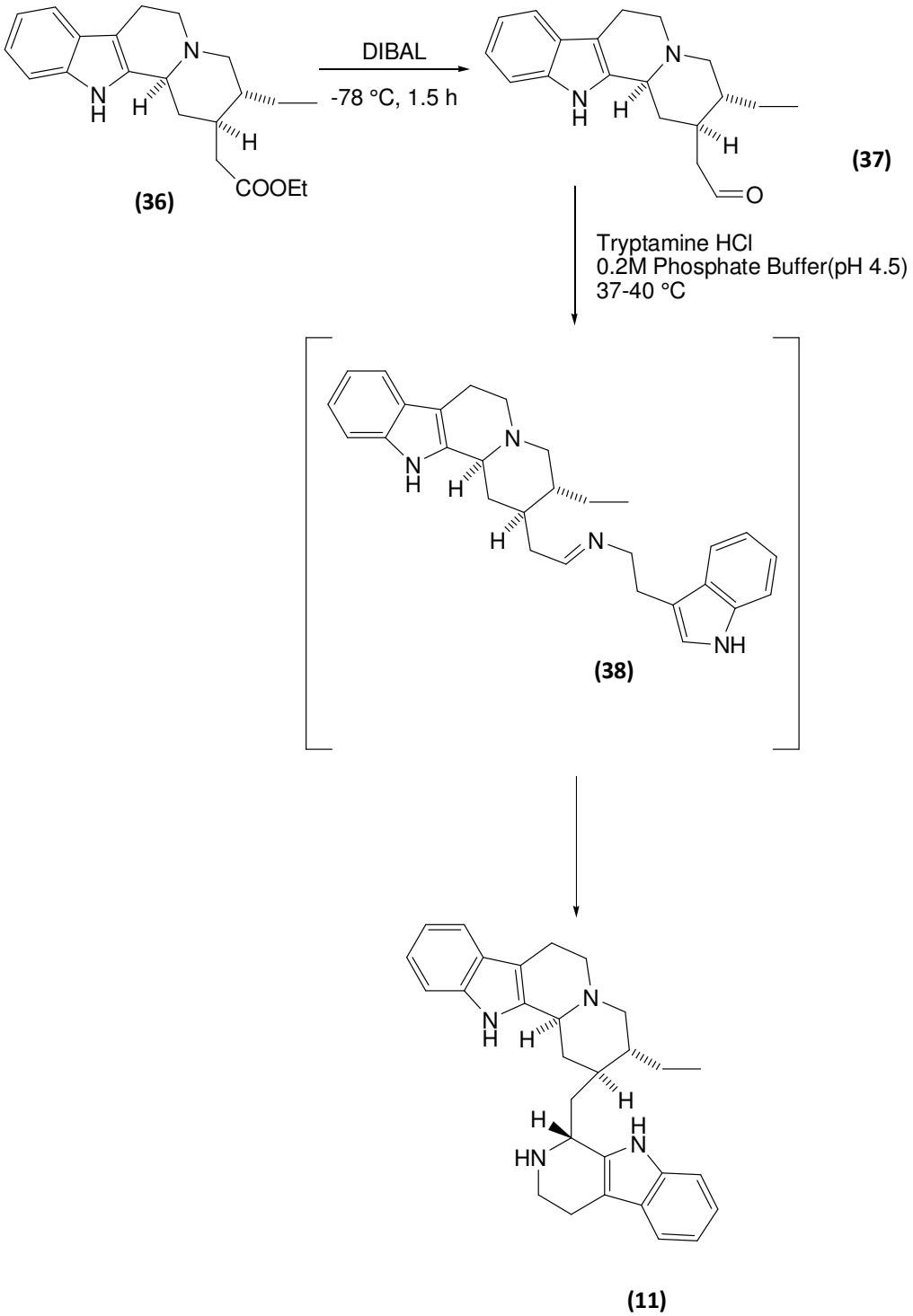


Scheme 3

1.2.5 Synthesis of bis-indole compounds

Although the synthesis of the indolizino[2,3-*a*]quinolizidine core and its derivatisation to natural compounds has been well investigated and reported, synthesis of bis-indole compounds have not. One that has been investigated is ochrolifuanine A **(11),** and it's full synthesis was reported by Malhorta *et al.* in 1999. [41]

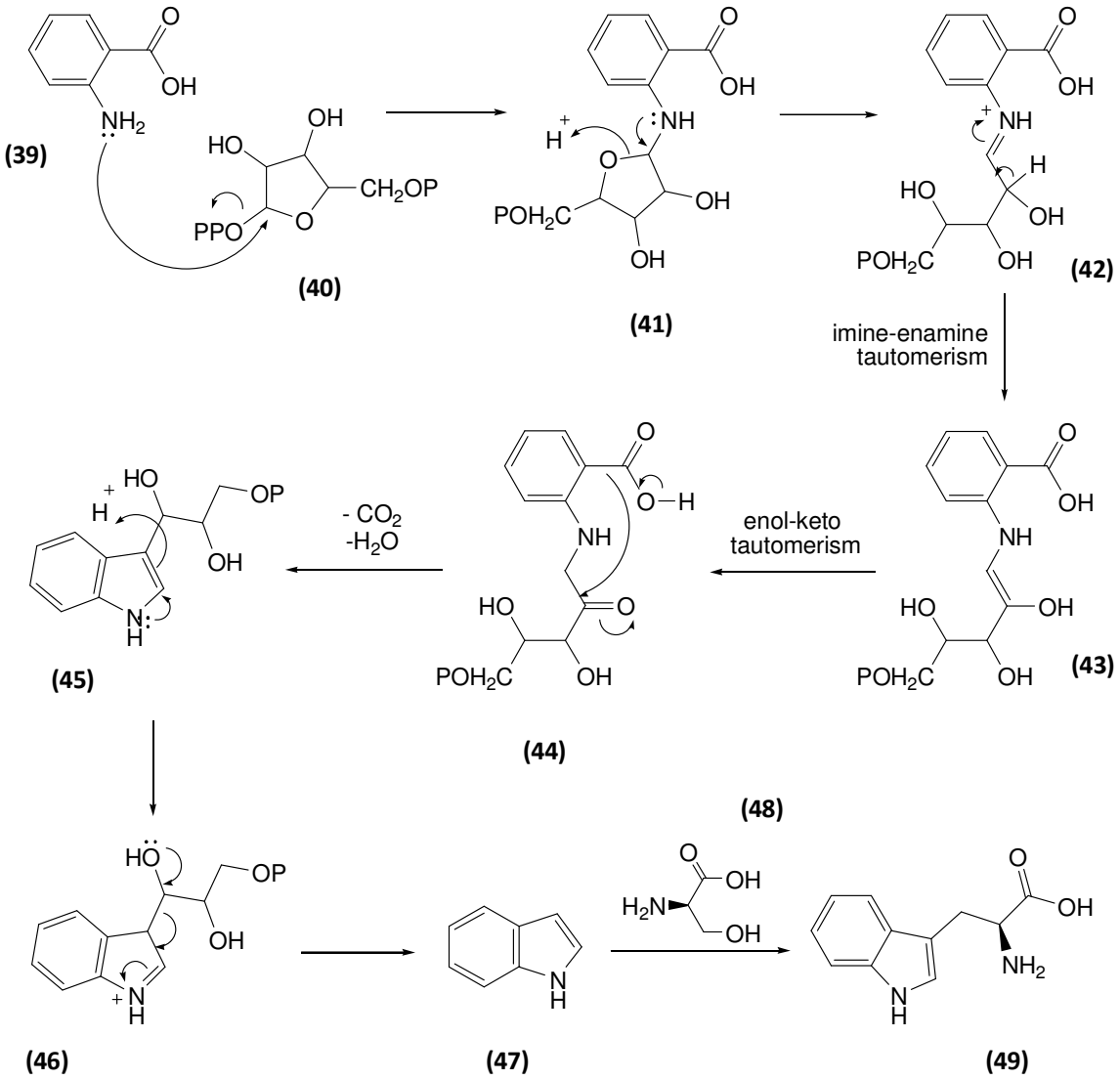
The bottom ring system was introduced by a condensation reaction between tryptamine hydrochloride and aldehyde **(37)** to give imine **(38)** which was then cyclised to give a β-carboline unit, forming ochrolifuanine A **(11)**. The reaction was carried out at 37-40 °C in the presence of a 0.2 M phosphate buffer at pH 4.5 (Scheme 4).


DIBAL
-78 °C, 1.5 h
(36)
(37)
Tryptamine HCl
0.2M Phosphate Buffer(pH 4.5)
37-40 °C
(38)
(11)

Scheme 4 [41]

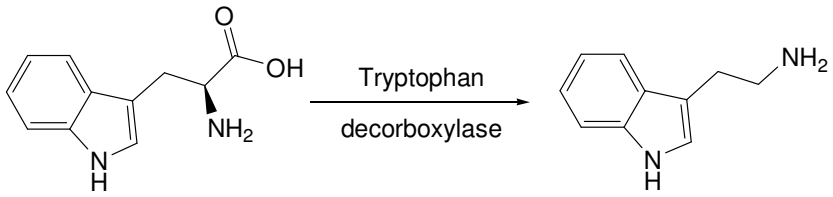
1.2.6 Biosynthesis of indole alkaloids

L-Tryptophan is the most common precursor in the biosynthesis of indole alkaloids. It is formed from the shikimate pathway (Scheme 5), *via* the intermediate anthranilic acid **(39)** which is converted into indole **(47)**, and subsequent reaction with L-serine **(48)** and the enzyme tryptophan synthase, gives L-tryptophan **(49)**. [42]



Scheme 5

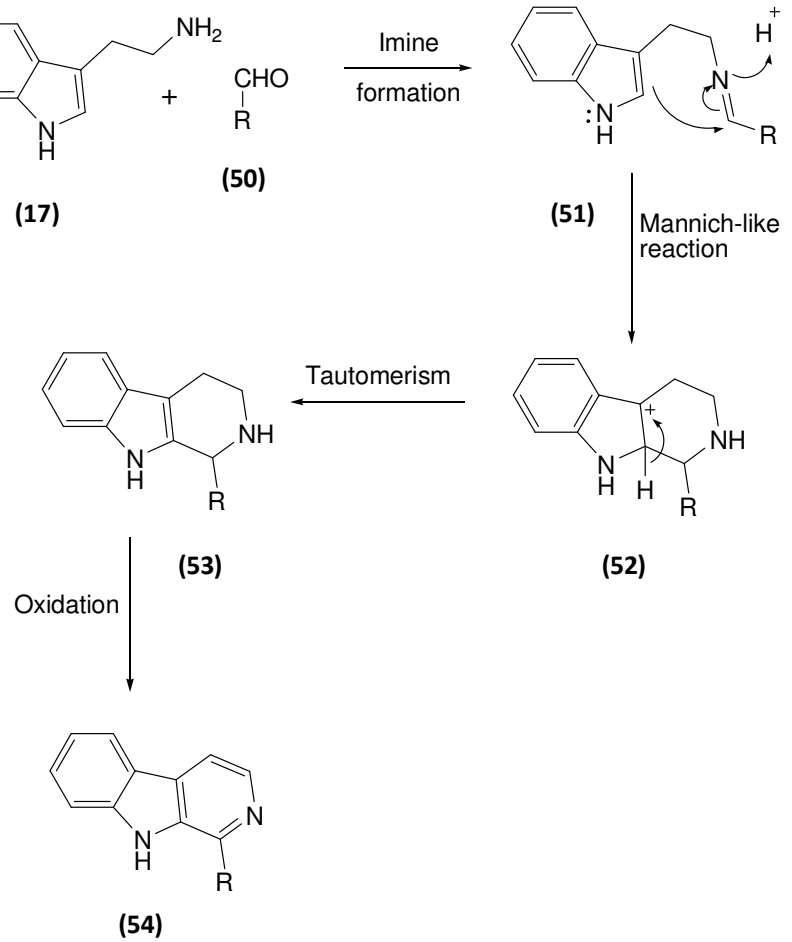
L-Tryptophan **(49)** is then converted into tryptamine **(17)** by a decarboxylation reaction assisted by the enzyme tryptophan decarboxylase (Scheme 6).



(48) **(17)**

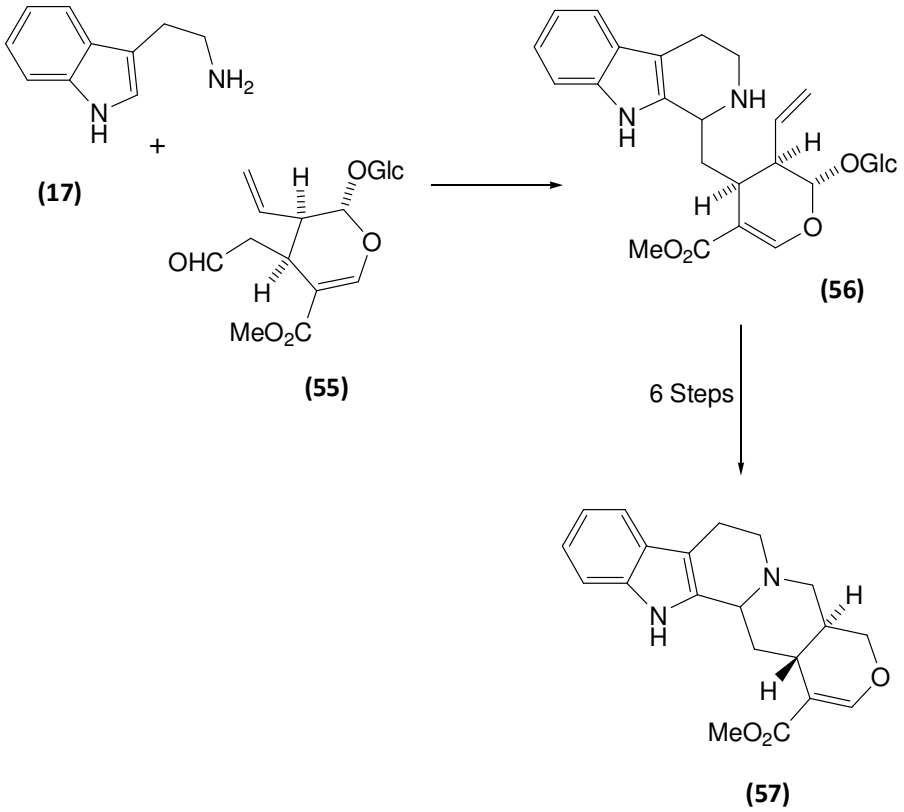
Scheme 6

The C2 carbon of the indole ring is nucleophilic due to the adjacent nitrogen atom, and can therefore participate in Mannich and Pictet-Spengler type reactions, forming a β-carboline **(52)** (Scheme 7).



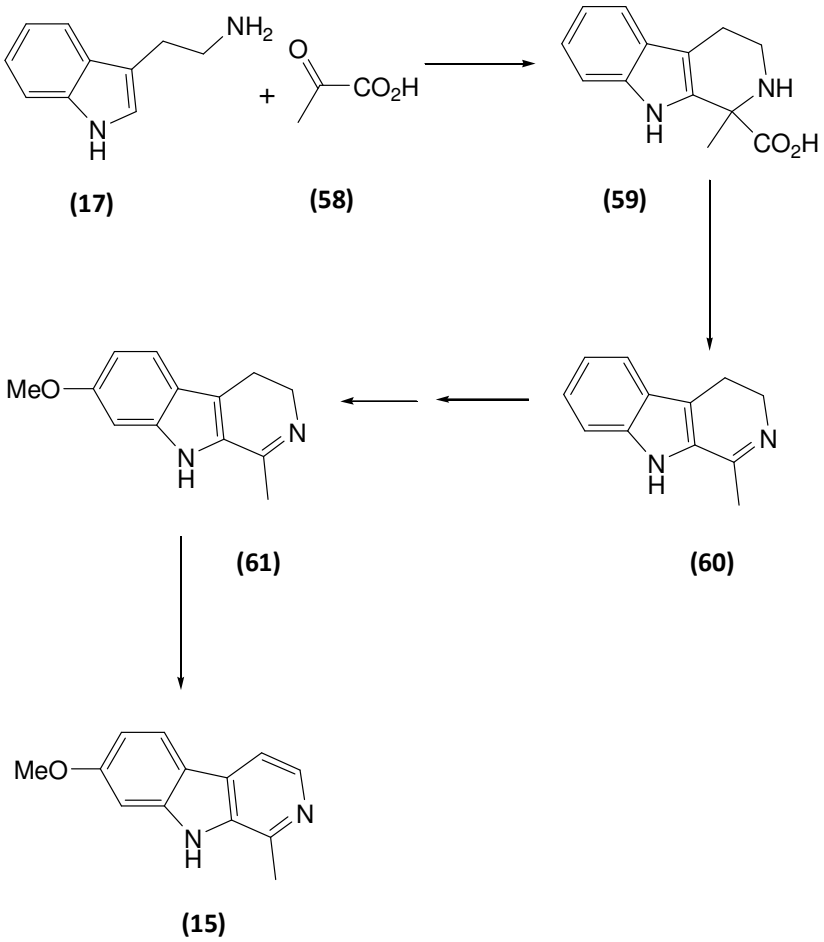
Scheme 7

Extra carbon atoms and rings are supplied by reacting tryptamine **(17)** with various aldehydes and ketones, depending on the complexity of the desired target. More complex compounds use an aldehyde such as secologanin **(55)**, shown below (Scheme 8) in the synthesis of strictosidine **(56)**, a precursor to complex indole alkaloids such as ajmalicine **(57)**, an antihypertensive drug used in the treatment of high blood pressure. [43]



Scheme 8

More simple compounds like harmine **(15)** described above, use ketoacids as precursors (Scheme 9).



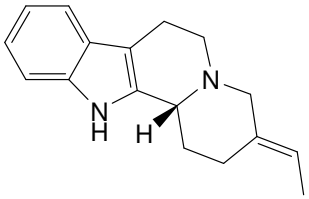
(17) **(58)** **(59)** **(60)** **(61)** **(15)**

Scheme 9

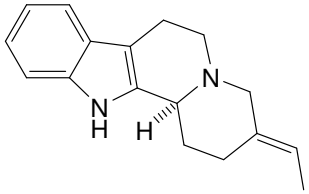
1.3 Allin Group Synthesis of the Indolizino[2,3-a]quinolizidine ring system

1.3.1 *N*-Acyliminium ions

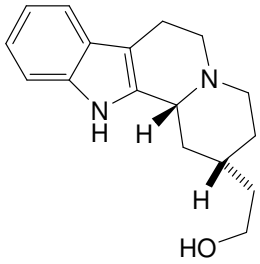
Previous work in the Allin group has focused on the application of *N*-acyliminium ions in the asymmetric synthesis of indole alkaloids. This has led to the synthesis of the indolizino[2,3-a]quinolizidine ring system [44] and some natural product targets derived from it, such as both enantiomers of deplancheine **(35a, 35b)** [45] and (+)-12*b*-epidevinylantirhine **(62)**. [46]



(35a)

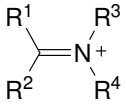


(35b)

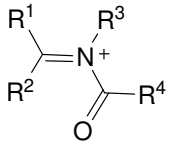


(62)

Cyclisation reactions proceeding *via* *N*-acyliminium ions **(64)** is a relatively new area compared to those that proceed *via* iminium ions **(63)** such as the Mannich [47] reaction, the Bischler-Napieralski [48] reaction, and the Pictet-Spengler [49] reaction.

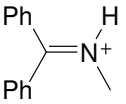


(63)

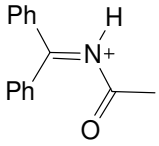


(64)

Both iminium and *N*-acyliminium ions are used extensively as electrophiles in the synthesis of alkaloidal and related systems; however, it seems possible that *N*-acyliminium ions are more reactive. A study on ^{13}C NMR spectra by Wurthwein *et al.* [50] showed that substitution of an *N*-methyl **(65)** by an *N*-acetyl group **(66)** led to a downfield shift of the imino carbon absorption of approximately 5 ppm. This could suggest that *N*-acyliminium ions are more electrophilic and therefore more reactive, than iminium ions.[45]



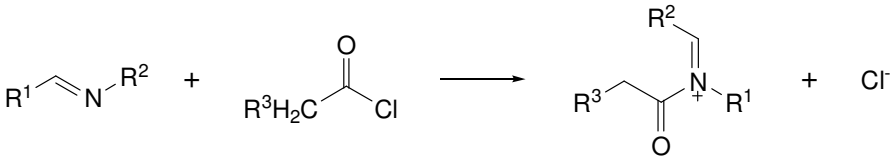
(65)



(66)

Due to their limited stability and high reactivity, *N*-acyliminium ions are generated *in situ*. There are five principal mechanisms for this: [45]

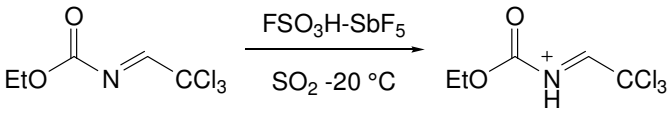
1) ***N*-Acylation of imines**: Condensation of aldehydes or ketones with amines can produce imines in high yields. These can then be acylated with reactive carboxylic acid derivatives such as anhydrides or acid chlorides.



(67) **(68)** **(69)**

Scheme 10 [51]

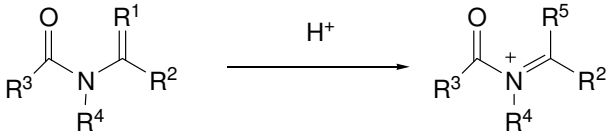
2) ***N*-Protonation of *N*-acylimines**: This method is not synthetically useful due to the requirement of forceful conditions and the instability of *N*-acylimines, as they tautomerise to the corresponding enamide.



(70) **(71)**

Scheme 11 [52]

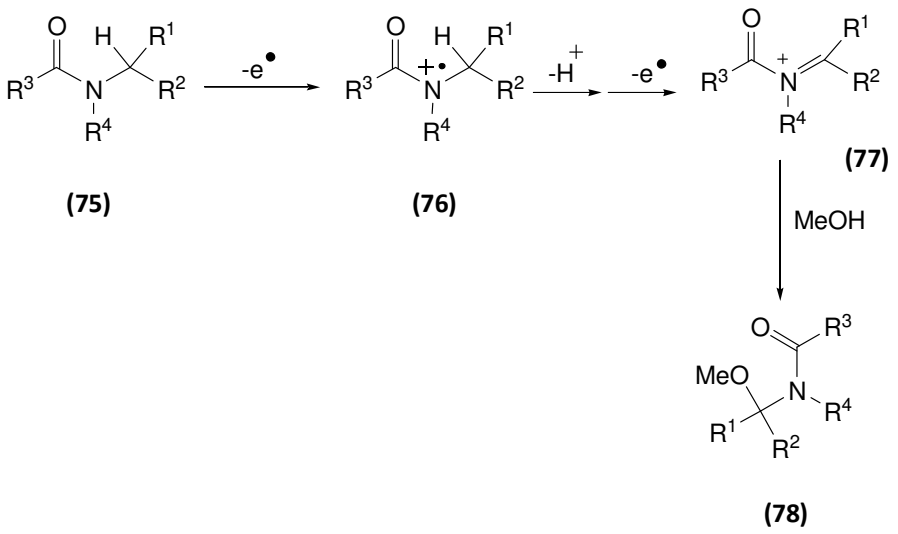
3) ***N*-Protonation of enamides**: *N*-acyliminium ions can be formed by the protonation of enamides.



(73) **(74)**

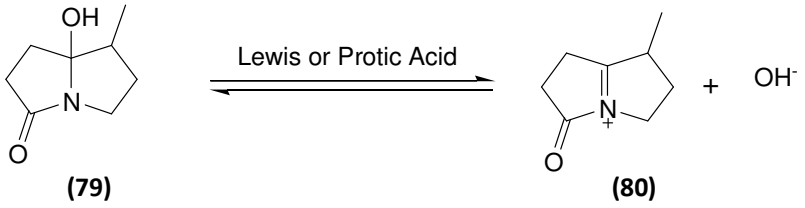
Scheme 12 [45]

4) **Oxidation of amides:** This method involves initial removal of an electron from the lone pair on the nitrogen atom of compound **(75)**, followed by loss of a proton and another electron from compound **(76)**. This gives the corresponding *N*-acyliminium ion **(77)**. These oxidations are generally carried out in the presence of a nucleophile, usually methanol, in order to trap the ion as soon as it is formed (Scheme 13).



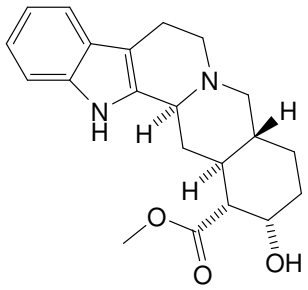
Scheme 13 [45]

5) **Heterolysis of amides with a leaving group on the α-carbon**: This is the most common method, and the one employed in Allin group's work. The leaving group is usually an oxygen substituent but could also be a halogen, nitrogen, sulfur or phosphorus substituent. Generally, an acid (Brönsted or Lewis) is used to generate the ion.

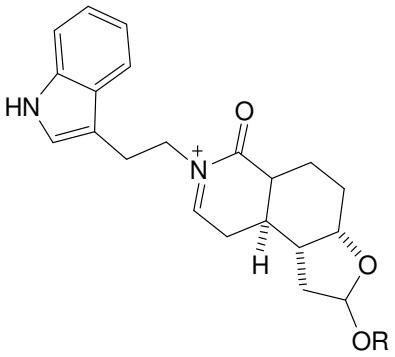


Scheme 14 [53]

Tamelen *et al.* [54] described a synthesis of yohimbine **(81)**, a naturally occurring alkaloid from the plant *Pausinystalia yohimbe* which has stimulant and aphrodisiac effects. The essential step in this synthesis is the acid-catalysed ring closure of an *N*-acyliminium ion **(82)**. The reaction proceeds extremely quickly due to the high reactivity of the electron-rich aromatic ring as a nucleophile, in this case an indole ring.



(81)



(82)

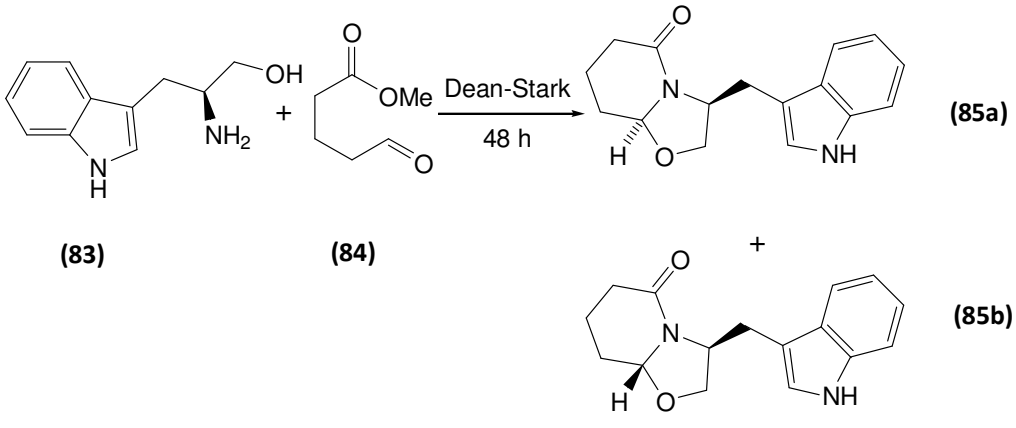
Reactions involving iminium ions or *N*-acyliminium ions are reversible, the reverse process being examples of the Grob fragmentation. [55] The product of a reaction involving an iminium ion is an amine, however with an *N*-acyliminium ion the product is an amide. Amides are far less susceptible to fragmentation than amines and therefore reactions involving *N*-acyliminium ions can be viewed as less reversible. [56]

1.3.2 Synthesis of the (+)- indolizino[2,3-a]quinolizidine ring system (86)

Cyclisation reactions using an *N*-acyliminium ion and an indole ring have played an important role in the Allin group's previous work and have been utilised in the

stereoselective synthesis of the indoloizino[2,3-α]quinolizidine template, which, as noted above, comprises a tetracyclic core present in a plethora of bioactive indole alkaloids.

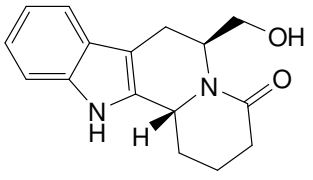
The synthesis of this ring system was achieved by reacting the β-amino alcohol derivative of L-tryptophan **(83)** with an appropriate keto acid **(84)** under Dean-Stark conditions for 48 hours, to give the expected bicyclic lactam product **(85)** as a 5:1 mixture of diasteroisomers, with **(85a)** being the major isomer (Scheme 15). [57]



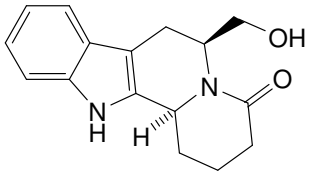
(83) **(84)**

Scheme 15

Originally both diastereoisomers of **(85)** were treated with titanium tetrachloride to give the desired cyclised product **(86)** as a 5:2 mixture of diasteroisomers. [57]

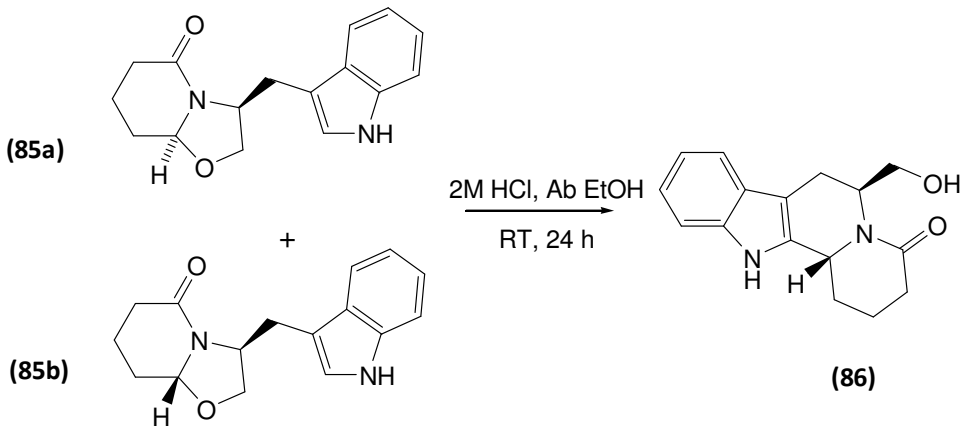


(86a) Major



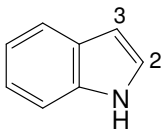
(86b) Minor

In an attempt to improve the selectivity, the bicyclic lactam diastereoisomers **(85a, b)** were treated with 2M hydrochloric acid in absolute ethanol. This gave the desired product **(86)** as a single diastereoisomer (Scheme 16). [44]



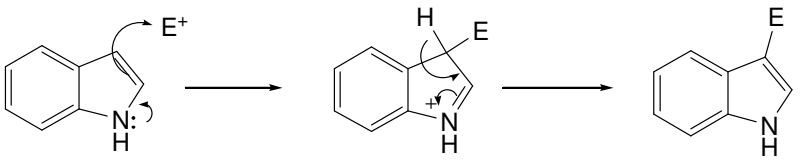
Scheme 16

1.3.3 Cyclisation reactions of indole (46)



(46)

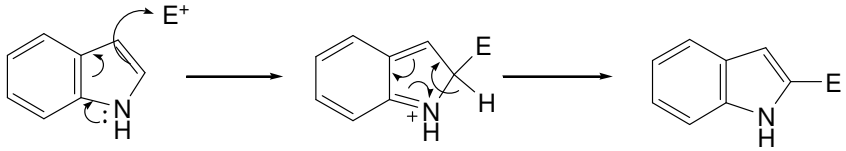
When undergoing electrophilic substitution, despite the C2 carbon being more strongly nucleophilic, reaction generally occurs at the C3 carbon. This is most likely due to the unwillingness of the benzene ring to lose aromaticity. Reaction at the C3 carbon does not require the benzene ring to be involved (Scheme 17).



(47) **(87a)** **(88a)**

Scheme 17

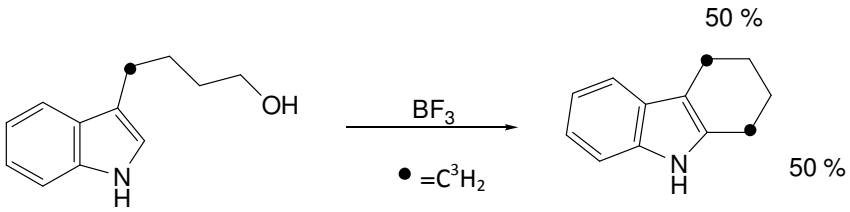
Reaction at the C2 carbon does involve the benzene ring (Scheme 18).



(47) **(87b)** **(88b)**

Scheme 18

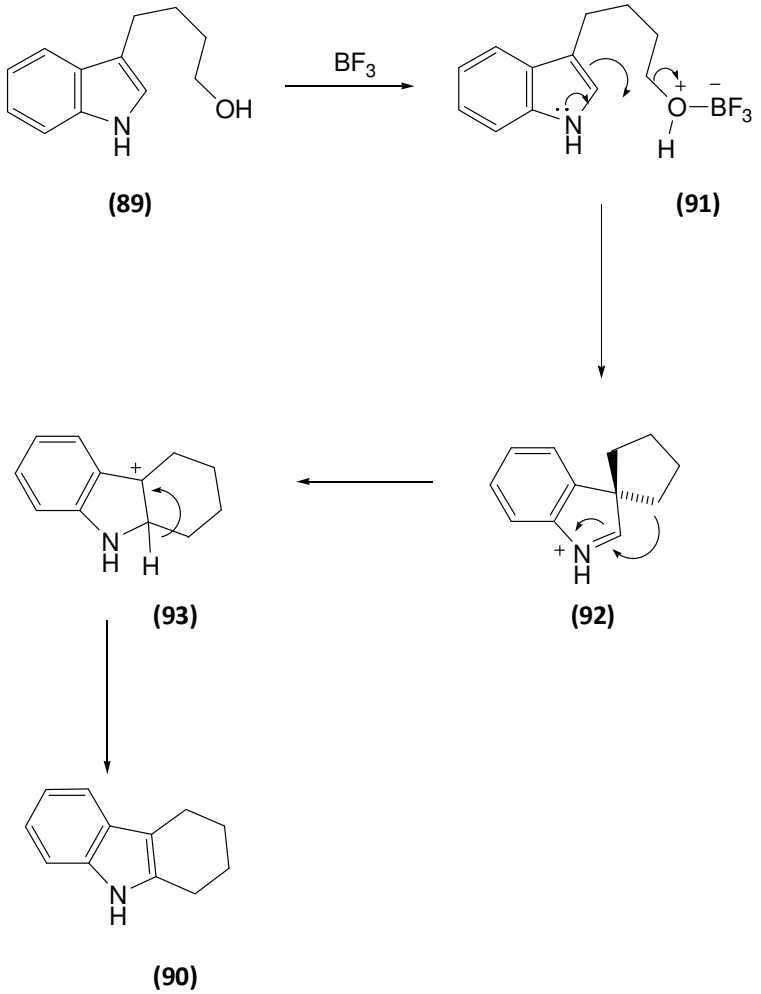
If the C3 position is blocked, one would assume the reaction would be forced to occur at the C2 position, and benzene would be forced to take part. However a study has shown that this is not the case. The compound shown below **(89)** was labelled with tritium (^{3}H) on the carbon attached in the C3 position **(89)**. When the cyclisation reaction had occurred it was found that not all the radio-labelled hydrogen was where it was expected to be: 50% resided adjacent to the C3 of indole as expected and 50% had migrated as shown (Scheme 19).



(89) **(90)**

Scheme 19

For this to occur, the reaction intermediate must be symmetrical, which results from attack at the 3-position. This forms a *spiro* compound, a 5 remembered ring at right angles to the indole moiety. Bond migration then occurs to give the observed product **(90)**; each CH_2 group has an equal chance of migrating (Scheme 20). [58]



Scheme 20

In cyclisations such as the one shown in Scheme 20, depending on the substituents of the compound, a new chiral centre can be introduced. A mixture of stereoisomers is possible, the composition of which is determined by the locations of substituents on the addition product. [56] When treating both bicyclic lactams **(85a, b)** with 2M hydrochloric acid only one diastereoisomer is observed **(86a)** (Scheme 16).

1.3.4 Rationalisation of the Stereochemical Outcome

Conformational models (Figure 2) were used by the Allin group to rationalise the stereochemical outcome of this cyclisation reaction. [45] Both diastereoisomers of the bicyclic lactam form the same *N*-acyliminium species as an intermediate. In the first conformation **(A)**, the carbonyl moiety is eclipsed in a 1,3-fashion by the hydrogen atom at the β-amino alcohol chiral centre. This gives the observed diastereoisomer. This is possible as the hydrogen atom at the iminium carbon is small and therefore provides no significant steric bulk to interfere with the positioning of the hydroxymethyl or indole groups. Bond rotation around the C-N^+ bond leads to conformation **(B)** which would give the unobserved diastereoisomer. This conformation has the hydroxymethyl group positioned closer to the carbonyl which appears to bring an unfavourable 1, 3-interaction between the two groups.

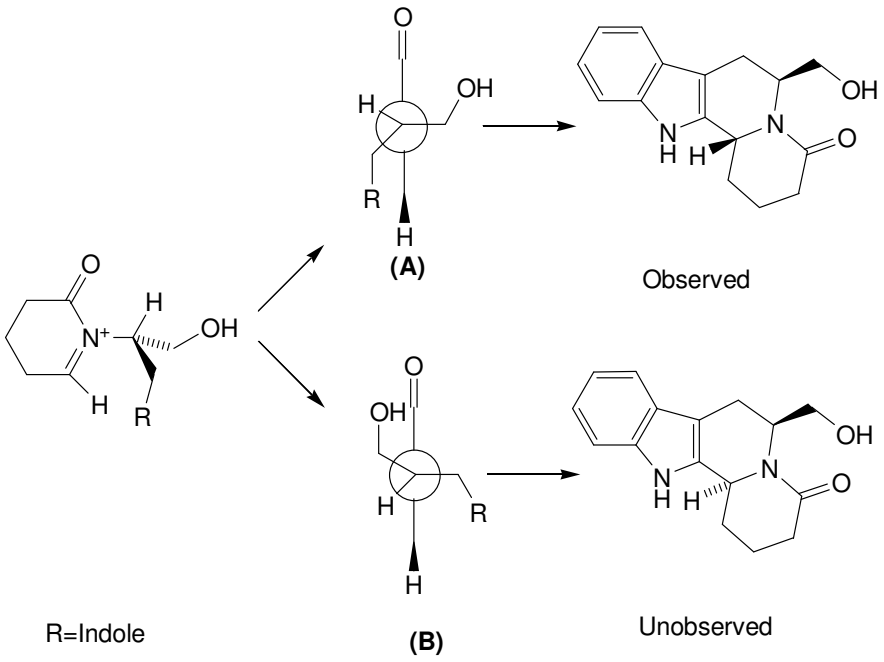
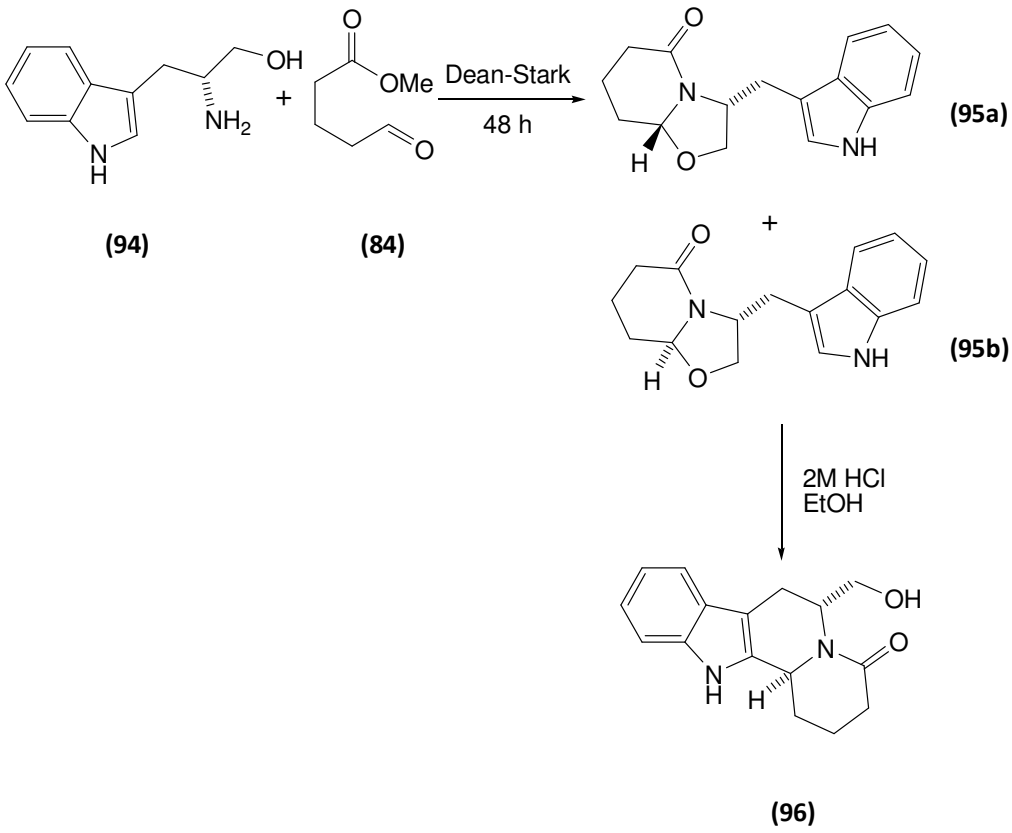


Figure 2

It is thought by the Allin group that conformation **(B)** leading to the minor diastereoisomer observed upon treatment with titanium tetrachloride is stabilised by chelation between the Lewis acid-complexed oxymethyl group and the carbonyl moiety. [45]

1.3.5 Synthesis of the (-)- indolizino[2,3-a]quinolizidine ring system (96)

Access to both enantiomers of the indoloizino[2,3-α]quinolizidine template was highly desirable as both stereochemistries appear in natural compounds. This was achieved using the same method as shown in Scheme 15, but using the β-amino alcohol derivative of D-tryptophan **(94)** as the starting material (Scheme 21).



(94) **(84)** **(96)**

Scheme 21

1.4 Functionalisations of the Indolizino[2,3-a]quinolizidine Ring System

With both enantiomers accessible, work then focused on functionalisations of the template. Due to the cost of unnatural D-tryptophan compared to its inexpensive enantiomer, the template formed from L-tryptophan was more commonly used. Figure 3 below shows possible functionalisations of the template.

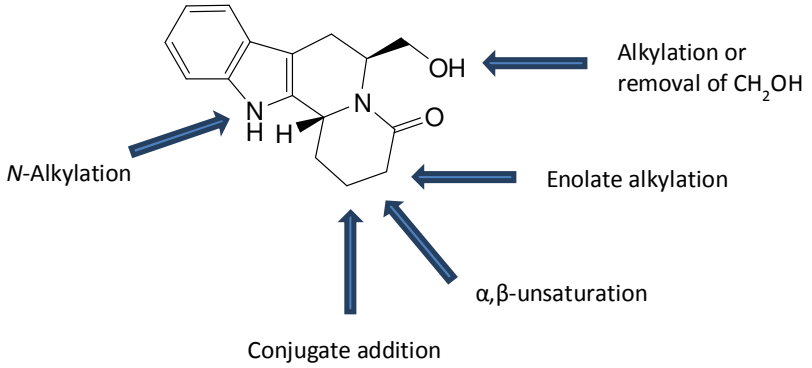
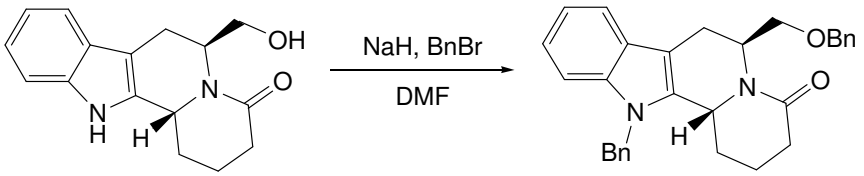


Figure 3: Functionalisations of the indolizino[2,3-a]quinolizidine ring system

1.4.1 Alkylations

The simplest of these functionalisations is an alkylation at the indole NH and the free hydroxyl group. These were carried out initially to prevent unwanted side reactions during further experiments (*i.e. N*-protection). A benzyl moiety was added to both N and O atoms as a protecting group using 2 equivalents of sodium hydride and benzyl bromide in anhydrous dimethylformamide (Scheme 22). Other groups could potentially be added using the same methodology.



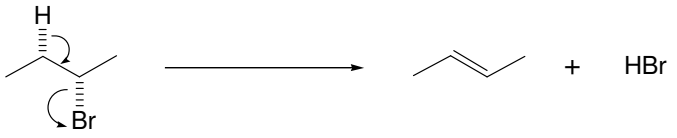
(86) **(97)**

Scheme 22

1.4.2 α,β-Unsaturation and conjugate additions

With protecting groups in place, the compound could be further functionalised (Scheme 24). α,β-Unsaturation was achieved using a method employed by Reich *et al.* [59] that showed selenoxides undergo clean *syn* elimination to form olefins at or below room temperature.

Syn elimination is where the substituents a removed from the same face of the bond as shown in Scheme 23.

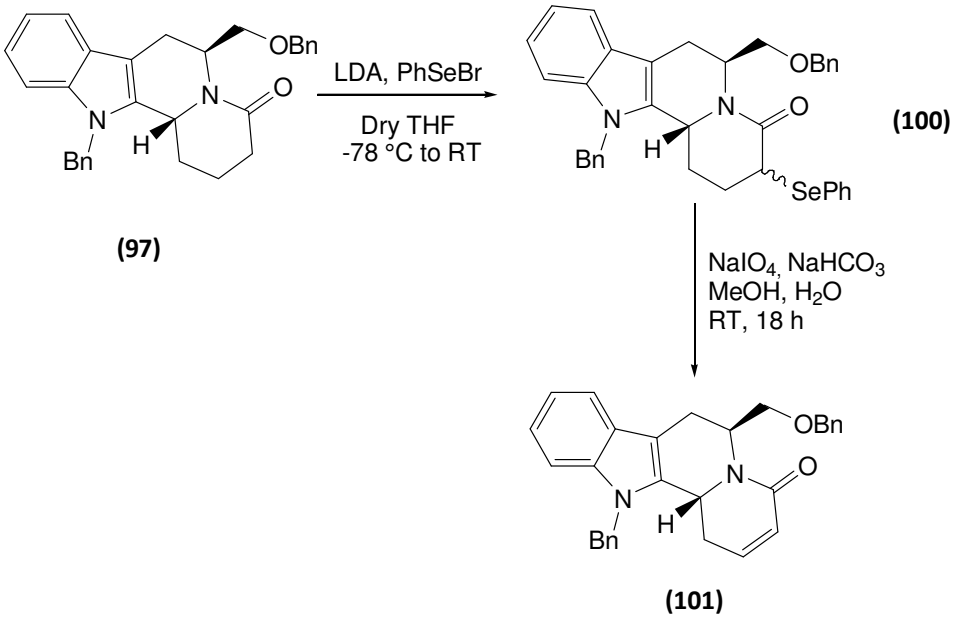


(98) **(99)**

Scheme 23

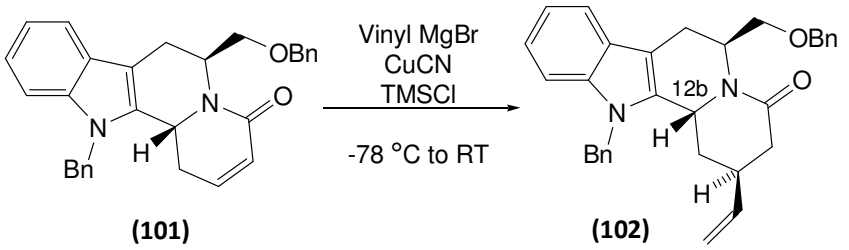
Lithium di*iso*propylamide was used to generate the enolate of the bis-protected template **(97)** which was then reacted with phenyl selenyl bromide at -78 °C to give selenide **(100)**. The crude selenide was dissolved in methanol and water, and treated with sodium

metaperiodate to give the desired α,β-unsaturated compound **(101)**, which could now be used to explore conjugate addition reactions.



Scheme 24

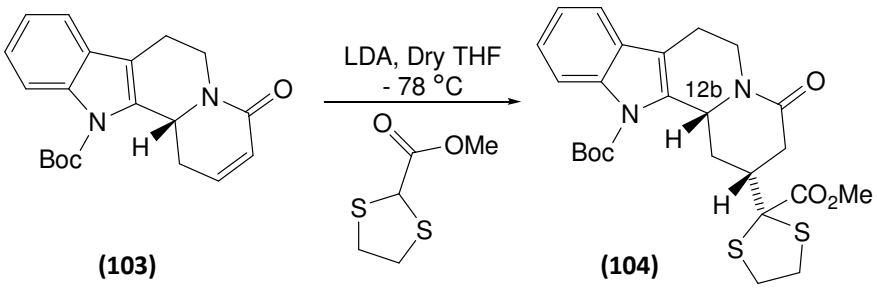
Various nucleophiles were investigated as reagents for conjugate addition reactions, with vinyl magnesium bromide being the most successful (Scheme 25), giving a 65% yield of compound **(102)**. It is important to note that the relative stereochemistry of the nucleophile at the newly created chiral centre remains the same regardless of what nucleophile is used and is *cis* to the H atom adjacent to the nitrogen on carbon 12b. This was also found to be true when conjugate additions were performed on the opposite enantiomer of the template. [60] This *cis* chemistry can also be produced when there is no hydroxymethyl moiety on the compound and the indole NH remains unprotected.



(101) **(102)**

Scheme 25

The opposite stereochemistry can be introduced as shown in Scheme 26. [60]



(103) **(104)**

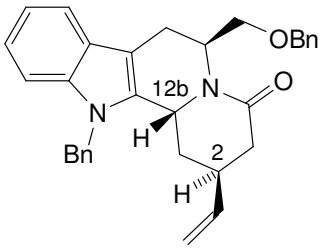
Scheme 26

In this case the relative stereochemistry of the nucleophile is always *trans* to the H atom. This stereocontrol could prove useful in the synthesis of natural compounds as both relative stereochemistries are found in natural products.

1.4.3 Rationalisation of the Stereochemical Outcome

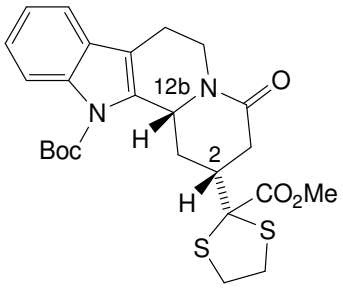
The stereochemical induction in compound **(102)** that gives rise to the protons at position 12b and position 2 having *trans* relative stereochemistry appears to result from the nucleophile approaching the face of the amide that carries the bulky benzyloxymethyl-

substituent, which is unexpected. However, the conformation of **(102)** is bowl-like and the nucleophile is actually approaching from the outer (possibly less hindered) face of the bowl. [60]



(102)

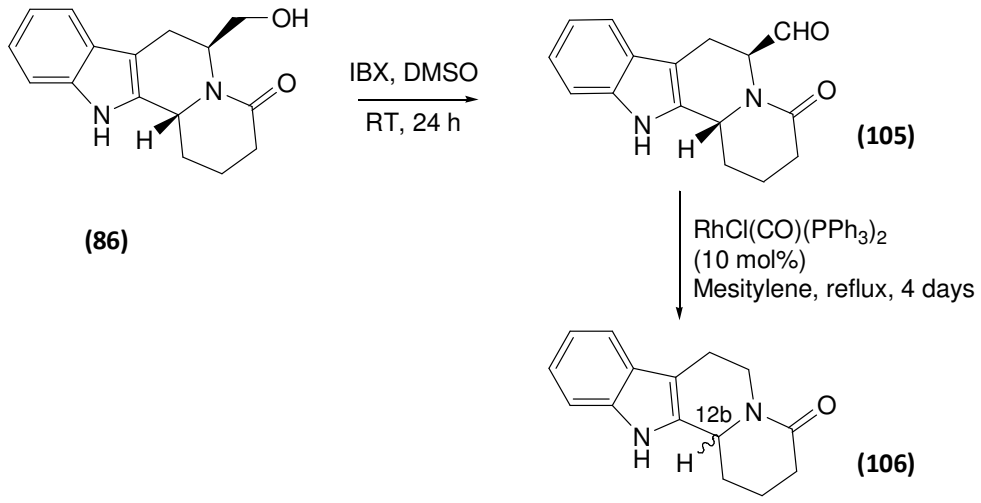
With no hydroxymethyl group, and a Boc-protected indole nitrogen, conjugate addition reactions yielded compounds with the protons at 12b and 2 having *cis* relative stereochemistry, as in compound **(104)**. Without the bulky benzyloxymethyl-substituent, the opposite face is most likely the less hindered.



(104)

1.4.4 Removal of hydroxymethyl moiety

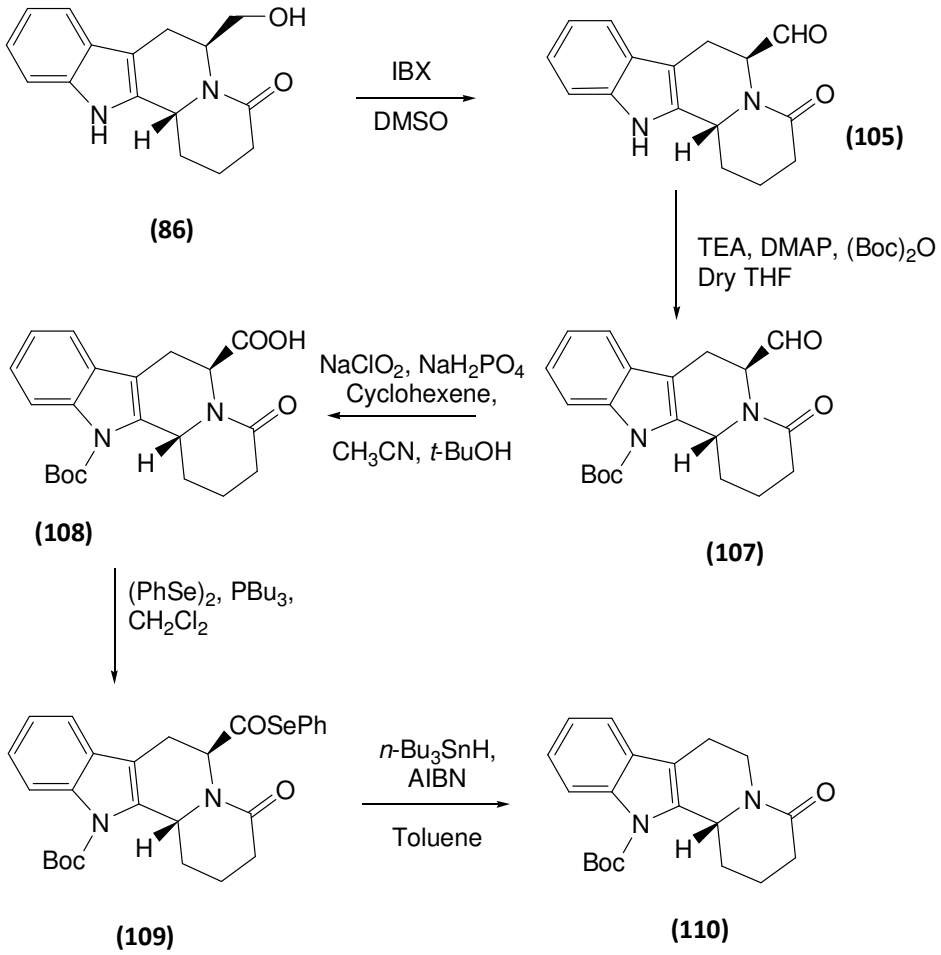
A rhodium-induced decarbonylation method for the removal of the hydroxyl methyl group (Scheme 27) was investigated to demonstrate the potential of synthetic utility of the indolizino[2,3-α]quinolizidine template as a precursor to natural compounds, none of which contain this moiety. [45]



(86)

Scheme 27

Compound **(86)** is oxidised to the corresponding aldehyde which is then subjected to the rhodinium decarbonylation protocol using mesitylene as a solvent. Unfortunately, possibly due to long reaction times and high temperatures, this method caused racemisation of the hydrogen on carbon 12b and was therefore abandoned. An alternative procedure was adopted using the radical decarbonylation of phenylseleno esters (Scheme 28). [60]

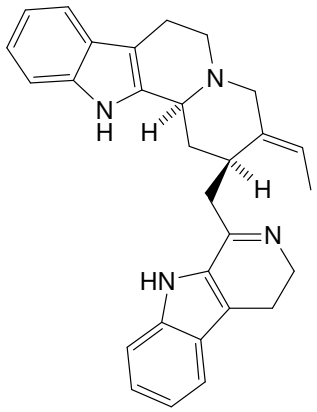


Scheme 28

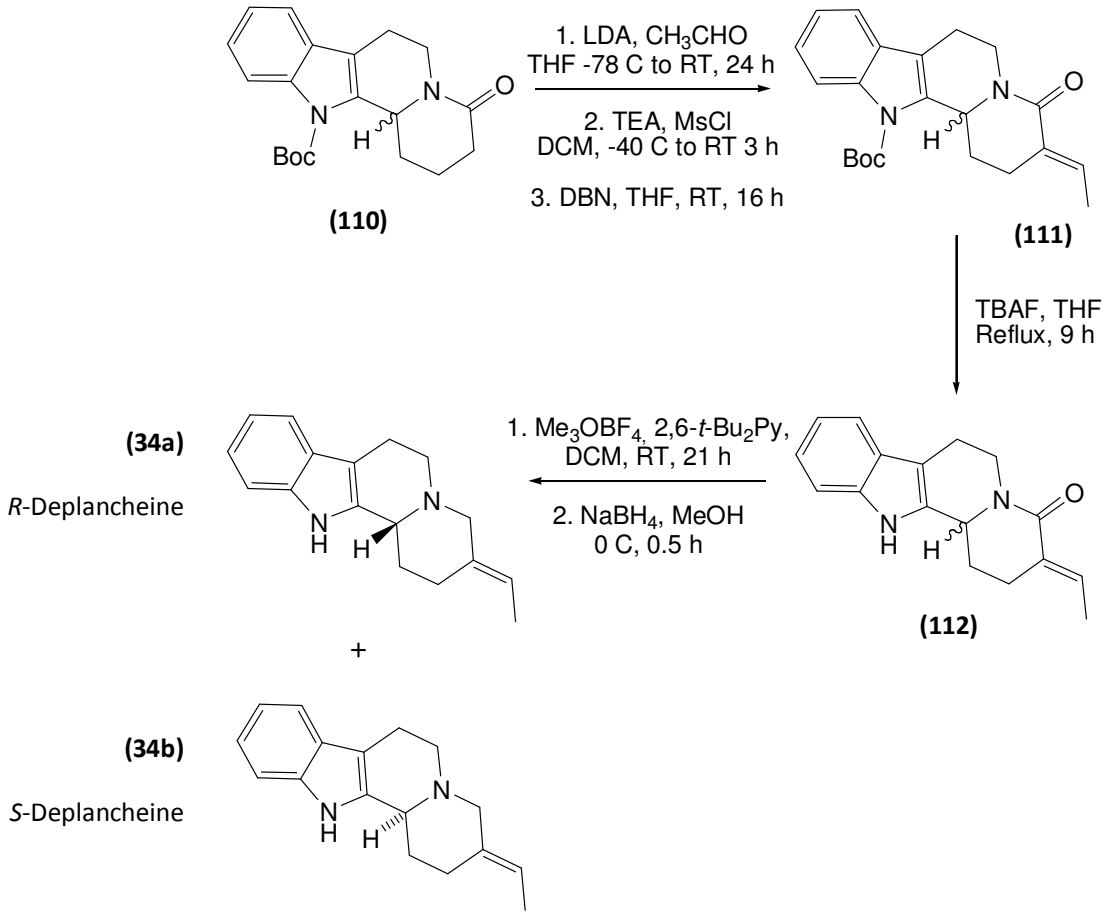
Compound **(86)** is converted to the corresponding aldehyde as in Scheme 27. The indole nitrogen is then Boc-protected **(107)**, and the aldehyde is further oxidised to the carboxylic acid **(108).** Compound **(108)** is converted into a phenylseleno ester **(109)** which is then eliminated using a tin-mediated radical de-carbonylation reaction to give the desired compound **(110)**.

1.4.5 Synthesis of both enantiomers of deplancheine

Enolate addition to the indolizino[2,3-α]quinolizidine has been used to introduce an ethylidene group. This was achieved using acetaldehyde and lithium di*iso*propylamide, followed by activation of the hydroxyl group and a DBN-induced elimination procedure (Scheme 29). This was done as part of the synthesis [45] of the natural compound deplancheine **(35)**, isolated from the plant *Alstonia deplanchei*. As one of the simplest indole alkaloids that shares the indoloisoquinoline tetracyclic core, numerous syntheses have been reported that test out different methodologies, with many giving racemic mixtures. The Allin group utilised their new methodology and control over stereochemistry to synthesise both enantiomers of deplancheine individually (Scheme 29) starting from the respective enantiomer of compound **(107).** [45] This introduction of the ethylidene group is important as it is found in natural compounds such as dihydrousambarensine **(13)**. [26]



(13)

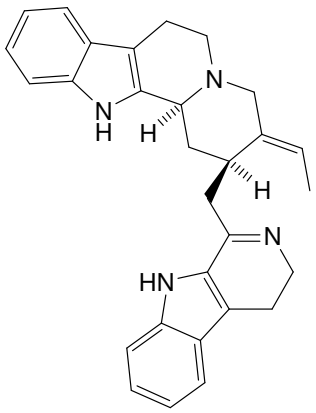


Scheme 29

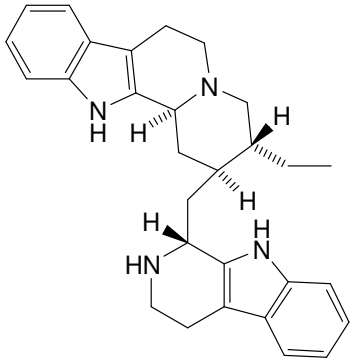
The enolate of compound **(110)** is generated using lithium di*iso*propylamide which then reacts with acetaldehyde. The OH group formed as a result of this reaction is activated using mesyl chloride and subsequently eliminated to give the ethylidene moiety, forming compound **(111)**. The Boc protecting group is removed by heating under reflux with tetrabutylammoniunm fluoride to give compound **(112)**. The removal of the carbonyl group is carried out using an adaption of the Borch protocol, [61] in which trimethyloxonium tetrafluoroborate is used as a methylating agent to add a methyl group to the carbonyl oxygen, forming a methoxy group, which is then eliminated.

1.4.6 Synthesis of (+)-12*b*-epidevinylantirhine

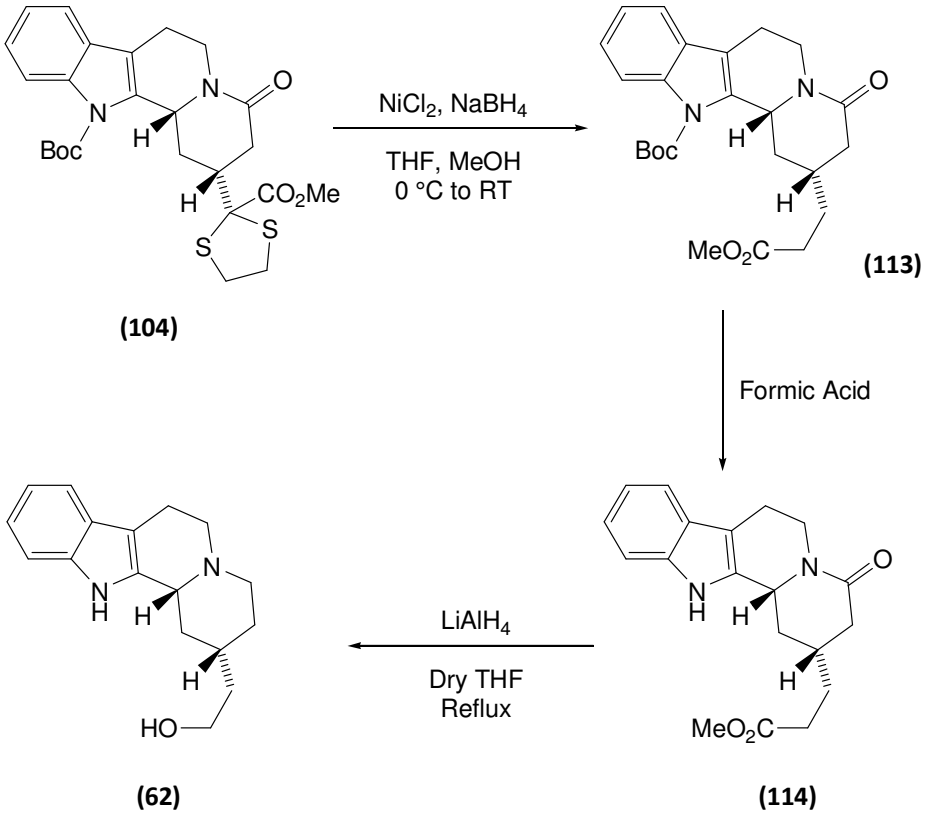
Another indole alkaloid synthesised by the Allin group is (+)-12*b*-epidevinylantirhine **(62)**. [46] This compound was first isolated by Wenkert [62] during studies towards a synthesis of geissoschizol. The production of this compound (Scheme 30) is significant as it introduces a free OH that can be used for extra functionality, with the potential to try to build a bis-indole compound such as dihydrousambarensine **(13)** or ochrolifuanine A **(11)**.



(13)



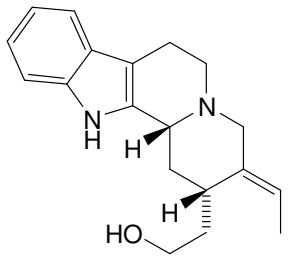
(11)



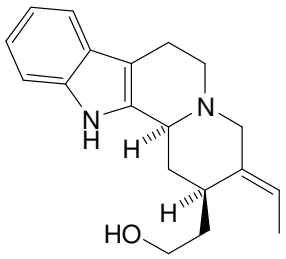
Scheme 30

The dithiolane ring is removed using sodium borohydride with a nickel chloride catalyst in a similar way as using Raney nickel. Formic acid is used to remove the Boc protecting group and the amide and ester are both reduce by heating under reflux with lithium aluminium hydride in anhydrous tetrahydrofuran.

It should be noted that addition of an ethylidene group to (+)-12*b*-epidevinylantirhine **(62)** would give access to the compound (+)-geissoschizol [63] **(115a)**, the opposite enantiomer of a naturally occurring indole alkaloid. Utilising the Allin group's stereocontrol and the incorporation of the ethylidene group, this could provide a new, asymmetric synthesis of (-)-geissoschizol **(115b)**, the naturally occurring enantiomer.

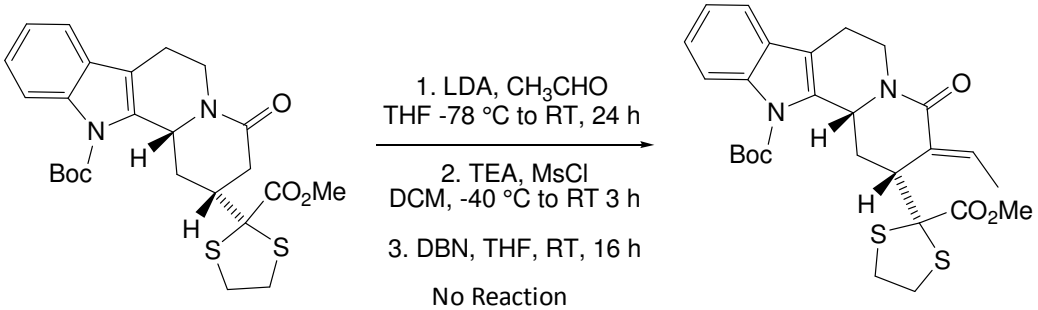


(115a)



(115b)

An attempt to incorporate this moiety was made *en route* to (+)-12*b*-epidevinylantirhine as shown (Scheme 31), but was unsuccessful,[64] possibly due to the steric hindrance of the bulky dithiolane ring.



(104) **(116)**

Scheme 31

The methodologies previously employed in the Allin group give an indication of;

- How to control stereochemistry
- Conjugate additions, how to build towards bis-indole compounds
- Enolate additions, introduction of ethylidene group
- How to remove the hydroxymethyl group

All of the above are important for building a structure-activity relationship picture of the indoloizino[2,3-α]quinolizidine template and for the synthesis of a bis-indole compound.

1.5 Determination of Anti-Malarial Activity

1.5.1 Introduction

The half maximal inhibitory concentration (IC_{50}) is a measure of biological activity that shows the required concentration of a compound needed for 50% inhibition of parasite growth. The compounds described below in this thesis need to be tested for anti-malarial activity to help guide us in understanding what aspects of their structure influence their activity (structure-activity relationship).

Several methods can be employed to measure anti-malarial activity. These all fundamentally rely on the determination of parasite growth. Intraerythrocytic development takes 48 hours to complete one growth cycle. Therefore, by exposing intraerythrocytic cultures for 48 hours to a range of drug concentrations, the concentration of drug that inhibits growth by 50% (IC_{50}) can be determined. The methods to monitor parasite growth are varied:

- Microscopic examination. [65]
- Colorimetric assays of enzyme production e.g. lactase dehydrogenase assay. [66]
- Radiometric monitoring of DNA replication by incorporation of triated hypoxanthine (a nucleotide precursor). This method is the most widely publicised and accepted.[67]
- Fluorescence assay of DNA replication using intercalating agents such as Syber Green I. [68]

All the above methods are accurate and have a variety of advantages (low cost, sensitivity, ease of use, no need for specialist equipment) and disadvantages (expensive, time consuming, high cost, lack of sensitivity, storage and disposal of radioactive materials).

Keele University Institute for Science and Technology in Medicine (ISTM) have developed a transgenic *P. falciparum* line that expresses a bioluminescent reporter gene, luciferase, during replication. [69] They have also reported improvements in the bioluminescence assay format to improve reproductivity of the assay data developed. [70] Similar developments in luciferase expressing parasites have been reported by other groups. [71] Bioluminescence assays provide data comparable to the other assay formats, but the absence of background signals and a high signal/noise ratio offers significant advantages in determination of the IC_{50} data.

In collaboration with Dr Paul Horrocks' group in the ISTM the IC_{50} data for all the compounds described in Chapter 2 of this thesis were determined following the method outlined in section 1.5.2.

1.5.2 Determining the IC_{50}

Stock culture [4% haematocrit, 2% trophozite stage parasitaemia of $Dd2^{luc}$ (transgenic luciferase parasite)] [69] was provided by a collaborating laboratory. 6 ml was required per plate.

A 96 well plate was set up as shown in Figure 4. Columns 10 and 11 served as controls; a positive (+) control, containing parasites only with no drug, and a negative (-) control which contained no parasites or drugs, just culture medium.

100 µl of stock culture was added to each well apart from column 2. To this, 150 µL of medium was added followed by 12 µL of the chosen drug at 10 mM concentration in dimethyl sulfoxide. These were mixed together using an auto-pipette and 50 µL was transferred from column 2 to column 3. The process was repeated with 50 µL being transferred across to each column. When column 9 was reached 50 µL was taken and discarded. This created a series of 3 fold dilutions of drug concentration ranging from 400

µM to 0.1 µM. The outside wells were filled with 200 µl of incomplete medium to minimise edge effects from evaporation during the following incubation period in a gassed chamber (1% O_2, 3%, CO_2, 96% N_2) at 37 °C for 48 hours (Figure 5).

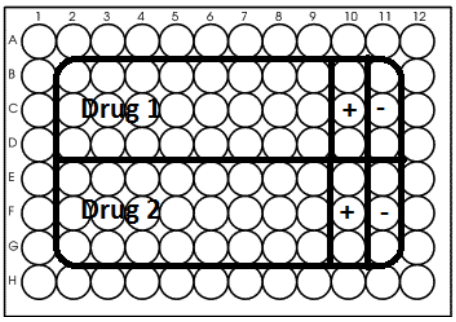


Figure 4: 96 well plate

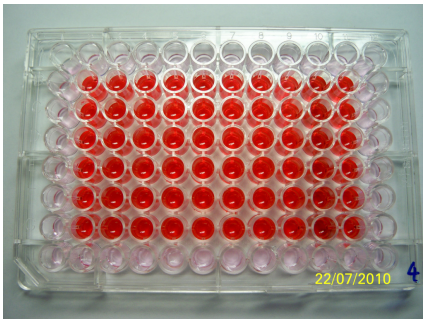


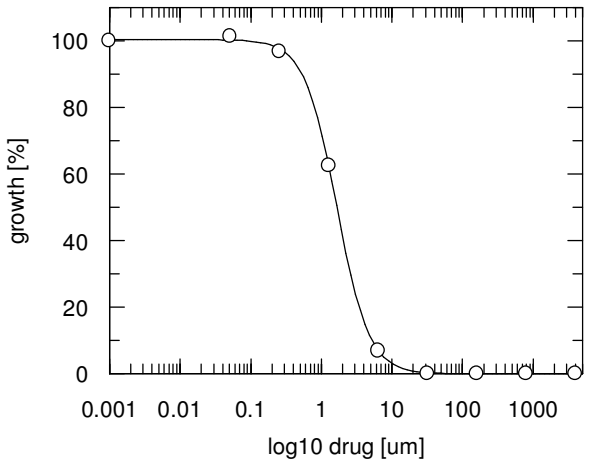
Figure 5: Drug inhibition plate after 48 hours [72]

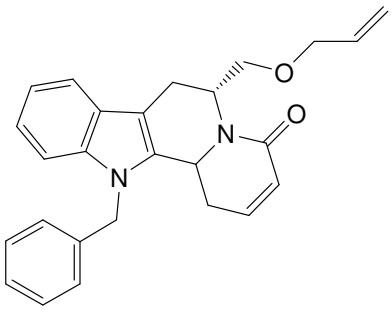
After the incubation period, 40 µL from each well was added to 10 µL of passive lysis buffer (Promega UK) on a white, 96 well plate. 50 µL of standard luciferase substrate (Promega UK) was added and the bioluminescence was measured (in relative light units) for 2 seconds using a MultiGloMax luminometer by Promega.

Each drug is done in triplicate on one plate and three biological replicas were conducted to provide data for analysis.

The relative light units calculated were converted into % growth and plotted against $\log_{10}$-transformed drug concentration. The IC_{50} values were determined from a sigmoidal dose-response curve (Figure 6) in GraphPad Prism v5. (GraphPad Software, Inc., San Diego, CA). This methodology has been submitted for publication to The Malarial Journal. [73]

Figure 6 shows the sigmoidal dose-response curve for a compound that will be described further in Chapter 2. The graph can be used to determine to the concentration of this compound needed to inhibit parasitic growth by 50% and hence, the IC_{50} value (1.7 µM).





(117)

Figure 6: Graph to determine the anti-malarial activity of **(114)** (1.7 μM).

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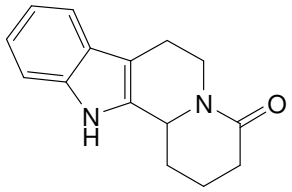
Chapter 2

Results and Discussion

2.1 Synthesis of the Indolizino[2,3-α]quinolizidine Ring System

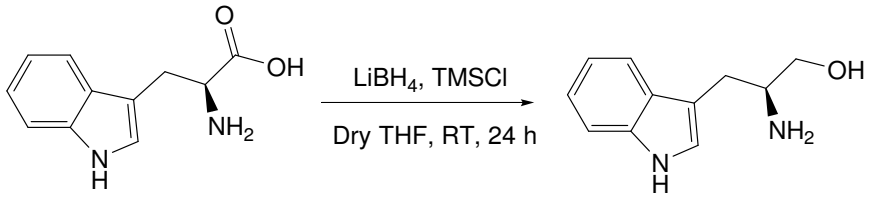
2.1.1 Synthesis of (+)-indolizino[2,3-α]quinolizidine

A full investigation into the structure-activity relationship of the indolizino[2,3-α]quinolizidine ring system as a starting point for the development of a novel series of anti-malarials required both enantiomers of the template **(1)** to be synthesised.



(1)

The requisite β-amino alcohol derivative of L-tryptophan was prepared by reducing L-tryptophan **(2)** with an excess of lithium borohydride and trimethylchlorosilane in anhydrous tetrahydrofuran (Scheme 1). [1]

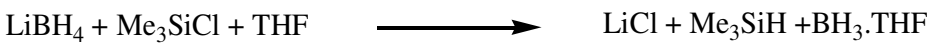


(2) **(3)**

Scheme 1

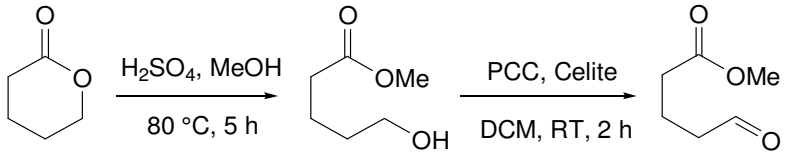
Metal borohydrides alone cannot reduce α-amino acid substrates; however in the presence of trimethylchlorosilane reduction becomes possible. The exact mechanism for this reaction has yet to be determined, but it is thought that a borane – tetrahydrofuran

complex is formed (Equation 1) and it is this species, along with excess trimethylchlorosilane, that acts as the reducing agent. [2]



Equation 1

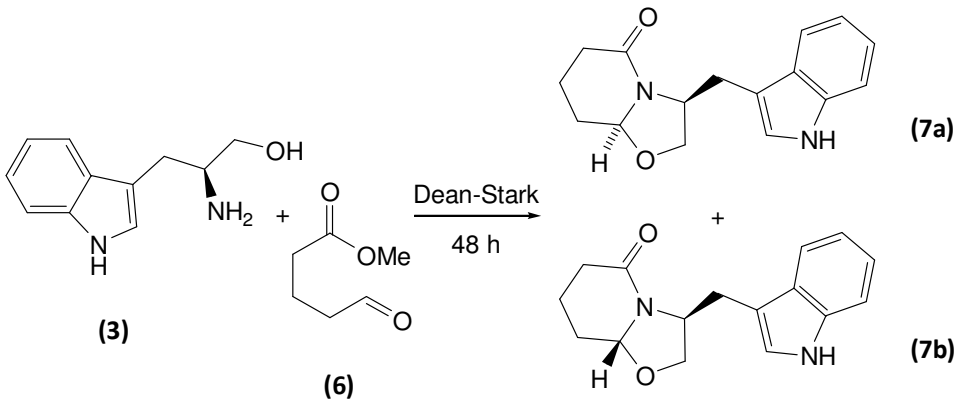
The desired keto acid **(6)** was formed by an acid-catalysed transesterification of δ-valerolactone, followed by oxidation to the corresponding aldehyde using a suspension of pyridinium chlorochromate and celite in dichloromethane (Scheme 2). No purification was carried out as re-lactonisation occurs on aqueous work up. [3]



(4) **(5)** **(6)**

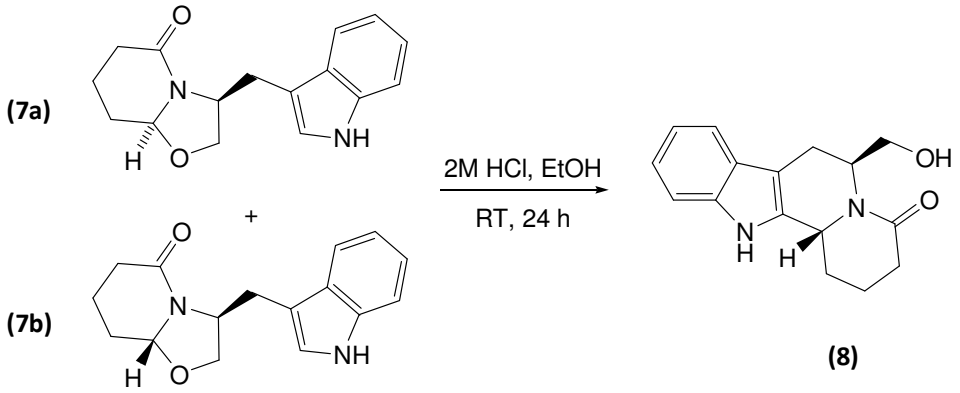
Scheme 2

The products of the reactions shown in Schemes 1 and 2 were then combined for 48 hours under Dean-Stark conditions, to give the expected bicyclic lactams **(7a, b)** in 20% yield as a mixture of diastereoisomers, with **(7a)** being the major isomer (Scheme 3).



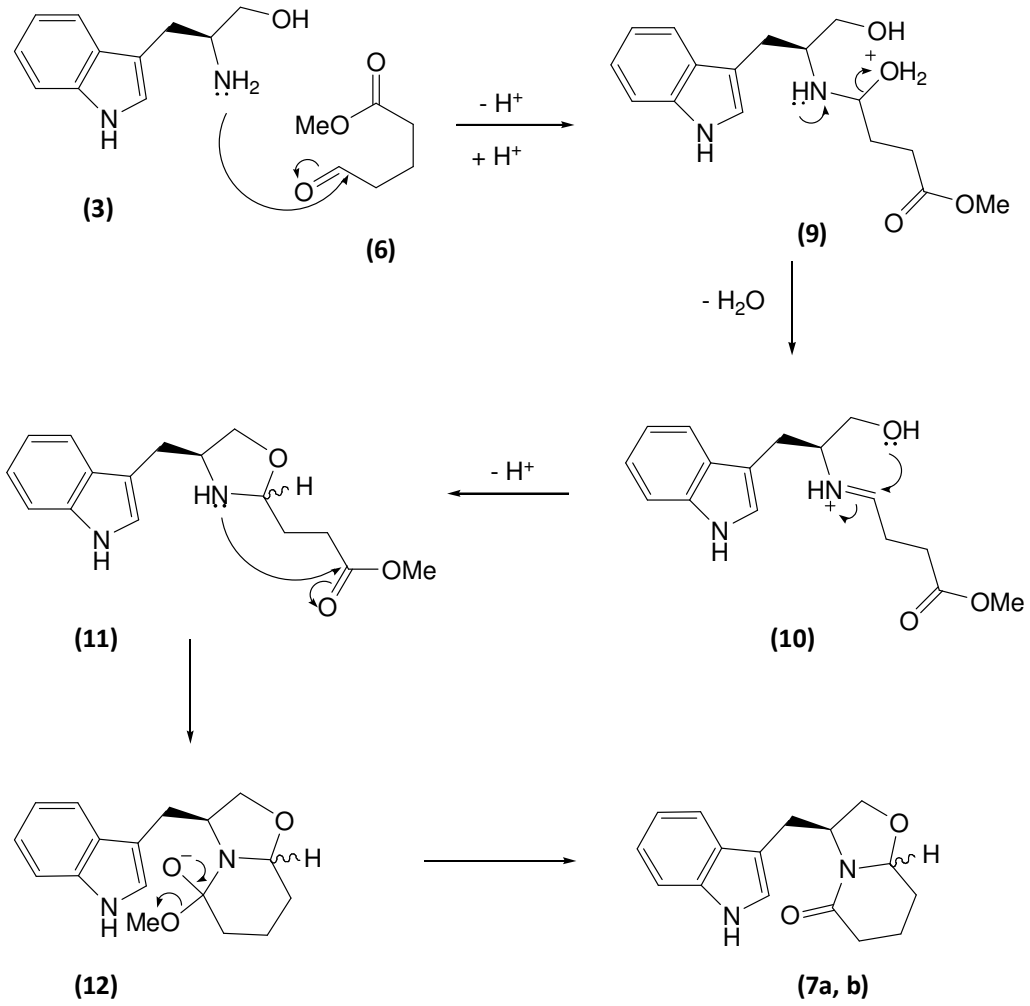
Scheme 3

The diasteroisomers were purified, but not separated. Instead, they were treated with 2M hydrochloric acid in absolute ethanol at room temperature to give the desired cyclised product **(8)** as a single diastereoisomer in 95% yield (Scheme 4). The rationalisation for this stereochemical outcome is described in Chapter 1.



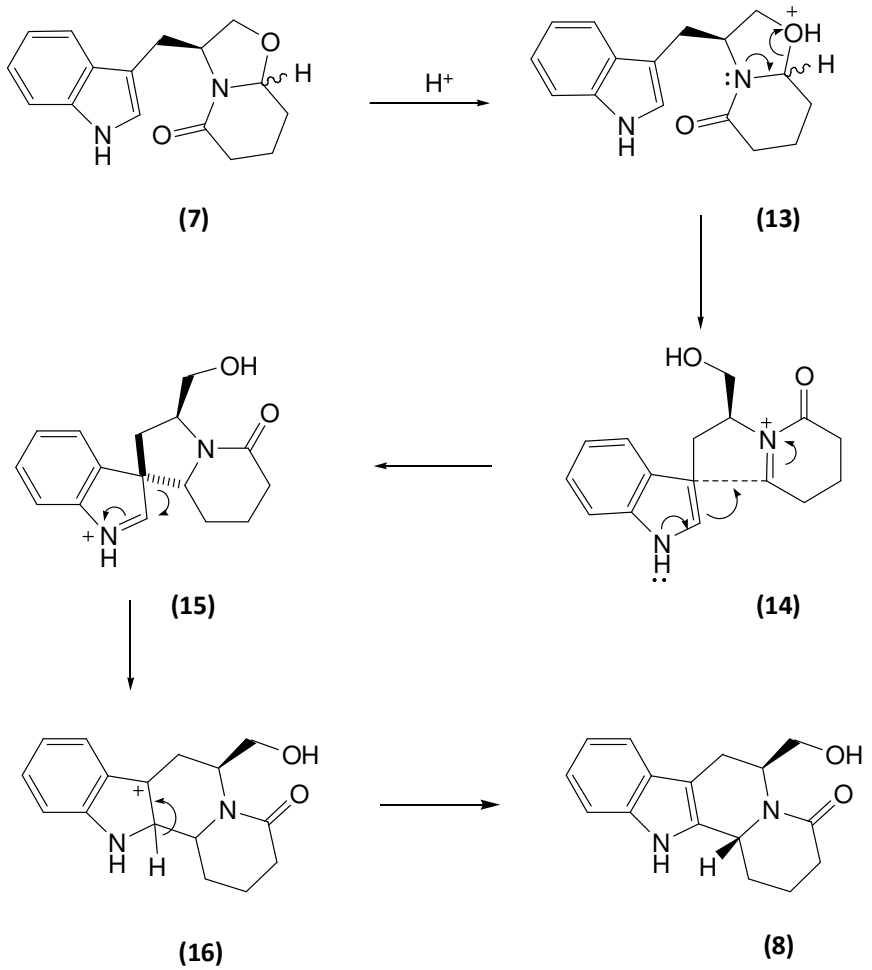
Scheme 4

The proposed mechanisms for the reactions described above are shown in Schemes 5 and 6.



Scheme 5

A mixture of diastereoisomers is formed from this reaction as the iminium ion intermediate **(10)** is planar and can be attacked from either side by the hydroxyl nucleophile.

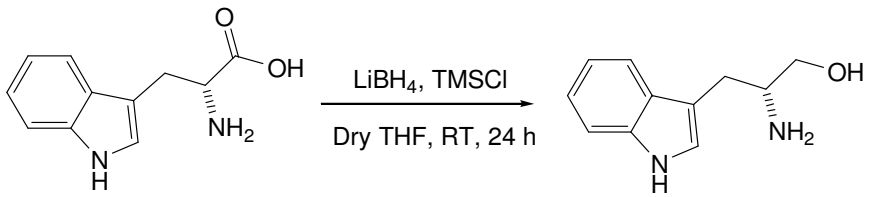


Scheme 6

As mentioned in section 1.3, attack is occurring at the 3 position of the indole moiety, followed by a subsequent rearrangement reaction, due to the unwillingness of the benzene ring in the indole moiety to lose aromaticity. [4]

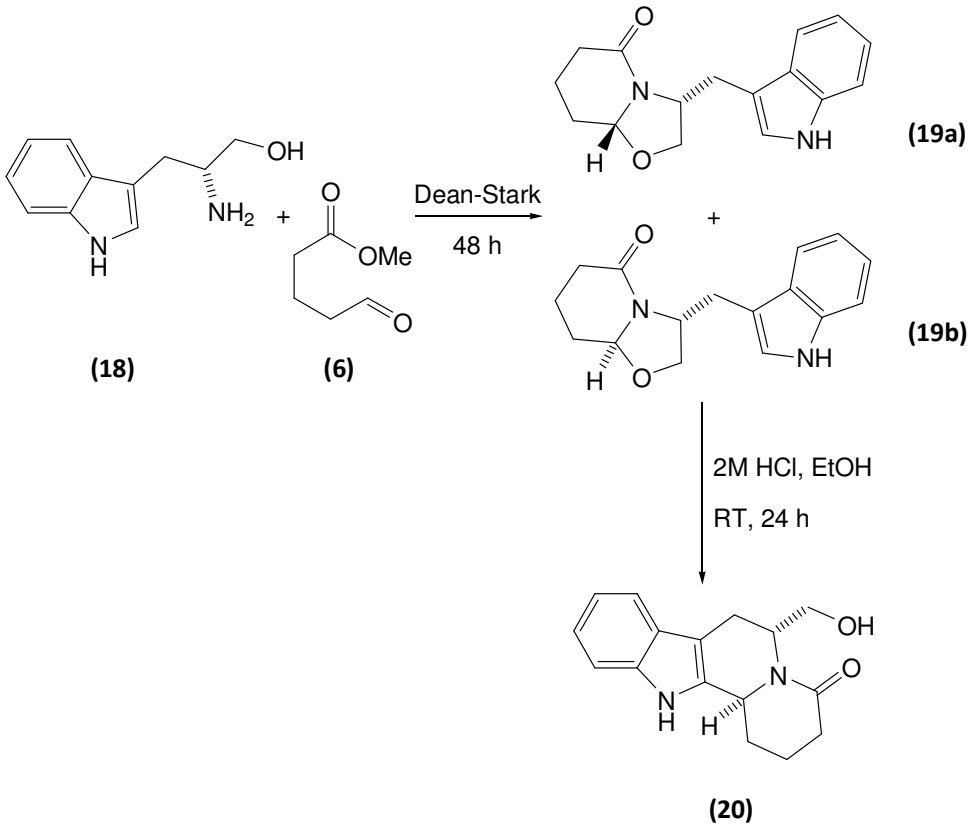
2.1.2 Synthesis of (-)-indolizino[2,3-α]quinolizidine

To obtain the opposite enantiomer of (+)-indolizino[2,3-α]quinolizidine, the synthesis outlined in the above section (2.1.1) was repeated using D-tryptophan as the starting material. The expected bicyclic lactams **(19a**, **b)** were obtained in 32% yield and were cyclised to give the (-)-enantiomer **(20)** in 95% yield (Schemes 7 and 8).



(17) **(18)**

Scheme 7



(18) **(6)** **(19a)** **(19b)** **(20)**

Scheme 8

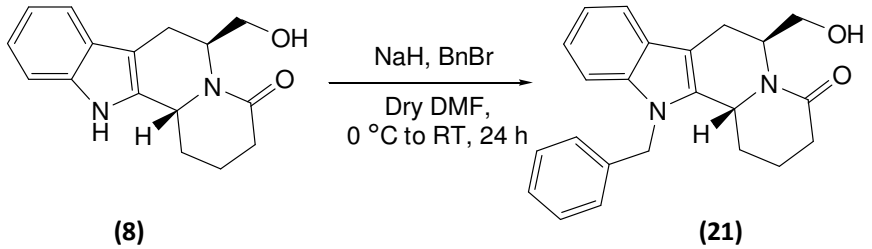
With both enantiomers in hand, the effects of ring stereochemistry on biological activity could now be investigated, along with a range of structural modifications of the indolizino[2,3-α]quinolizidine ring system.

2.2 Alkylations

2.2.1 Mono-alkylations of (+)-indolizino[2,3-α]quinolizidine

Protecting groups positioned on the indole NH and the OH of the template are a necessity to stop unwanted side reactions during further functionalisations, however a variety of different groups could be added and this prompted an investigation into whether or not the addition of groups may increase or decrease anti-malarial activity and subsequently which groups give the best improvement. The first groups to be tested were methyl, allyl and benzyl.

Due to the weakly acidic nature of the indole NH, a strong base was needed to deprotonate it. Sodium hydride (1.5 eq) in anhydrous dimethylformamide was used, followed by addition of benzyl bromide (1.5 eq) to give the mono-benzylated compound **(21)**, in 63% yield (Scheme 9).

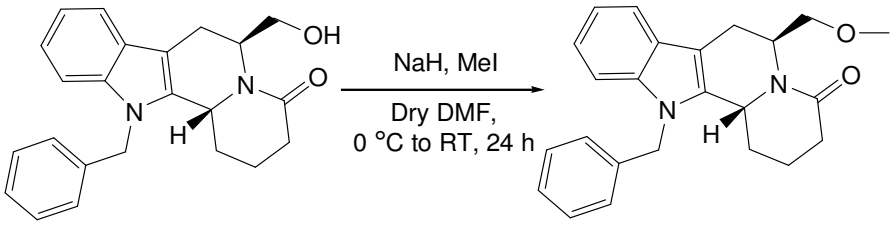


(8) **(21)**

Scheme 9

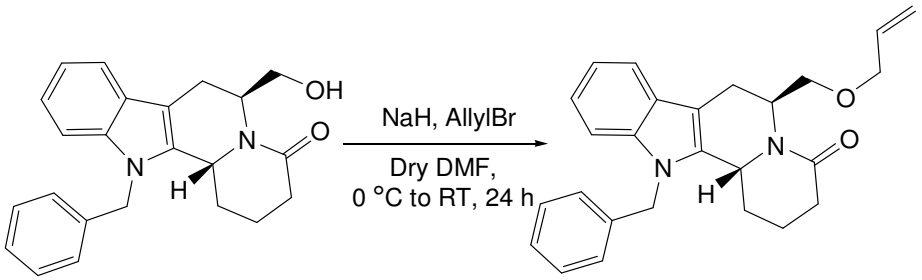
2.2.2 Bis-alkylations of (+)-indolizino[2,3-α]quinolizidine

Groups were then added to the oxygen atom of compound **(21)**. In the first instance, a methyl group (Scheme 10), and secondly an allyl group (Scheme 11). 2 Equivalents of sodium hydride were used followed by 2 equivalents of the appropriate alkylating agent.



(21) **(22)**

Scheme 10

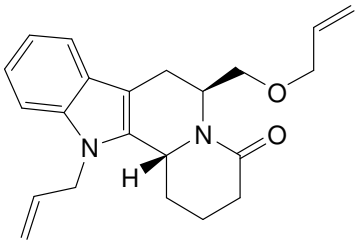


(21) **(23)**

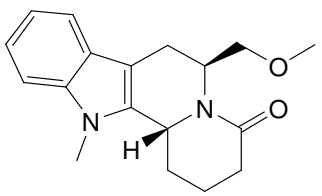
Scheme 11

Compounds **(22)** and **(23)** were produced in 14% and 37% yields respectively.

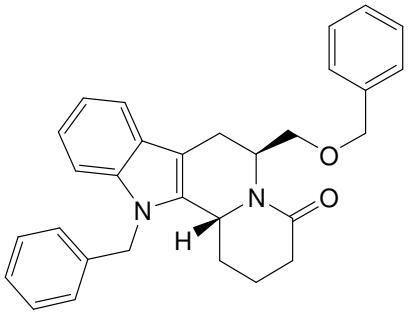
Other compounds in this series were previously synthesised by our group. These were prepared using similar methods to those described above and gave access to compounds **(24)** to **(26)**. [3]



(24)



(25)

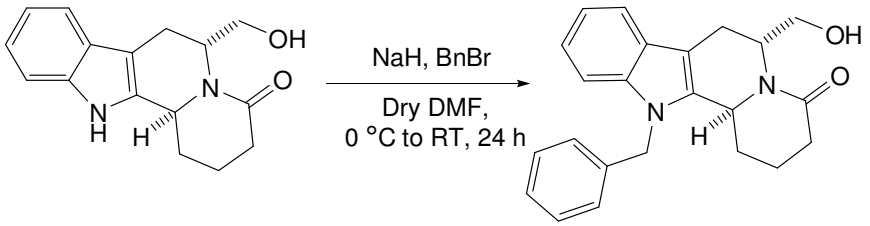


(26)

2.2.3 Mono-alkylations of (-)-indolizino[2,3-α]quinolizidine

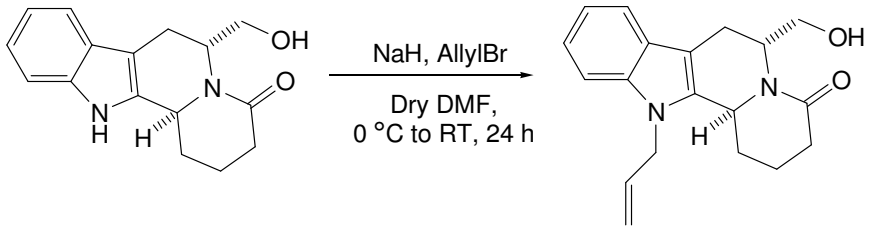
It was important to synthesise analogous compounds to those in section 2.2.1 starting from the template derived from D-tryptophan **(20)** so that their respective activities could be compared to determine if absolute stereochemistry is important for anti-malarial activity in this series of compounds.

A benzyl group and an allyl group were added to the indole NH of template **(20)** as described in Schemes 12 and 13. Compound **(27)** was formed in 41% yield and **(28)** in 74%.



(20) **(27)**

Scheme 12

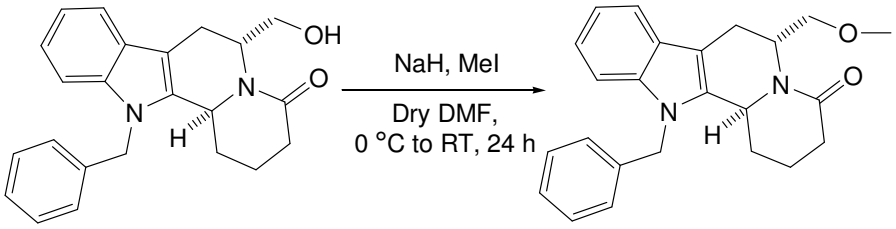


(20) **(28)**

Scheme 13

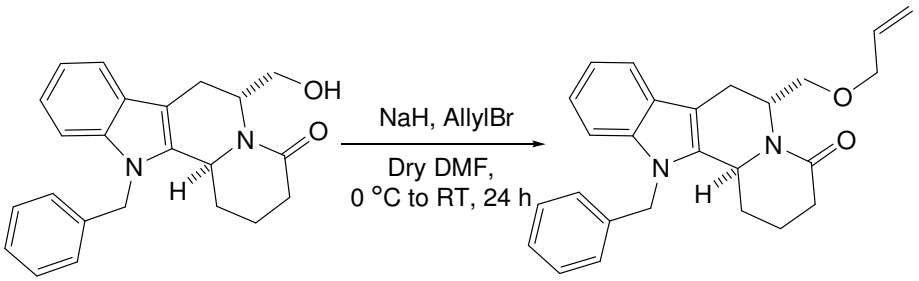
2.2.4 Bis-alkylations of (-)-indolizino[2,3-α]quinolizidine

The following compounds shown in Schemes 14 and 15 were synthesised as described in section 2.2.2, starting from compound **(27)**. By these routes compound **(29)** was formed in 51% yield and compound **(30)** in 93% yield.



(27) **(29)**

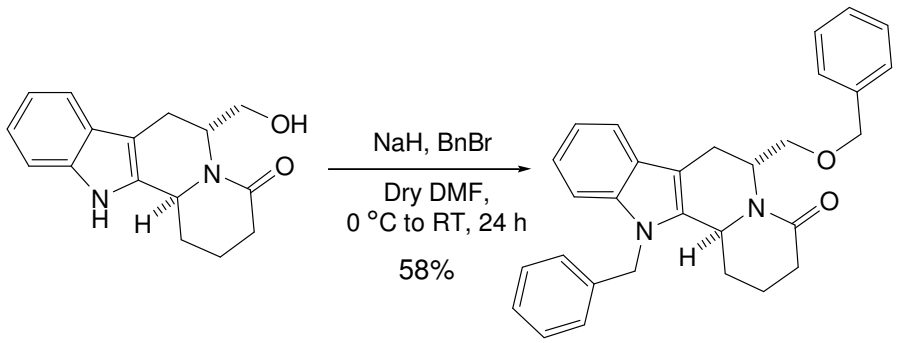
Scheme 14



(27) **(30)**

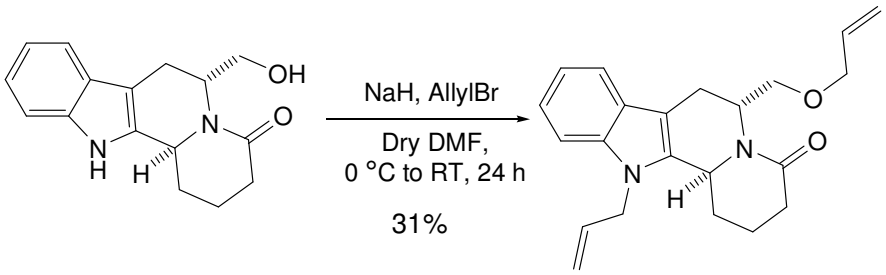
Scheme 15

When the same group was required on both the nitrogen and oxygen atoms, an excess of sodium hydride (4.4 eq) and the corresponding alkylating agent (3 eq) was used. This gave rise to the targets shown below in Schemes 16, 17 and 18.



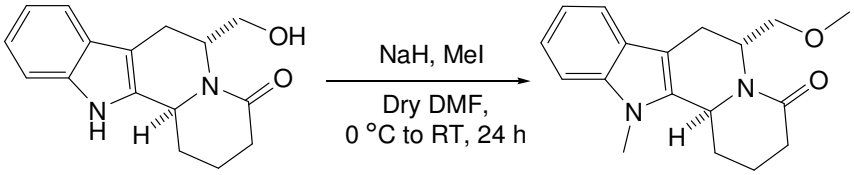
(20) **(31)**

Scheme 16



(20) **(32)**

Scheme 17



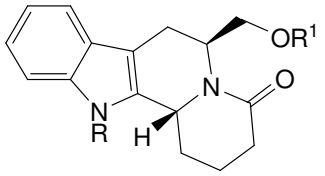
(20) **(33)**

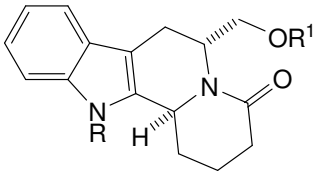
Scheme 18

2.2.5 Biological activities

The IC_{50} values (against *P. falciparum* strain $Dd2^{luc}$) for the range of compounds previously described are shown below in Table 1. Compounds in the L-series are those derived from L-tryptophan and compounds in the D-series are those derived from D-tryptophan.

Table 1:





L-Series	R	R^1	IC_{50} (µM) $Dd2^{luc}$	**D-Series**	R	R^1	IC_{50} (µM) $Dd2^{luc}$
(8)	H	H	71	(20)	H	H	56
(25)	Methyl	Methyl	30	(33)	Methyl	Methyl	29
(24)	Allyl	Allyl	16	(32)	Allyl	Allyl	13
(26)	Benzyl	Benzyl	12	(31)	Benzyl	Benzyl	3.5
(22)	Benzyl	Methyl	32	(29)	Benzyl	Methyl	1.3
(23)	Benzyl	Allyl	35	(30)	Benzyl	Allyl	1.3
				(27)	Benzyl	H	5
				(28)	Allyl	H	41

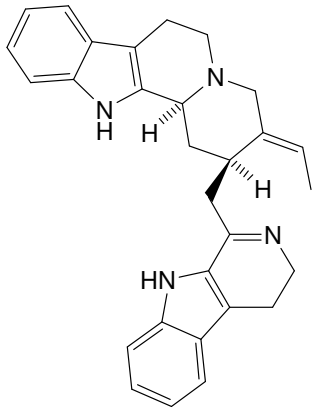
The first conclusion that can be drawn from these results is that the addition of a group has led to an increase in activity. In both the L and the D-series, the substituted compounds have lower IC_{50} values (and therefore higher anti-malarial activity) than the parent compounds **(8)** and **(20)**. The results of the D-series compounds also show that the addition of a second group is desirable. Compound **(27)** is mono-benzylated at the indole nitrogen and has an IC_{50} value of 5 µM. Addition of an allyl, methyl or benzyl group to the oxygen atom on compound **(27)** gave compounds **(31), (29)** and **(30)**, all of which have higher anti-malarial activity than **(27)**. Comparison of compounds **(28)** and **(32)** also show this trend. Compound **(28)** has an allyl group on the indole nitrogen and an IC_{50} value of 41 µM. Addition of a second allyl group gives compound **(32)**, which has a lower IC_{50} value of 13 µM and therefore has a higher anti-malarial activity.

This shows two things: (1) that the hydrogen bonding properties of the NH and OH groups are not important for the binding of the compound to its target and (2) that a reduction in the polarity of templates **(8)** and **(20)** is desirable. They are polar compounds and

reducing this will allow them to pass through cell membranes more freely. [5, 6] It is also possible that there are hydrophobic regions in the target binding site that the compounds could not bind to without substitutions at the N and O atoms. In section 1.2.3 it is proposed that a free indole NH is needed for high anti-malarial activity; however, this does not appear to be the case in this series of compounds.

The second conclusion is that compounds in the D-series (those derived from D-tryptophan) have better activity than their L-series analogues. This trend is apparent for all the compounds tested. In some cases the difference is marginal (compounds **(25)** and **(33)**, and compounds **(24)** and **(32)**), but in others, the increase in activity is highly significant. Compounds **(29), (30)** and **(31)** all show a marked increase in activity compared to their L-series analogues, giving IC_{50} values as low as 1.3 µM.

This increase in activity as we move from the L-series to the D-series highlights the importance of absolute stereochemistry of compounds derived from this indoloisoquinoline template. The more active D-series shares the same absolute stereochemistry at the ring junction within the heterocyclic core as the natural anti-malarial compound dihydrousambarensine **(34)**.

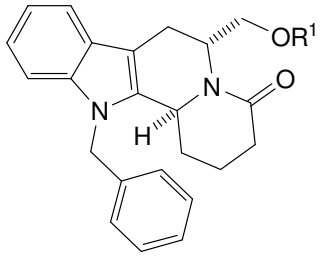


(34)

This relationship between stereochemistry and anti-malarial activity has also been reported by Frederich *et al.* [7] and is discussed in section 1.2.

These results also show a preference for a benzyl moiety at the indole nitrogen and either a methyl or allyl substituent at the oxygen atom (compounds **(29)** and **(30)**). Both of these have IC_{50} values of 1.3 µM, the highest level of activity in this set of compounds. An increase in steric bulk at R^1 (compound **(31)** which has a benzyl group) is not favourable.

Free rotation round the single bond of the benzyl moiety could be causing it to mimic the presence of a second ring system **(35)** such as that found in the naturally occurring anti-malarial dihydrousambarensine **(34)**.



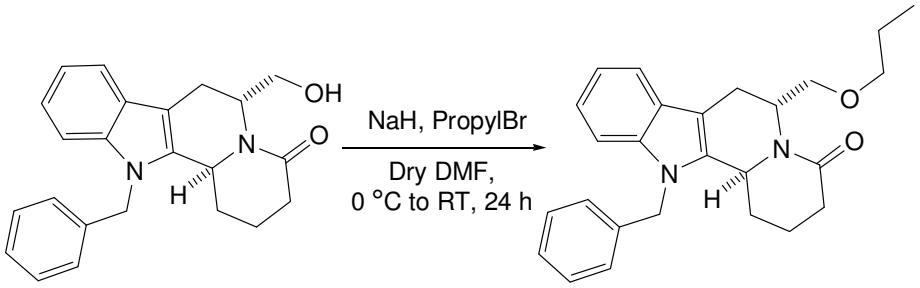
(35)

2.2.6 Investigations into ring size, electronic and steric properties

Due to the results gathered in section 2.2.5, we decided to continue synthesising only compounds in the D-series, as it has been shown that these have higher anti-malarial activity.

To try to understand which properties of the substituents added to the template were responsible for giving rise to better biological activity, and to try to improve on these findings, a further set of analogues was prepared.

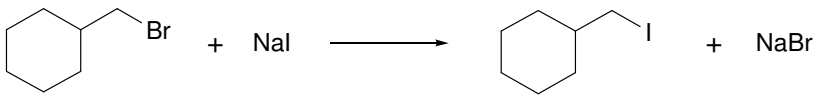
A propyl group was added to the free hydroxyl group of compound **(27)** as shown (Scheme 19) in 50% yield.



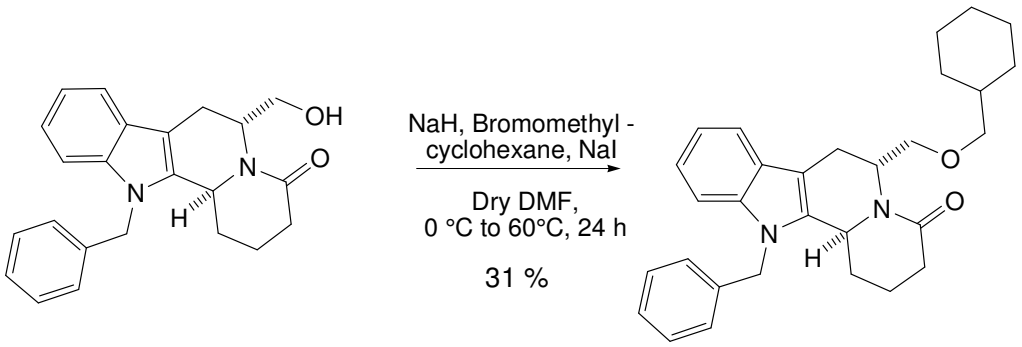
(27) **(36)**

Scheme 19

Bromomethyl cyclohexane and bromomethyl cylcobutane were both used as alkylating agents, along with benzyl bromide and allyl bromide to produce compounds **(37)**, **(39)** and **(41)**. Addition of these new groups was not as straightforward as the previous examples. Under our usual alkylation conditions no reaction occurred, so sodium iodide was added and the reaction heated at 60 °C for 24 hours. Addition of sodium iodide causes a displacement reaction *in situ* (Scheme 20) giving a more reactive alkylating agent. This is desirable as the iodide ion is a much better leaving group due to the weak C-I bond which requires less energy to break than the C-Br bond and therefore the desired reaction can proceed. [4]

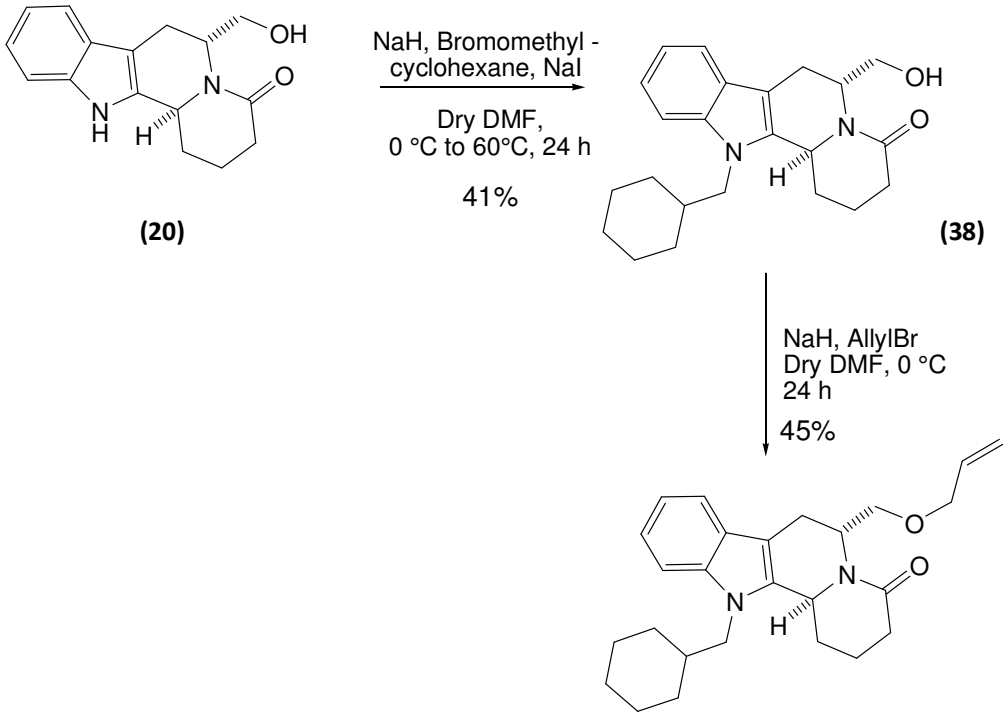


Scheme 20



(27) **(37)**

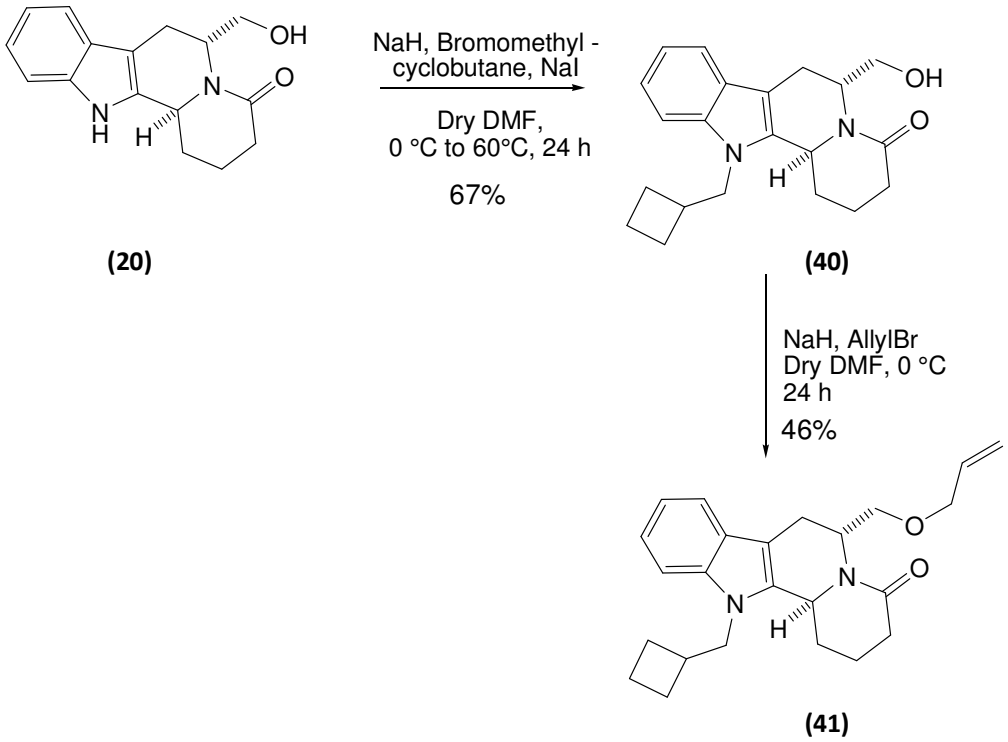
Scheme 21



(20) **(38)**

(39)

Scheme 22



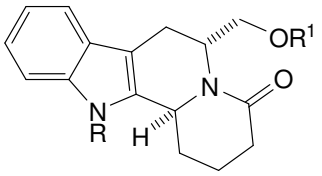
(20) **(40)** **(41)**

Scheme 23

2.2.7 Biological activities

Table 2 shows the IC_{50} values of the compounds synthesised in section 2.2.6 and their parent compounds from 2.2.5.

Table 2:



Parent Compounds				**Analogues**			
	R	R^1	IC_{50} (μM) $Dd2^{luc}$		R	R^1	IC_{50} (μM) $Dd2^{luc}$
(31)	Benzyl	Benzyl	3.5	(37)	Benzyl	Cyclohexylmethyl	19
(30)	Benzyl	Allyl	1.3	(36)	Benzyl	Propyl	2.8
				(39)	Cyclohexylmethyl	Allyl	9
				(41)	Cyclobutylmethyl	Allyl	7

Comparison of these compounds shows that an increase in steric bulk at the R^1 position leads to a decrease in anti-malarial activity. Compound **(30)** has an IC_{50} value of 1.3 μM. When the allyl group at the R^1 position is changed to a benzyl **(31)**, a cyclohexylmethyl **(37)** or a propyl group **(36)**, the activity drops to 3.5 μM, 19 μM and 2.8 μM respectively.

Due to the lack of the double bond in the propyl group, it is not planar and therefore it can rotate around, increasing steric bulk. It also lacks the electron density of the allyl group in compound **(30)** due to the absence of the π-bond. This finding is similar to that observed with the decrease in activity when the R^1 substituent is changed from a benzyl **(31)** to a cyclohexylmethyl **(37)**. Again there is a reduction in electron density and a reduction in planarity as the saturated cyclohexyl ring would be expected to favour a chair conformation.

Compounds **(39)** and **(41)** also show a decrease in activity. Here the R substituent has been changed from a benzyl to a cyclobutylmethyl in **(41)** and a cyclohexylmethyl in **(39)** and the IC_{50} values have dropped from 1.3 μM to 7 μM and 9 μM respectively. In both cases it seems that the steric and electronic properties of benzyl are preferred for higher activity. A change in ring size from an unsaturated six membered ring to an unsaturated

four membered ring does show a slight increase in activity, but it is negligible compared to that of having a benzyl moiety in the R position.

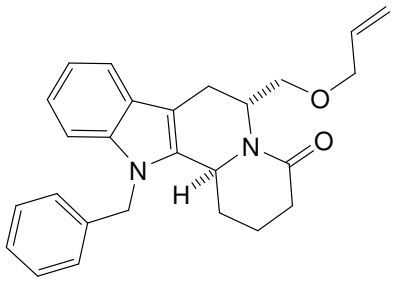
The properties of the benzyl substituent may explain why it is preferred to its saturated analogue. As a planar, hydrophobic structure, it can bind to flat hydrophobic regions of the target binding site (Figure 1). Neither a methyl or cyclohexylmethyl group would be able to bind in the same way; this is reflected in the IC_{50} values. The methyl group is too small for much interaction and a cyclohexylmethyl group as mentioned above, is not planar, and would have a larger steric influence (Figure 1). Both methyl and cyclohexylmethyl lack aromaticity and cannot π-bond. An allyl group might undergo π-bonding but it is also small and would not bind to the binding site as proficiently as a benzyl moiety.

Figure 1

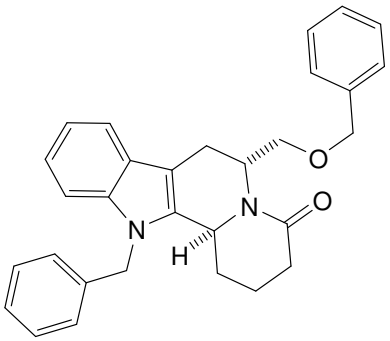
2.3 Introduction of α,β – Unsaturation to the Indolizino[2,3-α]quinolizidine ring system

2.3.1 α,β – Unsaturation

α,β – Unsaturation of compounds was achieved following the Reich method mentioned in Chapter 1. [8] This technique was performed on two of the compounds, **(30)** and **(31),** introduced in the previous section.

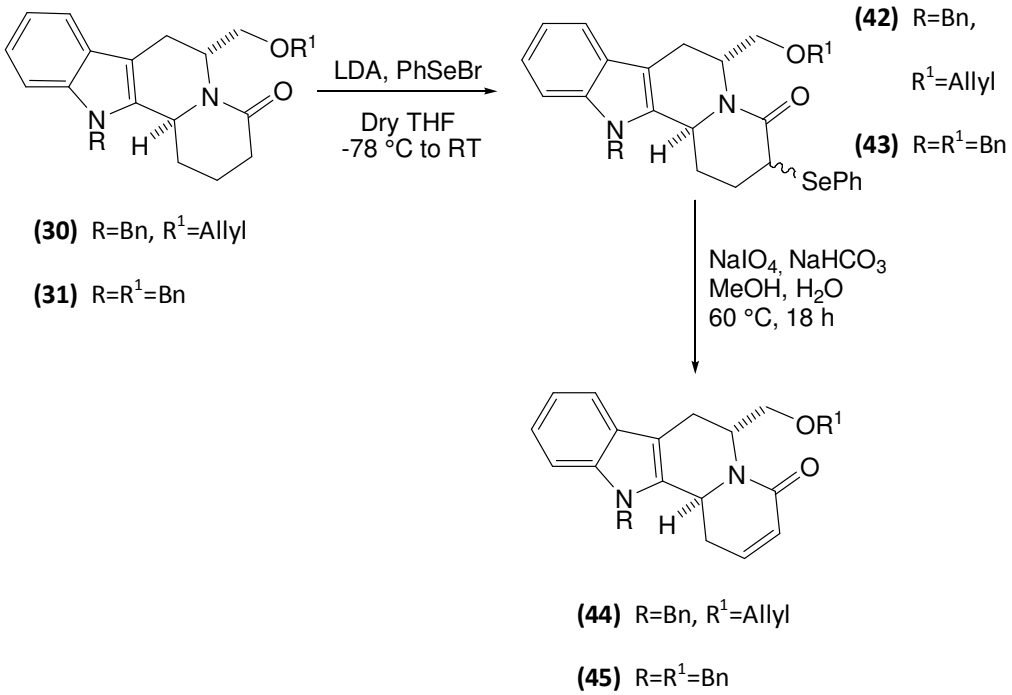


(30)



(31)

Addition of *n*-butyl lithium to di*iso*propylamine in anhydrous tetrahydrofuran at -78 °C formed lithium di*iso*propylamide, which in turn, was used to generate the enolates of these compounds. Phenyl selenyl bromide was then added to give the α-selenide required for the subsequent elimination reaction. Sodium metaperiodiate and sodium bicarbonate dissolved in methanol and water were used as an oxidant to promote elimination of the selenoxide, introducing a double bond (Scheme 24). Due to solubility issues, the elimination reaction was carried out at 60 °C instead of room temperature.



(30) R=Bn, R^1=Allyl

(31) R=R^1=Bn

(42) R=Bn, R^1=Allyl

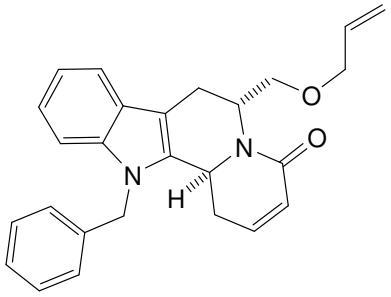
(43) R=R^1=Bn

(44) R=Bn, R^1=Allyl

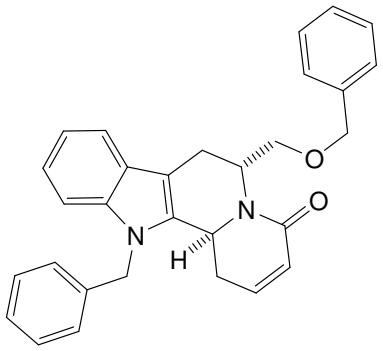
(45) R=R^1=Bn

Scheme 24

This methodology gave rise to compounds **(44)** and **(45)** in 40% and 25% yields respectively. The IC_{50} value of each compound is shown in Table 3 along with the activity of the saturated analogues.



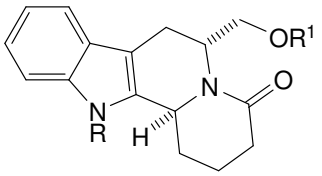
(44)



(45)

2.3.2 Biological activities

Table 3:



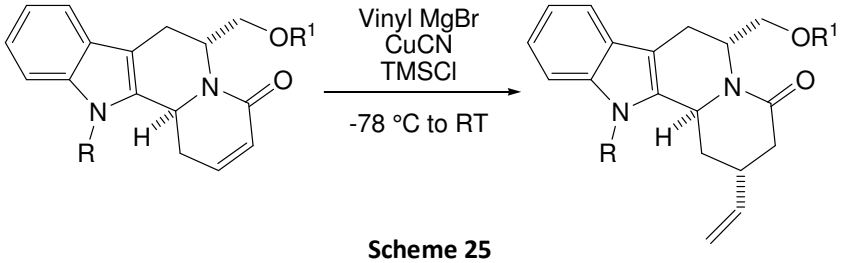
Saturated Analogues				**Unsaturated Analogues**			
	R	R^1	IC_{50} (μM) $Dd2^{luc}$		R	R^1	IC_{50} (μM) $Dd2^{luc}$
(30)	Benzyl	Allyl	1.3	(44)	Benzyl	Allyl	1.7
(31)	Benzyl	Benzyl	3.5	(45)	Benzyl	Benzyl	3.6

Introduction of unsaturation to the lactam ring could cause a change in ring conformation. In both cases shown in Table 3 this difference has not led to improved activity on comparison with the saturated analogues.

2.4 Conjugate Additions

2.4.1 Addition of a vinyl group

With α,β-unsaturation of the lactam ring achieved, conjugate additions to the ring were now possible. Vinyl groups were added to unsaturated compounds **(44)** and **(45)** using a reagent system of vinyl magnesium bromide and copper cyanide. This gave rise to compounds **(46)** and **(47)** in 37% and 49% yields respectively. The rationalisation for the stereochemical outcome of the newly created chiral centre is discussed in Chapter 1.



Scheme 25

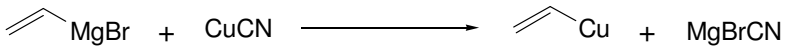
(44) R=Bn, R^1=Allyl

(45) R=R^1=Bn

(46) R=Bn, R^1=Allyl

(47) R=R^1=Bn

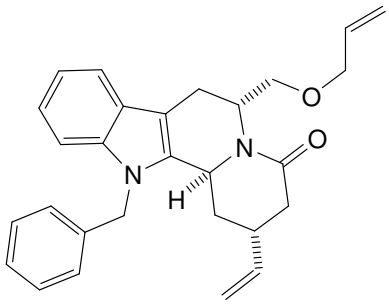
The copper cyanide is added in order to transmetalate the Grignard reagent, and create a copper vinyl species that will add to the C=C bond rather than the C=O bond.



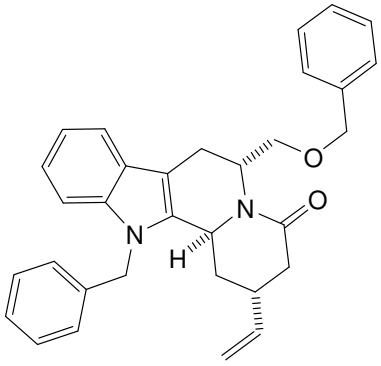
Scheme 26

The exact structure of the vinyl copper species is unknown and more complicated than Scheme 26 shows, as that species is far too un-reactive for the reaction to occur. [4]

Compounds **(46)** and **(47)** were formed by this method. Their activities are shown in Table 4, compared with their parent compounds. Compound **(48)** is an analogue of **(47)**, synthesised by another member of the group from L-tryptophan, and its anti-malarial activity is also shown in Table 4.



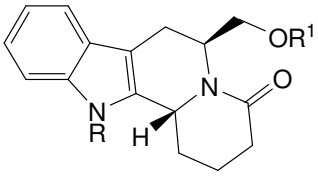
(46)

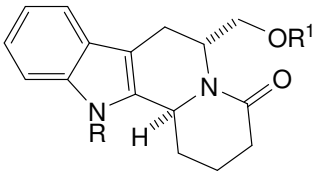


(47)

2.4.2 Biological Activities

Table 4:

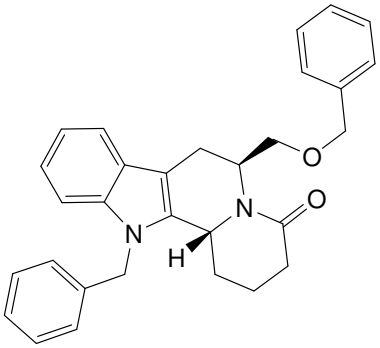




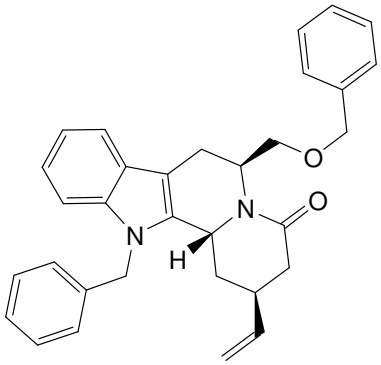
Parent Compounds				**Analogues with vinyl substituent**			
(D-Series)				**(D-Series)**			
	R	R^1	IC_{50} (μM) $Dd2^{luc}$		R	R^1	IC_{50} (μM) $Dd2^{luc}$
(30)	Benzyl	Allyl	1.3	(46)	Benzyl	Allyl	1.1
(31)	Benzyl	Benzyl	3.5	(47)	Benzyl	Benzyl	13
L-Series				**L-Series**			
(26)	Benzyl	Benzyl	12	(48)	Benzyl	Benzyl	20

Table 4 shows conflicting results. Introduction of a vinyl group to the bis-benzyl compound **(31)** to give compound **(47)** has caused a decrease in activity (3.5 μM to 13 μM). This

finding is supported by IC_{50} values of the enantiomers of these compounds. Compound **(26)** [3] has an IC_{50} value of 12 μM; addition of a vinyl group to give compound **(48)**, [3] causes this to fall to 20 μM.



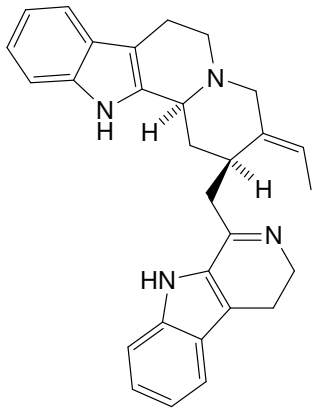
(26)



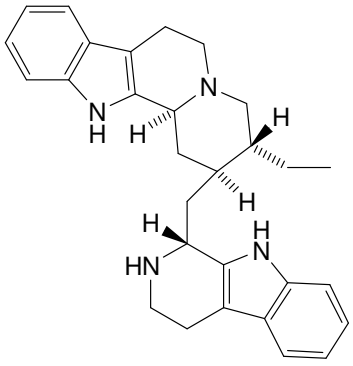
(48)

Contrary to this, addition of a vinyl group to the compound **(30)**, with a benzyl at R and an allyl at R^1, has caused an increase in activity, giving the most active compound presented so far. As mentioned before it is likely that increased steric bulk, particularly at the R^1 position is unfavourable. With two benzyl moieties and a vinyl group, it could be that compound **(47)** is now too big and bulky and is not readily accommodated into the binding site, that there is limited space. In compound **(46)**, with a less bulky allyl group at R^1, the addition of the vinyl group causes a favourable increase in activity. This suggests that addition to the β-position of the lactam ring could be highly beneficial for anti-malarial activity; however there is limited space, and adding a substituent larger than a vinyl group could cause a decrease in activity. Natural anti-malarial compounds such as dihydrousambarensine **(34)**, ochrolifuanine A **(49a)** and a plethora of others all have β-carboline type ring systems in this position. What they lack however, is a substituent in the R^1 position, in fact the hydroxy methyl moiety is not present at all and it is perhaps this that gives enough reduction in size to allow these natural compounds to have large structures bonded to the lactam ring and still retain high anti-malarial activity.

Therefore, it appears that removing the hydroxymethyl group from our indoloisoquinoline template is desirable, as it will allow us to investigate further additions to the lactam ring. The methodology for this is described in section 2.6.



(34)

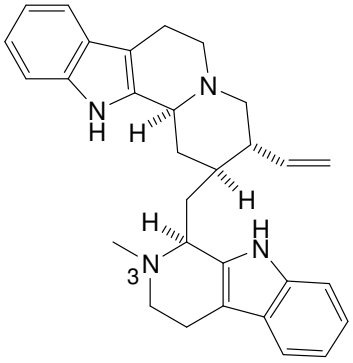


(49a)

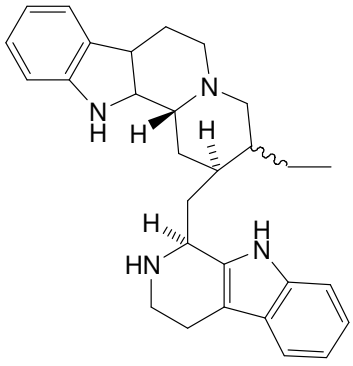
2.5 Basicity

2.5.1 Introduction

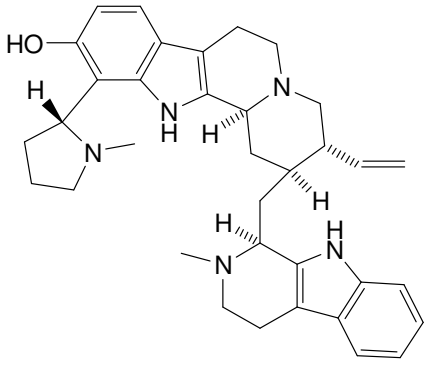
The activity of chloroquine has, in part, been attributed to its basic nature, causing it to accumulate in the acidic digestive vacuole of the *Plasmodium* parasite. [9,10] Similar findings have been reported in compounds from various *Strychnos* species, exhibiting anti-plasmodial activity. [7] Despite the similarities in structures, usambarine **(50)** (possessing a methyl substituent at N3, giving a tertiary amine) was found to be less active (IC_{50} = 2.5 µM) than ochrolifuanine A **(49a)** (IC_{50} = 0.12 µM) and E **(51)** (IC_{50} = 0.28 µM), which both possess an H atom instead, which would make the compound slightly more basic. Isostrychnopentamine **(52)** is also structurally similar to usambarine, yet possesses a third basic nitrogen atom in the pyrrolidine ring, and accordingly was found to be more active against the *Plasmodium* lines tested (IC_{50} = 0.12 µM).



(50)



(51)

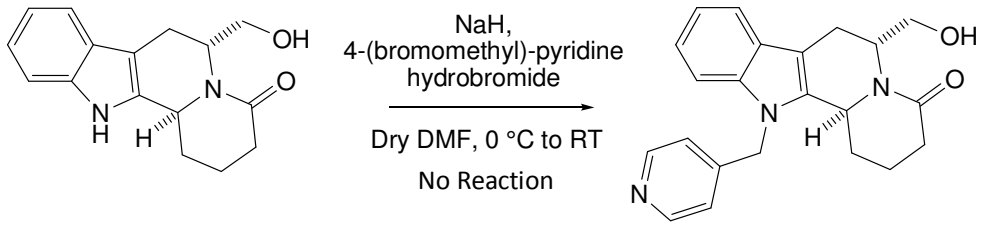


(52)

With this is mind, modifications were made to see if increasing the basicity of these compounds could have a similar effect.

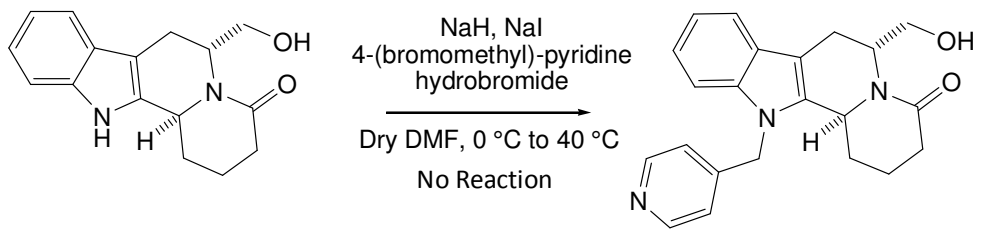
2.5.2 Increasing the basicity

Replacing the benzyl moiety at the R position with a pyridylmethyl group, and keeping the R^1 substituent an allyl group, gives an analogue to compound **(30)** with very similar steric properties, but with an increase in basicity due to the extra nitrogen atom. The addition of the pyridylmethyl group was not as straightforward as with the other alkylations. Previous methods developed successfully with previous alkylating agents failed to yield the desired product in this series (Schemes 27 and 28).



(20) **(53)**

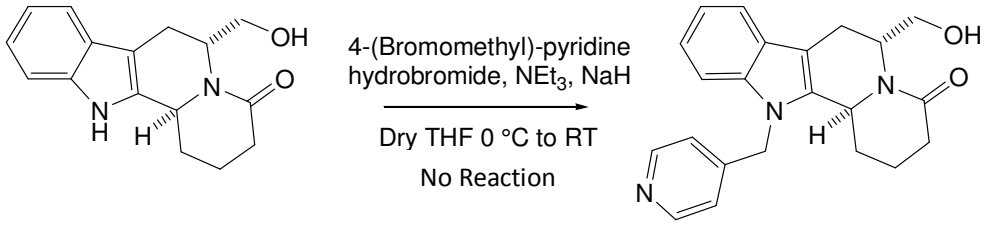
Scheme 27



(20) **(53)**

Scheme 28

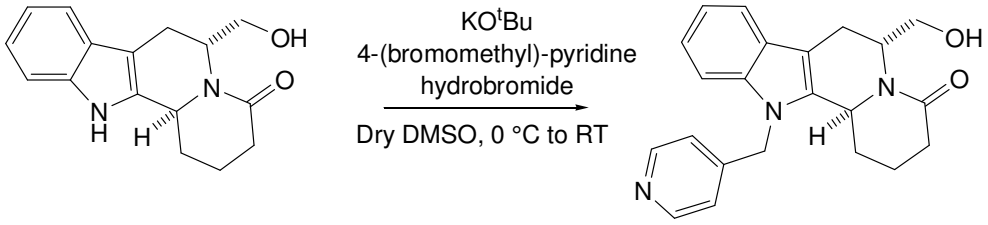
A method reported by Jilka *et al.* [11] for the dialkylation of carbocyclic analogues of Tröger's base with pyridyl groups was investigated. Two methods were described; the first, in which the pyridine salt was deprotonated with triethylamine and added as a solution to anhydrous tetrahydrofuran, followed by sodium hydride (Scheme 29), was attempted by another member of the Allin group and was found to be unsuccessful.



(20) **(53)**

Scheme 29

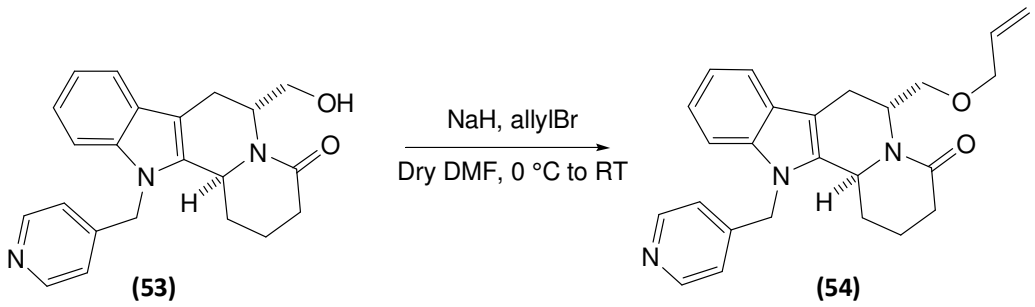
The second method described by Jilka *et al.* involved the bromomethylpyridine being treated with potassium *tert*-butoxide in dry dimethyl sulfoxide (Scheme 30). [11] This method proved to be successful and gave the desired compound **(53)** in 22% yield.



(20) **(53)**

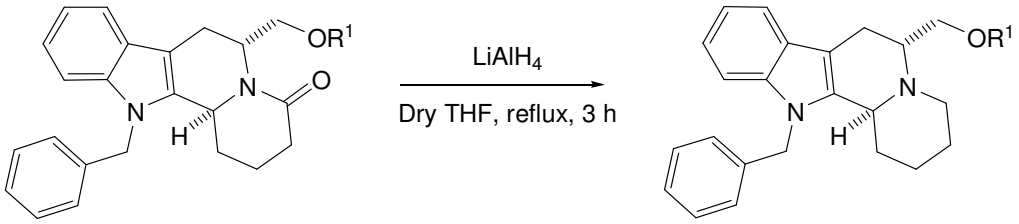
Scheme 30

An allyl group was then added to **(53)** in the usual way to give compound **(54)** in 90% yield**.**



Scheme 31

Another method investigated to increase the basicity of these compounds was to remove the carbonyl group, converting the amide functional group to a tertiary amine. Amides are very weak bases compared to amines since the adjacent carbonyl group is electron-withdrawing and causes the lone pair on the nitrogen to become delocalised. The carbonyl group was removed using 1 equivalent of lithium aluminium hydride in dry tetrahydrofuran under reflux conditions for 3 hours. The reaction was then stirred at room temperature overnight. This reaction was carried out on the two most biologically active compounds, **(29)** and **(30),** described in section 2.1 (Scheme 32).



(29) R^1=Me

(30) R^1=Allyl

(55) R^1=Me 45%

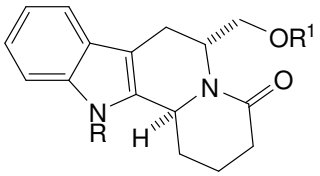
(56) R^1=Allyl 12%

Scheme 32

Table 5 shows the anti-malarial activity of the compounds described in this section, compared to the parent compounds.

2.5.3 Biological activity

Table 5:



Parent Compounds				**Analogues**			
	R	R^1	IC_{50} (µM) $Dd2^{luc}$		R	R^1	IC_{50} (µM) $Dd2^{luc}$
(30)	Benzyl	Allyl	1.3	(54)	4- Pyridyl methyl	Allyl	2.1
				(56)	Benzyl	Allyl	1.5
(29)	Benzyl	Methyl	1.3	(55)	Benzyl	Methyl	3.3

None of these compounds show any improvement in activity compared to the parent compounds **(29)** and **(30)** (both with an IC_{50} of 1.3 µM). Therefore it seems conclusive that the basicity of these compounds is not contributing to their activity. The drop in activity from the amide compounds **(29)** and **(30)** to the corresponding amines **(55)** and **(56)** is

small. Amides have the ability to form a hydrogen bond to a binding site through the carbonyl oxygen, if this particular binding was important for the compound's activity, a larger drop in activity might be expected. The nitrogen atom of an amide functional group cannot form a hydrogen bond since the lone pair of electrons will delocalise across with the neighbouring carbonyl group. However when converted into a tertiary amine, the N-atom can form hydrogen bonds through the free lone pair of electrons. It may be possible that the amine N-atom is binding by hydrogen bonds to the same site that the carbonyl oxygen of the amide does, and this is why very little change in activity has occurred. Alternatively, the binding site is not influenced by this particular functionality and no bonding is occurring in this location at all.

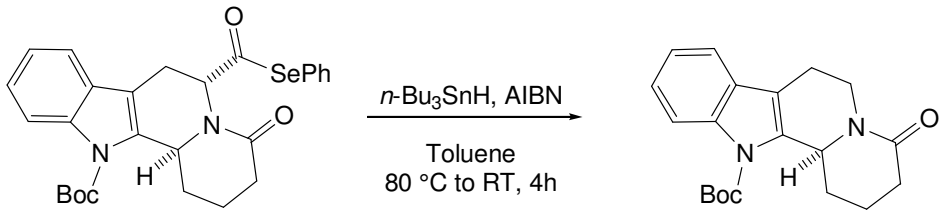
The addition of a nitrogen atom caused by swapping the benzyl moiety in compound **(30)** with a pyridyl group **(54)** gives the compound the ability to form new hydrogen bonds. Due to the decline in activity there seem to be no H bond donors in this region of the binding site.

2.6 Synthesis of a New Template

2.6.1 Removal of the hydroxy methyl group

The hydroxyl methyl moiety within the indolizino[2,3-α]quinolizidine ring system under investigation is not found in any natural compounds that exhibit anti-malarial activity. In section 2.4 it was also proposed that this moiety was preventing high levels of biological activity from being achieved by taking up chemical space.

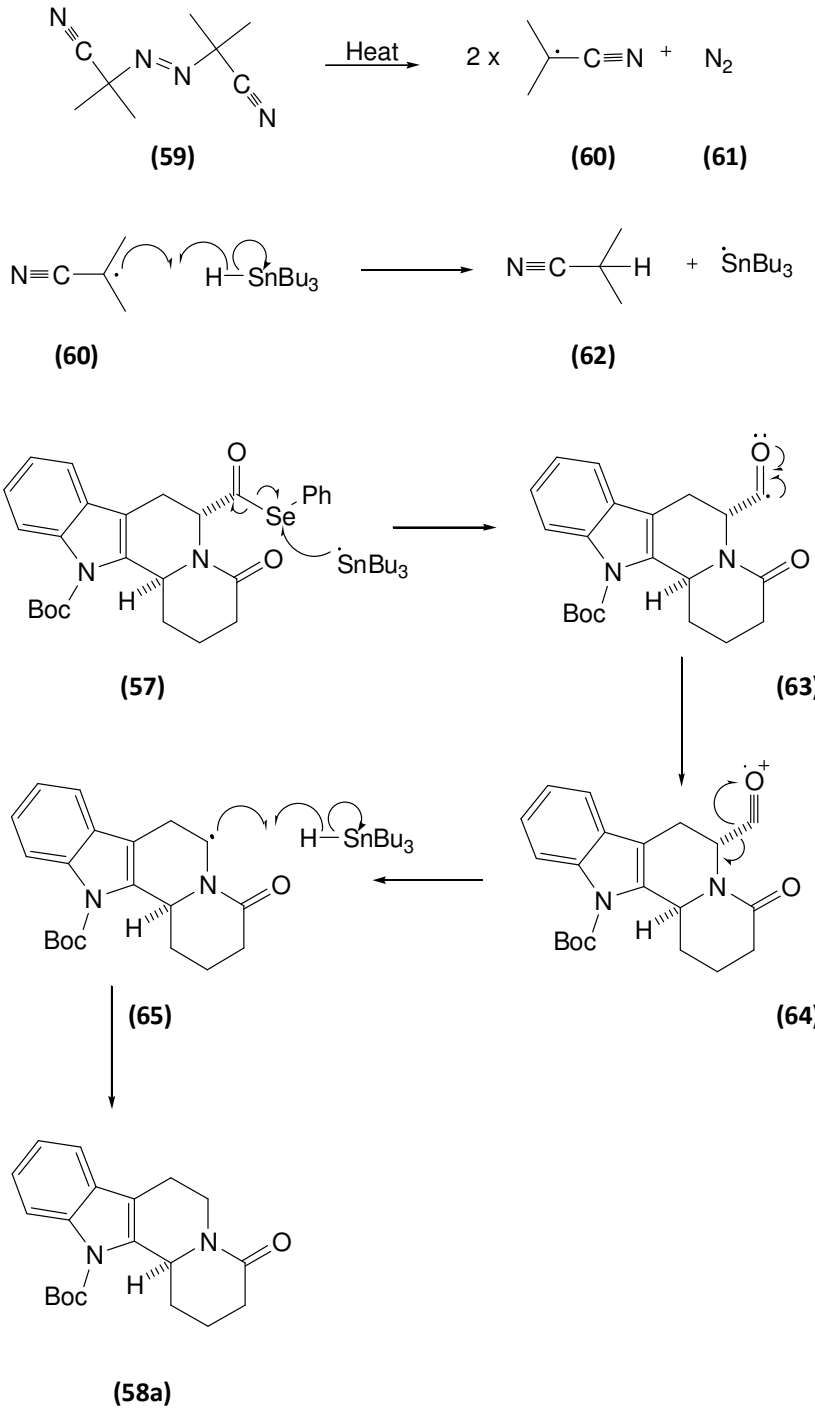
For these reasons, it was removed by a radical decarbonylation of an acyl selenide [12] using tributyltin hydrideand azobis*iso*butyronitrile, giving the desired product **(58a)** in 72% yield.



(57) **(58a)**

Scheme 33

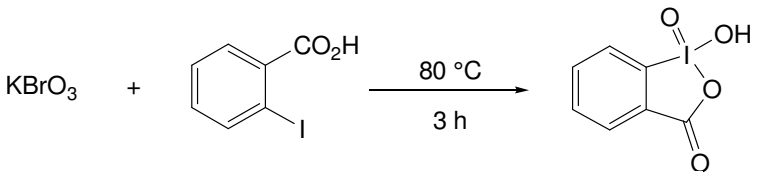
Heating azobis*iso*butyronitrile **(59)** causes its decomposition, eliminating a molecule of nitrogen gas and forming two 2-cyanoprop-2-yl radicals **(60)**. These can then initiate further reactions (Scheme 34).



Scheme 34

In order to create the phenylseleno ester, the OH group must be oxidised to the corresponding carboxylic acid. Previous research in the group showed that direct oxidation was not possible due the indole NH being sensitive to the use of all oxidising agents tried. [13] An indirect route of oxidising the alcohol to the aldehyde, followed by a protection of the indole nitrogen and then subsequent oxidation to the acid was proposed. The only oxidising agent found able to successfully give the aldehyde with our substrate was IBX. [13]

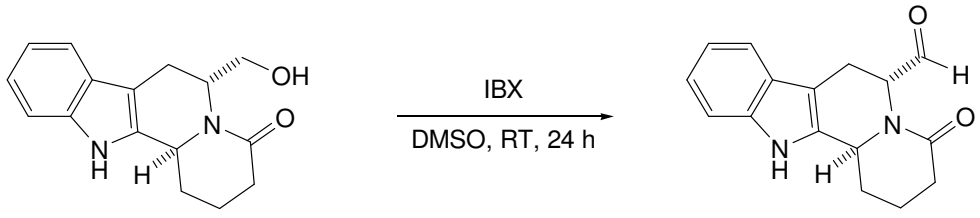
IBX **(68)** was synthesised from 2-iodobenzoic acid **(67)** and potassium bromate **(66)** (Scheme 35). [14]



(66) **(67)** **(68)**

Scheme 35

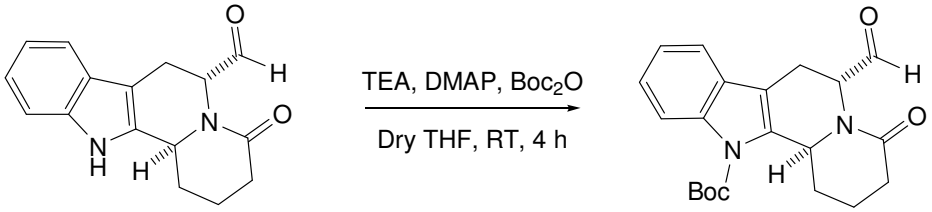
The oxidation reaction was performed in anhydrous dimethyl sulfoxide at room temperature and yielded aldehyde **(69)** as a white solid. This reaction worked well on a small (0.1 g) scale, but attempts to scale up to a larger (1.0 g) scale failed. Commercial stabilised IBX was then used and gave the aldehyde in 80% yield on a 1.0 g scale.



(20) **(69)**

Scheme 36

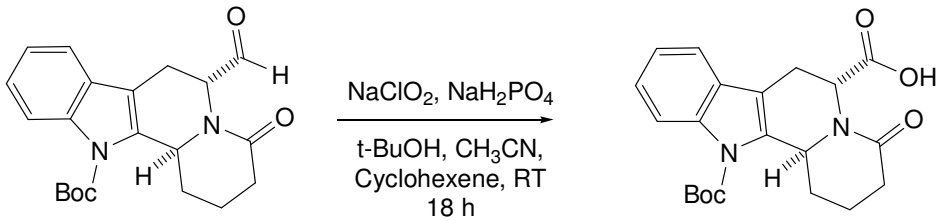
Protection of the indole NH was achieved by using di-*tert*-butyl carbonate in anhydrous tetrahydrofuran with triethylamine and 4-(dimethylamino)pyridine.



(69) **(70)**

Scheme 37

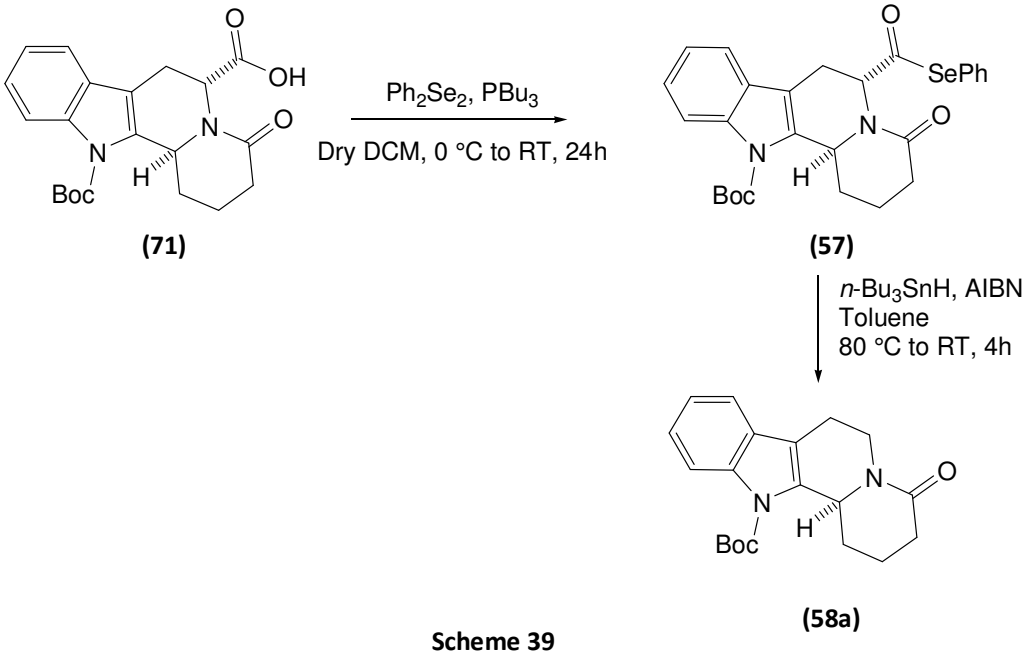
To obtain the carboxylic acid, a Pinnick oxidation [15] was performed using sodium chlorite and sodium dihydrogen phosphate dissolved in a mixture of acetonitrile and *tert*-butyl alcohol (Scheme 38). This gave the desired acid in 88% yield. Cyclohexene was used as a chlorine scavenger to catch any hypochlorite ions formed, as these may cause unwanted side reactions.



(70) **(71)**

Scheme 38

Selenation of the carboxylic acid was achieved in 55% yield by reacting the carboxylic acid with diphenyl diselenide and tributylphosphine in anhydrous dichloromethane.

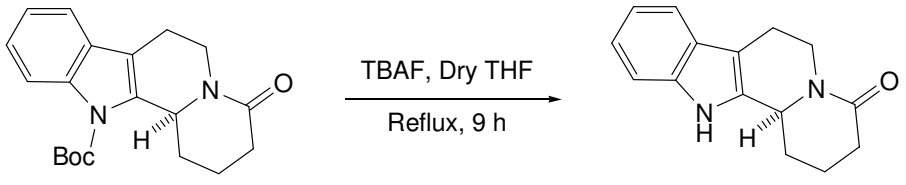


(71) **(57)** **(58a)**

Scheme 39

2.6.2 Functionalisation of template (72a)

The Boc protecting group was removed by heating under reflux with tetrabutylammonium fluoride in anhydrous tetrahydrofuran for 9 hours. This gave the free indole compound in 79% yield (Scheme 40).

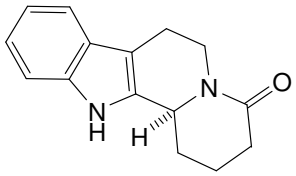


(58a) **(72a)**

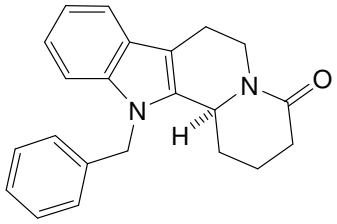
Scheme 40

A set of compounds derived from this new template **(72a)** were designed and synthesised. The aim was to test them against analogues that have the hydroxymethyl group in place to see if removal of this moiety leads to better anti-malarial activity and hence gain information on the necessity of functionality in this chemical space.

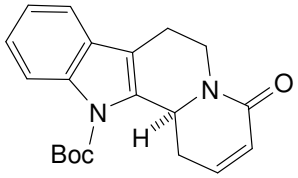
The other compounds in this series were synthesised using methods already described above in this thesis.



(72a) 79%



(73a) 80%

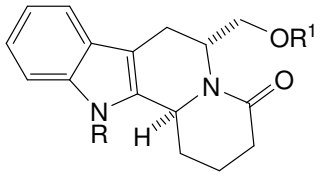


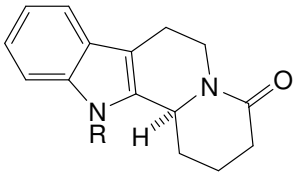
(74a) 26%

Table 6 compares the activities of compounds **(72a)** and **(73a)** with their analogues.

2.6.3 Biological activities

Table 6:





Parent Compounds				Analogues			
	R	R^1	IC_{50} (μM) $Dd2^{luc}$		R	R^1	IC_{50} (μM) $Dd2^{luc}$
(20)	H	H	56	(72a)	H	N/A	36.7
(27)	Benzyl	H	5	(73a)	Benzyl	N/A	5.3

In the first instance (compounds **(20)** and **(72a)**, with no modifications to either template, the removal of the hydroxymethyl group has caused a notable increase in activity, however it is still not comparable to the activity of compounds **(29), (30)** and **(46)**. This increase in activity is most probably due to the OH group having been removed and lowering the overall polarity of the compound.

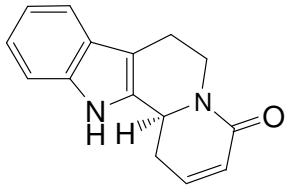
Addition of a benzyl group (compound **(73a)**) to template **(72a)** causes the same high increase in activity seen previously when a benzyl group was added to template **(20)**. Compound **(73a)** has an IC_{50} value very similar to analogue **(27)**, showing that in this case, the removal of the hydroxymethyl moiety has caused no significant change in anti-malarial activity. This would suggest that functionality in this region is not necessary for high activity.

The α,β-unsaturated compound **(74a)** shows an increase in activity (IC_{50} = 15.2 μM) compared to its saturated analogue **(58a)** (IC_{50} = 22.5 μM). This finding is different from the results described in section 2.3 which suggest that α,β-unsaturated compounds have a lower activity (albeit by only a small amount). The change from a benzyl **(73a)** to a Boc

(58a) protecting group on the indole nitrogen induces a decrease in activity. This result is unsurprising, as a Boc group shares none of benzyl's steric or electronic properties.

The Boc group on the α,β-unsaturated compound **(74a)** was removed using formic acid [16] as the tetrabutylammonium fluoride Boc removal method described above (Scheme 40) has been shown to reduce the unsaturated lactam ring.[3] This yielded the desired compound **(75a)**.

The structure of this compound **(75a)** was confirmed by ^{1}H NMR spectroscopy (showing that the $C(CH_3)_3$ peak, characteristic of a Boc group, was not present, and that an indole NH peak was present at 8.23 ppm). This compound was found to be unstable and no further data could be collected.



(75a)

Despite no real increase in anti-malarial activity, we felt it was desirable to continue making compounds without a hydroxymethyl substituent, due to the stronger resemblance to natural indole alkaloids. As the most active natural compounds (such as dihydrousambarensine **(34)** and ochrolifuanine A **(49a)**) have a free indole NH, the use of a Boc group in this position is better than a benzyl with our compound, due to its ease of removal.

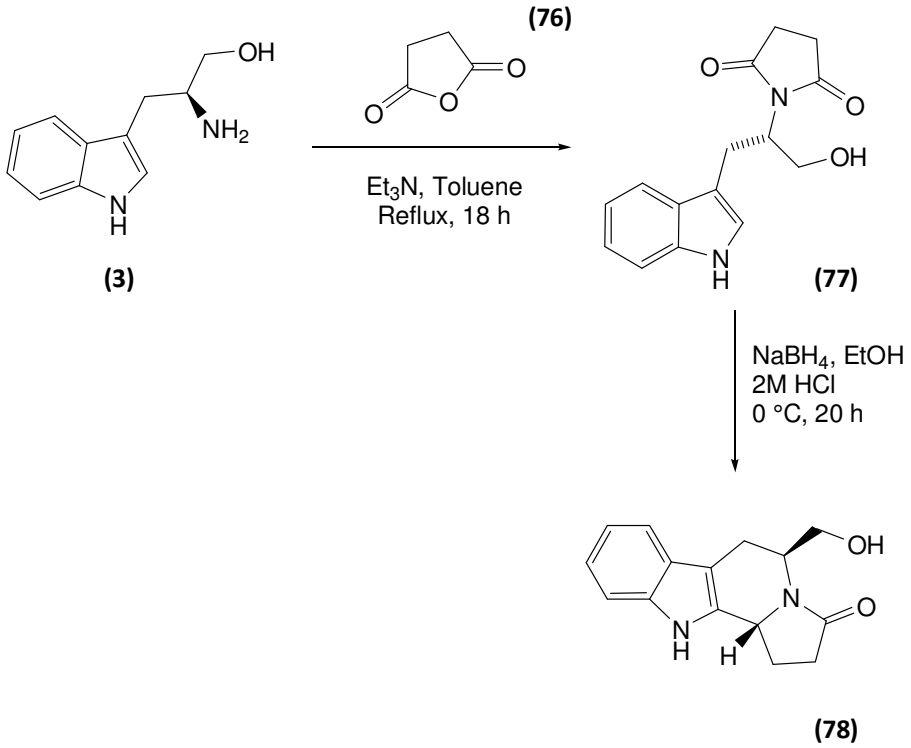
The synthesis of the indolizino[2,3-α]quinolizidine core requires five steps, and a further five steps to remove the hydroxymethyl moiety. This requires large amounts of starting material and reagents, and the reactions to be scaled up to give enough template for a series of compounds. Due to the high cost of D-tryptophan and the time required for a 10

step synthesis, an alternative route was sought for creating an indolizino[2,3-α]quinolizidine core.

2.7 Alternative Synthesis to the Indolizino[2,3-α]quinolizidine Ring System.

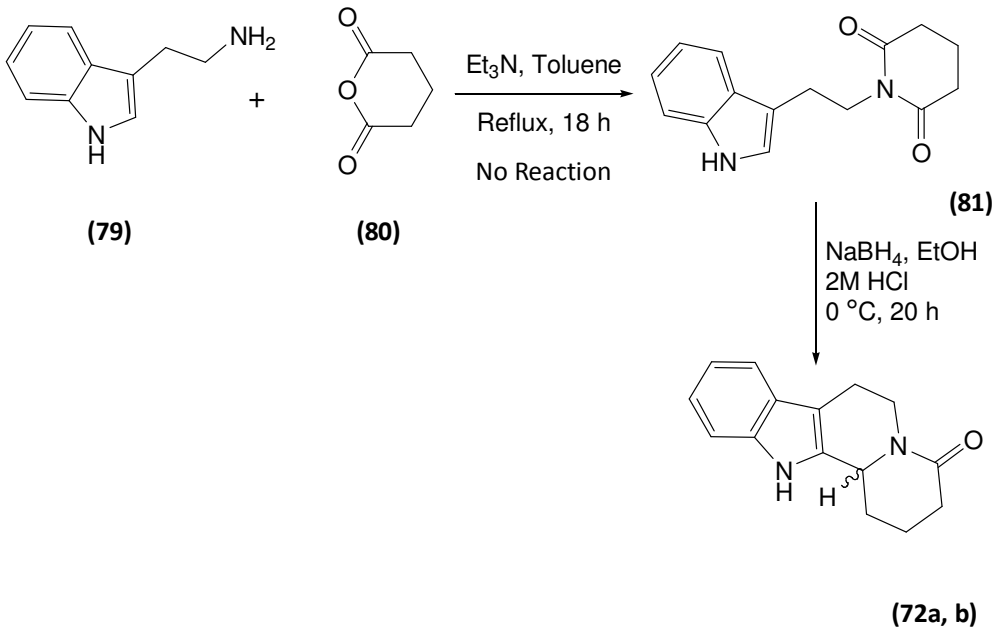
2.7.1 Previous work

Previous work in the Allin group towards the synthesis of the indoloizino[8,7-*b*]indole ring system **(78)** had shown that this template, similar to the indolizino[2,3-α]quinolizidine ring system, could be synthesised in two steps by reaction of the β-amino derivative of L-tryptophan **(3)** with succinic anhydride **(76)**, followed by a reductive cyclisation reaction. [17]



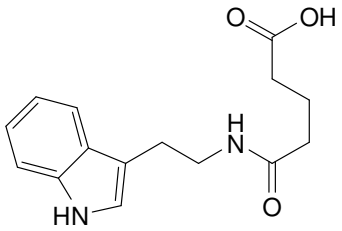
Scheme 41

We thought that this approach, *via* the corresponding imide **(81)**, could be used to access the racemic indolizino[2,3-α]quinolizidine ring system **(72a, b)** using tryptamine **(79)** and glutaric anhydride **(80)** (Scheme 42).



Scheme 42

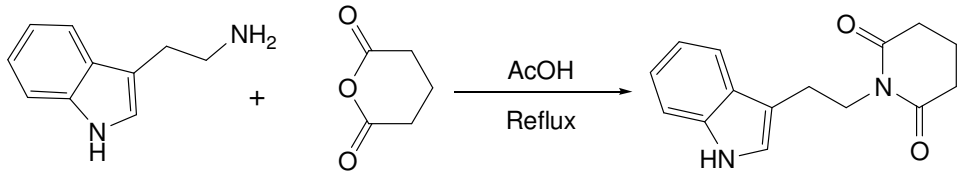
Key features of product **(72a, b)** are that it is racemic and that it lacks the hydroxylmethyl substituent. The first step of this proposed synthesis is the formation of an imide derivative from tryptamine and glutaric anhydride. The method described in Scheme 42 was used, but proved unsuccessful in this case as the reagents did not dissolve. The method was repeated but with replacing toluene with anhydrous tetrahydrofuran as a solvent. This gave rise to the formation of undesired compound **(82)**, showing that under these conditions the reaction is not reaching completion, but rather stopping half way. Different methods for the formation of this imide were then investigated.



(82)

2.7.2 Imide formation

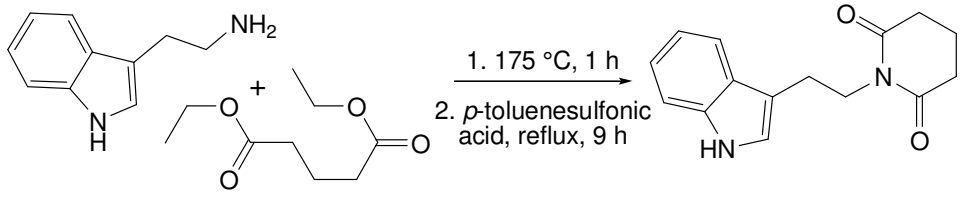
Ardeo *et al.* achieved the desired imide **(81)** in 67% yield, during their investigations into constructing the fused β-carboline framework found in corynanthe-type indole alkaloids. [18] They reacted tryptamine with glutaric anhydride while heating under reflux in acetic acid.



(79) **(80)** **(81)**

Scheme 43 [18]

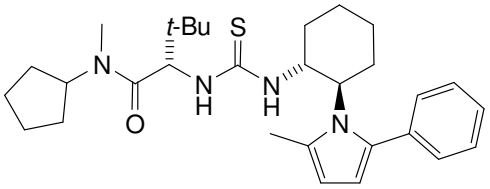
Morrison *et al.* accessed the same compound by a different method involving diethyl glutarate **(83)** as a substrate. [19] This was reacted with tryptamine at 175 °C for 1 hour, and subsequently heated under reflux in xylene with *p*-toluenesulfonic acid for 9 hours.



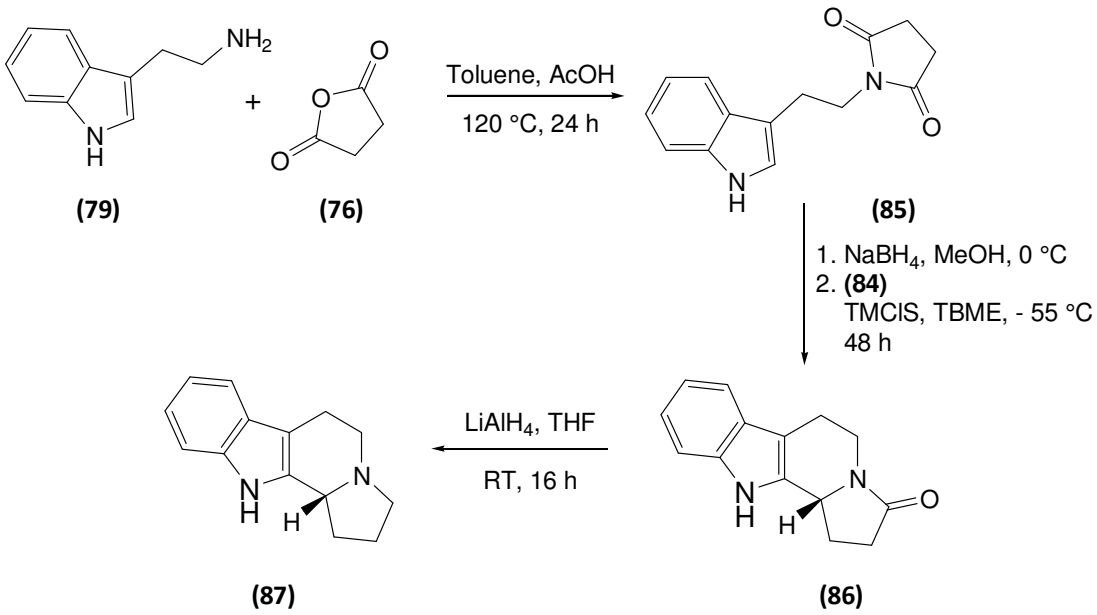
(79) **(83)** **(81)**

Scheme 44 [19]

Raheem *et al.* formed a similar imide *en route* to the natural compound (+)-harmicine **(87)**. [20] Tryptamine **(79)** was combined with succinic anhydride **(76)** in a mixture of toluene and acetic acid (1:3) and heated under reflux at 120 °C for 24 hours. The imide **(85)** was then cyclised to give compound **(86)** using sodium borohydride in methanol at 0 °C followed by trimethylchlorosilane, methyl *tert*-butyl ether and a chiral thiourea catalyst **(84)**. This method gave (+)-harmicine **(87)** in 97% e,e. All compounds reported by Raheem which were synthesised this way gave a high percentage enantiomeric excess.



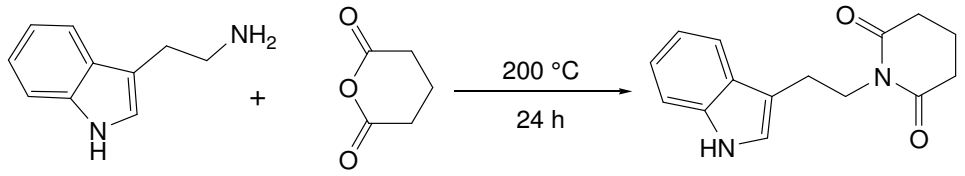
(84)



(79) **(76)** **(85)** **(87)** **(86)**

Scheme 45 [20]

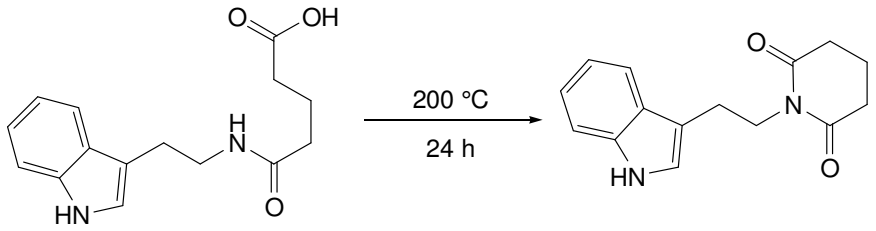
A method reported by Heaney, [21] that showed that an imide could be formed by heating tryptamine with glutaric anhydride at 200 °C with no solvent, was tried and found to be successful in our studies. Thin layer chromatography showed that after 2 hours there was still plenty of starting material left, so the reaction was left overnight and gave the desired imide **(81)** in 66% yield.



(79) **(80)** **(81)**

Scheme 46

We found that under these conditions, the undesired intermediate **(82)** could also be converted into the imide **(81)**.

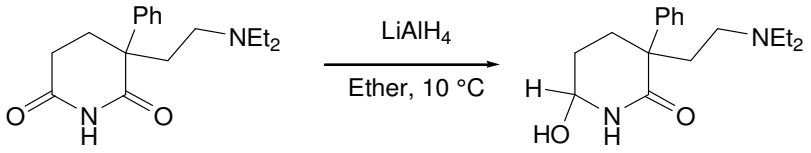


(82) **(81)**

Scheme 47

2.7.3 *N*-Acyliminium cyclisation

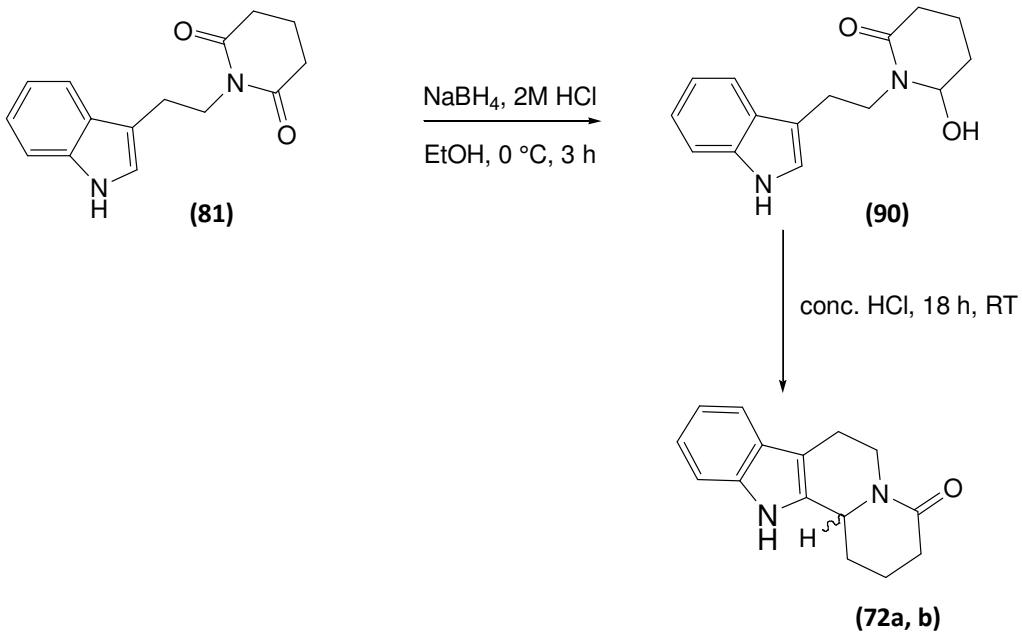
For cyclisation to occur, one of the carbonyl groups on the piperidinone ring must be reduced. In 1954, Tagmann *et al* showed that under mild conditions lithium aluminium hydride could be used to reduce compound **(88)** to the hydroxyl piperidinone compound **(89)**. [22]



(88) **(89)**

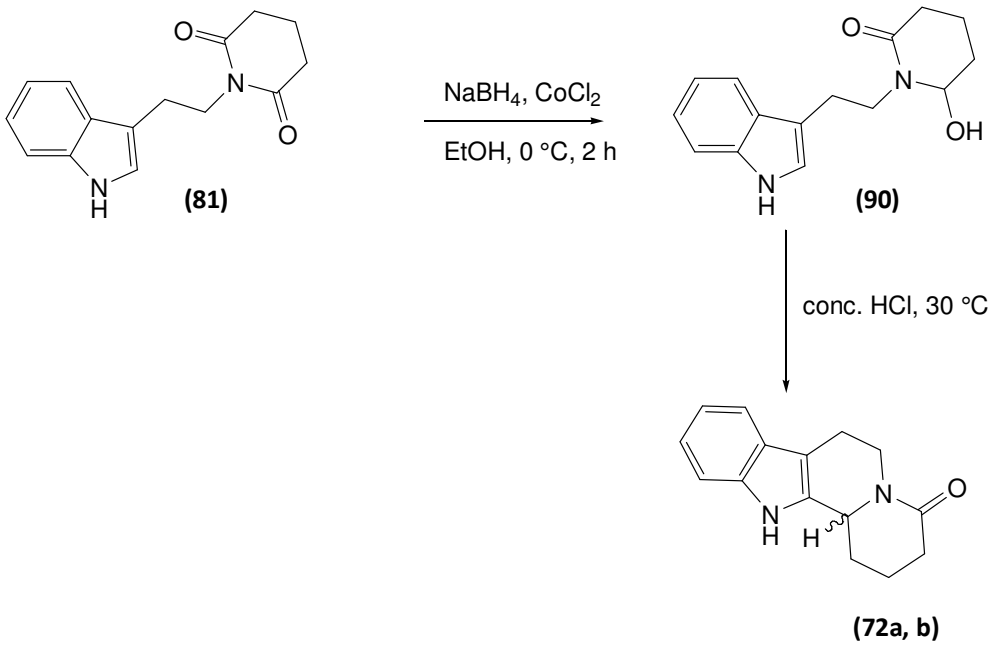
Scheme 48 [22]

Speckamp *et al.* developed this partial reduction of cyclic imides into high yielding procedures by using excess sodium borohydride in ethanol. [23, 24] During the reaction, dilute hydrochloric acid is added to stop the medium becoming too basic, as this would cause undesired ring opening. The reaction is performed at a low temperature which also helps to avoid ring opening. It has been reported that the addition of acid is not necessary if the reaction is performed using methanol as a solvent and carried out at -4 °C. Cyclisation of the reduced imide is achieved by addition of hydrochloric acid to the reaction (Scheme 49).



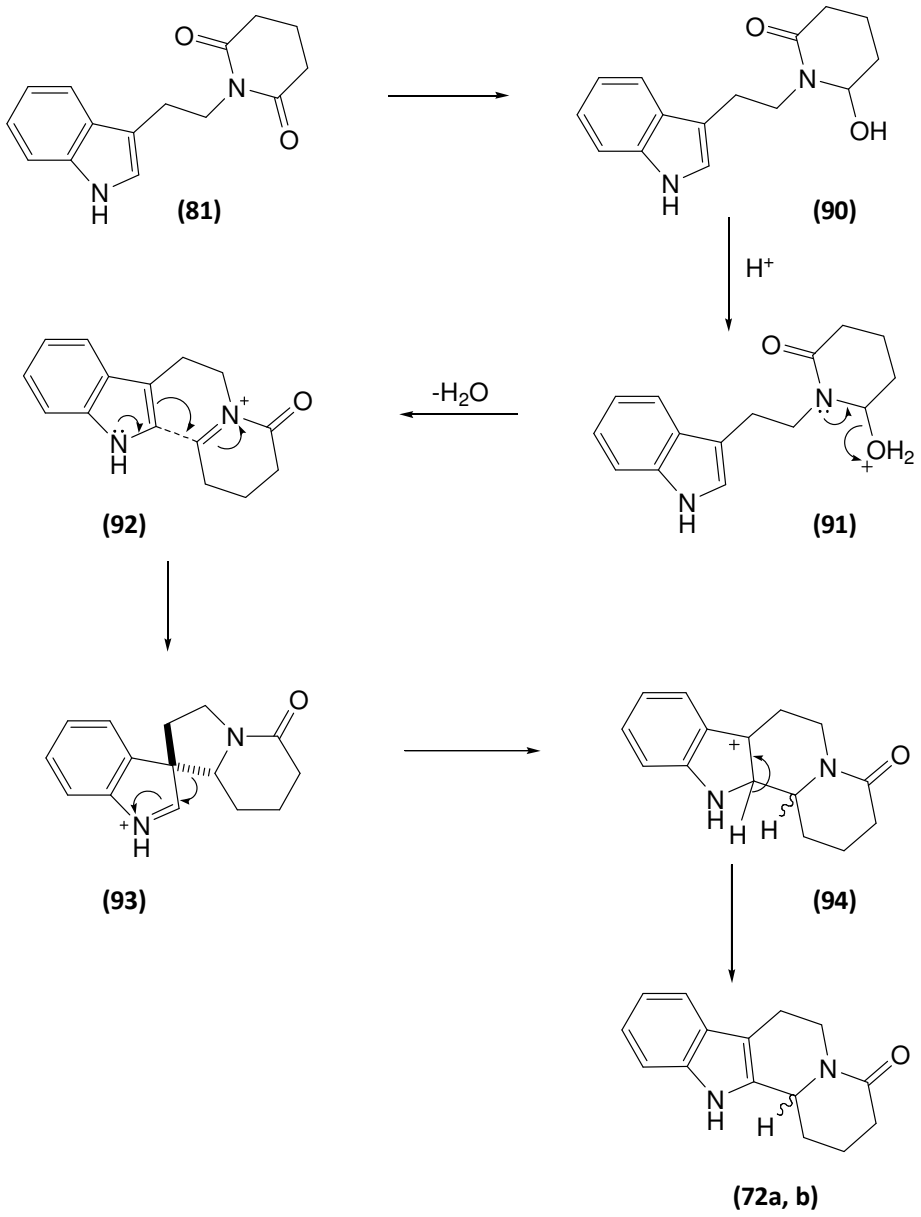
Scheme 49 [23, 24]

Rahman *et al.* in their synthesis towards β-carbolines, [25] report success using a variety of metals to catalyse reduction by sodium borohydride (Scheme 50). The imide was treated with 5 equivalents of sodium borohydride, and 1 equivalent of cobalt chloride in ethanol at 0 °C. After two hours a 95% yield of the desired product was obtained. This was then cyclised using concentrated hydrochloric acid. Similar yields were achieved using nickel chloride, chromium chloride and stannous chloride.



Scheme 50 [25]

The method employed in this current project was adapted from that of Speckamp *et al*. [23, 24] The imide was dissolved in absolute ethanol and cooled to 0 °C degrees. Sodium borohydride was added followed by 2M hydrochloric acid in absolute ethanol which was added portionwise over a period of 3 hours. The reaction was then acidified to pH 1-3 by further addition of 2 M hydrochloric acid in absolute ethanol and the reaction mixture was stirred at room temperature overnight. The addition of further 2M hydrochloric acid is needed to promote the cyclisation reaction. After work-up the crude product was washed with absolute ethanol to give an off-white solid **(72a, b)** in 68% yield (Scheme 49). The proposed mechanism for this cyclisation reaction is shown in Scheme 51.

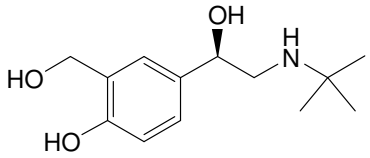


Scheme 51

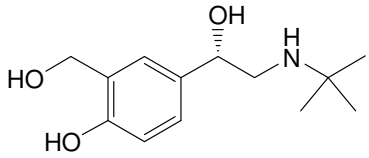
2.7.4 Racemic compounds

Using a racemic template, although quicker and cheaper to synthesise, could pose problems when testing for anti-malarial activity. A racemic mixture could be considered comparable with administering two different drugs with differing pharmacological

properties; it is possible only one enantiomer has biological activity. One enantiomer may act as an agonist or an antagonist causing an increase, or decrease, in the overall effectiveness of the drug. In the case of the asthma treating drug salbutamol, only one enantiomer (the R enantiomer **(95)**) has any advantageous effect. [26]

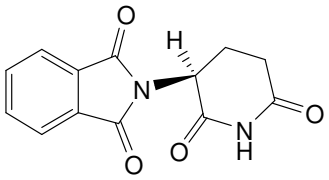


(95)
R -Salbutomol

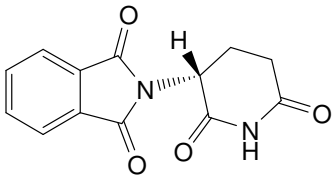


(96)
S- Salbutamol

Studies into the chiral drug thalidomide, commonly prescribed in the late 1950's for morning sickness, but withdrawn a few years later after being linked to serious birth-defects, have shown some evidence that the two isomers have differing biological effects. The R-enantiomer **(98)** has been shown to be responsible for the drugs sedative effects and the S-enantiomer **(97)** has been shown to be responsible for its imunnomodulatory effects. [27, 28] It is important to test each enantiomer of a chiral drug separately to see exactly what biological effect it might produce. Thalidomide is racemised *in vivo* [29] and therefore administering a single enantiomer would not avoid the undesirable side effects of this drug. Therefore it is important to know what effects a racemic mixture could have.



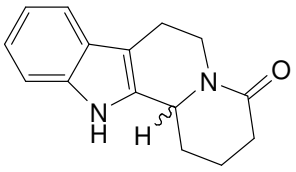
(97)
S-Thalidomide



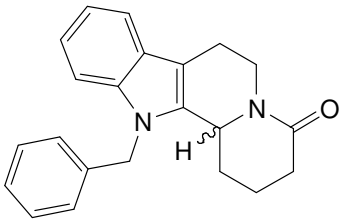
(98)
R-Thalidomide

The *in vitro* testing done on the compounds produced in this project may show if using a racemic mixture will have any effect on the compounds anti-malarial activities.

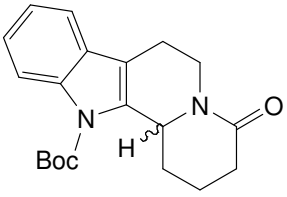
A series of compounds similar to the ones in section 2.6 were synthesised using methods described above. This was carried out to see what effect (if any) using a racemic template instead of an enantiomerically pure one might have on the activity of the compounds. Table 7 shows the IC_{50} values of the compounds **(72a, b)**, **(73a, b),** and **(58a, b)** compared with their enantiomerically pure analogues; Table 8 shows the unsaturated compound **(74a, b)** compared to its enantiomerically pure analogue **(74a)**.



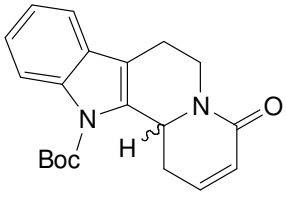
(72a, b) 68%



(73a, b) 58%



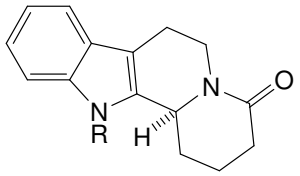
(58a, b) 55%

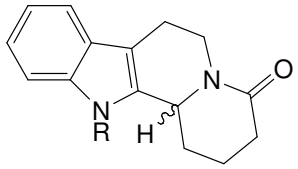


(74a, b) 37%

2.7.5 Biological activities

Table 7:





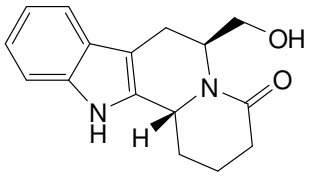
D-Series	R	IC_{50} (µM) $Dd2^{luc}$	**Racemic**	R	IC_{50} (µM) $Dd2^{luc}$
(72a)	H	36.7	(72a, b)	H	38.5
(73a)	Benzyl	5.3	(73a, b)	Benzyl	8.26
(58a)	Boc	22.5	(58a, b)	Boc	23.6

Table 8:

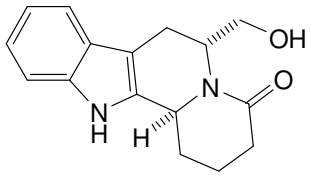
D-Series	R	IC_{50} (µM) $Dd2^{luc}$	**Racemic**	R	IC_{50} (µM) $Dd2^{luc}$
(74a)	Boc	15.2	(74a, b)	Boc	10.1

The above tables show that the IC_{50} values of the racemic compounds are not especially different from those of their pure enantiomer analogues. Out of the four comparisons, three show the pure enantiomer is slightly more active and the other one shows the racemate to be more active. There are a few possible reasons for this lack of difference in activity.

The racemic compounds contain both (-/+)-enantiomers; while previous results have shown compounds derived from (-)-enantiomer **(20)** to be far more active, there are some cases in which compounds derived from the (+)-enantiomer **(8)** have been shown to be active in the 10-30 µM range (**(24)**, **(25)** and **(26)**), suggesting that compounds with this stereochemistry could, with structural modifications become more active.



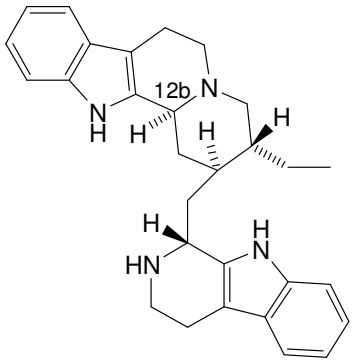
(8)



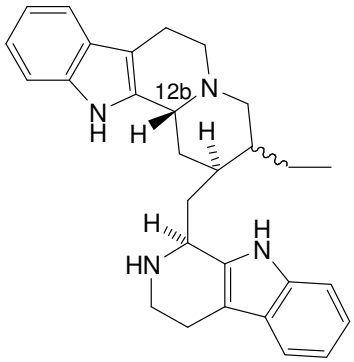
(20)

The enantiomerically pure compounds from section 2.6 were derived from the (-)-template **(20)**, they have not been synthesised with the opposite stereochemistry and therefore their biological activity is unknown. They could have been more potent than the compounds previously tested, and therefore their presence would not cause a decline in activity as would be expected. It is possible that stereochemistry is less important now the hydroxymethyl group has been removed and only one chiral centre is now present. It could be that it was the relative stereochemistry (*cis/trans*) at the chiral centre containing the hydroxymethyl moiety that made such a difference in the activity of the compounds in section 2.1.

Previously mentioned in section 1.2 was the potent anti-malarial compound ochrolifuanine A **(49a)** and its far less potent enantiomer ochrolifuanine E **(51)**.[7] The stereochemistry of the hydrogen atom at position 12b in ochrolifuanine A is the same as that of the our more potent (-)-enantiomer **(20)**, whereas E shares the same stereochemistry as our less potent (+)-enantiomer **(8)**, therefore we thought that the absolute stereochemistry at this position was important. However ochrolifuanine A and E have three asymmetric carbon atoms and the difference in their anti-malarial activities could be attributed to these other chiral centres.

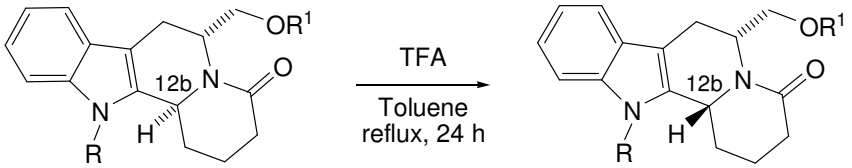


(49a)



(51)

This theory could be tested by deriving compounds from template **(99)**, a diastereoisomer of the (-)-template **(20).** Previous work in our group shows that **(99)** could be synthesised from compound **(20)** by refluxing with trifluoroacetic acid in toluene (Scheme 52). [3]



(20) **(99)**

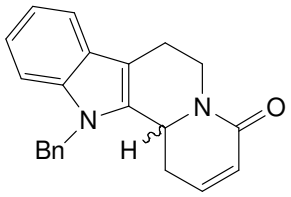
Scheme 52

Groups could then be added to template **(99)** using methods described above to give compounds analogous to those derived from template **(20)**. The IC_{50} values could then be compared to the parent compounds to see if there were any significant differences in activity arising from the change of stereochemistry at the hydrogen atom at position 12b. This would demonstrate whether the absolute stereochemistry at this asymmetric centre is important for anti-malarial activity.

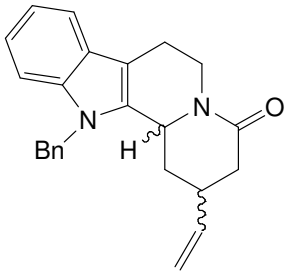
Due to the little difference in the activities between racemic compounds and their enantiomerically pure forms, the project was moved forward using the more readily accessible racemic template. Even if activity of the racemic compounds is lower, they will still give an indication of what modifications lead to increased anti-malarial activity. If a significant lead compound is developed, we have the knowledge base to synthesise an enantiomerically pure analogue.

2.7.6 Other racemic derivatives and biological activities

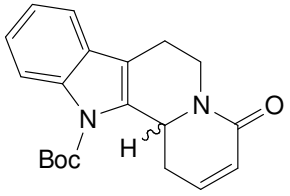
Other compounds in the racemic series that were synthesised using previously developed methods are shown. Their activities are displayed in Table 9.



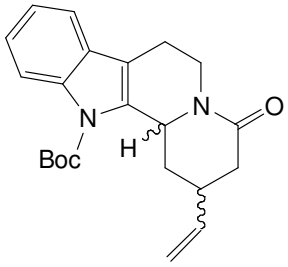
(100a, b) 58%



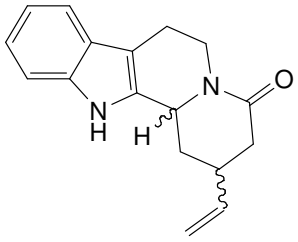
(101a, b) 20%



(74a, b) 37%

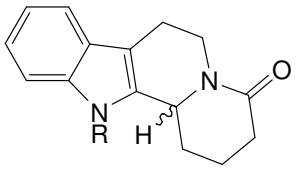


(102a, b) 45%



(103a, b) 46%

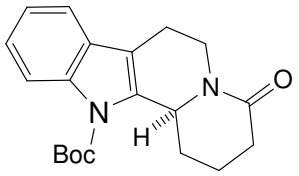
Table 9:



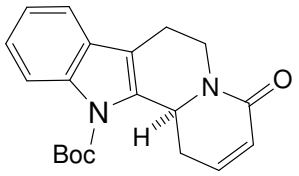
Unsaturated Compounds			**Compounds with a Vinyl substituent**		
	R	IC_{50} (μM) $Dd2^{luc}$		R	IC_{50} (μM) $Dd2^{luc}$
(100a, b)	Benzyl	1.9	(101a, b)	Benzyl	4.7
(74a, b)	Boc	10.1	(102a, b)	Boc	14.5
			(103a, b)	H	69.6

The structure-activity relationships of these racemic compounds follow similar trends to those that were found with the enantiomerically pure series in section 2.6.

Both compounds **(100a, b)** and **(74a, b)** show that introduction of α,β-unsaturation has caused an increase in activity when compared to their saturated analogues **(73a, b)** and **(58a, b)** (IC_{50} values of 8.26 μM and 23.6 μM respectively). This is also the case with the enantiomerically pure compound **(74a)** (IC_{50} = 15.2 μM) when compared to its saturated analogue **(58a)** ((IC_{50} = 22.5 μM).

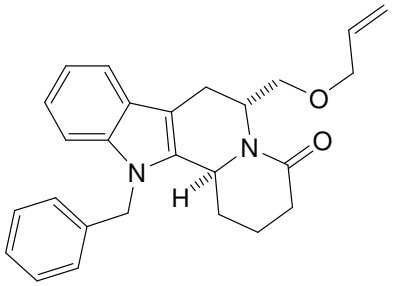


(58a)

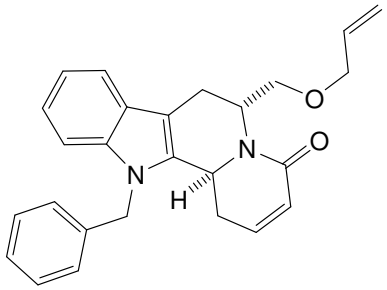


(74a)

The IC_{50} values of the compounds described in section 2.3 showed that when the hydroxymethyl group is present, introduction of α,β-unsaturation of the lactam ring did not lead to an increase in activity. Compound **(30)** has an IC_{50} value of 1.3 µM, whereas its unsaturated analogue **(44)** has a value of 1.7 µM.

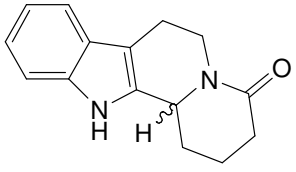


(30)

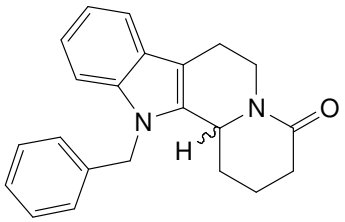


(44)

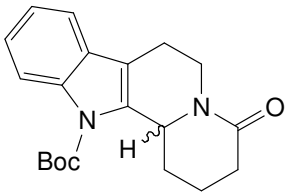
Addition of a group to the indole nitrogen atom in the racemic template **(72a, b)** has caused an increase in activity, from **(72a, b)** (IC_{50} = 38.5 µM) to **(73a, b)** (IC_{50} = 8.26 µM) and **(58a, b)** (IC_{50} = 23.6 µM), a trend seen in section 2.2 and section 2.6.



(72a, b)



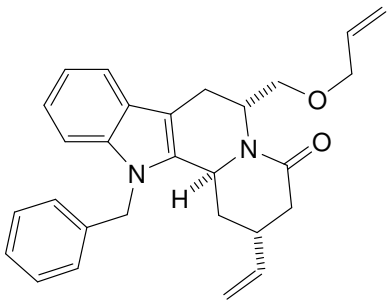
(73a, b)



(58a, b)

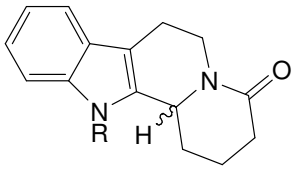
The IC_{50} values of these compounds show that a benzyl substituent causes a greater increase in activity than a Boc group, as seen with the enantiomerically pure compounds **(58a)** and **(73a)** in section 2.6.

The introduction of a vinyl group (compounds **(101a, b)** and **(102a, b)**) causes an increase in anti-malarial activity when compared to their parent compounds **(73a, b)** and **(58a, b)** (Table 10). This is consistent with the results from section 2.4, in which addition of a vinyl group to compound **(30)** (IC_{50} = 1.3 µM) gave compound **(46)** which has an IC_{50} value of 1.1 µM.



(46)

Table 10:



Parent Compounds			Vinyl Compounds		
	R	IC_{50} (μM) $Dd2^{luc}$		R	IC_{50} (μM) $Dd2^{luc}$
(73a, b)	Benzyl	8.26	(101a, b)	Benzyl	4.7
(58a, b)	Boc	23.6	(102a, b)	Boc	14.5
(72a, b)	H	38.5	(103a, b)	H	69.4

Both the enantiomerically pure series and the racemic series of compounds follow similar structure-activity trends. This provides evidence that further structural modifications to the easily obtainable racemic template (and the anti-malarial activities obtained) will be a good indication of the bio-activity of the enantiomerically pure compounds.

2.7.7 Conclusion to the structure-activity relationship investigation of the indolizino[2,3-α]quinolizidine ring system

Thus far in this project, we conducted an investigation into the structure-activity relationship of the indolizino[2,3-α]quinolizidine ring system. Structural modifications that have been explored include; stereochemistry, alkylations, α,β-unsaturation followed by conjugate addition, removal of the hydroxymethyl moiety and the use of a racemic template.

From this we have discovered that:

- Compounds derived from (-)-Indolizino[2,3-α]quinolizidine are more active than those derived from (+)-Indolizino[2,3-α]quinolizidine.

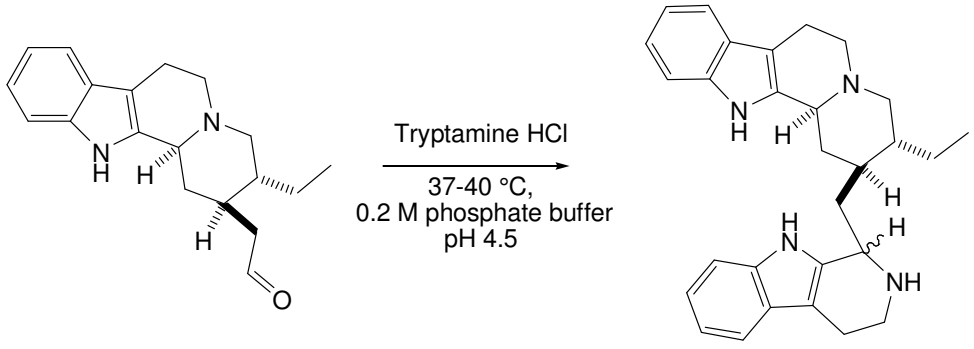
- That the addition of groups to the indole nitrogen and the oxygen atom increases activity.
- A benzyl group on the indole nitrogen and a methyl or allyl group on the oxygen atom give the best anti-malarial activity (IC_{50} = 1.3 μM)
- Introduction of α,β-unsaturation causes a decrease in anti-malarial activity in compounds with the hydroxymethyl moiety, but an increase in compounds without.
- Addition of a vinyl group, in general, causes an increase in activity and led to compound **(46)**, our most active compound so far (IC_{50} = 1.1 μM).
- Increasing the basicity of the compounds in this project did not increase anti-malarial activity.
- Using a readily accessible racemic template **(72a, b)** instead of an enantiomerically pure one gives a good indication of anti-malarial activity in this series.

2.8 Towards a Bis-Indole Derivative

2.8.1 Ochrolifuanine A

With the structure-activity relationships of the indolizino[2,3-α]quinolizidine template investigated, the project moved towards the synthesis of a compound containing an additional ring system. The most active compounds found in nature are those containing two indole ring systems. The total synthesis of the bis-indole anti-malarial compound ochrolifuanine A has been reported **(49a)**, [30] but to the best of our knowledge no others have. The synthesis and subsequent structure-activity relationship investigation of bis-indole compounds could provide novel leads with high anti-malarial activity.

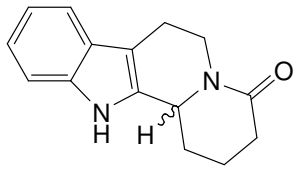
The key step in this reaction is a Pictet-Spengler [31] reaction between tryptamine and an aldehyde group.



(104) **(49a, b)**

Scheme 53

This gives the desired compound **(49a, b)** as a mixture of diastereoisomers that could then be separated. The Pictet-Spengler reaction provides a route for deriving compounds with an additional ring system. In order for this to be successful, aldehyde functionality must be introduced to the lactam ring of compound **(72a, b)**.

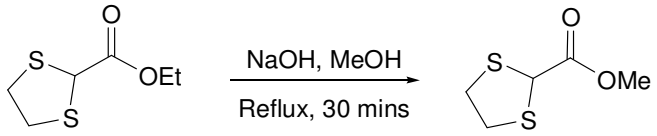


(72a, b)

2.8.2 Aldehyde functionalisation of racemic indolizino[2,3-α]quinolizidine

Using the racemic version of the indolizino[2,3-α]quinolizidine ring system, the previously reported synthesis of (+)-12*b*-epidevinylantirhine was followed. [32]

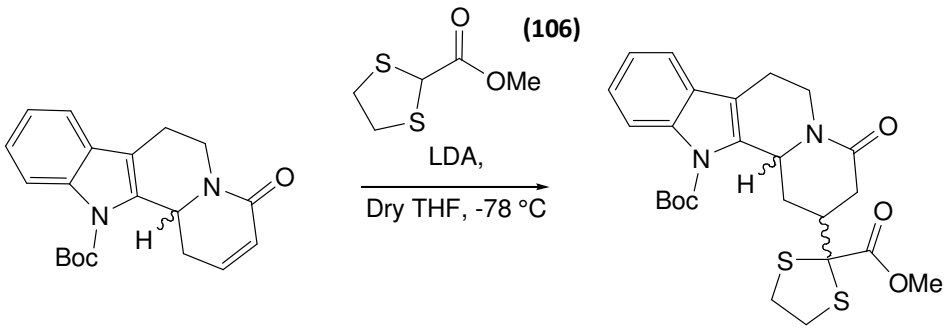
The first step involves a conjugate addition reaction between α,β-unsaturated compound **(74a, b)**, and methyl-1,3-dithiolane-2-carboxylate **(106)**. This compound is not readily available and therefore was prepared from ethyl-1,3-dithiolane-2-carboxylate **(105)** by refluxing in methanol with sodium hydroxide for 30 minutes with the exclusion of light. [33] This gave the desired compound as a yellow oil.



(105) **(106)**

Scheme 54

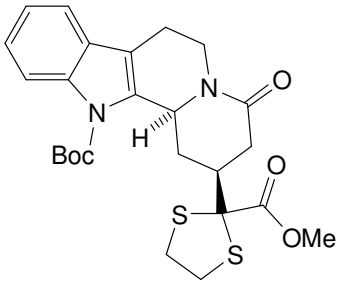
(106) was then reacted with **(74a, b)** using lithium di*iso*propylamide as a base. This gave compound **(107)** in 54% yield (Scheme 55).



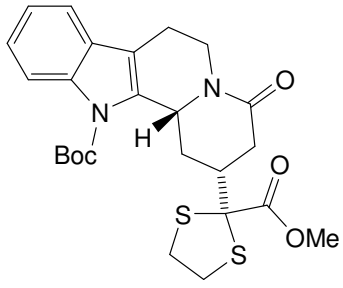
(74a, b) **(107a, b)**

Scheme 55

Previous work has shown that with no hydroxymethyl group and a protecting group on the indole nitrogen atom, the incoming nucleophile always attacks one (the less hindered) face of the compound, giving only one relative stereochemistry. [32] This is discussed earlier in Chapter 1. The two enantiomers now present in the racemic mixture are as shown **(107a)** and **(107b)**.

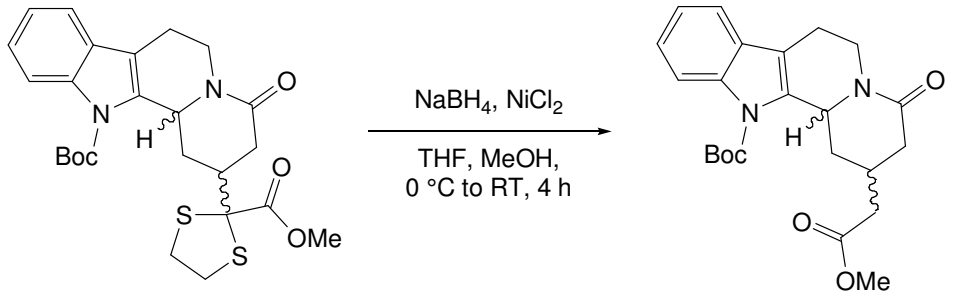


(107a)



(107b)

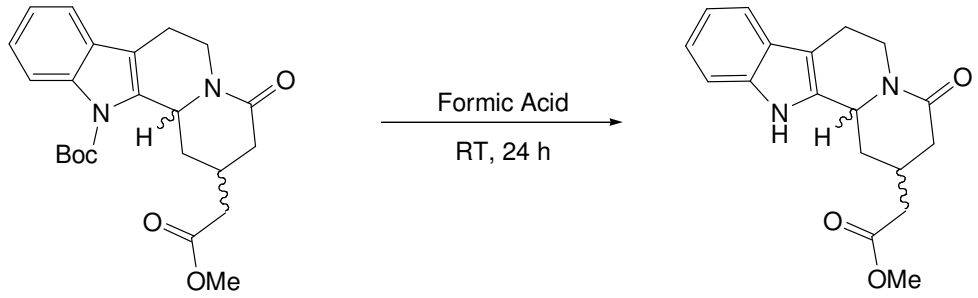
The removal of the dithiolane ring from **(107a, b)** was achieved using sodium borohydride with a nickel chloride catalyst in 61% yield. [34] As mentioned above in Chapter 1 the borohydride and nickel catalyst acts like Raney nickel, reducing the C-S bonds. [4]



(107a, b) **(108a, b)**

Scheme 56

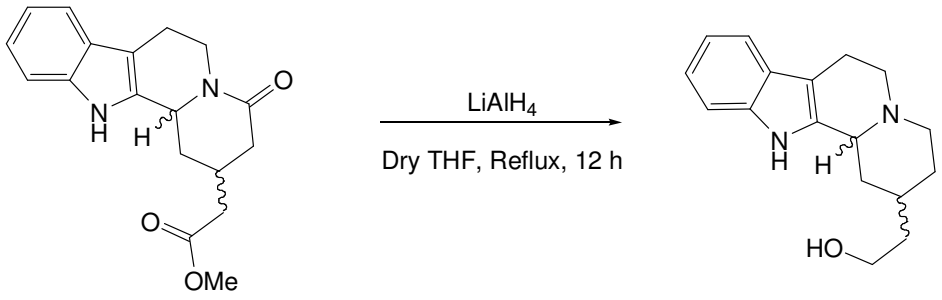
The removal of the Boc group from **(108a, b)** was subsequently achieved cleanly in 82% yield on stirring with formic acid for 24 hours.



(108a, b) **(109a, b)**

Scheme 57

After removal of the Boc protecting group, the compound was treated with lithium aluminium hydride in dry tetrahydrofuran and heated under reflux for 3 hours, followed by 9 hours stirring at room temperature. An excess of reducing agent was used to convert, in one pot, the ester to an alcohol and the amide to the corresponding amine, giving (+/-) -12*b*-epidevinylantirhine **(110a, b)** in 76% yield (Scheme 58).

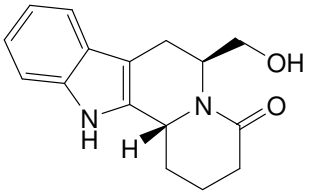


(109a, b) **(110a, b)**

Scheme 58

2.8.3 Oxidation

To obtain the aldehyde group, the primary alcohol must be oxidised. There are numerous agents that could be used to oxidise an alcohol to an aldehyde. PDC, [35] PCC, [36] Dess-Martin Periodinane [37] and the Swern oxidation [38] are a few, however all these except PCC were attempted unsuccesfully on compound **(8)** by another member of the Allin group. The lack of success was possibly due to the indole NH, known to be unstable under oxidising conditions. [13, 39]



(8)

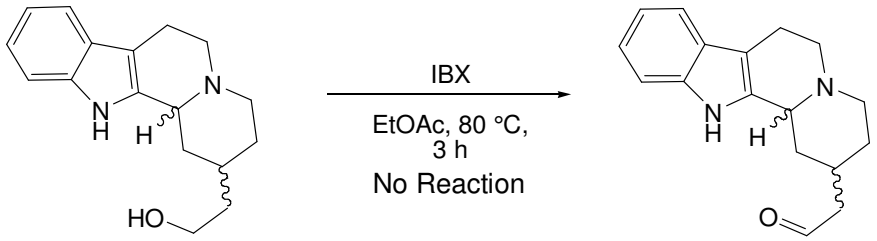
Due to the similarities between PCC and PDC we assumed that PCC would also be an unsuitable oxidising agent. The oxidising agent IBX has been reported not to affect indoles with a free NH, [40] therefore an IBX oxidation was attempted (Scheme 59).

(110a, b) **(111a, b)**

Scheme 59

An aqueous work up was performed and the compound extracted into ethyl acetate. ^{1}H NMR analysis of the crude material showed only the presence of by-products of the oxidising agent. We thought that, due to the insolubility of the starting material, the aldehyde would also be fairly insoluble and therefore was not extracted. The experiment was repeated but without the aqueous work up. Instead, after 24 hours stirring the reaction was put on a freeze dryer to remove the dimethyl sulfoxide. Unfortunately, this did not yield any positive results either.

A different method was then employed that required no extraction or aqueous work up. IBX oxidations are usually carried out in dimethyl sulfoxide due to the inability of the oxidising agent to dissolve in any other solvent, but Moore *et al.* [41] reported that at elevated temperatures, IBX can be used to convert alcohols cleanly into the corresponding aldehydes in excellent yields. Once the solution has cooled, the reduced IBX can be filtered out and the solvent removed. They reported a 90% yield when using ethyl acetate. This reaction was attempted with compound **(110a, b)** (Scheme 60).

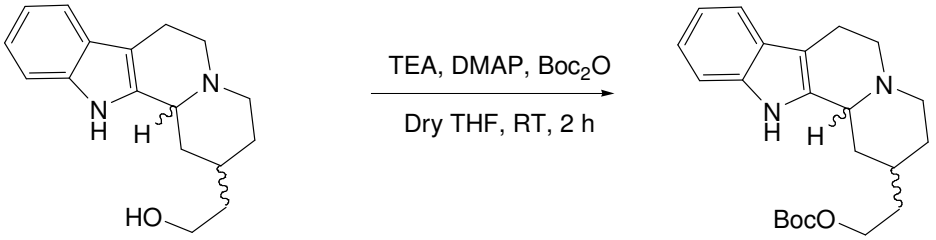


(110a, b) **(111a, b)**

Scheme 60

^{1}H NMR spectroscopy of the crude reaction mixture showed no characteristic aldehyde peak or starting material.

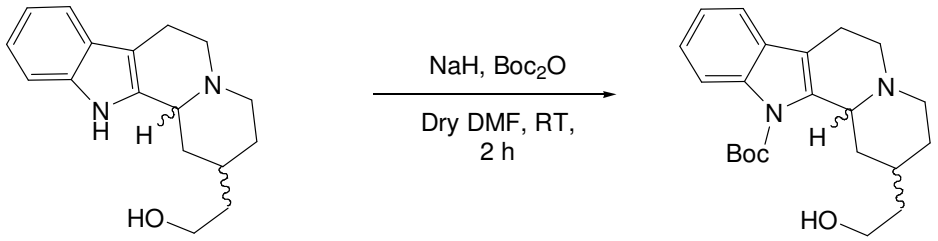
We then decided to protect the indole NH, to see if this would make the oxidation reaction successful. Triethylamine with 4-(dimethylamino)pyridine and di-*tert*-butyl carbonate in anhydrous tetrahydrofuran was used. After 2 hours, analysis by TLC showed complete conversion of starting material to the product shown in Scheme 61.



(110a, b) **(112a, b)**

Scheme 61

The base was changed to sodium hydride which previously had shown selectivity for the indole NH due to the reaction proceeding by a different mechanism, and the Boc protection was successful.



(110a, b) **(113a, b)**

Scheme 62

Oxidation attempts on compound **(113a, b)** also failed. This may be due to the amide in the lactam ring having been converted to an amine, an unforeseen problem, as IBX has been reported to be chemoselective towards alcohol groups in the presence of primary, secondary and tertiary amines. [40] Frigaro *et al.* successfully oxidised compound **(114)** to its corresponding aldehyde **(115)** in 91% yield, [40] in the presence of a tertiary amine and an unprotected indole moiety.

(114) **(115)**

Scheme 63 [40]

Due to the success of this reaction and the compound's similarity to ours **(110a, b)**, the effectiveness of the IBX being used was called into question. A test reaction, known to work and described in section 2.6 was performed. ^{1}H NMR spectroscopy of the crude

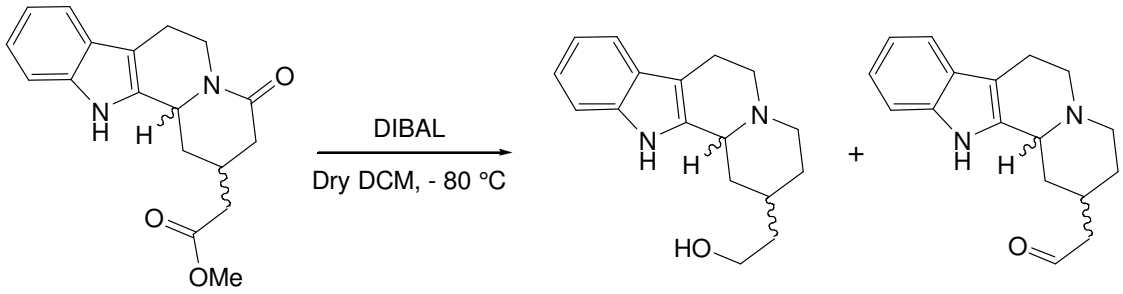
material showed clean conversion from the alcohol to the aldehyde, with a strong C*H*O peak at 9.4 ppm.

(20) **(69)**

Scheme 64

2.8.4 DIBAL reduction

After several unsuccessful methods trying to obtain the desired aldehyde through oxidation, a reduction method was employed. Di*iso*butylaluminium hydride is known for reducing esters to aldehydes at low temperatures. [4] A short reaction time was used and a cold reaction temperature to try to prevent over-reduction to the alcohol. 1 Equivalent of DIBAL was used at – 80 °C in anhydrous dichloromethane. After 1 hour the reaction was quenched with methanol. ^{1}H NMR spectroscopy of the crude reaction mixture showed only starting material. The experiment was repeated using 3 equivalents of DIBAL. This gave a mixture of the desired aldehyde **(111a, b)** and the alcohol **(110a, b)**. The amide was also reduced.



(109a, b) **(110a, b)** **(111a, b)**

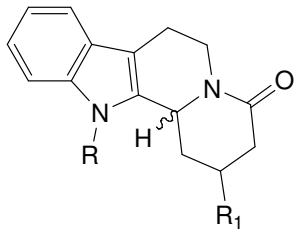
Scheme 65

As expected the major product was the alcohol **(110a, b)**. Unfortunately, as the amide was also reduced, the alcohol could not be oxidised to the aldehyde **(111a, b)** as originally planned. We had thought that the amide would not be reduced at such a low temperature. [42]

2.8.5 Biological activities

The compounds described in this section were tested for anti-malarial activity, and their IC_{50} values are displayed in Table 11. As these compounds are intermediates towards a bis-indole target, high anti-malarial activity was not expected, but was sought to provide a more complete structure-activity profile.

Table 11:



Compound	R	R^1	IC_{50} (μM) $Dd2^{luc}$
(107a, b)	Boc	Ethyl-1,3-dithiolane-2-carboxylate	9.17
(108a, b)	Boc	$CH_2C{=}OOCH_3$	20.3
(109a, b)	H	$CH_2C{=}OOCH_3$	55.1
(110a, b)	H	CH_2CH_2OH	27.2
(111a, b)	H	CH_2CHO	24.7
(112a, b)	H	CH_2CH_2Boc	65.1

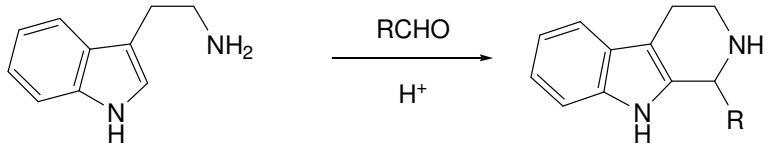
The results of these compounds show that **(107a, b)** had the highest anti-malarial activity, most probably due to having a 5 membered ring at the R^1 position. This resembles an additional ring system more closely than the other R^1 substituents of the compounds in this series. The removal of the Boc group from compound **(108a, b)** to **(109a, b)** shows a decrease in activity. This is expected as previous results showed that addition of a group at the indole nitrogen always increased activity.

With aldehyde functionality in place **(111a, b),** a Pictet-Spengler reaction could now be performed.

2.9 Addition of a Second Ring System

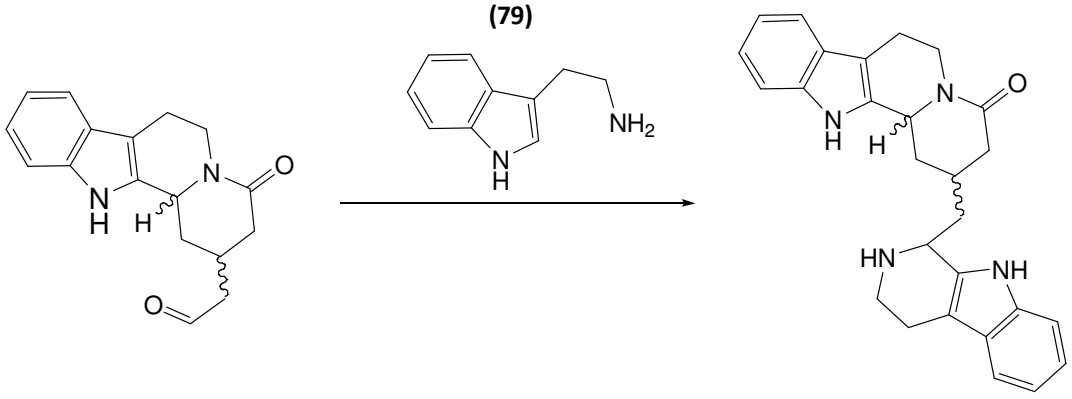
2.9.1 Pictet-Spengler reaction

The Pictet-Spengler reaction is one in which a β-arylethylamine such as tryptamine **(79)** undergoes a condensation reaction with an aldehyde or ketone, followed by ring closure (Scheme 66). Usually heat and the presence of an acidic catalyst are required. This forms a β-carboline unit **(116)**, which we would like to add our compound **(111a, b)** (Scheme 67)**.**



(79) **(116)**

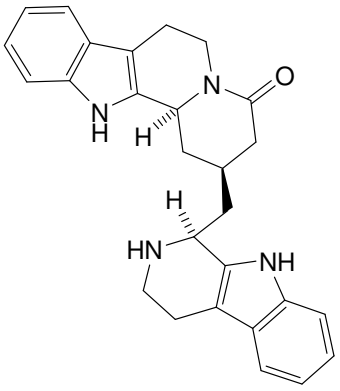
Scheme 66



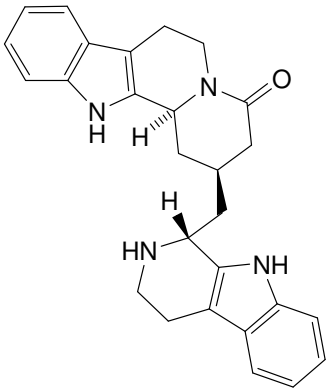
(111a, b) **(117)**

Scheme 67

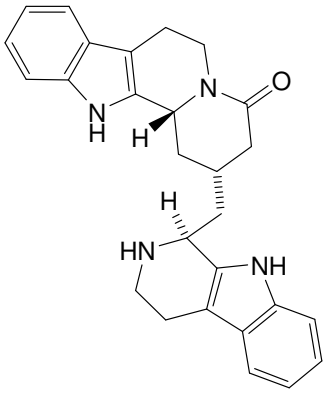
This would potentially give a mixture of 4 diastereoisomers.



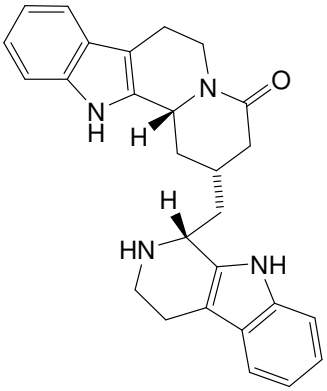
(117a)



(117b)

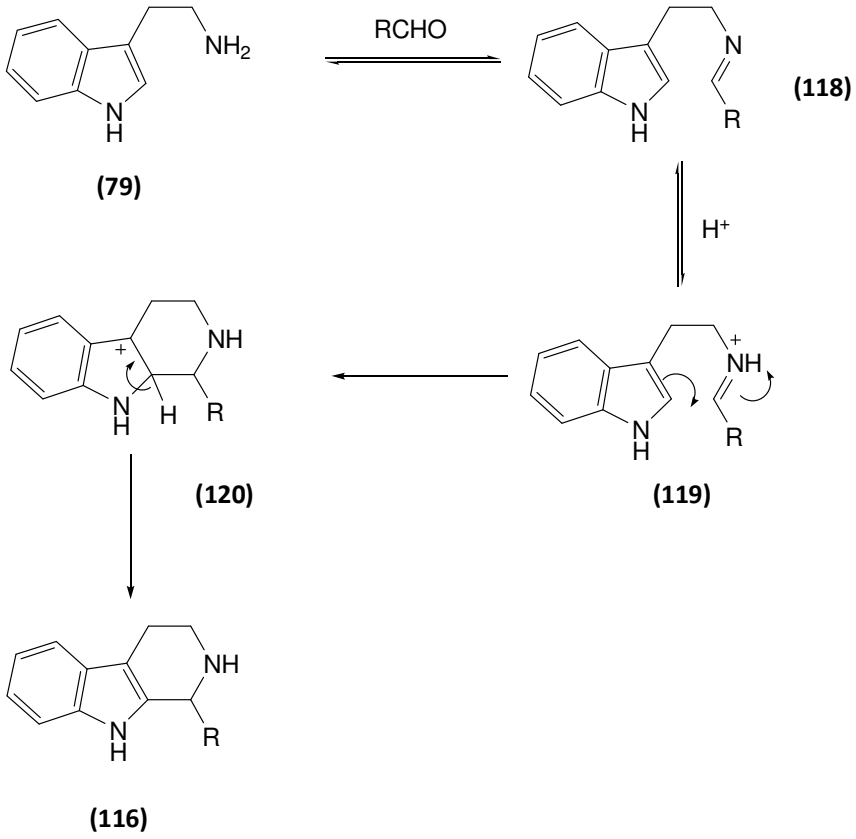


(117c)



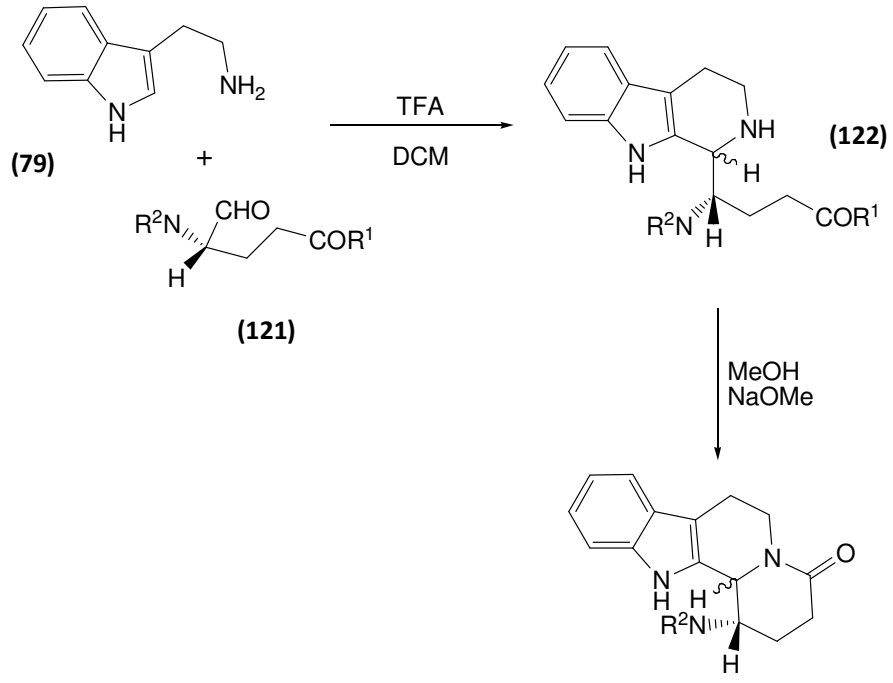
(117d)

More nucleophilic rings such as indole give better reaction yields under mild conditions. The mechanism for this reaction is shown in Scheme 68.



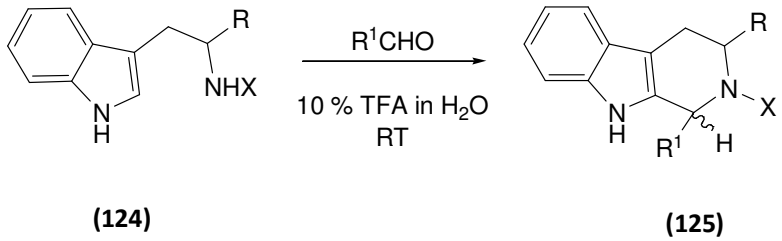
Scheme 68

The Pictet-Spengler reaction has been employed by various research groups as a route towards β-carbolines, it is commonly carried out using trifluoroacetic acid in anhydrous dichloromethane. Ducrot *et al.* [43] used it to generate β-carboline precursors to 1-aminoindole[2,3-α]quinolizidines **(123)** (Scheme 69).



Scheme 69 [43]

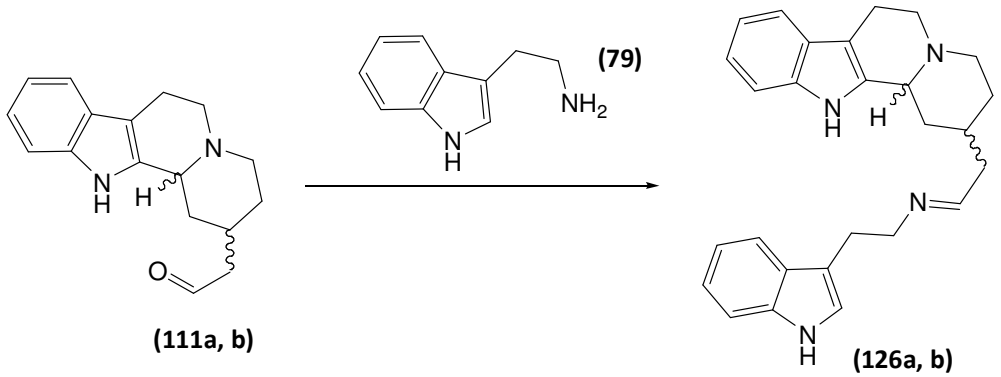
Kundu *et al.* [44] used a novel varient of the Pictet-Spengler reaction using trifluoroacetic acid in water as a method of synthesising β-carbolines in high yields (Scheme 70).



Scheme 70 [44]

2.9.2 Imine formation

The first step of this reaction was to form an imine between compound **(111a, b)** and tryptamine **(79)**.



Scheme 71

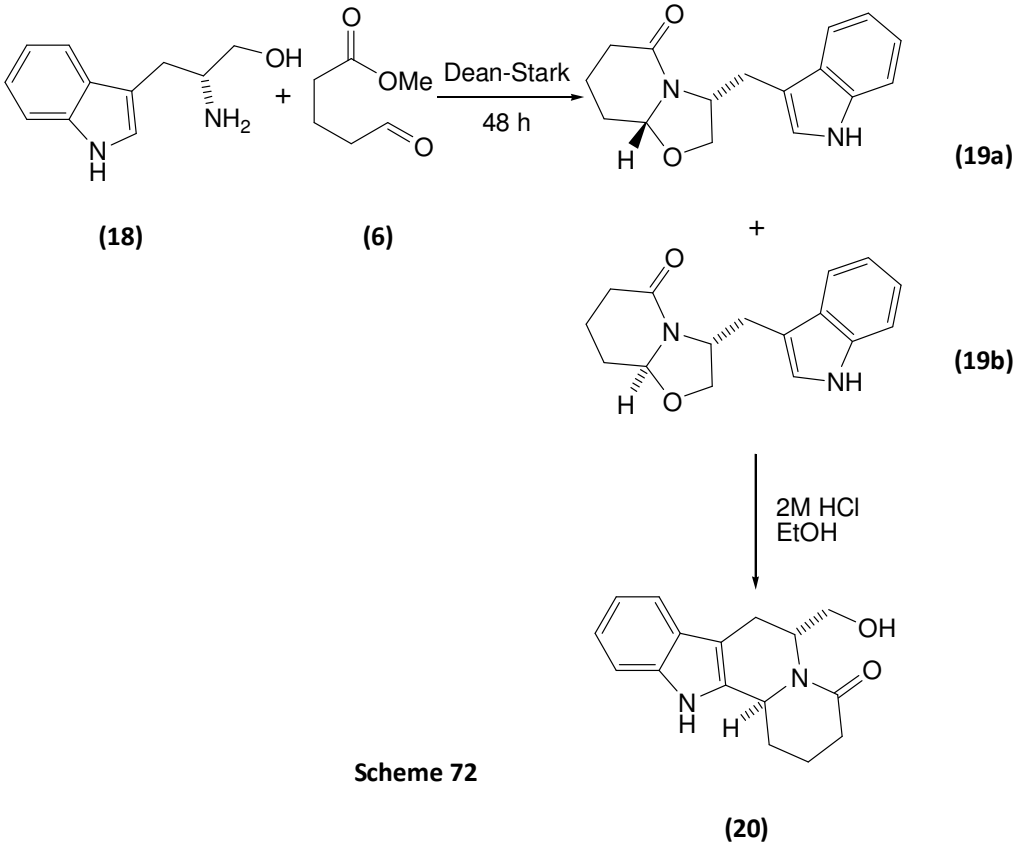
The conditions employed were those described by Bailey *et al.* [45] in which the imine is formed by combining tryptamine and the aldehyde under a nitrogen atmosphere in anhydrous dichloromethane. 3Å molecular sieves were added since the by-product of this reaction is water. Once the imine is formed, trifluoroacetic acid is added.

Compound **(111a, b)** and tryptamine **(79)** were combined as described. After 30 minutes it was noted that the compounds had not dissolved and therefore the reaction was heated to 30 °C. After 4 hours a small portion of the reaction mixture was extracted, the solvents removed and a ^{1}H NMR spectrum obtained, which showed the presence of tryptamine. The reaction was left overnight. Thin layer chromatography showed presence of both tryptamine **(79)** and aldehyde **(111a, b).** The reaction was run again at a higher temperature using anhydrous tetrahydrofuran for 12 hours. After this time, analysis by thin layer chromatography showed no presence of tryptamine. Column chromatography

was performed, but unfortunately none of the desired product could be obtained. No further action could be taken due to the project finishing.

2.10 Validity of these Compounds as Anti-Malarials

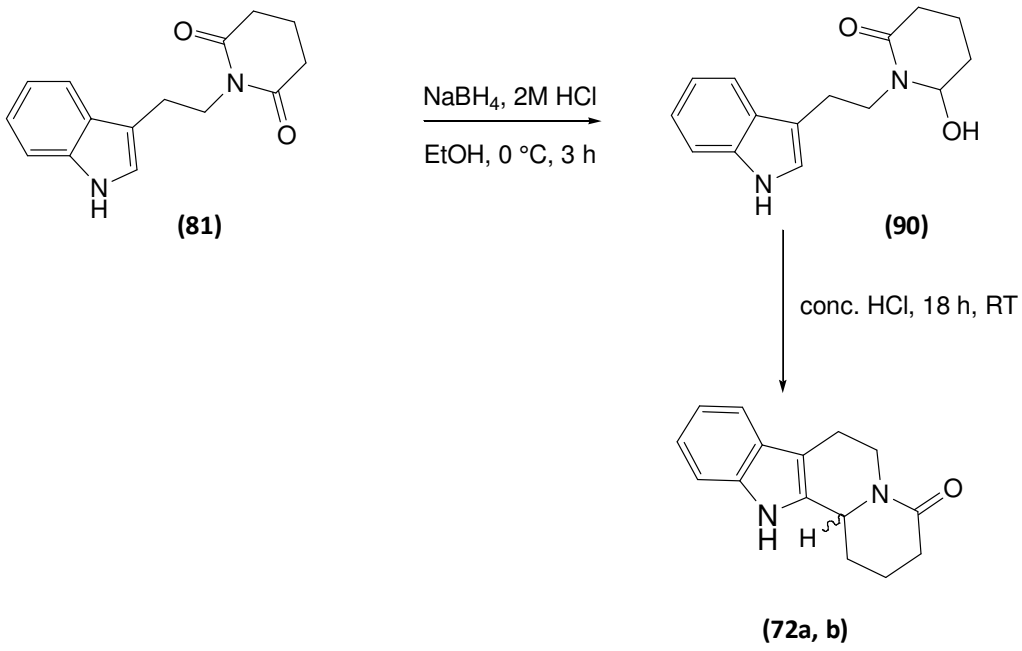
For a compound to be considered a viable candidate for a drug, it must adhere to three main criteria. It must be cost effective, effective and safe. The first synthetic method towards the targets described in this thesis (Scheme 72), starting from D-tryptophan is highly costly and time consuming.



(18) (6) (20)

Scheme 72

However the second, shorter method (Scheme 73) starting from tryptamine and resulting in racemic compounds is much more affordable, the IC_{50} results show that using racemic compounds gives very similar activity to that of the pure enantiomers and therefore provides a quicker, cheaper route to the compounds described in this project.



Scheme 73

These compounds, although they display anti-malarial activity, are not yet effective enough to be seriously considered as drug candidates (an IC_{50} value in the low nM range is desirable). They do however comply with three out of four of Lipinski's rule of 5, a standard guideline for developing drugs. [46]

The rule of 5 states that, in general, for a drug to be orally active, it must have no more than one violation of the following criteria:

1) No more that 5 hydrogen bond donors
2) No more than 10 hydrogen bond acceptors
3) A molecular mass less than 500
4) An octanol-water partition coefficient (log P) less than five

The compounds described in this thesis definitely comply with rules 1-3. The octanol-water partition coefficient has not been tested. It is therefore likely that with further

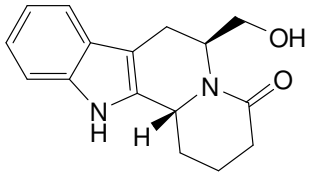
structural modifications to increase activity, this class of compounds could make viable drug candidates for malaria.

The safety of these compounds has not been fully assessed as they are not active enough to warrant much further investigation. However, cytotoxicity screening of natural structurally related indole alkaloids has been conducted and selectivity index (SI) determined. The selectivity index can be defined as the ratio of cytotoxicity over anti-plasmodial activity; a high number displays selectivity for the plasmodium parasite and therefore makes a compound a more desirable drug candidate. The selectivity index for dihydrousambarensine has been reported as 375 (with an IC_{50} value of 0.032 µM) against the chloroquine resistance strain W2, [447] showing high anti-malarial activity, and low cytotoxicity. It is therefore possible for structurally related compounds to have this selectivity too.

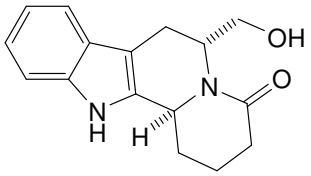
Preliminary toxicity screenings were conducted for 5 of the compounds described in this thesis and they were found to be non-toxic (see appendix 4.2). [48]

2.11 Conclusion

In summary, we have developed several anti-malarial compounds derived from both enantiomers of the indolizino[2,3-α]quinolizidine template **(8)** and **(20)**. Those derived from **(20)** were more active. We have investigated several structural modifications to the templates to try to obtain a compound with high anti-malarial activity.

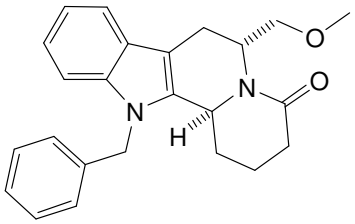


(8)

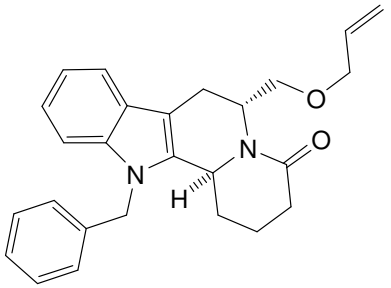


(20)

Addition of protecting groups to both the indole nitrogen and the hydroxyl group increase anti-malarial activity. The combination of N-benzyl, O-methyl **(29)** or N-benzyl, O-allyl **(30)** yielded the most potent compounds.

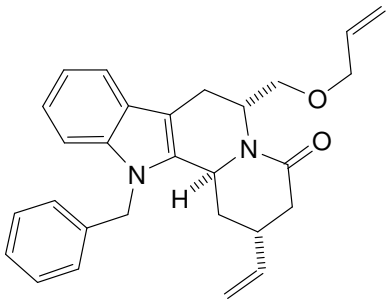


(29)



(30)

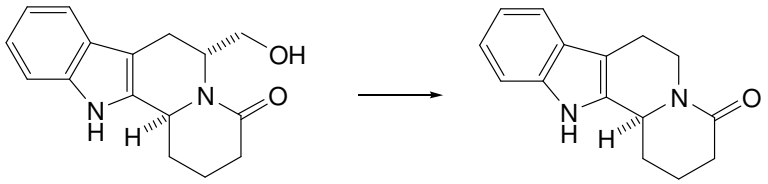
With the hydroxymethyl moiety present, incorporation of α,β-Unsaturation in the lactam ring does not increase activity, however when followed by a conjugate addition reaction using vinyl magnesium bromide gave the most active compound in this series **(46)** (IC_{50}=1.1 μM).



(46)

Increasing the basicity of the compounds by converting the amide into an amine and by adding a pyridyl group to the indole NH did not increase activity.

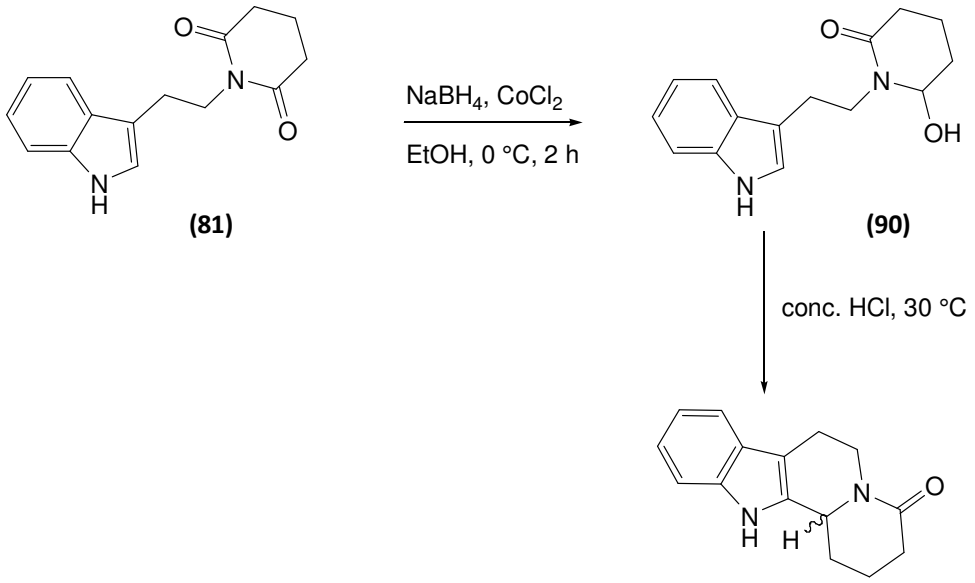
Following previously described methodology, the hydroxyl methyl group was removed from **(20)** and a series of compounds were derived from this new template **(72a)**, which bore a closer resemblance to natural anti-malarials.



(20) **(72a)**

Scheme 74

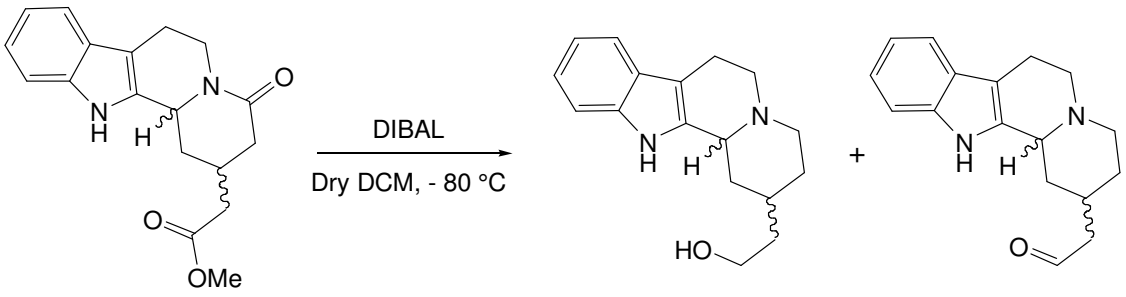
We have developed a novel synthetic route to a racemic template that is quicker and cheaper to make than **(72a)** and a set of compounds derived from it showed that the racemic versions of these compounds are comparable in anti-malarial activity with the enantiomerically pure ones.



(81) **(90)** **(72a, b)**

Scheme 75

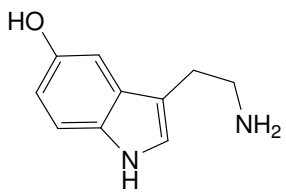
We have developed a novel route to the aldehyde **(111a, b)**, which could then undergo a Pictet-Spengler reaction with tryptamine **(79)** to give a bis-indole compound. Unfortunately our attempts so far have proved unsuccessful.



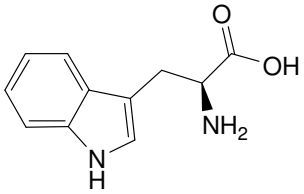
(109a, b) **(110a, b)** **(111a, b)**

Scheme 76

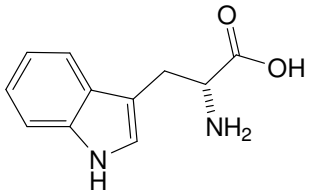
Future work will focus on developing bis-indole compounds. The method reported by Malhorta [30] in the synthesis of ochrolifuanine A should prove successful in attaching a second ring system to our aldehyde **(111a, b)** to give the desired bis-indole compound **(117)**. Derivatives of tryptamine, such as 5-hydroxytryptamine **(127)** or tryptophan **(2)**, **(17)** could also be used to form a second ring system and to give insight as to whether extra functionality in this area could affect anti-malarial activity.



(127)



(2)



(17)

The functional groups on the above compounds could also be further functionalised, and in the case of tryptophan, effects of stereochemistry could be investigated.

Chapter 3

Experimental

3.1 General Information

3.1.1 Solvents and reagents

All solvents, where necessary, were dried and stored over 3 Å molecular sieve prior to use.

Reagent chemicals were purchased from the following companies: Alfa Aesar UK, Sigma-Aldrich UK, Fischer Scientific and Acros Organics.

3.1.2 Chromatographic procedures

Analytical thin layer chromatography (TLC) was conducted using aluminium backed plates with 0.20 mm silica gel with fluorescent indicator UV_{254} and using polyester backed plates with 0.20 mm aluminium oxide with fluorescent indicator $N/UV_{254.}$

Plates were examined under UV light (254 nm).

Column chromatography over silica gel was conducted using Geduran Si 60 (40-63 µm). Samples were pre-absorbed onto the minimum amount of silica.

Column chromatography over alumina was conducted using Acros Organics aluminium oxide, basic, Brockman 1 for chromatography. 50-200 µm, 60 A. Samples were pre-absorbed onto the minimum amount of alumina.

Pressure was applied by the use of hand bellows.

3.1.3 Spectra

Infrared spectra (IR) was conducted in the range of 4000-500 cm^{-1}, using a Thermo Scientific Nicolet is10 with a Pike Technologies GladiATR. Samples were applied directly to the ATR plate.

Nuclear Magnetic Resonance (NMR) spectra (^{1}H and ^{13}C) were recorded at 300 MHz using a Bruker Spectrospin DPX 300 Spectrometer. Multiplicities were recorded as broad peaks (br), singlets (s), doublets (d), triplets (t), quartets (q), quintets (qu), sextets (si), septets (se) double doublets (dd), doublet of double doublets (ddd), doublet of triplets (dt), doublet of quartets (dq), triplet of doublets (td), quartet of quartets (qq), sextet of doublets (sid) and multiplets (m). All NMR samples were made up in deuterated solvents with all values quoted in ppm relative to tetramethylsilane (TMS) as an internal reference. Coupling constants (*J* values) are reported in Hertz (Hz).

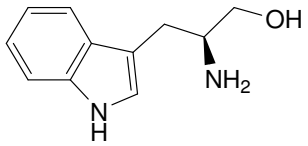
Mass Spectrometry Samples were sent for EI/CI and Accurate Mass measurement to EPSRC National Mass Spectrometry Centre at the University of Wales, Swansea.

3.1.4 Other data

Melting points were determined using a Stuart Scientific SMP3 Melting point instrument. Specific Rotations were performed using a Rudolph Research Analytical Autopol Automatic Polarimeter and are reported in units of deg° $dm^{-1}cm^{3}\ g^{-1}$.

3.2 Synthesis of Indolizino[2,3-α]quinolizidines

(2*S*)-2-Amino-3-(1*H*-indole-3-yl)propan-1-ol **(3)** [3]



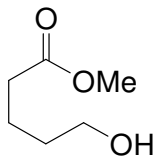
Anhydrous tetrahydrofuran (75 ml) was added to lithium borohydride (1.07 g, 49.0 mmol) under a nitrogen atmosphere. Trimethylsilyl chloride (12.4 ml, 97.9 mmol) was added over the course of two minutes, followed by L-tryptophan **(2)** (5 g, 24.5 mmol) which was added slowly over the course of 5 minutes. The reaction was left stirring at room temperature for 24 hours.

Methanol (30 ml) was added cautiously to quench the reaction. The solvents were evaporated and the resulting oil was treated with 20% potassium hydroxide solution (40 ml). The solution was washed with ethyl acetate (3 x 100 ml) and the organic extracts were combined and dried using anhydrous magnesium sulphate. The drying agent was then removed by filtration and the solvent removed under reduced pressure to yield a brown oil (3.36 g 72.2%). No further purification was carried out.

δ_H(300 MHz; DMSO) 2.50-2.60 (1H, m, C*H*(H)CHNH$_2$), 2.78 (1H, dd, *J* 14.1, 6, C*H*(H)CHNH$_2$), 2.93-3.01 (1H, m, CH$_2$C*H*NH$_2$), 3.18-3.37 (2H, m, C*H*$_2$OH), 6.95 (1H, t, *J* 6.9, Ar*H*), 7.05 (1H, t, *J* 7.2, Ar*H*), 7.14 (1H, s, C=C*H*NH), 7.33 (1H, d, *J* 7.8, Ar*H*), 7.53 (1H, d, *J* 7.8, Ar*H*), 10.83 (1H, br s, N*H*).

OH proton was not observed.

Methyl 5-hydroxypentanoate **(5)** [3]

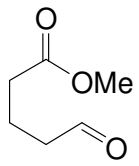


Concentrated sulfuric acid (25 drops) was added to a solution of δ-valerolactone **(4)** (25.8 g, 257.7 mmol) in methanol (250ml) and heated under reflux at 80°C for 5 hours. The mixture was cooled using an ice/salt bath and sodium hydrogen carbonate (2.5 g) was added and stirred for 10 minutes. This was then filtered and the solvent removed under reduced pressure to yield a colourless oil (30.23 g, 89%). The product was not purified as re-lactonisation occurs on aqueous work up or distillation.

δ_H(300 MHz; $CDCl_3$) 1.52-1.59 (2H, m, C*H*$_2$CH$_2$OH), 1.62-1.69 (2H, m, C*H*$_2$CH$_2$CH$_2$OH), 2.31 (2H, t, *J* 7.5, CH_2COOCH_3), 3.56-3.60 (2H, m, C*H*$_2$OH), 3.63 (3H, s, OC*H*$_3$).

OH proton was not observed

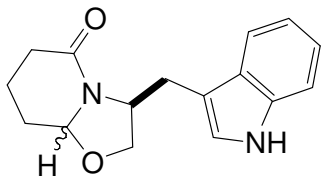
Methyl 5-oxopentanoate **(6)** [3]



Methyl 5-hydroxypentanoate **(5)** (11.8 g, 89.4 mmol) was added slowly to a suspension of pyridinium chlorochromate (29.0 g, 134.5 mmol) and celite (29.0 g) in dichloromethane (200 ml) and stirred at room temperature for 4 hours. The solution was decanted and the solids were washed with diethyl ether (4 x 100 ml) until the solvent ran clear. The combined organic solutions were filtered through an alumina column and the solvent was removed under reduced pressure to give a green oil (9.65 g, 83%) which was not purified further.

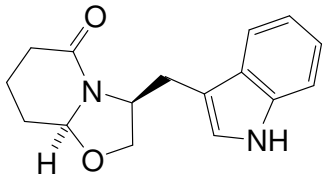
δ_H(300 MHz; $CDCl_3$) 1.62-1.65 (2H, m, C*H*$_2$CH$_2$CHO), 1.86-1.95 (2H, m, C*H*$_2$CHO), 2.26-2.31 (2H, m, C*H*$_2$CH$_2$CH$_2$CHO), 3.63 (3H, s, OC*H*$_3$), 9.73 (1H, s, C*H*O).

(3S)-3-((1H-Indol-3-yl)methyl)-hexahydrooxazolo[3,2-a]pyridin-5-one **(7a, b)** [3]



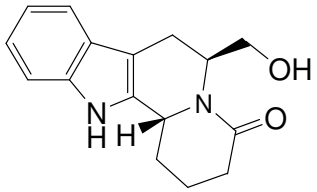
(2*S*)-2-Amino-3-(1*H*-indole-3-yl)propan-1-ol **(3)** (7.36 g, 38.70 mmol) and methyl 5-oxopentanoate **(6)** (5.04 g, 38.70 mmol) were combined in toluene (150 ml) and refluxed under Dean-Stark conditions for 48 hours.

The mixture was cooled and the remaining toluene was removed under reduced pressure. The resulting brown oil was absorbed onto silica and purified by flash column chromatography over silica using ethyl acetate as the eluent to produce the desired compound as a mixture of diastereoisomers which were not separated (2.13 g, 20%). The ^{1}H NMR data for the major isomer **(7a)** is shown below.



δ_H(300 MHz; $CDCl_3$) 1.29-2.00 (4H, m, C*H*$_2$C*H*$_2$CH$_2$CO), 2.31-2.42 (2H, m, C*H*$_2$CO), 2.57-2.60 (1H, m, C*H*(H)C=C), 3.61-3.70 (1H, m, C*H*(H)C=C), 3.95-4.07 (2H, m, C*H*$_2$O), 4.21-4.24 (1H, m, NC*H*CH$_2$O), 4.60-4.65 (1H, m, NC*H*OCH$_2$), 6.98 (1H, s, C=C*H*NH), 7.04-7.20 (2H, m, Ar*H*), 7.30 (1H, d, *J* 7.8 Ar*H*), 7.77 (1H, d, *J* 8.1 Ar*H*), 8.02 (1H, br s, N*H*).

(6S,12bR)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(8)** [3]

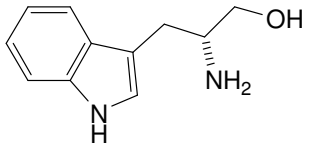


(3*S*,8a*S*)-3-(1H-Indol-3-ylmethyl)-hexahydro-oxazolo[3,2-a]pyridine-5-one **(7a, b)** (1.39 g, 5.10 mmol) was dissolved in absolute ethanol (70 ml) and acidified to pH 1 using 2 M hydrochloric acid in absolute ethanol. This was then stirred at room temperature for 20 hours.

The reaction was quenched by the addition of saturated sodium hydrogen carbonate solution and extracted with ethyl acetate (3 x 100 ml). The organic extracts were combined, dried using anhydrous magnesium sulphate, and the drying agent was removed by filtration. The solvent was removed under reduced pressure to give a brown solid (1.33 g, 95%). A portion of this was re-crystallised from absolute ethanol to give a white solid.

δ_H(300 MHz; DMSO) 1.57-1.65 (1H, m, C*H*(H)CH_2CH_2CO), 1.78-1.85 (2H, m, C$H_2CH_2CH_2CO$), 2.30-2.42 (2H, m, CH_2CO), 2.55 (1H, s, C*H*(H)CH_2CH_2CO), 2.64-2.74 (1H, m, C=CC*H*(H)), 2.85 (1H, d, *J* 15, C=CC*H*(H)), 3.41 (2H, s, CH_2OH), 4.70 (1H, d, *J* 8.7, NC*H*C=C), 4.84 (1H, br s, OH), 5.27 (1H, dt, *J* 6, 6, NC*H*CH_2OH), 6.97 (1H, t, *J* 7.4, Ar*H*), 7.11 (1H, t, *J* 7.4, Ar*H*), 7.37 (1H, d, *J* 7.8, Ar*H*), 7.45 (1H, d, *J* 7.8, Ar*H*), 10.95 (1H, br s, N*H*).

(2*R*)-2-Amino-3-(1*H*-indole-3-yl)propan-1-ol **(18)** [3]



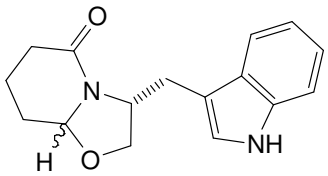
Anhydrous tetrahydrofuran (75 ml) was added to lithium borohydride (1.07 g, 49.0 mmol) under a nitrogen atmosphere. Trimethylsilyl chloride (12.4 ml, 97.9 mmol) was added over the course of two minutes, followed by D-tryptophan **(17)** (5 g, 24.5 mmol) which was added slowly over the course of 5 minutes. The reaction was left stirring at room temperature for 24 hours.

Methanol (30 ml) was added cautiously to quench the reaction. The solvents were evaporated and the resulting oil was treated with 20% potassium hydroxide solution (40 ml). The solution was washed with ethyl acetate (3 x 100 ml) and the organic extracts were combined and dried using anhydrous magnesium sulphate. This was then filtered and the solvent removed under reduced pressure to yield a brown oil (4.14 g, 89%). No further purification was carried out.

δ_H(300 MHz; DMSO) 2.58 (1H, dd, *J* 14.1, 7.2, C*H*(H)CHNH$_2$), 2.79 (1H, dd, *J* 14.1, 6, C*H*(H)CHNH$_2$), 2.94-3.02 (1H, m, CH$_2$C*H*NH$_2$), 3.19-3.30 (1H, m, C*H*(H)OH), 3.31-3.39 (1H, m, C*H*(H)OH) 6.94 (1H, t, *J* 6.9, Ar*H*), 7.03 (1H, td, *J* 8.1, 1.2, Ar*H*), 7.13 (1H, s, C*H*NH), 7.32 (1H, d, *J* 7.8, Ar*H*), 7.52 (1H, d, *J* 7.8, Ar*H*), 10.89 (1H, br s, N*H*).

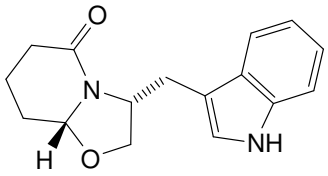
OH proton was not observed.

(3R)-3-((1H-Indol-3-yl)methyl)-hexahydrooxazolo[3,2-a]pyridin-5-one **(19a, b)** [3]



(2*R*)-2-Amino-3-(1*H*-indole-3-yl)propan-1-ol **(18)** (5.97 g, 31.42 mmol) and methyl 5-oxopentanoate **(6)** (4.08 g, 31.42 mmol) were combined in toluene (150 ml) and refluxed under Dean-Stark conditions for 48 hours.

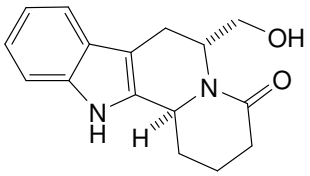
The mixture was cooled and the remaining toluene removed by rotary evaporation. The resulting brown oil was absorbed onto silica and purified by flash column chromatography over silica using ethyl acetate as the eluent to produce the desired compound as a mixture of diastereoisomers that were not separated (2.76 g, 33%). The ^{1}H NMR data for the major isomer **(19a)** is shown below.



δ_H(300 MHz; $CDCl_3$) 1.34-1.47 (1H, m, C*H*(H)CH_2CH_2CO), 1.59-1.65 (1H, m, CH_2C*H*(H)CH_2CO), 1.74-1.90 (1H, m, CH_2C*H*(H)CH_2CO), 2.27-2.30 (1H, m, C*H*(H)CH_2CH_2CO), 2.44-2.49 (2H, m, CH_2CO), 2.54-2.63 (1H, m, C*H*(H)C=C), 3.60-3.68 (1H, m, C*H*(H)C=C), 3.93 (1H, d, *J* 9, C*H*(H)O), 3.98-4.02 (1H, m, C*H*(H)O), 4.20-4.27

(1H, m, NC*H*CH_2CO), 4.58 (1H, dd, *J* 9.6, 2.7, NC*H*OCH_2), 6.92 (1H, s, C=C*H*NH), 7.03-7.26 (2H, m, Ar*H*), 7.60 (1H, d, *J* 7.8, Ar*H*), 7.72 (1H, d, *J* 7.5, Ar*H*), 8.52 (1H, br s N*H*).

(6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** [3]



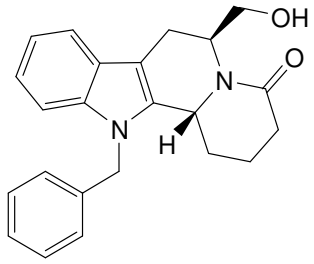
(3*R*,8a*R*)-3-(1H-Indol-3-ylmethyl)-hexahydro-oxazolo[3,2-a]pyridine-5-one **(19a, b)** (0.60 g, 2.11 mmol) was dissolved in absolute ethanol (50 ml) and acidified to pH 1 using 2 M hydrochloric acid in absolute ethanol and stirred at room temperature for 20 hours.

The reaction was quenched by the addition of saturated sodium hydrogen carbonate solution and extracted with ethyl acetate (3 x 100 ml). The organic extracts were combined, dried using anhydrous magnesium sulphate and the drying agent was removed by filtration. The solvent was removed under reduced pressure to yield a brown solid (0.57 g, 95%). A small portion was re-crystallised from absolute ethanol to give white crystals.

δ_H(300 MHz; DMSO) 1.57-1.70 (1H, m, C*H*(H)CH_2CH_2CO), 1.81-1.90 (2H, m, CH_2CH_2CO), 2.30-2.43 (2H, m, CH_2CO), 2.56-2.58 (1H, m, C*H*(H)= C*H*(H)CH_2CH_2CO), 2.64-2.79 (1H, m, C=CC*H*(H)), 2.86 (1H, d, *J* 15.9, C=CC*H*(H)), 3.39-3.45 (2H, m, CH_2OH), 4.71 (1H, d, *J* 7.2, NC*H*C=C), 4.85 (1H, t, *J* 5.4, O*H*), 5.28 (1H, dt, *J* 6, 6, NC*H*CH_2OH), 7.03 (1H, t, *J* 6.9, Ar*H*), 7.12 (1H, t, *J* 7.2, Ar*H*), 7.38 (1H, d, *J* 7.8, Ar*H*), 7.45 1H, d, *J* 7.5, Ar*H*), 10.95 (1H, br s, N*H*).

3.3 Synthesis of Indolizino[2,3-α]quinolizidine Substrates

(6S,12bR)-12-Benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(21)** [13]



Sodium hydride (60% dispersion in mineral oil, 0.13 g, 3.35 mmol) and (6S,12bR)-6-hydroxymethyl-2,3,6,7,12,12b-hexahydro-1H-indole[2,3-a]quinolizin-4-one **(8)** (0.60 g, 2.23 mmol) were combined under a nitrogen atmosphere and cooled to 0 °C using an ice bath. Anhydrous dimethylformamide (10 ml) was added and the ice bath removed. The reaction was then stirred at room temperature for 30 minutes. After this time, benzyl bromide (0.4 ml, 3.35 mmol) was added and the reaction was stirred for 24 hours.

The reaction was quenched by the addition of ice water (15 ml), washed with ethyl acetate (3 x 100 ml) and brine (3 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This yielded a white solid (0.51 g, 63%).

Mp: 235-237 °C; $[\alpha]_D = +150.99$ ($c = 0.01$, $CHCl_3$); ν_{max} (cm^{-1}) 3290 OH, 1591 NC=O

δ_H (300 MHz; $CDCl_3$) 1.55-1.69 (1H, m, C*H*(H)CH_2CH_2CO), 1.77-1.91 (2H, m, C*H*$_2CH_2CO$), 2.33-2.42 (1H, m, C*H*(H)CH_2CH_2CO), 2.44-2.64 (2H, m, C*H*$_2CO$), 2.87 (1H, d *J* 15.9, C=CC*H*(H)), 3.03 (1H, ddd *J* 15.9, 6, 2.1, C=CC*H*(H)), 3.47-3.52 (1H, m,

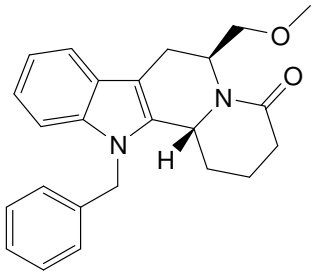
C*H*(H)OH), 3.59-3.64 (1H, m, C*H*(H)OH), 4.61 (1H, d, *J* 12, NC*H*C=C), 5.26 (1H, d, *J* 17.5 NC*H*(H)Ph), 5.38 (1H, d, *J* 17.5 NC*H*(H)Ph), 5.46-5.53 (1H, m, NC*H*CH_2OH), 6.94-6.97 (2H, m, Ar*H*), 7.13-7.19 (3H, m, Ar*H*), 7.26-7.33 (3H, m, Ar*H*), 7.52-7.56 (1H, m, Ar*H*)

OH proton was not observed

δ_C (300 MHz; $CDCl_3$) 19.34 (*C*H_2), 21.78 (*C*H_2), 30.48 (*C*H_2), 31.89 (*C*H_2), 47.71 (*C*H), 49.04 (*C*H_2), 51.14 (*C*H), 62.91 (*C*H_2), 107.41 (*C*), 109.92 (*C*H), 118.46 (*C*H), 119.88 (*C*H), 122.30 (*C*H), 125.73 (2x*C*H), 126.77 (*C*), 127.64 (*C*H), 128.98 (2x*C*H), 133.02 (*C*), 137.15 (*C*), 138.24 (*C*), 172.25 (N*C*=O)

MS (CI) *m/z* 361 [MH^+, 100%]; (Found: MH^+, 361.1909. $C_{23}H_{24}N_2O_2$ requires 361.1911).

(6S,12bR)-12-Benzyl-6-(methoxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(22)**



Sodium hydride (60% dispersion in mineral oil, 0.071 g, 1.77 mmol) and (6S,12bR)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(21)** (0.29 g, 0.803 mmol) were combined under a nitrogen atmosphere and cooled to 0°C. Anhydrous dimethylformamide (5 ml) was added and the reaction was stirred at room temperature for 30 minutes. After this time, methyl iodide (0.17 ml, 1.20 mmol) was added and the reaction was stirred for 24 hours.

The reaction was quenched by the addition of ice water (15 ml), washed with ethyl acetate (3 x 100 ml) and brine (3 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, which was then removed by filtration and solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent to give a yellow solid (0.043 g, 14%).

Mp: 194-195 °C; $[\alpha]_D$ = +161.25 (*c* = 0.008, $CHCl_3$); v_{max} (cm^{-1}) 1259 O-Me, 1629 NC=O.

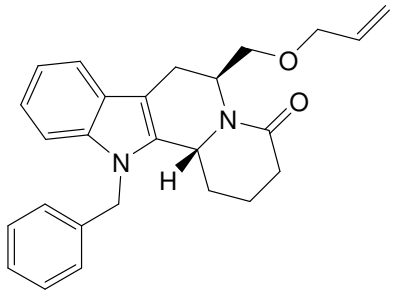
δ_H (300 MHz; $CDCl_3$) 1.44-1.57 (1H, m, C*H*(H)CH_2CH_2CO), 1.62-1.82 (2H, m, C*H*$_2CH_2CO$), 2.28-2.36 (1H, m, C*H*(H)CH_2CH_2CO), 2.38-2.42 (1H, m, C*H*(H)CO), 2.46-2.54 (1H, m, C*H*(H)CO), 2.76 (1H, d *J* 15.6, C=CC*H*(H)), 2.92 (1H, ddd, *J* 15.9, 5.7, 2.1, C=CC*H*(H)), 3.12-3.26 (2H, m, C*H*$_2$OMe), 3.16 (3H, s, OC*H*$_3$), 4.40 (1H, d, *J* 12, NC*H*C=C), 5.17 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.33 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.57 (1H,

dt, *J* 6, 6, NC*H*CH_2OMe), 6.85-6.87 (2H, m, Ar*H*), 7.04-7.08 (3H, m, Ar*H*), 7.16-7.24 (3H, m, Ar*H*), 7.45-7.49 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.31 (*C*H_2), 20.95 (*C*H_2), 29.32 (*C*H_2), 30.98 (*C*H_2), 44.53 (*C*H), 46.71 (*C*H_2), 50.26 (*C*H), 57.74 (*C*H_2), 70.13 (*C*H_3), 106.87 (*C*), 108.73 (*C*H), 117.46 (*C*H), 118.74 (*C*H), 121.15 (*C*H), 124.70 (2x*C*H), 125.87 (*C*), 126.55 (*C*H), 127.86 (2x*C*H), 132.39 (*C*), 136.21 (*C*), 137.28 (*C*), 169.25 (N*C*=O).

MS (CI) *m/z* 375 [MH^+, 100%]; (Found: MH^+, 375.2064. $C_{24}H_{27}N_2O_2$ requires 375.2067).

(6S,12bR)-6-(Allyloxymethyl)-12-benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(23)**



Sodium hydride (60% dispersion in mineral oil, 0.07 g, 1.65 mmol) and (6S,12bR)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(21)** (0.27 g, 0.75 mmol) were combined under a nitrogen atmosphere and cooled to 0°C. Anhydrous dimethylformamide (5 ml) was then added the reaction was stirred for 30 minutes at room temperature. After this time allyl bromide (0.10 ml, 1.12 mmol) was added and the reaction was left to stir for 24 hours.

The reaction was quenched by the addition of ice water (15 ml), washed with ethyl acetate (3 x 100 ml) and brine (3 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, which was then removed by filtration and solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent to give a yellow solid (0.11 g, 37%).

Mp: 147-149 °C; $[\alpha]_D$ = +140.00 (*c* = 0.011); v_{max} (cm^{-1}) 1633 NC=O.

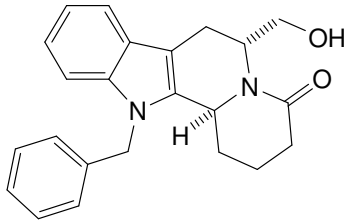
δ_H (300 MHz; $CDCl_3$) 1.44-1.57 (1H, m, C*H*(H)CH_2CH_2CO), 1.60-1.82 (2H, m, CH_2CH_2CO), 2.28-2.34 (1H, m, C*H*(H)CH_2CH_2CO), 2.36-2.42 (1H, m, C*H*(H)CO), 2.45-2.54 (1H, m, C*H*(H)CO), 2.80 (1H, d *J* 15.9, C=CC*H*(H)), 2.92 (1H, ddd, *J* 15.9, 5.7, 2.1, C=CC*H*(H)), 3.20-3.30 (2H, m, CH_2OAllyl), 3.74-3.88 (2H, m , OCH_2CH=CH_2), 4.42 (1H,

d, *J* 12, NC*H*C=C), 4.98-5.12 (2H, m, $OCH_2CH=CH_2$), 5.17 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.32 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.52-5.59 (1H, m, NC*H*CH_2OAllyl), 5.64-5.77 (1H, m, $NCHCH_2OCH_2CH$), 6.85-6.87 (2H, m, Ar*H*), 7.05-7.09 (3H, m, Ar*H*), 7.17-7.23 (3H, m, Ar*H*), 7.46-7.49 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.31 (*C*H_2), 20.89 (*C*H_2), 29.34 (*C*H_2), 30.95 (*C*H_2), 44.74 (*C*H), 46.69 (*C*H_2), 50.31 (*C*H), 67.40 (*C*H_2), 70.59 (*C*H_2), 106.83 (*C*), 108.73 (*C*H), 115.78 (*C*H_2), 117.47 (*C*H), 118.74 (*C*H), 121.14 (*C*H), 124.70 (2x*C*H), 125.92 (*C*), 126.53 (*C*H), 127.86 (2x*C*H), 132.37 (*C*), 133.68 (*C*H), 136.23 (*C*), 137.27 (*C*), 169.28 (N*C*=O).

MS (CI) *m/z* 401 [MH^+, 100%]; (Found: MH^+, 401.2222. $C_{26}H_{29}N_2O_2$ requires 401.2224).

(6R,12bS)-12-Benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(27)**



(6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.60 g, 2.23 mmol) and sodium hydride (60% dispersion in mineral oil, 0.13 g, 3.35 mmol) were combined under a nitrogen atmosphere. An ice bath was placed underneath and anhydrous dimethylformamide (5 ml) was added. The ice bath was removed and the reaction was left to stir at room temperature for half an hour. Benzyl bromide (0.40 ml, 3.35 mmol) was added and the reaction was left for 24 hours.

The reaction was quenched using ice water (15 ml), washed with ethyl acetate (3 x 50 ml) then brine (3 x 50 ml) and dried using anhydrous magnesium sulphate, which was then removed by filtration. The solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a yellow solid (0.33 g, 41%).

Mp: 233-235 °C; $[\alpha]_D$ = -119.62 (*c* = 0.004, $CHCl_3$); v_{max} (cm^{-1}) 3290 (OH), 1591 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.49-1.63 (1H, m, C*H*(H)CH_2CH_2CO), 1.70-1.82 (2H, m, C*H*$_2CH_2CO$), 2.26-2.31 (1H, m, C*H*(H)CH_2CH_2CO), 2.35-2.58 (2H, m, C*H*$_2CO$), 2.80 (1H, d *J* 15.9, C=CC*H*(H)), 2.96 (1H, ddd *J* 15.6, 5.7, 1.8, C=CC*H*(H)), 3.41 (1H, m, C*H*(H)OH), 3.52-3.58 (1H, m, C*H*(H)OH), 4.54 (1H, d, *J* 10.8, NC*H*C=C), 5.19 (1H, d, *J* 17.5,

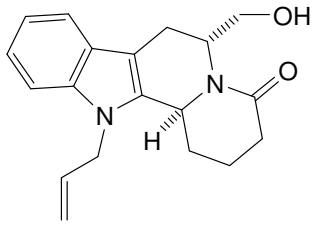
NC*H*(H)Ph), 5.31 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.39-5.46 (1H, m, NC*H*CH_2OH), 6.87-6.90 (2H, m, Ar*H*), 7.06-7.11 (3H, m, Ar*H*), 7.19-7.26 (3H, m, Ar*H*), 7.47-7.50 (1H, m, Ar*H*).

OH proton was not observed.

δ_C (300 MHz; $CDCl_3$) 19.35 (*C*H_2), 21.76 (*C*H_2), 30.49 (*C*H_2), 31.87 (*C*H_2), 47.70 (*C*H_2), 49.01 (*C*H), 51.13 (*C*H), 63.03 (*C*H_2), 107.39 (*C*), 109.91 (*C*H), 118.45 (*C*H), 119.88 (*C*H), 122.31 (*C*H), 125.72 (2x*C*H), 126.77 (*C*), 127.65 (*C*H), 128.97 (2x*C*H), 133.00 (*C*), 137.14 (*C*), 138.25 (*C*), 172.26 (N*C*=O).

MS (CI) *m/z* 361 [MH^+, 100%]; (Found: MH^+, 361.1913. $C_{23}H_{24}N_2O_2$ requires 361.1911).

(6R,12bS)-12-Allyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(28)**



Sodium hydride (60% dispersion in mineral oil, 0.043 g, 1.11 mmol) was added to (6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.2 g, 0.74 mmol) under a nitrogen atmosphere. The reaction was cooled with an ice bath and dry dimethylformamide (5 ml) was added. The ice bath was removed and the reaction was left stirring at room temperature for half an hour. After this time, allyl bromide (0.097 ml, 1.11 mmol) was added and the reaction was left overnight.

The reaction was quenched using ice water (20 ml) and extracted into ethyl acetate (3 x 20 ml). The combined organic extracts were washed with brine (3 x 50 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a pale yellow solid (0.17 g, 74%).

Mp: 161-164 °C; $[\alpha]_D$ = -138.25 (*c* = 0.00217 $CHCl_3$); v_{max} (cm^{-1}) 1603 NC=O, 3332 OH.

δ_H (300 MHz; $CDCl_3$) 1.58-1.66 (1H, m, C*H*(H)CH_2CH_2CO), 1.78-1.89 (2H, m, C*H*$_2CH_2CO$), 2.39-2.50 (1H, m, C*H*(H)CH_2CH_2CO), 2.39-2.50 (1H, m, C*H*(H)CO), 2.53-2.62 (1H, m, C*H*(H)CO), 2.75 (1H, d *J* 16.2, C=CC*H*(H)), 2.93 (1H, dd, *J* 15.9, 4.2, C=CC*H*(H)), 3.43 (1H, t, *J* 10.5 C*H*(H)OH), 3.53-3.59 (1H, m, C*H*(H)OH), 4.61-4.69 (1H,

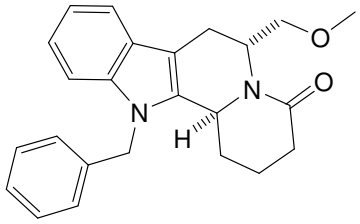
m, NC*H*C=C), 4.61-4.69 (1H, m, NC*H*(H)CHCH$_2$), 4.61-4.69 (1H, m, NCH$_2$CHC*H*(H)), 4.83 (1H, d, *J* 17.1, NC*H*(H)CHCH$_2$), 5.15 (1H, d, *J* 10.5, NCH$_2$CHC*H*(H)), 5.39-5.46 (1H, m, NC*H*CH$_2$OH), 5.83-5.93 (1H, m, NCH$_2$C*H*), 7.05-7.19 (3H, m, Ar*H*), 7.45 (1H, d, *J* 7.5, Ar*H*).

OH proton was not observed.

δ_C (300 MHz; $CDCl_3$) 18.34 (*C*H$_2$), 20.70 (*C*H$_2$), 29.50 (*C*H$_2$), 30.88 (*C*H$_2$), 45.40 (*C*H$_2$), 48.02 (*C*H), 50.01 (*C*H), 61.94 (*C*H$_2$), 105.91 (*C*), 108.80 (*C*H), 115.91 (*C*H$_2$), 117.34 (*C*H), 118.68 (*C*H), 121.05 (*C*H), 125.68 (*C*), 131.75 (*C*), 131.93 (*C*H), 136.90 (*C*), 171.32 (N*C*=O).

MS (CI) *m/z* 311 [MH^+, 100%]; (Found: MH^+, 311.1757. $C_{19}H_{23}N_2O_2$ requires 311.1754).

(6R,12bS)-12-Benzyl-6-(methoxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(29)**



Sodium Hydride (60% dispersion in mineral oil, 0.046 g, 1.16 mmol) was combined with (6R,12bS)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(27)** (0.21 g, 0.581 mmol) under a nitrogen atmosphere and cooled to 0°C using an ice bath. Dimethylformamide (5 ml) was added and the ice bath removed. The reaction was stirred at room temperature for 30 minutes. Methyl Iodide (0.073 ml, 1.16 mmol) was added and the reaction was stirred for 24 hours at room temperature.

The reaction was quenched by the addition of ice water (15 ml), washed with ethyl acetate (3 x 50 ml) and brine (3 x 50 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed by rotary evaporation. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent to give a yellow solid (0.11g, 51%).

Mp: 191-192 °C; $[\alpha]_D$ = -144.23 (*c* = 0.00208 $CHCl_3$); v_{max} (cm^{-1}) 1629 NC=O.

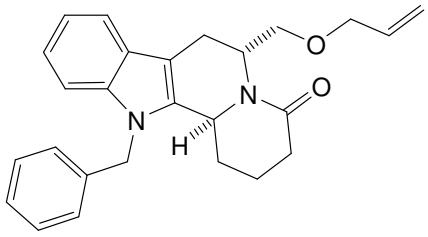
δ_H (300 MHz; $CDCl_3$) 1.41-1.56 (1H, m, C*H*(H)CH_2CH_2CO), 1.59-1.82 (2H, m, C$H_2CH_2CH_2CO$), 2.27-2.42 (1H, m, C*H*(H)CH_2CH_2CO), 2.46-2.49 (1H, m, C*H*(H)CO), 2.52-2.55 (1H, m, C*H*(H)CO), 2.76 (1H, d *J* 15.9, C=CC*H*(H)), 2.92 (1H, ddd, *J* 15.9, 5.7, 2.1, C=CC*H*(H)), 3.12-3.27 (2H, m, CH_2OMe), 3.17 (3H, s, OCH_3), 4.41 (1H, d, *J* 11.1,

NC*H*C=C), 5.18 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.34 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.57 (1H, dt, *J* 6.9, 6 NC*H*CH_2OMe), 6.85-6.88 (2H, m, Ar*H*), 7.03-7.09 (3H, m, Ar*H*), 7.17-7.24 (3H, m, Ar*H*), 7.46-7.50 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 19.39 (*C*H_2), 22.03 (*C*H_2), 30.41 (*C*H_2), 32.06 (*C*H_2), 45.62 (*C*H), 47.80 (*C*H_2), 51.35 (*C*H), 58.82 (*C*H_2), 71.22 (*C*H_2), 107.97 (*C*), 109.81 (*C*H), 118.54 (*C*H), 119.83 (*C*H), 122.24 (*C*H), 125.79 (2x*C*H), 126.97 (*C*), 127.63 (*C*H), 128.95 (2x*C*H), 133.46 (*C*), 137.30 (*C*), 138.37 (*C*), 170.36 (N*C*=O).

MS (CI) *m/z* 375 [MH^+, 100%]; (Found: MH^+, 375.2069. $C_{24}H_{27}N_2O_2$ requires 375.2067).

(6R,12bS)-6-(Allyloxymethyl)-12-benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(30)**



Sodium hydride (60% dispersion in mineral oil, 0.13 g, 3.27 mmol) and (6R,12bS)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(27)** (0.59 g, 1.63 mmol) were combined under a nitrogen atmosphere. An ice bath was placed underneath the flask and anhydrous dimethylformamide (10 ml) was added. The ice bath was removed and the reaction was stirred at room temperature for 30 minutes. After this time, allyl bromide (0.28 ml, 3.27 mmol) was added and the reaction was left for 24 hours.

The reaction was quenched with the addition of ice water (30 ml) and washed with ethyl acetate (3 x 50 ml) and brine (3 x 50 ml). The organic extracts were combined and dried using anhydrous magnesium sulphate. This was then filtered out and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This produced a yellow solid (0.61 g, 93%).

Mp: 140-142 °C; $[\alpha]_D$ = -110.76 (*c* = 0.032 $CHCl_3$); ν_{max} (cm^{-1}) 1633 NC=O.

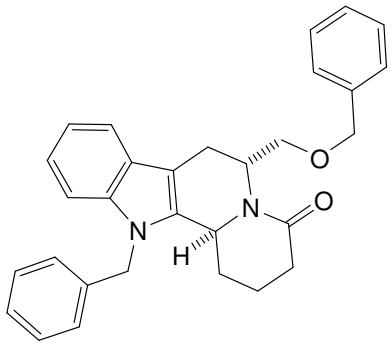
δ_H (300 MHz; $CDCl_3$) 1.45-1.58 (1H, m, C*H*(H)CH_2CH_2CO), 1.62-1.83 (2H, m, CH_2CH_2CO), 2.27-2.35 (1H, m, C*H*(H)CH_2CH_2CO), 2.40-2.43 (1H, m, C*H*(H)CO), 2.46-2.55 (1H, m, C*H*(H)CO), 2.80 (1H, d *J* 15.6, C=CC*H*(H)), 2.92 (1H, ddd, *J* 15.9, 5.7, 2.1, C=CC*H*(H)), 3.20-3.30 (2H, m, CH_2OAllyl), 3.74-3.89 (2H, m , OCH_2CH=CH_2), 4.43 (1H,

d, *J* 10.8, NC*H*C=C), 5.06 (2H, m, $OCH_2CH=CH_2$), 5.18 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.33 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.52-5.59 (1H, m, NC*H*CH_2OAllyl) 5.65-5.78 (1H, m, $NCHCH_2OCH_2CH$), 6.85-6.88 (2H, m, Ar*H*), 7.05-7.10 (3H, m, Ar*H*), 7.17-7.22 (3H, m, Ar*H*), 7.46-7.50 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.30 (*C*H_2), 20.91 (*C*H_2), 29.35 (*C*H_2), 30.93 (*C*H_2), 44.79 (*C*H), 46.72 (*C*H_2), 50.33 (*C*H), 67.43 (*C*H_2), 70.61 (*C*H_2), 106.87 (*C*), 108.74 (*C*H), 115.81 (*C*H_2), 117.49 (*C*H), 118.76 (*C*H), 121.17 (*C*H), 124.71 (2x*C*H), 125.94 (*C*), 126.55 (*C*H), 127.88 (2x*C*H), 132.35 (*C*), 133.67 (*C*H), 136.23 (*C*), 137.30 (*C*), 169.38 (N*C*=O).

MS (CI) *m/z* 401 [MH^+, 100%]; (Found: MH^+, 401.2222. $C_{26}H_{29}N_2O_2$ requires 401.2224).

(6R,12bS)-12-Benzyl-6-(benzyloxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(31)** [49]



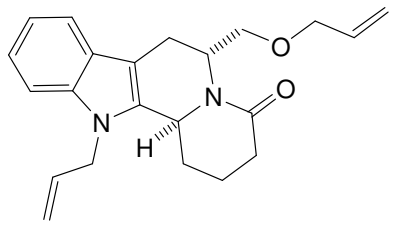
Sodium hydride (60% dispersion in mineral oil, 1.28 g, 32.09 mmol) and (6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (1.97 g, 7.29 mmol) under a nitrogen atmosphere. This was cooled to 0°C using an ice bath. Anhydrous dimethylformamide (40 ml) was added and the ice bath removed. The reaction was left stirring at room temperature for 30 minutes. After this time benzyl bromide (2.61 ml, 21.83 mmol) was added and the reaction left for 24 hours.

The reaction was quenched with the addition of ice water (30 ml) and washed with ethyl acetate (3 x 100 ml) and brine (3 x 100 ml). The organic extracts were combined and dried over anhydrous magnesium sulphate, which was then filtered out, and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with ethyl acetate as the eluent. This gave a pale yellow foam (1.90 g, 58%).

δ_H (300 MHz; $CDCl_3$) 1.47-1.67 (1H, m, C*H*(H)CH_2CH_2CO), 1.70-1.80 (2H, m, $CH_2CH_2CH_2CO$), 2.23-2.35 (1H, m, C*H*(H)CH_2CH_2CO), 2.38-2.43 (1H, m, CH_2CH_2CH(H)CO), 2.45-2.53 (1H, m, CH_2CH_2CH(H)CO), 2.85 (1H, d, *J* 15.9,

C=CC*H*(H)), 2.94 (1H, ddd, *J* 15.6, 5.4, 1.8, C=CC*H*(H)), 3.23-3.33 (2H, m, CH_2OBn), 4.31 (1H, d, *J* 10.8, NC*H*C=C), 4.33 (1H, d, *J* 12, OC*H*(H)Ph), 4.40 (1H, d, *J* 12.3, OC*H*(H)Ph), 5.12 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.25 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.58-5.65 (1H, m, C=CCH_2C*H*). 6.80-6.82 (2H, m, Ar*H*), 7.06-7.19 (11H, m, Ar*H*), 7.47-7.51 (1H, m, Ar*H*).

(6R,12bS)-12-Allyl-6-(allyloxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(32)**



(6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.20 g, 0.74 mmol) and sodium hydride (60% dispersion in mineral oil, 0.13 g, 3.26 mmol) were combined under a nitrogen atmosphere. An ice bath was placed underneath and anhydrous dimethylformamide (5 ml) was added. The ice bath was then removed and the reaction was stirred at room temperature for 30 minutes. After this time, allyl bromide (0.20 ml, 2.22 mmol) was added and the reaction was left stirring for 24 hours.

Ice water (15 ml) was added to quench the reaction, which was then washed with ethyl acetate (3 x 50 ml) and brine (3 x 50 ml). The organic extracts were combined and dried using anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent to give an orange solid (0.08 g, 31%).

Mp: 74-76°C; $[\alpha]_D$ = -140.00 (*c* = 0.016, $CHCl_3$); v_{max} (cm^{-1}) 1624 NC=O.

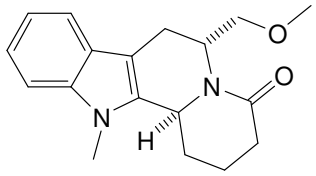
δ_H (300 MHz; $CDCl_3$) 1.48-1.62 (1H, m , C*H*(H)CH_2CH_2CO), 1.71-1.92 (2H, m, C*H*$_2CH_2CO$), 2.37-2.48 (1H, m, C*H*(H)CH_2CH_2CO), 2.52-2.55 (1H, m, C*H*(H)CO), 2.58-2.61 (1H, m, C*H*(H)CO), 2.76 (1H, d, *J* 15.9, C=CC*H*(H)), 2.90 (1H, ddd, *J* 15.6, 5.7, 2.1,

C=CC*H*(H)), 3.21-3.37 (2H, m, C*H*$_2$OAllyl), 3.81 (1H, ddt, *J* 12.9, 5.7, 1.2, NCHCH$_2$OC*H*(H)), 3.92 (1H, ddt, *J* 12.6, 5.4, 1.2, NCHCH$_2$OC*H*(H)), 4.53-4.64 (2H, m, NC*H*$_2$), 4.81 (1H, d, *J* 17.1, NC*H*C=C), 5.00-5.09 (2H, m, CHOCH$_2$CHC*H*$_2$), 5.11-5.14 (2H, m, NCH$_2$CHC*H*$_2$), 5.57 (1H, dt, *J* 6, 6, NC*H*CH$_2$OAllyl), 5.67-5.80 (1H, m, CHOCH$_2$C*H*) 5.82-5.94 (1H, m, NCH$_2$C*H*), 7.03-7.19 (3H, m Ar*H*), 7.45 (1H, d, *J* 7.8, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 19.45 (*C*H$_2$), 21.89 (*C*H$_2$), 30.52 (*C*H$_2$), 32.07 (*C*H$_2$), 45.73 (*C*H) 46.51 (*C*H$_2$), 51.31 (*C*H), 68.49 (*C*H$_2$), 71.62 (*C*H$_2$), 107.39 (*C*), 109.76 (*C*H), 116.86 (*C*H$_2$), 116.95 (*C*H$_2$), 118.48 (*C*H), 119.66 (*C*H), 122.01 (*C*H), 126.94 (*C*), 133.05 (*C*H), 133.20 (*C*), 134.74 (*C*H), 137.97 (*C*), 170.45 (N*C*=O).

MS (CI) *m/z* 351 [MH^+, 100%]; (Found: MH^+, 351.2062. $C_{22}H_{27}N_2O_2$ requires 351.2067).

(6R,12bS)-6-(Methoxymethyl)-12-methyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(33)**



(6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.2 g, 0.74 mmol) and sodium hydride (60% dispersion in mineral oil, 0.13 g, 3.26 mmol) were combined under a nitrogen atmosphere and cooled to 0°C using and ice bath. Anhydrous dimethylformamide (5 ml) was added and the ice bath removed. The reaction was left stirring at room temperature for 30 minutes. After this time methyl iodide (0.14 ml, 2.22 mmol) was added and the reaction was left to stir for a further 24 hours.

The reaction was quenched with the addition of ice water (15 ml) and washed with ethyl acetate (3 x 50 ml) and then brine (3 x 50 ml). The organic extracts were combined and dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified by flash chromatography over silica using 5% methanol in ethyl acetate as the eluent. This gave a bronze solid (0.17 g, 77%).

Mp: 156-157 °C; $[\alpha]_D$ = -230.77 (c = 4.42 x 10^{-3}, $CHCl_3$); ν_{max} (cm^{-1}) 1624 NC=O.

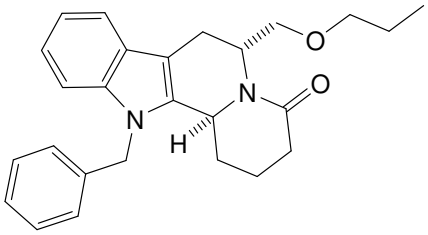
δ_H (300 MHz; $CDCl_3$) 1.49-1.63 (1H, m , C*H*(H)CH_2CH_2CO), 1.75-1.92 (2H, m, CH_2CH_2CO), 2.36-2.46 (2H, m, CH_2CO), 2.48-2.63 (1H, m, C*H*(H)CH_2CH_2CO), 2.70 (1H, d, *J* 15.6, C=CC*H*(H)), 2.88 (1H, ddd, *J* 15.6, 5.4, 1.8, C=CC*H*(H)), 3.13-3.19 (1H, m, C*H*(H)OCH_3), 3.21 (3H, s, OCH_3), 3.29-3.35 (1H, m, C*H*(H)OCH_3), 3.63 (3H, s, NCH_3),

4.62 (1H, d, *J* 11.1, NC*H*C=C), 5.57 (1H, dt, *J* 6.9, 6, NC*H*CH_2OCH_3), 7.04 (1H, t, *J* 7.5, Ar*H*), 7.12-7.22 (2H, m, Ar*H*), 7.42 (1H, d, *J* 7.8, Ar*H*).

δ_C(300 MHz; $CDCl_3$) 18.35 (*C*H_2), 20.76 (*C*H_2), 29.11 (*C*H_2), 30.24 (*C*H_3), 31.02 (*C*H_2), 44.65 (*C*H), 50.18 (*C*H), 57.74 (*C*H_3), 70.09 (*C*H_2), 105.62 (*C*), 107.92 (*C*H), 117.36 (*C*H), 118.34 (*C*H), 120.81 (*C*H), 125.52 (*C*), 132.18 (*C*), 137.13 (*C*), 169.40 (N*C*=O).

MS (CI) *m/z* 299 [MH^+, 100%]; (Found: MH^+, 299.1750. $C_{18}H_{23}N_2O_2$ requires 299.1754).

(6R,12bS)-12-Benzyl-6-(propoxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(36)**



Sodium hydride (60% dispersion in mineral oil, 0.05 g, 1.31 mmol) was added to (6R,12bS)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(27)** (0.23 g, 0.60 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and anhydrous dimethylformamide (10 ml) was added. The reaction was stirred at room temperature for half an hour. After this time, propyl bromide (0.12 ml, 1.20 mmol) was added and the reaction was left stirring overnight.

Ice cold water (20 ml) was added to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a yellow solid (0.12 g, 50%).

Mp: 153-155 °C; $[\alpha]_D$ = -127.36 (*c* = 0.00212 $CHCl_3$); v_{max} (cm^{-1}) 1634 NC=O.

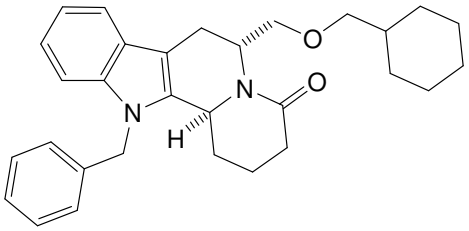
δ_H (300 MHz; $CDCl_3$) 1.15-1.80 (1H, m, C*H*(H)CH_2CH_2CO), 1.15-1.80 (2H, m, C*H*$_2$$CH_2CO$), 1.15-1.80 (2H, m, $OCH_2CH_2CH_3$), 1.15-1.80 (3H, m, $OCH_2CH_2CH_3$), 2.28-2.42 (1H, m, C*H*(H)CH_2CH_2CO), 2.28-2.42 (1H, m, C*H*(H)CO), 2.44-2.53 (1H, m, C*H*(H)CO), 2.78 (1H, d *J* 15.9, C=CC*H*(H)), 2.91 (1H, ddd, *J* 15.6, 5.4, 1.8, C=CC*H*(H)), 3.13-3.18 (1H, m, C*H*(H)OC_3H_7) 3.13-3.18 (1H, m, OC*H*(H)CH_2CH_3), 3.23-3.31 (1H, m,

C*H*(H)OC_3H_7), 3.23-3.31 (1H, m, OC*H*(H)CH_2CH_3), 4.42 (1H, d, *J* 9.6, NC*H*C=C), 5.17 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.33 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.55 (1H, dt, *J* 7.5, 6, NC*H*$CH_2OC_3H_7$), 6.85-6.88 (2H, m, Ar*H*), 7.03-7.08 (3H, m, Ar*H*), 7.17-7.24 (3H, m, Ar*H*), 7.46-7.49 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 10.58 (*C*H_3), 19.40 (*C*H_2), 21.88 (*C*H_2), 22.87 (*C*H_2), 30.37 (*C*H_2), 32.05 (*C*H_2), 45.77 (*C*H), 47.81 (*C*H_2), 51.39 (*C*H), 68.98 (*C*H_2), 72.52 (*C*H_2), 108.01 (*C*), 109.78 (*C*H), 118.55 (*C*H), 119.81 (*C*H), 122.21 (*C*H), 125.79 (2x*C*H), 127.05 (*C*), 127.60 (*C*H), 128.94 (2x*C*H), 133.49 (*C*), 137.33 (*C*), 138.39 (*C*), 170.34 (*C*).

MS (CI) *m/z* 403 [MH^+, 100%]; (Found: MH^+, 403.2375. $C_{26}H_{31}N_2O_2$ requires 403.2380).

(6R,12bS)-12-Benzyl-6-((cyclohexylmethoxy)methyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(37)**



Sodium hydride (60% dispersion in mineral oil, 0.034 g, 0.853 mmol) was added to (6R,12bS)-12-benzyl-6-(hydroxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(27)** (0.14 g, 0.388 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and anhydrous dimethylformamide (10 ml) was added. The reaction was stirred at room temperature for half an hour. After this time, (bromomethyl)cyclohexane (0.108 ml, 0.775 mmol) and sodium iodide (0.12 g, 0.775 mmol) were added and the reaction was heated to 60 °C and left overnight.

Ice cold water (20 ml) was added to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a yellow oil (0.11 g, 31%).

$[\alpha]_D$ = -34.55 (*c* = 0.011 $CHCl_3$); ν_{max} (cm^{-1}) 1633 NC=O.

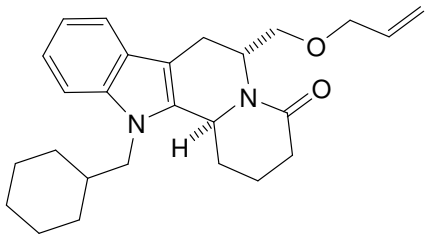
δ_H (300 MHz; $CDCl_3$) 1.05-1.82 (1H, m, C*H*(H)CH_2CH_2CO), 1.05-1.82 (2H, m, C$H_2$$CH_2CO$), 1.05-1.82 (11H, m, $OCH_2C_6H_{11}$), 2.29-2.37 (1H, m C*H*(H)CH_2CH_2CO), 2.39-2.48 (1H, m, C*H*(H)CO), 2.51-2.53 (1H, m, C*H*(H)CO), 2.77 (1H, d *J* 15.9, C=CC*H*(H)),

2.89 (1H, dd, *J* 5.7, 2.1, OC*H*(H)C_6H_{11}), 2.93-2.98 (1H, m, C=CC*H*(H)), 3.10-3.15 (1H, m, C*H*(H)$OCH_2C_6H_{11}$), 3.10-3.15 (1 H, m, OC*H*(H)C_6H_{11}), 3.22 (1H, dd, *J* 7.5, 2.4, C*H*(H)$OCH_2C_6H_{11}$), 4.41 (1H, d, *J* 10.2, NC*H*C=C), 5.18 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.34 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.55 (1H, dt, *J* 7.2, 6.3 NC*H*$CH_2OCH_2C_6H_{11}$), 6.87-6.92 (2H, m, Ar*H*), 7.04-7.09 (3H, m, Ar*H*), 7.19-7.24 (3H, m, Ar*H*), 7.46-7.49 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 19.40 (*C*H_2), 21.84 (*C*H_2), 25.84 (*C*H_2), 25.87 (*C*H_2), 26.60 (*C*H_2), 29.88 (*C*H_2), 29.92 (*C*H_2), 30.32 (*C*H_2), 32.04 (*C*H_2), 37.99 (*C*H), 45.70 (*C*H), 47.81 (*C*H_2), 51.42 (*C*H), 69.17 (*C*H_2), 76.69 (*C*H_2), 108.05 (*C*), 109.73 (*C*H), 118.53 (*C*H), 119.79 (*C*H), 122.18 (*C*H), 125.79 (2xCH), 127.03 (*C*), 127.61 (*C*H), 128.94 (2xCH), 133.49 (*C*), 137.33 (*C*), 138.36 (*C*), 170.32 (N*C*=O).

MS (CI) *m/z* 457 [MH^+, 100%]; (Found: MH^+, 457.2843. $C_{30}H_{37}N_2O_2$ requires 457.1754).

(6R,12bS)-6-(Allyloxymethyl)-12-(cyclohexylmethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(39)**



Sodium hydride (60% dispersion in mineral oil, 0.04 g, 1.11 mmol) was added to (6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.25 g, 0.925 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and anhydrous dimethylformamide (10 ml) was added. The reaction was stirred at room temperature for half an hour. After this time, (bromomethyl)cyclohexane (0.16 ml, 1.11 mmol) and sodium iodide (0.17 g, 1.11 mmol) were added and the reaction was heated to 60 °C and left overnight.

Ice cold water (20 ml) was added to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a white foam **(38)** (0.14 g, 41%).

Sodium hydride (60% dispersion in mineral oil 0.034 g, 0.842 mmol) was added to the product **(38)** (0.14 g, 0.383 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and dry dimethylformamide (10 ml) was added. The reaction was stirred at room temperature for half an hour. After this time allyl bromide (0.07 ml, 0.765 mmol) was added and the reaction was left overnight.

Ice cold water (20 ml) was used to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a yellow solid (0.07 g, 45%).

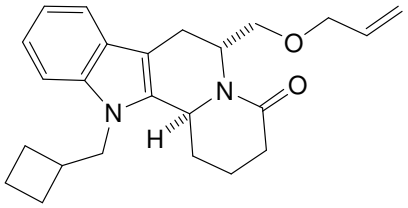
Mp: 130-133 °C; $[\alpha]_D$ = - 70.80 (*c* = 0.00226); ν_{max} (cm^{-1}) 1636 NC=O.

δ_H (300 MHz; $CDCl_3$) 0.77-1.99 (1H, m, C*H*(H)CH_2CH_2CO), 0.77-1.99 (2H, m, CH_2CH_2CO), 0.77-1.99 (11H, m, $NCH_2C_6H_{11}$), 2.38-2.50 (1H, m, C*H*(H)CH_2CH_2CO), 2.38-2.50 (1H, m, C*H*(H)CO), 2.53-2.63 (1H, m, C*H*(H)CO), 2.77 (1H, d *J* 15.9, C=CC*H*(H)), 2.84-2.91 (1H, m, C=CC*H*(H)), 3.42 (2H, si, *J* 9.9, CH_2OAllyl), 3.65 (1H, q, *J* 8.7, NC*H*(H)C_6H_{11}), 3.78-3.92 (2H, m , OCH_2CH=CH_2), 4.06 (1H, dd, *J* 14.4, 5.7, NC*H*(H)C_6H_{11}), 4.64 (1H, d, *J* 10.8, NC*H*C=C), 4.99-5.13 (2H, m, OCH_2CH=CH_2), 5.54 (1H, dt, *J* 6.6, 6.6, NC*H*CH_2OAllyl), 5.67-5.78 (1H, m, $NCHCH_2OCH_2CH$), 7.04 (1H, t, *J* 6.9, Ar*H*), 7.13 (1H, t, Ar*H*), 7.21 (1H, t, *J* 6.9, Ar*H*), 7.43 (1H, d, *J* 7.8, Ar*H*)

δ_C (300 MHz; $CDCl_3$) 19.36 (*C*H_2), 21.98 (*C*H_2), 25.64 (*C*H_2), 25.79 (*C*H_2), 26.22 (*C*H_2), 30.41 (*C*H_2), 31.04 (2x*C*H_2), 31.93 (*C*H_2), 38.14 (*C*H), 45.89 (*C*H), 50.82 (*C*H_2), 51.49 (*C*H), 68.67 (*C*H_2) 71.78 (*C*H_2), 107.01 (*C*), 110.20 (*C*H), 116.96 (*C*H_2), 118.44 (*C*H), 119.29 (*C*H), 121.60 (*C*H), 126.91 (*C*), 133.48 (*C*), 134.75 (*C*H), 137.72 (*C*), 170.60 (N*C*=O).

MS (CI) *m/z* 407 [MH^+, 100%]; (Found: MH^+, 407.2694. $C_{26}H_{35}N_2O_2$ requires 407.2693).

(6R,12bS)-6-(Allyloxymethyl)-12-(cyclobutylmethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(41)**



Sodium hydride (60% dispersion in mineral oil, 0.04 g, 1.11 mmol) was added to (6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.25 g, 0.925 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and anhydrous dimethylformamide (10 ml) was added. The reaction was stirred at room temperature for half an hour. After this time, (bromomethyl)cyclobutane (0.13 ml, 1.11 mmol) and sodium iodide (0.17 g, 1.11 mmol) were added and the reaction was heated to 60 °C and left overnight.

Ice cold water (20 ml) was used to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a solid **(40)** (0.21 g, 67%).

Sodium hydride (60 % dispersion in mineral oil, 0.055g, 1.37 mmol) was added to the product **(40)** (0.21 g, 0.621 mmol) under a nitrogen atmosphere. This was cooled to 0 °C using an ice bath and anhydrous dimethylformamide (10 ml) was added. The reaction was

stirred at room temperature for half an hour. After this time allyl bromide (0.11 ml, 1.24 mmol) and the reaction was left overnight.

Ice cold water (20 ml) was used to quench the reaction which was then extracted into ethyl acetate (3 x 50 ml). The organic extracts were combined and washed with brine (3 x 50 ml). These were then dried over anhydrous magnesium sulphate, this was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 5% methanol in ethyl acetate as the eluent. This gave a yellow oil (0.15 g, 46%).

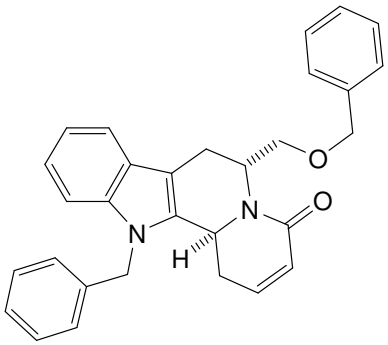
$[\alpha]_D$ = - 62.86 (*c* = 0.007); ν_{max} (cm^{-1}) 1636 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.48-1.95 (1H, m, C*H*(H)CH_2CH_2CO), 1.48-1.95 (2H, m, CH_2CH_2CO), 1.48-1.95 (6H, m, $NCH_2CHC_3H_6$), 2.39-2.50 (1H, m, C*H*(H)CH_2CH_2CO), 2.39-2.50 (1H, m, C*H*(H)CO), 2.54-2.69 (1H, m, C*H*(H)CO), 2.54-2.69 (1H, m, $NCH_2CHC_3H_6$), 2.75 (1H, d *J* 15.9, C=CC*H*(H)), 2.87 (1H, ddd, *J* 15.9, 5.7, 1.8, C=CC*H*(H)), 3.27 (2H, m, *J* 7.2, CH_2OAllyl), 3.78-3.95 (2H, m, OCH_2CH=CH_2), 3.78-3.95 (1H, m, NC*H*(H)C_4H_7), 4.18 (1H, dd, *J* 14.7, 6, (NC*H*(H)C_4H_7), 4.67 (1H, d, *J* 11.1, NC*H*C=C), 5.07 (2H, m, $OCH_2CH=CH_2$), 5.55 (1H, dt, *J* 6.6, 6.6, NC*H*CH_2OAllyl), 5.67-5.80 (1H, m, $NCHCH_2OCH_2CH$), 7.01-7.06 (1H, m, Ar*H*), 7.13 (1H, td, *J* 15.3, 8.1, 1.2, Ar*H*), 7.25 (1H, d, *J* 8.1, Ar*H*), 7.42 (1H, d, *J* 7.5, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.32 (*C*H_2), 19.39 (*C*H_2), 21.97 (*C*H_2), 26.42 (*C*H_2), 27.46 (*C*H_2). 30.47 (*C*H_2), 31.96 (*C*H_2), 36.12 (*C*H), 45.80 (*C*H), 49.66 (*C*H_2), 51.45 (*C*H), 68.54 (*C*H_2), 71.66 (*C*H_2), 107.31 (*C*), 109.97 (*C*H), 116.96 (*C*H_2), 118.41 (*C*H), 119.33 (*C*H), 121.66 (*C*H), 126.97 (*C*), 133.03 (*C*), 134.75 (*C*H), 137.84 (*C*), 170.56 (N*C*=O).

MS (CI) *m/z* 379 [MH^+, 100%]; (Found: MH^+, 379.2383. $C_{24}H_{31}N_2O_2$ requires 379.2380).

(6R,12bS)-12-Benzyl-6-(benzyloxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(45)** [13]



Di*iso*propylamine (0.67 ml, 4.79 mmol) was added to dry tetrahydrofuran (5 ml) under a nitrogen atmosphere at 0 °C. n-Butyllithium (2.5 M in hexanes, 1.92 ml, 4.79 mmol) was added and the reaction stirred for 15 minutes. This was then cooled to -78 °C. (6R,12bS)-12-benzyl-6-(benzyloxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(31)** (1.9 g, 1.60 mmol) in dry tetrahydrofuran (15 ml) was added *via* cannula. The reaction was stirred at -78 °C for one hour. Following this, phenyl selenium bromide (0.57 g, 2.40 mmol) in dry tetrahydrofuran (10ml) was added *via* cannula and the reaction was left stirring for 24 hours.

The reaction was quenched with saturated ammonium chloride solution (40 ml) and extracted into diethyl ether (3 x 75 ml). The organic extracts were combined and washed with saturated ammonium chloride solution (100 ml), dried over anhydrous magnesium sulphate, which was removed by filtration and the solvents were removed under reduced pressure. This produced a brown oil, which was used with no further purification.

The crude selenide (1.12 g, 1.85 mmol) was dissolved in methanol (220 ml) and water (45 ml). Sodium metaperiodate (0.91 g, 4.25 mmol) and sodium bicarbonate (0.19 g, 2.22 mmol) were added and the reaction was heated at 60 °C for 24 hours.

The reaction was quenched with saturated sodium bicarbonate solution (100 ml) and diethyl ether (150 ml). The organic layer was washed with water (100 ml) followed by

brine (100 ml), dried over anhydrous magnesium sulphate which was removed by filtration and the solvents were removed under reduced pressure.

The crude product was absorbed onto silica and purified by flash column chromatography over silica using ethyl acetate as the eluent to give a pale yellow solid (0.33 g, 40%).

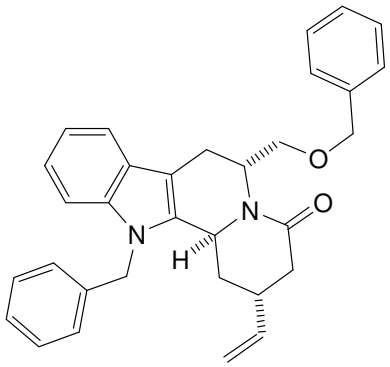
Mp: 60-61 °C; $[\alpha]_D$ = -254.77 (*c* = 0.00577 $CHCl_3$) v_{max} (cm^{-1}) 1662 NC=O.

δ_H (300 MHz; $CDCl_3$) 2.13-2.24 (1H, m, C*H*(H)CH_2CH_2CO), 2.49 (1H, dq, *J* 17.4, 6.3, 3.9, C*H*(H)CH_2CH_2CO), 2.95 (1H, ddd, *J* 15.6, 5.4, 1.8, C=CC*H*(H)), 3.08 (1H, d, *J* 15.9, C=CC*H*(H)), 3.22-3.32 (2H, m, CH_2OBn), 4.38 (2H, s, OCH_2Ph), 4.52-4.57 (1H, m, NC*H*C=C), 5.11 (1H, d, *J* 17.5, C*H*(H)Ph), 5.20 (1H, d, *J* 17.5, C*H*(H)Ph), 5.34-5.40 (1H, m, NC*H*CH_2OBn), 5.99 (1H, dd, *J* 9.6, 2.7, CH_2C*H*CHCO), 6.41-6.48 (1H, m, CH_2CHC*H*CO), 6.76-6.79 (2H, m, Ar*H*), 7.06-7.17 (11H, m, Ar*H*), 7.51-7.56 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 22.00 (*C*H_2), 32.03 (*C*H_2), 46.07 (*C*H), 47.31 (*C*H_2), 49.84 (*C*H), 68.10 (*C*H_2), 72.60 (*C*H_2), 107.70 (*C*), 109.85 (*C*H), 118.73 (*C*H), 119.96 (*C*H), 122.34 (*C*H), 125.61 (2x*C*H), 126.01 (*C*H), 127.03 (*C*), 127.37 (3x*C*H), 127.60 (*C*H), 128.25 (2xCH), 128.96 (2xCH), 132.62 (*C*), 137.07 (*C*), 138.19 (*C*), 138.26 (*C*H), 138.30 (*C*), 164.92 (N*C*=O).

MS (CI) *m/z* 449 [MH^+, 100%]; (Found: MH^+, 449.2222. $C_{30}H_{29}N_2O_2$ requires 449.2224).

(2R,6R,12bS)-12-Benzyl-6-(benzyloxymethyl)-2-vinyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(47)** [13]



A 0.7 M solution of vinyl magnesium bromide in tetrahydrofuran (10.6 ml, 7.43 mmol) was added to a suspension of copper cyanide (0.34 g, 3.75 mmol) in anhydrous tetrahydrofuran (20 ml) at -78 °C while stirring. The reaction was warmed to 0 °C for 3 minutes then re-cooled to -78 °C. (6R,12bS)-12-benzyl-6-(benzyloxymethyl)-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizin-4(12H)-one **(45)** (0.33 g, 0.736 mmol) in anhydrous tetrahydrofuran (15 ml) was added *via* cannula at -78 °C. After 5 minutes, chlorotrimethylsilane (0.48 ml, 3.75 mmol) was added and the reaction was left to warm slowly to room temperature overnight.

The reaction as quenched with the addition of saturated ammonium chloride solution (20 ml) and water (20 ml) and left stirring for 20 minutes. A 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (6 ml) was added and the stirring continued for 15 minutes. The reaction was extracted into ethyl acetate (3 x 50 ml) and the combined organic extracts were dried over anhydrous magnesium sulphate. This was then filtered out and the solvents removed under reduced pressure.

The crude product was absorbed onto silica and purified by flash column chromatography over silica with 1:1 ethyl acetate: petroleum ether as the eluent. This gave a pale yellow solid (0.17 g, 49%).

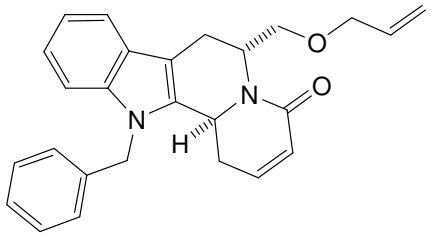
Mp: 133-136 °C; $[\alpha]_D$ = -52.81 (*c* = 0.00303 $CHCl_3$); ν_{max} (cm^{-1}) 1636 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.74-1.84 (1H, m, C*H*(H)CHCH$_2$CO), 2.08-2.18 (1H, m, C*H*(H)CHCH$_2$CO), 2.40-2.57 (2H, m, CH$_2$CHC*H$_2$*CO), 2.40-2.57 (1H, m, CH$_2$C*H*CH$_2$CO), 2.83 (1H, d, *J* 15.9, C=CC*H*(H)), 2.94 (1H, ddd, *J* 15.6, 5.4, 1.8, C=CC*H*(H)), 3.24 (2H, d, *J* 7.5, C*H$_2$*OBn), 4.33 (2H, s, OC*H$_2$*Ph), 4.39-4.44 (1H, m, NC*H*C=C), 4.84-4.95 (2H, m, CHCH=C*H$_2$*), 5.10 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.24 (1H, d, *J* 17.5, C*H*(H)Ph), 5.53-5.59 (1H, m, NC*H*CH$_2$OBn), 5.60-5.72 (1H, m, CHC*H*=CH$_2$), 6.79-6.82 (2H, m, Ar*H*), 7.05-7.17 (11H, m, Ar*H*), 7.47-7.50(1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 20.90 (*C*H$_2$), 31.63 (*C*H), 33.83 (*C*H$_2$), 35.10 (*C*H$_2$), 45.06 (*C*H), 46.40 (*C*H), 46.71 (*C*H$_2$), 67.22 (*C*H$_2$), 71.54 (*C*H$_2$), 107.16 (*C*), 108.64 (*C*H), 114.61 (*C*H$_2$), 117.38 (*C*H), 118.81 (*C*H), 121.16 (*C*H), 124.75 (2xCH), 125.97 (*C*), 126.39 (*C*H), 126.42 (2xCH), 126.57 (*C*H), 127.17 (2xCH), 127.88 (2xCH), 132.26 (*C*), 136.16 (*C*), 137.13 (*C*), 137.36 (*C*), 138.10 (*C*H), 169.06 (N*C*=O).

MS (CI) *m/z* 477 [MH^+, 100%]; (Found: MH^+, 477.2530. $C_{32}H_{33}N_2O_2$ requires 477.2537).

(6R,12bS)-6-(Allyloxymethyl)-12-benzyl-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizin-4(12H)-one **(44)**



Di*iso*propylamine (0.14 ml, 0.999 mmol) was added to anhydrous tetrahydrofuran (5 ml) under a nitrogen atmosphere at 0 °C. n-Butyllithium (2.5 M in hexanes, 0.4 ml, 0.999 mmol) was added and the reaction stirred for 15 minutes. This was then cooled to -78 °C. (6R,12bS)-6-(allyloxymethyl)-12-benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(30)** (0.4 g, 0.999mmol) in anhydrous tetrahydrofuran (10 ml) was added *via* cannula. The reaction was stirred at -78 °C for one hour. Following this, phenyl selenium bromide (0.26 g, 1.099 mmol) in anhydrous tetrahydrofuran (10ml) was added *via* cannula and the reaction was left stirring for 24 hours.

The reaction was quenched with saturated ammonium chloride solution and extracted into diethyl ether (3 x 20 ml). The organic extracts were combined and washed with saturated ammonium chloride solution (100 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure. This produced a brown oil, which was used with no purification.

The crude selenide (0.54 g, 0.972 mmol) was dissolved in methanol (55 ml) and water (11 ml). Sodium metaperiodate (0.48 g, 2.24 mmol) and sodium bicarbonate (0.098 g, 1.17 mmol) were added and the reaction was heated at 60 °C for 24 hours.

The reaction was quenched with saturated sodium bicarbonate solution (50 ml) and diethyl ether (75 ml). The organic layer was washed with water (50 ml) followed by brine

(50 ml), dried over anhydrous magnesium sulphate which was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography on silica using ethyl acetate as the eluent to give a yellow oil (0.10 g, 25%).

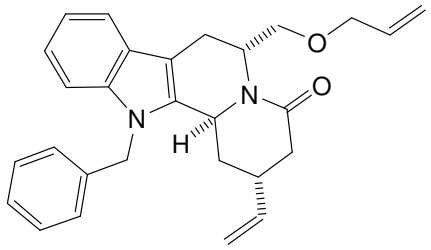
$[\alpha]_D$ = - 129.00 (*c* = 0.01); ν_{max} (cm^{-1}) 1663 NC=O.

δ_H (300 MHz; $CDCl_3$) 2.13-2.26 (1H, m, C*H*(H)CH_2CH_2CO), 2.54 (1H, dq, *J* 17.7, 6.6, 3.9, C*H*(H)CH_2CH_2CO), 2.94 (1H, ddd, *J* 15.6, 5.1, 1.8, C=CC*H*(H)), 3.03 (1H, d *J* 15.9, C=CC*H*(H)), 3.19-3.31 (2H, m, CH_2OAllyl), 3.81-3.85 (2H, m , OCH_2CH=CH_2), 4.63-4.69 (1H, m, NC*H*C=C), 4.98-5.12 (2H, m, OCH_2CH=CH_2), 5.16-5.23 (2H, m, NCH_2Ph), 5.28-5.35 (1H, m, NC*H*CH_2OAllyl), 5.65-5.78 (1H, m, $NCHCH_2OCH_2$C*H*), 6.00 (1H, dd, *J* 9.9, 3, CH_2C*H*=CHCO), 6.45-6.51 (1H, m, CH_2CH=C*H*CO), 6.84-6.89 (2H, m, Ar*H*), 7.05-7.14 (3H, m, Ar*H*), 7.19-7.25 (3H, m, Ar*H*), 7.51-7.54 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.88 (*C*H_2), 32.07 (*C*H_2), 45.99 (*C*H), 47.38 (*C*H_2), 49.83 (*C*H), 68.25 (*C*H_2), 71.79 (*C*H_2), 107.77 (*C*H), 109.87 (*C*), 116.76 (*C*H_2), 118.78 (*C*H), 119.91 (*C*H), 122.36 (*C*H), 125.65 (2xCH), 126.04 (*C*H), 127.03 (*C*), 127.65 (*C*H), 128.99 (2xCH), 132.59 (*C*), 134.73 (*C*H), 137.12 (*C*), 138.19 (*C*H), 164.90 (N*C*=O).

MS (CI) *m/z* 399 [MH^+, 100%]; (Found: MH^+, 399.2057 $C_{26}H_{27}N_2O_2$ requires 399.2067).

(2R,6R,12bS)-6-(Allyloxymethyl)-12-benzyl-2-vinyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(46)**



A 0.7 M solution of vinyl magnesium bromide in tetrahydrofuran (3.6 ml, 2.53 mmol) was added to a suspension of copper cyanide (0.12 g, 1.28 mmol) in anhydrous tetrahydrofuran (10 ml) at -78 °C while stirring. The reaction was warmed to 0 °C for 3 minutes then re-cooled to -78 °C. (6R,12bS)-6-(allyloxymethyl)-12-benzyl-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizin-4(12H)-one **(44)** (0.10 g, 0.251 mmol) in anhydrous tetrahydrofuran (5 ml) was added *via* cannula at -78 °C. After 5 minutes trimethylsily chloride (0.16 ml, 1.28 mmol) was added and the reaction was left to warm slowly to room temperature overnight.

The reaction was quenched with the addition of saturated ammonium chloride solution (10 ml) and water (10 ml) and then left stirring for 20 minutes. A 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (6 ml) was added and the stirring continued for a further 15 minutes. The reaction was extracted into ethyl acetate (3 x 50 ml) and the combined organic extracts were dried over anhydrous magnesium sulphate. This was then filtered out and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica with ethyl acetate as the eluent. This gave a brown oil (0.04 g, 37%).

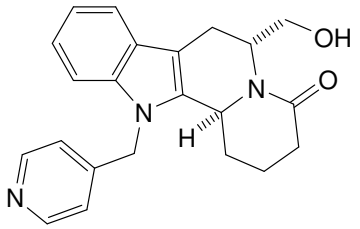
$[\alpha]_D$ = -29.41 (*c* =0.00068 $CHCl_3$); v_{max} (cm^{-1}) 1633 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.76-1.86 (1H, m, CHC*H*(H)CHCH$_2$CO), 2.15-2.20 (1H, m, CHC*H*(H)CHCH$_2$CO), 2.48-2.61 (2H, m, CHC*H*$_2$CO), 2.48-2.61 (1H, m, C*H*CH$_2$CO), 2.78 (1H, d, *J* 15.9, C=CC*H*(H)), 2.95 (1H, ddd, *J* 13.8, 3.9, 1.8, C=CC*H*(H)), 3.17-3.23 (2H, m, C*H*$_2$OAllyl), 3.79 (2H, m, OC*H*$_2$CH=CH$_2$), 4.49 (1H, d, *J* 8.7, NC*H*C=C), 4.94-5.11 (2H, m, OCH$_2$CH=C*H*$_2$), 4.94-5.11 (2H, m, CHCH$_2$CHCH=C*H*$_2$), 5.16 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.31 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.52 (1H, dt, *J* 6.9, 6.3, NC*H*CH$_2$OAllyl), 5.64-5.77 (1H, m, NCHCH$_2$OCH$_2$C*H*=CH$_2$), 5.64-5.77 (1H, m, CHCH$_2$CHC*H*=CH$_2$), 6.87-6.90 (2H, m, Ar*H*), 7.05-7.11 (3H, m, Ar*H*), 7.20-7.23 (3H, m, Ar*H*), 7.46-7.50 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 20.88 (*C*H$_2$), 31.61 (*C*H), 33.66 (*C*H$_2$), 35.02 (*C*H$_2$), 44.97 (*C*H), 46.33 (*C*H), 46.82 (*C*H$_2$), 67.33 (*C*H$_2$), 70.67 (*C*H$_2$), 107.26 (*C*), 108.67 (*C*H), 114.72 (*C*H$_2$), 115.78 (*C*H$_2$), 117.38 (*C*H), 118.77 (*C*H), 121.18 (*C*H), 124.79 (2xCH), 125.95 (*C*), 126.60 (*C*H), 127.91 (2xCH), 132.28 (*C*), 133.60 (*C*H), 136.20 (*C*), 137.37 (*C*), 137.98 (*C*H).

MS (CI) *m/z* 427 [MH$^+$, 100%]; (Found: MH$^+$, 427.2379. $C_{28}H_{31}N_2O_2$ requires 427.2380).

(6R,12bS)-6-(Hydroxymethyl)-12-(pyridin-4-ylmethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(53)**



(6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (0.20 g, 0.740 mmol) was dissolved in anhydrous dimethyl sulfoxide under a nitrogen atmosphere. 4-(Bromomethyl)-pyridine hydrobromide (0.28 g, 1.11 mmol) was added and the reaction cooled to 0 °C before addition of potassium *tert*-butoxide (0.12 g, 1.11 mmol). The reaction then left to warm slowly overnight.

After this time the reaction was quenched with water (10 ml), extracted with ethyl acetate (3 x 20 ml) and washed with brine (3 x 50 ml). It was then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This yielded a white solid (0.06 g, 22%).

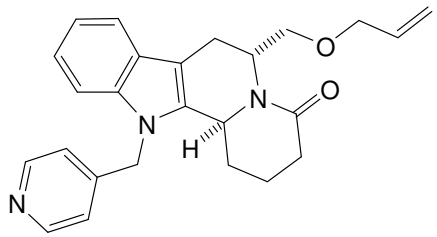
Mp: 227-229 °C; $[\alpha]_D$ = -67.80 (*c* = 0.00059 $CHCl_3$); v_{max} (cm^{-1}) 1591 NC=O, 3290 OH.

δ_H (300 MHz; $CDCl_3$) 1.52-1.66 (1H, m, C*H*(H)CH_2CH_2CO), 1.72-1.81 (2H, m, CH_2CH_2CO), 2.26-2.31 (1H, m, C*H*(H)CH_2CH_2CO), 2.35-2.54 (2H, m, CH_2CH_2CO), 2.79 (1H, d, *J* 15.9, C=CC*H*(H)), 2.96 (1H, ddd, *J* 15.9, 5.7, 1.8, C=CC*H*(H)), 3.41 (1H, t, *J* 10.5, CHC*H*(H)OH), 3.52-3.60 (1H, m, CHC*H*(H)OH), 4.54 (1H, d, *J* 11.1, C*H*C=C), 5.19 (1H, d, *J* 17.5, NC*H*(H)Pyr), 5.31 (1H, d, *J* 17.5, NC*H*(H)Pyr), 5.39-5.49 (1H, m,

C*H*CH_2OH), 6.87-6.90 (12H, m, Ar*H*), 7.06-7.12 (3H, m Ar*H*), 7.16-7.26 (2H, m, Ar*H*), 7.46-7.49 (1H, m, Ar*H*).

No further data was obtained due to this being an intermediate compound.

(6R,12bS)-6-(Allyloxymethyl)-12-(pyridin-4-ylmethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(54)**



Sodium hydride (60% dispersion in mineral oil, 0.013 g, 0.33 mmol) and (6R,12bS)-6-(hydroxymethyl)-12-(pyridin-4-ylmethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(53)** (0.06 g, 0.17 mmol) were combined under a nitrogen atmosphere. An ice bath was placed underneath the flask and anhydrous dimethylformamide (5 ml) was added. The ice bath was removed and the reaction stirred at room temperature for 30 minutes. After this time allyl bromide (0.03 ml, 0.33 mmol) was added and the reaction was left for 24 hours.

The reaction was quenched with the addition of ice water (10 ml) and washed with ethyl acetate (3 x 50 ml) and brine (3 x 50 ml). The organic extracts were combined and dried using anhydrous magnesium sulphate. This was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This produced a white oil (0.06 g, 90%).

$[\alpha]_D$ = -118.88 (*c* = 0.00077 $CHCl_3$); v_{max} (cm^{-1}) 1633 NC=O.

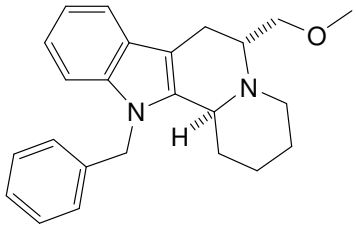
δ_H (300 MHz; $CDCl_3$) 1.45-1.58 (1H, m, C*H*(H)CH_2CH_2CO), 1.64-1.76 (2H, m, CH_2CH_2CO), 2.29-2.53 (1H, m, C*H*(H)CH_2CH_2CO), 2.29-2.53 (2H, m, CH_2CH_2CO), 2.80 (1H, d, *J* 15.3, C=CC*H*(H)), 2.93 (1H, dd, *J* 15.9, 3.3, C=CC*H*(H)), 3.20-3.30 (2H, m, CHCH_2OAllyl), 3.75-3.87 (2H, m, OCH_2CH=CH_2), 4.43 (1H, d, *J* 10.2, C*H*C=C), 4.99-5.12

(2H, m, $OCH_2CH=CH_2$), 5.18 (1H, d, *J*, 17.5, NC*H*(H)Pyr), 5.33 (1H, d, *J* 17.5, NC*H*(H)Pyr), 5.55-5.57 (1H, m, $C=CCH_2CH$), 5.67-5.82 (1H, m, $OCH_2CH=CH_2$), 6.86-6.88 (2H, m, Ar*H*), 7.08-7.09 (3H, m Ar*H*), 7.19-7.20 (2H, m, Ar*H*), 7.47-7.48 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.32 (CH_2), 20.91 (CH_2), 29.36 (CH_2), 30.96 (CH_2), 44.76 (*C*H), 46.72 (CH_2), 50.33 (*C*H), 67.43 (CH_2), 70.61 (CH_2), 106.87 (*C*), 108.74 (*C*H), 115.82 (CH_2), 117.50 (*C*H), 118.77 (*C*H), 121.17 (*C*H), 124.71 (2xCH), 125.94 (*C*), 126.56 (*C*H), 127.89 (2xCH), 132.36 (*C*), 133.68 (*C*H), 136.23 (*C*), 137.29 (*C*), 169.35 (N*C*=O).

MS (CI) *m/z* 401 [M^+, 100%]; (Found: M^+, 401.2224. $C_{25}H_{27}N_3O_2$ requires 401.2224)

(6R,12bS)-12-Benzyl-6-(methoxymethyl)-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine **(55)**



Anhydrous tetrahydrofuran (5 ml) was added to lithium aluminium hydride (0.032 g, 0.855 mmol) in a pre-dried, 3 necked flask under a nitrogen atmosphere and cooled to 0 °C. (6R,12bS)-12-benzyl-6-(methoxymethyl)-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(29)** (0.16 g, 0.427 mmol) in anhydrous tetrahydrofuran (5 ml) was added *via* cannula. The reaction was heated under reflux for 3 hours and then stirred at room temperature overnight.

The reaction was quenched with the addition of diethyl ether (10 ml) and sodium potassium tartrate solution (20 ml) and left to stir for one hour. The organic layer was separated and dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto alumina and purified by flash column chromatography over alumina using 1% methanol in chloroform as the eluent. This yielded a brown oil (0.07 g, 45%).

$[\alpha]_D$ = -22.86(*c* = 0.007, $CHCl_3$); ν_{max} (cm^{-1}) 2924 CH.

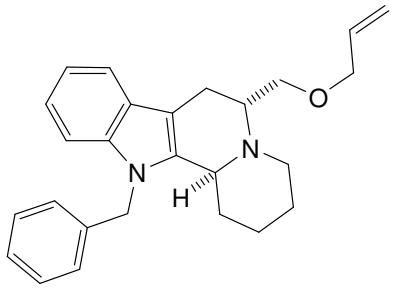
δ_H (300 MHz; $CDCl_3$) 1.32-1.63 (1H, m, CHC*H*(H)CH_2CH_2), 1.32-1.63 (2H, m, $CHCH_2CH_2CH_2CH_2$), 1.69-1.84 (1H, m, CHC*H*(H)CH_2CH_2), 2.27-2.97 (2H, m, $CHCH_2CH_2CH_2CH_2$), 3.10-3.16 (2H, m $CHCH_2CH_2CH_2CH_2$), 3.24 (3H, s, OCH_3), 3.26-3.36 (2H, m, C=CCH_2), 3.51-3.55 (2H, m, CH_2OCH_3), 3.64 (1H, d, *J* 9.6, NC*H*C=C), 5.14 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.26 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.11-5.29 (1H, m,

C=CCH_2C*H*), 6.88-6.91 (2H, m, Ar*H*), 7.00-7.04 (3H, m, Ar*H*), 7.15-7.21 (3H, m, Ar*H*), 7.43-7.47 (1H, m Ar*H*).

δ_C (300 MHz; $CDCl_3$) 23.96 (*C*H_2), 25.12 (*C*H_2), 25.23 (*C*H_2), 30.52 (*C*H_2), 47.49 (*C*H_2), 52.53 (*C*H_2), 55.59 (*C*H), 55.70 (*C*H), 59.02 (*C*H_3), 71.44 (*C*H_2), 107.35 (*C*), 109.64 (*C*H), 118.24 (*C*H), 119.19 (*C*H), 121.23 (*C*H), 125.95 (2xCH), 127.22 (*C*H), 127.52 (*C*), 128.72 (2xCH), 136.60 (*C*), 137.71 (*C*), 137.84 (*C*).

MS (CI) *m/z* 361 [MH^+, 100%]; (Found: MH^+, 361.2279. $C_{24}H_{29}N_2O$ requires 361.2274).

(6R,12bS)-6-(Allyloxymethyl)-12-benzyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine **(56)**



Anhydrous tetrahydrofuran (5 ml) was added to lithium aluminium hydride (0.028 g, 0.750 mmol) in a pre-dried, 3 necked flask under a nitrogen atmosphere and cooled to 0 °C. (6R,12bS)-6-(allyloxymethyl)-12-benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(30)** (0.15 g, 0.375 mmol) in anhydrous tetrahydrofuran (5 ml) was added *via* cannula. The reaction was heated under reflux for 3 hours and then stirred at room temperature overnight.

The reaction was quenched with the addition of diethyl ether (10 ml) and sodium potassium tartrate solution (20 ml) and left to stir for one hour. The organic layer was separated and dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto alumina and purified by flash column chromatography over alumina using 5% ethyl acetate in chloroform as the eluent. This yielded a brown oil (0.017 g, 12%).

$[\alpha]_D$ = -29.41 (*c* = 0.00068, $CHCl_3$); ν_{max} (cm^{-1}) 2920 CH.

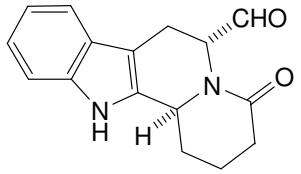
δ_H (300 MHz; $CDCl_3$) 1.33-2.22 (2H, m, CHC*H*$_2$CH$_2$CH$_2$CH$_2$), 1.33-2.22 (2H, m, CHCH$_2$C*H*$_2$CH$_2$CH$_2$), 2.74-3.00 (2H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$), 2.74-3.00 (2H, m, C=CC*H*$_2$), 3.13 (1H, d, *J* 12.3, NC*H*C=C), 3.31-3.36 (2H, m, C*H*$_2$OAllyl), 3.59-3.61 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$), 3.86 (2H, d, *J* 5.4, OC*H*$_2$CH=CH$_2$), 5.06-5.30 (1H, m,

NC*H*CH_2OAllyl), 5.06-5.30 (2H, m, NC*H_2*Ph), 5.06-5.30 (2H, m, $OCH_2CH=CH_2$), 5.73-5.88 (1H, m, $OCH_2CH=CH_2$), 6.90 (2H, d, *J* 7.8, Ar*H*), 7.01-7.04 (3H, m, Ar*H*), 7.15-7.23 (3H, m, Ar*H*), 7.44-7.47 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 23.15 (*C*H_2), 24.09 (*C*H_2), 24.27 (*C*H_2), 29.67 (*C*H_2), 46.45 (*C*H_2), 51.71 (*C*H_2), 54.63 (*C*H), 54.94 (*C*H), 67.83 (*C*H_2), 71.15 (*C*H_2), 106.33 (*C*), 108.58 (*C*H), 115.86 (*C*H_2), 117.17 (*C*H), 118.11 (*C*H), 120.16 (*C*H), 124.88 (2xCH), 126.15 (*C*H), 126.50 (*C*), 127.65 (2xCH), 133.77 (*C*H), 135.55 (*C*), 136.70 (*C*), 136.81 (*C*).

MS (CI) *m/z* 387 [MH^+, 100%]; (Found: MH^+, 387.2434. $C_{26}H_{31}N_2O$ requires 387.2431)

2-((6R,12bS)-4-Oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-6-yl)acetaldehyde **(69)** [3]

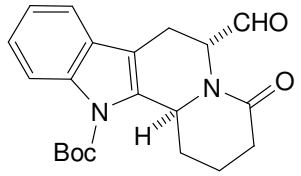


Commercial stabilised IBX 45% (5.00 g, 7.77 mmol) was added to a solution of (6*R*,12b*S*)-6-Hydroxymethyl-2,3,6,7,12,12b-hexahrydro-1Hindolo[2,3-a]quinolizim-4-one **(20)** (1.05 g, 3.89 mmol) in dimethyl sulfoxide (20 ml) under a nitrogen atmosphere. The reaction was stirred at room temperature for 24 hours.

After this time the reaction was poured into water (50 ml), extracted into ethyl acetate (3 x 20 ml) and washed with brine (3 x 20 ml). The combined organic fractions were dried with anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica using ethyl acetate as the eluent. This gave an off white solid (0.83 g, 80%).

δ_H (300 MHz; $CDCl_3$) 1.55-1.68 (1H, m, C*H*(H)CH_2CH_2CO), 1.87-1.96 (2H, m, $CH_2CH_2CH_2CO$), 2.34-2.47 (1H, m, C*H*(H)CH_2CH_2CO), 2.34-2.47 (1H, m, CH_2CH_2CH(H)CO), 2.61-2.68 (1H, m, CH_2CH_2CH(H)CO), 3.02 (1H, ddd, *J* 15.9, 6.6, 2.1, C=CC*H*(H)), 3.35 (1H, d, *J* 15.9, C=CC*H*(H)), 4.82 (1H, d, *J* 11.4, NC*H*C=C), 5.90 (1H, d, *J* 6.3, C=CCH_2CH), 7.03-7.13 (2H, m, Ar*H*), 7.22 (1H, t, *J* 6.3, Ar*H*), 7.47 (1H, d, *J* 7.5, Ar*H*), 8.32 (1H, s br, N*H*), 9.41 (1H, s, C*H*O).

(6R,12bS)-tert-Butyl 4-oxo-6-(2-oxoethyl)-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(70)** [3]

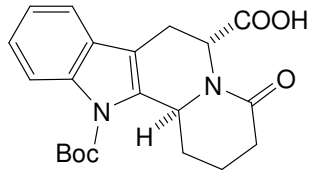


2-((6R,12bS)-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-6-yl)acetaldehyde **(69)** (2.59 g, 9.59 mmol) was dissolved in anhydrous tetrahydrofuran (30 ml) under a nitrogen atmosphere. Triethylamine (2.68 ml, 19.18 mmol), dimethylaminopyridine (0.59 g, 4.80 mmol) and di-tert-butyl dicarbonate (2.72 g, 12.47 mmol) were added in sequence. The reaction was left stirring for 5 hours.

After this time the volatiles were removed and the remaining oil was re-dissolved in ethyl acetate and washed with saturated ammonium chloride solution (2 x 50 ml), saturated sodium bicarbonate solution (2 x 50 ml) and brine (2 x 50 ml). The organic extract was dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified with flash column chromatography over silica using 1:1 ethyl acetate: petroleum ether as the eluent. This gave a yellow oil (2.08 g, 59%).

δ_H (300 MHz; $CDCl_3$) 1.29-1.44 (1H, m C*H*(H)CH_2CH_2CO), 1.50 (9H, s, $OC(CH_3)_3$), 1.87-1.96 (2H, m, C*H*$_2CH_2CO$), 2.39-2.48 (1H, m, CH_2C*H*(H)CO), 2.51-2.57 (1H, m C*H*(H)CH_2CH_2CO), 2.61-2.70 (1H, m, CH_2C*H*(H)CO), 2.86 (1H, ddd, *J* 16.5, 6.0, 2.4, C=CC*H*(H)), 3.29 (1H, d, *J* 16.2, C=CC*H*(H)), 5.18 (1H, dd, *J* 10.5, 1.8, NC*H*C=C), 5.84 (1H, d, *J* 5.1, C=CH_2C*H*), 7.15-7.25 (2H, m, Ar*H*), 7.39 (1H, d, *J* 6.9, Ar*H*), 7.96 (1H, d, *J* 7.8, Ar*H*), 9.43 (1H, s, C*H*O).

2-((6R,12bS)-12-(tert-Butoxycarbonyl)-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-6-yl)acetic acid **(71)** [3]

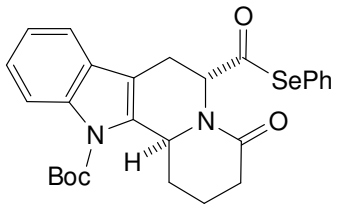


(6R,12bS)-tert-butyl4-oxo-6-(2-oxoethyl)-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(70)** (1.52 g, 4.12 mmol) was dissolved in acetonitrile (18 ml), tert-butyl alcohol (67.50 ml) and cyclohexene (33 ml). The reaction was cooled to 0°C using an ice bath. Sodium chlorite (2.87 g, 31.70 mmol) and sodium dihydrogen phosphate (3.47 g, 28.82 mmol) in water (67.50 ml) were added slowly at 0°C. The reaction was warmed to room temperature and left stirring for 18 hours.

The reaction was extracted into ethyl acetate and washed with 1 M sodium dithionite solution (2 x 50 ml). It was then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This gave a pale yellow foam (1.40 g, 88%).

δ_H (300 MHz; $CDCl_3$) 1.30-1.43 (1H, m C*H*(H)CH_2CH_2CO), 1.59 (9H, s, $OC(CH_3)_3$), 1.81-1.86 (2H, m, C*H*$_2CH_2CO$), 2.34-2.49 (1H, m, CH_2CH(H)CO), 2.53-2.59 (1H, m C*H*(H)CH_2CH_2CO), 2.62-2.65 (1H, m, CH_2CH(H)CO), 2.83 (1H, ddd, *J* 15.9, 6.0, 2.1, C=CC*H*(H)), 3.33 (1H, d, *J* 16.2, C=CC*H*(H)), 5.28 (1H, d, *J* 9, NC*H*C=C), 5.95 (1H, d, *J* 4.5, C=CH_2CH), 7.13-7.24 (2H, m, Ar*H*), 7.36-7.38 (1H, m, Ar*H*), 7.98 (1H, d, *J* 7.8, Ar*H*).

(6R,12bS)-tert-Butyl4-oxo-6-(2-oxo-2-(phenylselanyl)ethyl)-1,2,3,4,6,7 hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(57)** [3]

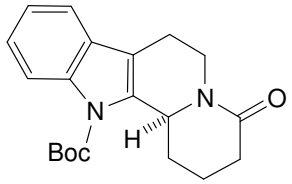


Anhydrous dichloromethane (20 ml) was added to 2-((6R,12bS)-12-(tert-butoxycarbonyl)-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-6-yl)acetic acid **(71)** (1.40 g, 3.64 mmol) under a nitrogen atmosphere. Diphenyl diselenide (1.71 g, 5.46 mmol) was added and the reaction cooled to 0°C. Tributyl phosphine (1.82 ml, 7.28 mmol) was added dropwise and the reaction was warmed to room temperature and left for 24 hours.

Dichloromethane (100 ml) and water (100 ml) were added and the aqueous layer was extracted further with dichloromethane. The combined organic extracts were washed with brine (100 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using ethyl acetate followed by 5% methanol in ethyl acetate to give pale yellow foam (1.06 g, 55%).

δ_H (300 MHz; $CDCl_3$) 1.34-1.47 (1H, m C*H*(H)CH_2CH_2CO), 1.60 (9H, s, $OC(CH_3)_3$), 1.88-2.07 (2H, m, C*H*$_2CH_2CO$), 2.45-2.60 (1H, m, CH_2CH(H)CO), 2.45-2.60 (1H, m C*H*(H)CH_2CH_2CO), 2.68-2.85 (1H, m, CH_2CH(H)CO), 2.68-2.85 (1H, m, C=CC*H*(H)), 3.41 (1H, d, *J* 16.5, C=CC*H*(H)), 5.50 (1H, d, *J* 10.5, NC*H*C=C), 6.08 (1H, d, *J* 4.8, C=CH_2CH), 7.13-7.24 (5H, m, Ar*H*), 7.28-7.32 (2H, m, Ar*H*), 7.36 (1H, d, *J* 7.2, Ar*H*), 7.97 (1H, d, *J* 8.1, Ar*H*).

(S)-tert-Butyl 4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(58a)** [3]

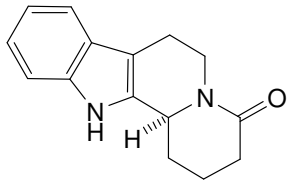


(6R,12bS)-tert-butyl4-oxo-6-(2-oxo-2-(phenylselanyl)ethyl)-1,2,3,4,6,7 hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(57)** (0.4 g, 0.764 mmol) in anhydrous toluene (10 ml) was added *via* cannula to a three necked flask under a nitrogen atmosphere, and flushed with nitrogen for 15 minutes. Tributylin hydride (0.82 ml, 3.06 mmol) was added *via* syringe. The reaction was then heated to 80 °C and azobis*iso*butyronitrile (0.03 g, 0.153 mmol) was added portion wise over two hours. The reaction was left for a further two hours and then cooled to room temperature and the solvent removed under reduced pressure.

The crude product was absorbed onto silica and purified using flash column chromatography over silica eluting with petroleum ether followed by ethyl acetate to give a colourless oil (0.19 g, 72%).

δ_H (300 MHz; $CDCl_3$) 1.25-1.40 (2H, m, C*H*$_2$CH$_2$CH$_2$CO), 1.60 (9H, s, *N*-Boc), 1.75-1.80 (2H, m, C*H*$_2$CH$_2$CO), 2.27-2.39 (1H, m, CH$_2$C*H*(H)CO), 2.48-2.75 (1H, m, CH$_2$C*H*(H)CO), 2.48-2.75 (2H, m, C=CC*H*$_2$), 2.48-2.75 (1H, m, C=CCH$_2$C*H*(H)), 4.98-5.09 (1H, m, C=CCH$_2$C*H*(H)), 4.98-5.09 (1H, m, NC*H*C=C), 7.13-7.23 (2H, m, Ar*H*), 7.34 (1H, d, *J* 7.2, Ar*H*), 7.96 (1H, d, *J* 8.1, Ar*H*).

(S)-1,2,3,6,7,12b-Hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(72a)** [13]



Anhydrous tetrahydrofuran (20 ml) was added to (S)-tert-butyl 4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(58a)** (0.2 g, 0.578 mmol) under a nitrogen atmosphere. Tetrabutylammonium fluoride 1.0 M solution (5.78 ml, 5.78 mmol) was added and the reaction heated under reflux for 9 hours.

After this time the reaction was cooled and water (20 ml) was added. The product was extracted into ethyl acetate (3 x 20 ml) and dried using anhydrous magnesium sulphate, which was then removed by filtration and the solvents were removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography using 5% methanol in ethyl acetate as the eluent. This yielded a white oil (0.11 g, 79%).

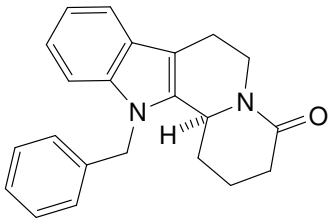
$[\alpha]_D$ = -136.36 (*c* = 0.00022 $CHCl_3$); v_{max} (cm^{-1}) 3233.33 NH, 1609 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.50-1.89 (1H, m, C*H*(H)CH_2CH_2CO), 1.50-1.89 (2H, m, C$H_2$$CH_2CO$), 2.28-2.42 (1H, m, C*H*(H)CH_2CH_2CO), 2.51-2.57 (2H, m, CH_2CH_2CO), 2.67-2.87 (1H, m, C=CCH_2C*H*(H)), 2.67-2.87 (2H, m, C=CCH_2), 4.69-4.74 (1H, m, NC*H*C=C), 5.06-5.16 (1H, m, C=CCH_2C*H*(H)), 7.09 (2H, sid, *J* 14.4, 7.2, 0.9, Ar*H*), 7.27 (1H, d, *J* 7.5, Ar*H*), 7.44 (1H, d, *J* 7.2, Ar*H*), 7.98 (1H, br s, N*H*).

δ_C (300 MHz; $CDCl_3$) 19.39 (*C*H_2), 21.01 (*C*H_2), 29.07 (*C*H_2), 32.42 (*C*H_2), 40.16 (*C*H_2), 54.39 (*C*H), 109.61 (*C*), 110.95 (*C*H), 118.44 (*C*H), 119.86 (*C*H), 122.18 (*C*H), 126.88 (*C*), 133.30 (*C*), 136.19 (*C*), 169.28 (N*C*=O).

MS (CI) *m/z* 241 [MH^+, 100%]; (Found: MH^+, 241.1337. $C_{15}H_{17}N_2O$ requires 241.13335).

(S)-12-Benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(73a)**



(S)-1,2,3,6,7,12b-Hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(72a)** (0.09 g, 0.375 mmol) and sodium hydride (60 % dispersion in mineral oil, 0.023 g, 0.750 mmol) were combined under a nitrogen atmosphere and cooled to 0 °C using an ice bath. Anhydrous dimethylformamide (20 ml) was added and the ice bath removed. The reaction was stirred at room temperature for 30 minutes. After this time benzyl bromide (0.07 ml, 0.750 mmol) was added and the reaction was left for 24 hours.

The reaction was quenched by the addition of ice water (20 ml) and extracted into ethyl acetate (3 x 50 ml). It was then washed with brine (2 x 50 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 5% methanol in ethyl acetate as the eluent to give a yellow oil (0.11 g, 80%).

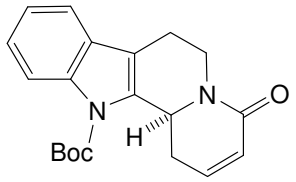
$[\alpha]_D$ = -181.82 (*c* = 0.00077); ν_{max} (cm^{-1}) 2922 CH, 1625 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.42-1.80 (1H, m, C*H*(H)CH_2CH_2CO), 1.42-1.80 (2H, m, CH_2CH_2CO), 2.26-2.37 (1H, m, C*H*(H)CH_2CH_2CO), 2.26-2.37 (1H, m, CH_2C*H*(H)CO), 2.44-2.52 (1H, m, CH_2C*H*(H)CO), 2.59-2.85 (2H, m, C=CCH_2), 2.59-2.85 (1H, m, C=CCH_2C*H*(H)), 4.58-4.62 (1H, m, NC*H*C=C), 5.06-5.12 (1H, m, C=CCH_2C*H*(H)), 5.20 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.30 (1H, d, *J* 17.5, NC*H*(H)Ph), 6.90-6.93 (2H, m, Ar*H*), 7.04-7.09 (3H, m, Ar*H*), 7.16-7.26 (3H, m, Ar*H*), 7.46-7.50 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 18.41 (*C*H_2), 20.52 (*C*H_2), 29.32 (*C*H_2), 31.01 (*C*H_2), 39.17 (*C*H_2), 46.87 (*C*H_2), 53.71 (*C*H), 108.77 (*C*), 110.00 (*C*H), 117.43 (*C*H), 118.83 (*C*H), 121.15 (*C*H), 124.73 (2xCH), 125.51 (*C*), 126.48 (*C*H), 127.87 (2xCH), 133.73 (*C*), 136.19 (*C*), 137.03 (*C*), 168.70 (N*C*=O).

MS (CI) *m/z* 331 [MH^+, 100%]; (Found: MH^+, 331.1809. $C_{22}H_{23}N_2O$ requires 331.1805).

(S)-tert-Butyl 4-oxo-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizine-12(4H)-carboxylate **(74a)** [3]



Di*iso*propylamine (0.72 ml, 5.04 mmol) was added to anhydrous tetrahydrofuran (5 ml) under a nitrogen atmosphere at 0 °C. n-Butyllithium (2.5 M in hexanes, 2.02 ml, 5.04 mmol) was added and the reaction stirred for 15 minutes. This was then cooled to -78 °C. (S)-tert-butyl 4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate) **(58a)** (0.58 g, 1.68 mmol) in anhydrous tetrahydrofuran (10 ml) was added *via* cannula. The reaction was stirred at -78 °C for one hour. Following this, phenyl selenium bromide (0.59 g, 2.52 mmol) in anhydrous tetrahydrofuran (10ml) was added *via* cannula and the reaction was left stirring for 24 hours.

The reaction was then quenched with saturated ammonium chloride solution and extracted into diethyl ether (3 x 20 ml). The organic extracts were combined and washed with saturated ammonium chloride solution (100 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents were removed under reduced pressure. This produced a brown oil, which was used with no further purification.

The crude selenide (1.04 g, 2.10 mmol) was dissolved in methanol (220 ml) and water (45 ml). Sodium metaperiodate (1.03 g, 4.83 mmol) and sodium bicarbonate (0.21 g, 2.52 mmol) were added and the reaction was heated at 60 °C for 24 hours.

The reaction was quenched with saturated sodium bicarbonate solution (100 ml) and ether (150 ml). The organic layer was washed with water (200 ml) followed by brine (200 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents were removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography on silica using ethyl acetate as the eluent to give a yellow oil (0.15 g, 26%).

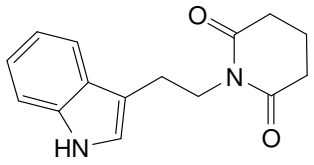
$[\alpha]_D$ = -410.26 (*c* = 0.00078 $CHCl_3$); v_{max} (cm^{-1}) 1725 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.61 (9H, s, $(CH_3)_3$), 2.09 (1H, tt, *J* 17.1, 4.8, 2.7, C*H*(H)CH=CHCO), 2.62-2.84 (1H, m, C=CCH_2C*H*(H)), 2.62-2.84 (2H, m, C=CC*H*$_2$), 2.95 (1H, dq, *J* 17.4, 3.9, (C*H*(H)CH=CHCO), 4.91-4.97 (1H, m, C=CCH_2C*H*(H)), 5.16-5.21 (1H, m, NC*H*C=C), 6.01 (1H, dd, *J* 9.9, 3, CH_2CH=C*H*CO), 6.58-6.64 (1H, m, CH_2C*H*=CHCO), 7.19-7.27 (2H, m, Ar*H*), 7.40 (1H, d, *J* 8.4, Ar*H*), 8.00 (1H, d, *J* 8.1, Ar*H*).

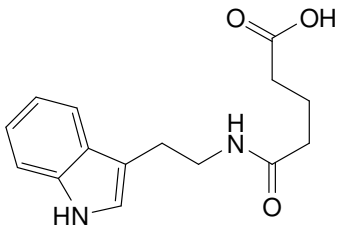
δ_C (300 MHz; $CDCl_3$) 20.52 (*C*H_2), 27.20 (*C*H_3)$_3$, 30.61 (*C*H_2), 36.50 (*C*H_2), 52.27 (*C*H), 83.51 (*C*), 114.77 (*C*H), 116.98 (*C*), 117.37 (*C*H), 122.08 (*C*H), 123.71 (*C*H), 124.41 (*C*H), 127.50 (*C*), 133.09 (*C*), 135.53 (*C*), 138.10 (*C*H), 149.01 (*C*), 163.81 (N*C*=O).

3.4 Racemic compounds

1-(2-(1H-Indol-3-yl)ethyl)piperidine-2,6-dione **(81)**



Anhydrous tetrahydrofuran (10 ml) was added to tryptamine **(79)** (1.0 g, 6.24 mmol) and glutaric anhydride **(80)** (0.71 g, 6.24 mmol) under a nitrogen atmosphere. Triethylamine (1 ml, 6.55 mmol) was added and the mixture was heated under reflux for 18 hours. After this time the volatiles were removed under reduced pressure to yield a brown oil. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 2:1 ethyl acetate: petroleum ether as the eluent. A compound was isolated, and then re-crystallised from chloroform to give and off white solid. This was the undesired compound shown below **(82)**.



Mp: 141- 142 °C; v_{max} (cm^{-1}) 1634 C=O, 1687 C=O, 3054 OH, 3317 NH, 3390 NH.

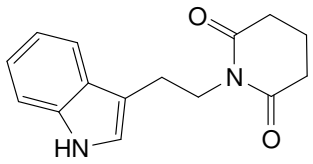
δ_H (300 MHz; DMSO) 1.72 (2H, qu, *J* 7.2, NHCOCH$_2$C*H$_2$*), 2.09 (2H, t, *J* 6.9, NHCOC*H$_2$*), 2.20 (2H, t, *J* 7.2, C*H$_2$*COOH), 2.80 (2H, t, *J* 7.2, NHCH=CC*H$_2$*), 3.27-3.34 (2H, m, NCH=CCH$_2$C*H$_2$*), 6.97 (1H, t, *J* 7.2, Ar*H*), 7.06 (1H, t, *J* 7.8, Ar*H*), 7.13 (1H, s, NHC*H*=C),

7.33 (1H, d, *J* 7.8, Ar*H*), 7.52 (1H, d, *J* 7.8, Ar*H*), 7.95 (1H, s, N*H*CO), 10.82 (1H, br s, N*H*).

δ_C (300 MHz; DMSO) 20.65 (*C*H_2), 25.24 (*C*H_2), 33.03 (*C*H_2), 34.45 (*C*H_2), 39.37 (*C*H_2), 111.31 (CH), 111.80 (C), 118.15 (CH), 118.20 (CH), 120.85 (CH), 122.53 (CH), 127.16 (C), 136.15 (C), 171.40 (C=O), 174.22 (NHC=O).

This was converted into the desired compound by heating at 200 °C for 24 hours with no solvent.

1-(2-(1H-Indol-3-yl)ethyl)piperidine-2,6-dione **(81)**



Tryptamine **(79)** (20 g, 124.83 mmol) and glutaric anhydride **(80)** (14.24 g, 124.83 mmol) were combined and heated at 200 °C for 24 hours. The brown oil formed was absorbed onto silica and purified by flash column chromatography over silica with 3:2 ethyl acetate: petroleum ether as the eluent. This yielded a burnt orange solid (21.21 g, 66%).

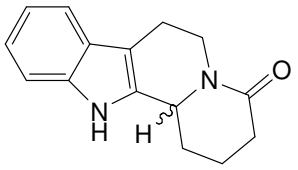
Mp: 171-172 °C; ν_{max} (cm^{-1}) 3324 NH, 1661 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.80 (2H, qu, *J* 6.6, $NCOCH_2CH_2$), 2.54 (4H, t, *J* 6.6, $NCOCH_2$), 2.91 (2H, t, *J* 7.8, CH_2CH_2N), 4.00 (2H, t, *J* 8.1, CH_2CH_2N), 6.98 (1H, d, *J* 1.8, NHCH=C), 7.09 (2H, qu, *J* 7.2, ArH), 7.27 (1H, d, *J* 7.5, ArH), 7.71 (1H, d, *J* 7.5, ArH), 8.01 (1H, br s, NH).

δ_C (300 MHz; $CDCl_3$) 16.08 (CH_2), 22.68 (CH_2), 31.81 (2xCH_2), 39.29 (CH_2), 110.03 (CH), 111.79 (C), 118.07 (CH), 118.36 (CH), 120.93 (CH), 121.12 (CH), 126.56 (C), 135.07 (C), 171.56 (2xNC=O).

MS (CI) *m/z* 257 [MH^+, 100%]; (Found: MH^+, 257.1289. $C_{15}H_{17}N_2O_2$ requires 257.1285).

1,2,3,6,7,12b-Hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(72a, b)**



1-(2-(1H-indol-3-yl)ethyl)piperidine-2,6-dione **(81)** (2 g, 7.81 mmol) was dissolved in absolute ethanol and cooled to 0 °C with an ice bath. Sodium borohydride (2.9 g, 78.1 mmol) was added, followed by 2M hydrochloric acid in absolute ethanol (3.9 ml), added portion wise over a three hour period *via* syringe. After this time the solution was acidified to pH 1-3 with 2M hydrochloric acid in absolute ethanol over a 15 minute period, the ice bath was removed and the reaction was left stirring at room temperature overnight.

Saturated sodium bicarbonate solution (200 ml) was used to quench the reaction which was then extracted into ethyl acetate (2 x 100 ml) and washed with brine (2 x 100 ml). It was then dried over anhydrous magnesium sulphate, which was removed by filtration and the solvent was removed under reduced pressure. The crude product was heated in absolute ethanol, cooled to 0 °C overnight and filtered to give an off white solid (1.27 g, 68%). A small portion of this was re-crystallised to give white crystals.

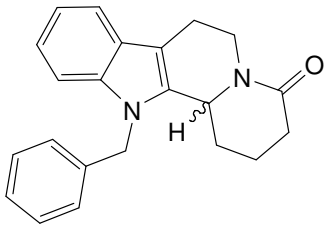
Mp: 245-247 °C; v_{max} (cm^{-1}) 3259 NH, 1592 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.74-2.04 (1H, m, C*H*(H)CH_2CH_2CO), 1.74-2.04 (2H, m, C*H*$_2CH_2CO$), 2.37-2.51 (1H, m, C*H*(H)CH_2CH_2CO), 2.37-2.51 (1H, m, CH_2C*H*(H)CO), 2.59-2.65 (1H, m, CH_2C*H*(H)CO), 2.76-2.90 (1H, m, C=CCH_2C*H*(H)), 2.76-2.90 (2H, m, C=CC*H*$_2$), 4.80-4.83 (1H, m, NC*H*C=C), 5.15-5.26 (1H, m, C=CCH_2C*H*(H)), 7.12-7.23 (2H, m, Ar*H*), 7.37 (1H, d, *J* 8.1, Ar*H*), 7.53 (1H, d, *J* 7.5, Ar*H*), 8.15 (1H, br s, N*H*).

δ_C (300 MHz; $CDCl_3$) 19.41 (*C*H_2), 21.01 (*C*H_2), 29.07 (*C*H_2), 32.44 (*C*H_2), 40.14 (*C*H_2), 54.39 (*C*H), 109.58 (*C*), 110.95 (*C*H), 118.43 (*C*H), 119.84 (*C*H), 122.16 (*C*H), 126.87 (*C*), 133.33 (*C*), 136.18 (*C*), 169.24 (N*C*=O).

MS (CI) *m/z* 241 [MH^+, 100%]; (Found: MH^+, 241.1338. $C_{15}H_{17}N_2O$ requires 241.1335).

12-Benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(73a, b)**



1,2,3,6,7,12b-Hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(72a, b)** (1.27 g, 5.29 mmol) and sodium hydride (60 % dispersion in mineral oil, 0.42 g, 10.58 mmol) were combined under a nitrogen atmosphere and cooled to 0 °C. Dimethylformamide (40 ml) was added and the solution stirred at room temperature for 30 minutes. After this time benzyl bromide (1.27 ml, 10 .58 mmol) was added and the solution was left stirring for 24 hours.

The reaction was quenched with the addition of ice cold water (30 ml) and extracted into ethyl acetate (3 x 50 ml), then washed with brine (2 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by column chromatography over silica using 5% methanol in ethyl acetate as the eluent. This gave an off white solid (1.02 g, 58%).

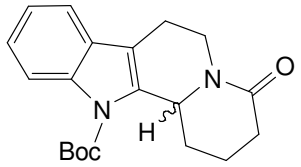
Mp: 188-190 °C; v_{max} (cm^{-1})1636 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.26-1.60 (1H, m, C*H*(H)CH_2CH_2CO), 1.26-1.60 (2H, m, C$H_2$$CH_2CO$), 2.10-2.22 (1H, m, C*H*(H)CH_2CH_2CO), 2.10-2.22 (1H, m, C*H*(H)CO), 2.30-2.37 (1H, m, C*H*(H)CO), 2.46-2.73 (2H, m, C=CCH_2), 2.46-2.73 (1H, m, C=CCH_2CH(*H*)), 4.45 (1H, d, *J* 8.7, NC*H*C=C), 5.00 (1H, dd, *J* 9.9, 2.7, C=CCH_2CH(*H*)), 5.30 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.40 (1H, d, *J* 17.5, NC*H*(H)Ph), 6.79 (2H, d, *J* 6.3, Ar*H*), 6.93-6.96 (3H, m, Ar*H*), 7.04-7.13 (3H, m, Ar*H*), 7.35-7.38 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 19.45 (*C*H_2), 21.68 (*C*H_2), 30.56 (*C*H_2), 32.16 (*C*H_2), 40.24 (*C*H_2), 47.89 (*C*H_2), 54.76 (*C*H), 109.94 (*C*H), 110.90 (*C*), 118.53 (*C*H), 119.91 (*C*H), 122.23 (*C*H), 125.82 (2x*C*H), 126.66 (*C*), 127.5665 (*C*H), 128.97 (2x*C*H), 134.97 (*C*), 137.39 (*C*), 138.14 (*C*), 169.65 (N*C*=O).

MS (CI) *m/z* 331 [MH^+, 100%]; (Found: MH^+, 331.1810. $C_{22}H_{23}N_2O$ requires 331.1805).

tert-Butyl 4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(58a, b)**



1,2,3,6,7,12b-Hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(72a, b)** (3.79 g, 15.77 mmol) was dissolved in anhydrous tetrahydrofuran (100 ml) under a nitrogen atmosphere. Triethylamine (9.29 ml, 66.58 mmol), dimethylaminopyridine (0.5 g, 8.32 mmol) and di-*tert*-butyl dicarbonate (7.27 g, 33.29 mmol) were subsequently added.

After three hours, thin layer chromatography was used to determine that the reaction had completed and the solvents were removed under reduced pressure to give a brown oil. This was dissolved in ethyl acetate (50 ml) and washed with saturated ammonium chloride solution (2 x 100 ml), saturated sodium bicarbonate solution (2 x 100 ml) and brine (100 ml). The organic layer was dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude oil was absorbed onto silica and purified by column chromatography over silica using ethyl acetate as the eluent. This yielded a white solid (2.98 g, 55%).

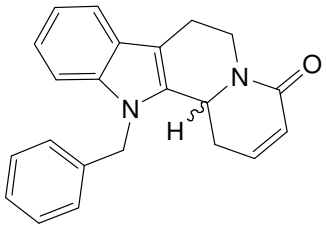
Mp: 153-154 °C; ν_{max} (cm^{-1}) 1639 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.26-1.43 (1H, m, C(H)*H*CH_2CH_2CO), 1.62 (9H, s, $NCOOC(CH_3)_3$), 1.74-1.83 (2H, m, C*H*$_2CH_2CO$), 2.27-2.39 (1H, m, CH_2CH(H)CO), 2.49-2.74 (1H, m, CH_2CH(H)CO), 2.49-2.74 (1H, m, C(H)*H*CH_2CH_2CO), 2.49-2.74 (2H, m, C=CC*H*$_2$), 2.49-2.74 (1H, m, C=CCH_2C*H*(H)), 4.99-5.08 (1H, m, C=CCH_2C*H*(H)), 4.99-5.08 (1H, m, NC*H*C=C), 7.19 (2H, si, *J* 6.3, Ar*H*), 7.33 (1H, d, *J* 7.2, Ar*H*), 8.00 (1H, d, *J* 8.4, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 19.49 (*C*H_2), 21.59 (*C*H_2), 28.14 (*C*H_3)$_3$, 30.15 (*C*H_2), 32.16 (*C*H_2), 38.80 (*C*H_2), 56.00 (*C*H), 84.16 (*C*), 115.45 (*C*H), 118.23 (*C*H), 118.26 (*C*H), 122.95 (*C*H), 124.55 (*C*H), 128.59 (*C*), 135.23 (*C*), 136.74 (*C*), 151.46 (*C*), 169.40 (N*C*=O).

MS (CI) *m/z* 341 [MH^+, 100%]; (Found: MH^+, 341.1864. $C_{20}H_{25}N_2O_3$ requires 341.1860).

12-Benzyl-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizin-4(12H)-one **(100a, b)**



Di*iso*propylamine (1.63 ml, 11.56 mmol) was added to anhydrous tetrahydrofuran (10 ml) under a nitrogen atmosphere at 0 °C. n-Butyllithium (2.5 M in hexanes, 4.62 ml, 11.56 mmol) was added and the reaction stirred for 15 minutes. After this time the reaction was cooled to -78 °C and 12-benzyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(73a, b)** (1.91 g, 5.78 mmol) dissolved in anhydrous tetrahydrofuran (30 ml) was added *via* cannula. The reaction was left to stir for one hour upon which phenyl selenium bromide (2.05 g, 8.67 mmol) dissolved in anhydrous tetrahydrofuran (20 ml) was added *via* cannula. The reaction was left to warm slowly to room temperature overnight.

Saturated ammonium chloride solution (50 ml) was used to quench the reaction. It was then extracted into diethyl ether (2 x 50 ml), washed with saturated ammonium chloride solution (2 x 50ml), and dried over anhydrous magnesium sulphate which was then removed by filtration. The solvents were removed under reduced pressure to give a brown oil which was used with no further purification.

The crude selenide (2.68 g, 5.52 mmol) was dissolved in methanol (220 ml) and water (45 ml). Sodium metaperiodite (5.43 g, 25.39 mmol) and sodium bicarbonate (1.11 g, 13.25 mmol) were added and the reaction was heated to 60 °C and left overnight.

The reaction was poured into saturated sodium bicarbonate solution (100 ml) and diethyl ether (150). The organic layer was washed with water (100 ml) and brine (100 ml), then dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure.

The crude product was absorbed onto silica and purified by flash column chromatography over silica with ethyl acetate as the eluent. This yielded a brown solid (1.05 g, 58%).

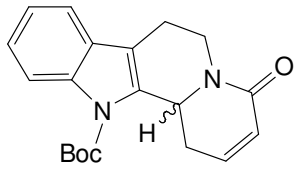
Mp: 121-124 °C; ν_{max} (cm^{-1}) 1658 NC=O.

δ_H (300 MHz; $CDCl_3$) 2.02-2.16 (1H, m, C*H*(H)CH_2CH_2CO), 2.46 (1H, dt, *J* 7.4, 4.8, C*H*(H)CH_2CH_2CO), 2.60-2.83 (2H, m, C=CCH_2), 2.60-2.83 (1H, m, C=CCH_2C*H*(H)), 4.68 (1H, dd, *J* 13.5, 3, NC*H*C=C), 4.87-4.94 (1H, m, C=CCH_2C*H*(H)), 5.10-5.14 (2H, m, NCH_2Ph), 5.92 (1H, dd, *J* 9.6, 2.1, CH_2C*H*CHCO), 6.35-6.40 (1H, m, CH_2CHC*H*CO), 6.84 (2H, d, *J* 6.9, Ar*H*), 7.00-7.04 (3H, m, Ar*H*), 7.13-7.17 (3H, m, Ar*H*), 7.44-7.47 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.51 (*C*H_2), 32.17 (*C*H_2), 38.76 (*C*H_2), 47.42 (*C*H_2), 51.68 (*C*H), 110.03 (*C*H), 110.65 (*C*), 118.65 (*C*H), 120.00 (*C*H), 122.37 (*C*H), 125.66 (*C*H), 125.77 (2xCH), 126.48 (*C*), 127.65 (*C*H), 129.02 (2xCH), 133.82 (*C*), 137.15 (*C*), 137.94 (*C*), 138.48 (*C*H), 164.93 (N*C*=O).

MS (CI) *m/z* 329 [MH^+, 100%]; (Found: MH^+, 329.1653. $C_{22}H_{21}N_2O$ requires 329.1648).

tert-Butyl 4-oxo-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizine-12(4H)-carboxylate **(74a, b)**



Di*iso*propylamine (2.49 ml, 17.60 mmol) was added to anhydrous tetrahydrofuran (20 ml) under a nitrogen atmosphere at 0 °C. n-Butyllithium (2.5 M in hexanes, 7.04 ml, 17.60 mmol) was added and the reaction stirred for 15 minutes. After this time the reaction was cooled to -78 °C and tert-butyl 4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(58a, b)** (2.00 g, 5.88 mmol) dissolved in anhydrous tetrahydrofuran (40 ml) was added *via* cannula. The reaction was left to stir for one hour upon which phenyl selenium bromide (2.78 g, 11.76 mmol) dissolved in anhydrous tetrahydrofuran (20 ml) was added *via* cannula. The reaction was left slowly to warm to room temperature overnight.

Saturated ammonium chloride solution (50 ml) was used to quench the reaction. It was then extracted into diethyl ether (2 x 100 ml), washed with saturated ammonium chloride solution (2 x 100 ml) and dried over anhydrous magnesium sulphate which was then removed by filtration. The solvents were removed under reduced pressure to give a brown oil which was used with no further purification.

The crude selenide (3.71 g, 7.49 mmol) was dissolved in methanol (220 ml) and water (45 ml). Sodium metaperiodite (7.36 g, 34.44 mmol) and sodium bicarbonate (1.51 g, 17.97 mmol) were added and the reaction was heated to 60 °C and left overnight.

The reaction was poured into saturated sodium bicarbonate solution (100 ml) and diethyl ether (150). The organic layer was washed with water (100 ml) and brine (100 ml), then

dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure.

The crude product was absorbed onto silica and purified by flash column chromatography over silica with 3:2 ethyl acetate: petroleum ether as the eluent. This yielded a yellow solid (0.55 g, 37%).

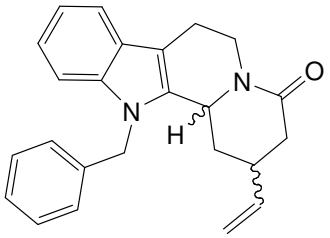
Mp: 187-189 °C; ν_{max} (cm^{-1}) 1662 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.60 (9H, s, NCOOC(CH_3)$_3$), 2.01-2.12 (1H, m, C*H*(H)CH_2CH_2CO), 2.64-2.78 (2H, m, C=CCH_2), 2.64-2.78 (1H, m, C=CCH_2C*H*(H)), 2.94 (1H, dq, *J* 17.1, 9.9, 3.6, C*H*(H)CH_2CH_2CO), 4.89-4.95 (1H, m, C=CCH_2C*H*(H)), 5.14-5.18 (1H, m *J* 12.3, NC*H*C=C), 5.99 (1H, dd, *J* 9.6, 2.7, CH_2C*H*CHCO), 6.56-6.62 (1H, m, CH_2CHC*H*CO), 7.15-7.25 (2H, m, Ar*H*), 7.37 (1H, d, *J* 7.5, Ar*H*), 7.99 (1H, d, *J* 8.1, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.56 (*C*H_2), 28.25 (*C*H_3)$_3$, 31.66 (*C*H_2), 37.54 (*C*H_2), 53.31 (*C*H), 84.55 (*C*), 115.82 (*C*H), 118.01 (*C*), 118.42 (*C*), 123.13 (*C*H), 124.77 (*C*H), 125.45 (*C*H), 128.53 (*C*), 134.12 (*C*), 136.57 (*C*), 139.17 (*C*H), 150.04 (*C*), 164.83 (N*C*=O).

MS (CI) *m/z* 339 [MH^+, 100%]; (Found: MH^+, 339.1708. $C_{20}H_{23}N_2O_3$ requires 339.1703).

12-Benzyl-2-vinyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(101a, b)**



A 1.0 M solution of vinyl magnesium bromide in tetrahydrofuran (15.20 ml, 15.20 mmol) was added to a suspension of copper cyanide (0.68 g, 7.61 mmol) in anhydrous tetrahydrofuran (20 ml) at -78 °C while stirring. The reaction was warmed to 0 °C for 3 minutes then re-cooled to -78 °C. 12-benzyl-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizin-4(12H)-one **(100a, b)** (0.50 g, 1.52 mmol) in anhydrous tetrahydrofuran (30 ml) was added *via* cannula at -78 °C. After 5 minutes trimethylsily chloride (1.00 ml, 7.61 mmol) was added and the reaction left to warm to room temperature overnight.

The reaction as quenched with the addition of saturated ammonium chloride solution (20 ml) and water (20 ml) and left stirring for 20 minutes. A 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (6 ml) was added and the stirring continued for 15 minutes. The reaction was extracted into ethyl acetate (3 x 50 ml) and the combined organic extracts were dried over anhydrous magnesium sulphate. This was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica with 2:1 ethyl acetate: petroleum ether as the eluent. This gave a yellow oil (0.11 g, 20%).

ν_{max} (cm^{-1}) 1662 NC=O.

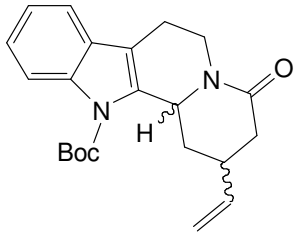
δ_H (300 MHz; $CDCl_3$) 1.76-1.85 (1H, m, C*H*(H)CHCH$_2$CO), 2.07-2.14 (1H, m, C*H*(H)CHCH$_2$CO), 2.43-2.85 (2H, m, CH$_2$CHC*H*$_2$CO), 2.43-2.85 (1H, m, CH$_2$C*H*CH$_2$CO),

2.43-2.85 (2H, m, C=CC*H*$_2$), 2.43-2.85 (1H, m, C=CCH$_2$C*H*(H)), 4.63-4.66 (1H, m, NC*H*C=C), 4.90-5.05 (1H, m, C=CCH$_2$C*H*(H)), 4.90-5.05 (2H, m, CHCH=C*H*$_2$), 5.16 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.25 (1H, d, *J* 17.5, NC*H*(H)Ph), 5.60-5.73 (1H, m, CHC*H*=CH$_2$), 6.90-6.93 (2H, m, Ar*H*), 7.02-7.07 (3H, m, Ar*H*), 7.16-7.25 (3H, m, Ar*H*), 7.45-7.48 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.57 (*C*H$_2$), 32.54 (*C*H), 34.42 (*C*H$_2$), 35.88 (*C*H$_2$), 40.55 (*C*H$_2$), 47.99 (*C*H$_2$), 51.14 (*C*H), 109.77 (*C*H), 111.44 (*C*), 115.70 (*C*H$_2$), 118.40 (*C*H), 119.90 (*C*H), 122.23 (*C*H), 125.83 (2xCH), 126.60 (*C*), 127.60 (*C*H), 128.99 (2xCH), 134.53 (*C*), 137.22 (*C*), 138.16 (*C*), 138.91 (*C*H), 169.33 (N*C*=O).

MS (CI) *m/z* 357 [MH^+, 100%]; (Found: MH^+, 357.1962. $C_{24}H_{25}N_2O$ requires 357.1961).

tert-Butyl 4-oxo-2-vinyl-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(102a, b)**



A 1.0 M solution of vinyl magnesium bromide in tetrahydrofuran (12.10 ml, 12.10 mmol) was added to a suspension of copper cyanide (0.54 g, 6.06 mmol) in anhydrous tetrahydrofuran (20 ml) at -78 °C while stirring. The reaction was warmed to 0 °C for 3 minutes then re-cooled to -78 °C. tert-butyl 4-oxo-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizine-12(4H)-carboxylate **(74a, b)** (0.41 g, 1.21 mmol) in anhydrous tetrahydrofuran (30 ml) was added *via* cannula at -78 °C. After 5 minutes trimethylsily chloride (0.77 ml, 6.06 mmol) was added and the reaction was left to warm to room temperature overnight.

The reaction as quenched with the addition of saturated ammonium chloride solution (20 ml) and water (20 ml) and left stirring for 20 minutes. A 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (6 ml) was added and the stirring continued for 15 minutes. The reaction was extracted into ethyl acetate (3 x 50 ml) and the combined organic extracts were dried over anhydrous magnesium sulphate. This was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica with 2:1 ethyl acetate: petroleum ether as the eluent. This gave a yellow oil (0.20 g, 45%).

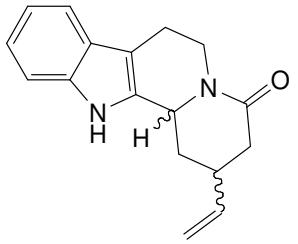
ν_{max} (cm^{-1}) 1639 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.61-1.74 (2H, m, C*H*$_2$CHCH$_2$CO), 1.61 (9H, s, NCOOC(C*H*$_3$)$_3$), 2.42-2.51 (1H, m, CH$_2$C*H*CH$_2$CO), 2.42-2.51 (1H, m, CH$_2$CHC*H*(H)CO), 2.57-2.78 (1H, m, CH$_2$CHC*H*(H)CO), 2.57-2.78 (1H, m, C=CCH$_2$C*H*(H)), 2.57-2.78 (2H, m, C=CC*H*$_2$), 4.98-5.14 (1H, m, NC*H*C=C), 4.98-5.14 (1H, m, C=CCH$_2$C*H*(H)), 4.98-5.14 (2H, m, CHCH=C*H*$_2$), 5.88-5.99 (1H, m, CHC*H*=CH$_2$), 7.14-7.25 (2H, m, Ar*H*), 7.34-7.37 (1H, m, Ar*H*), 7.91-7.94 (1H, m, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.67 (*C*H$_2$), 28.22 (*C*H$_3$)$_3$, 32.88 (*C*H), 33.98 (*C*H$_2$), 36.18 (*C*H$_2$), 39.26 (*C*H$_2$), 52.43 (*C*H), 84.41 (*C*), 115.40 (*C*H), 115.49 (*C*H$_2$), 118.26 (*C*H), 118.65 (*C*), 122.98 (*C*H), 124.55 (*C*H), 128.71 (*C*), 135.55 (*C*), 136.75 (*C*), 139.25 (*C*H), 150.28 (*C*), 169.33 (N*C*=O).

MS (CI) *m/z* 367 [MH$^+$, 100%]; (Found: MH$^+$, 367.2021. $C_{22}H_{27}N_2O_3$ requires 367.2016).

2-Vinyl-1,2,3,6,7,12b-hexahydroindolo[2,3-a]quinolizin-4(12H)-one **(103a, b)**



tert-Butyl 4-oxo-2-vinyl-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(102a, b)** (0.12 g, 0.327 mmol) was dissolved in formic acid (4.80 ml, 127.11 mmol) under a nitrogen atmosphere and stirred at room temperature for 24 hours.

After this time the solvent was evaporated and shaken with 10 % aqueous sodium carbonate solution then extracted into dichloromethane, dried using magnesium sulphate, which was then removed by filtration and the solvent was removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica eluting with 1:1 ethyl acetate: petroleum ether as the eluent to give a brown oil (0.04 g, 46%).

v_{max} (cm^{-1}) 1615 NC=O, 3271 NH.

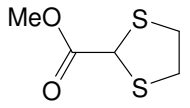
δ_H (300 MHz; $CDCl_3$) 2.09-2.18 (1H, m, C*H*(H)CHCH$_2$CO), 2.26-2.35 (1H, m, C*H*(H)CHCH$_2$CO), 2.55 (2H, t, *J* 6.9, CH$_2$CHC*H$_2$*CO), 2.70-2.83 (1H, m, CH$_2$C*H*CH$_2$CO), 2.70-2.83 (1H, m, C=CCH$_2$C*H*(H)), 2.86-2.99 (2H, m, C=CC*H$_2$*), 4.84-4.89 (1H, m, NC*H*C=C), 5.09-5.18 (1H, m, C=CCH$_2$C*H*(H)), 5.09-5.18 (2H, m, CHCH=C*H$_2$*), 5.92 (1H, se, *J* 5.7, CHC*H*=CH$_2$), 7.15 (2H, sd, *J* 18, 14.4, 7.2, 1.2, Ar*H*), 7.34 (1H, d, *J* 7.5, Ar*H*), 7.50 (1H, d, *J* 7.5, Ar*H*), 8.36 (1H, s br, N*H*).

δ_C (300 MHz; $CDCl_3$) 21.05 (*C*H_2), 32.86 (*C*H_2), 32.96 (*C*H), 36.63 (*C*H_2), 40.97 (*C*H_2), 51.77 (*C*H), 110.03 (*C*), 111.04 (*C*H), 115.75 (*C*H_2), 118.30 (*C*H), 119.80 (*C*H), 122.10 (*C*H), 127.03 (*C*), 133.18 (*C*), 136.13 (*C*), 138.93 (*C*H), 168.83 (N*C*=O).

MS (CI) *m/z* 267 [MH^+, 100%]; (Found: MH^+, 267.1496. $C_{17}H_{19}N_2O$ requires 267.1492).

3.5 Towards a bis-indole compound

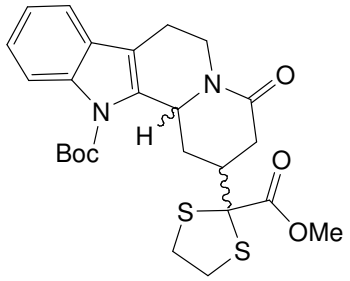
Methyl 1,3-dithiolane-2-carboxylate **(106)** [3]



Sodium hydroxide (0.08 g, 2.0 mmol), was dissolved in methanol (125 ml) and treated with ethyl-1,3-dithiolane carboxylate **(105)** (5 g, 28.2 mmol). The reaction was heated under reflux for 30 minutes with the exclusion of light. The solvent was removed under reduced pressure and the remaining liquid was dissolved in diethyl ether and washed with brine (30 ml). The ether layer was dried over anhydrous magnesium sulphate, which was then removed by filtration and the ether was removed under reduced pressure. This gave a yellow liquid (3.6 g, 78%).

δ_H (300 MHz; $CDCl_3$) 3.13-3.23 (2H, m, SCH_2), 3.26-3.34 (2H, m, SCH_2), 3.59 (3H, s, OCH_3), 4.71 (1H, s, CH_2SCH).

tert-Butyl 2-(2-(methoxycarbonyl)-1,3-dithiolan-2-yl)-4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(107a, b)**



Di*iso*propylamine (0.5 ml, 3.54 mmol) was dissolved in anhydrous tetrahydrofuran (20 ml) under a nitrogen atmosphere. After cooling to 0 °C, n-butyllithium (2.5 M in hexanes, 1.42 ml, 3.54 mmol) was added and the reaction stirred at 0 °C for 15 minutes. It was then re-cooled to -78 °C and methyl, 1-2 dithiolane carboxylate **(106)** (0.21 g, 1.18 mmol) in 20 ml of anhydrous tetrahydrofuran was added *via* cannula. The reaction was left stirring for a further 15 minutes and then tert-butyl 4-oxo-1,6,7,12b-tetrahydroindolo[2,3-a]quinolizine-12(4H)-carboxylate **(74a, b)** (0.2g, 0.591 mmol) in anhydrous tetrahydrofuran (40 ml) was added also *via* cannula. The reaction was then left to warm slowly to room temperature overnight.

The reaction was quenched with the addition of water (25 ml) and extracted into ethyl acetate (3 x 50 ml), dried over anhydrous magnesium sulphate, which was then removed by filtration and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 3:1 petroleum ether: ethyl acetate as the eluent. This yielded a brown oil (0.16 g, 54%).

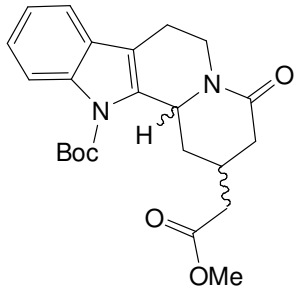
ν_{max} (cm^{-1}) 1635 NC=O.

δ_H (300 MHz; $CDCl_3$) 1.34 (1H, qu, *J* 6.9, C*H*(H)CHCH$_2$CO), 1.63 (9H, s, NCOOC(C*H*$_3$)$_3$), 2.47 (1H, dd, *J* 17.4, 11.7, CH$_2$CHC*H*(H)CO), 2.64-2.65 (1H, m, C=CC*H*(H)), 2.72-2.77 (1H, m, C=CC*H*(H)), 2.80-2.84 (1H, m, C*H*(H)CHCH$_2$CO), 2.80-2.84 (1H, m, C=CCH$_2$C*H*(H)), 2.87-2.96 (1H, m, CH$_2$CHC*H*(H)CO), 2.87-2.96 (1H, m, CH$_2$C*H*CH$_2$CO), 3.18-3.26 (2H, m, SC*H*$_2$), 3.28-3.36 (2H, m, SC*H*$_2$), 3.74 (3H, s, COOC*H*$_3$), 5.03-5.10 (1H, m, C=CCH$_2$C*H*(H)), 5.03-5.10 (1H, m, NC*H*C=C), 7.15-7.27 (2H, m, Ar*H*), 7.36-7.38 (1H, m, Ar*H*), 7.96 (1H. d, *J* 7.8, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.67 (*C*H$_2$), 28.13 (*C*H$_3$)$_3$, 33.77 (*C*H$_2$), 35.73 (*C*H$_2$), 38.39 (*C*H), 39.05 (*C*H$_2$), 40.03 (*C*H$_2$), 40.32 (*C*H$_2$), 53.57 (*C*H$_3$), 55.44 (*C*H), 74.09 (*C*), 84.57 (*C*), 115.52 (*C*H), 118.39 (*C*H), 118.68 (*C*), 123.08 (*C*H), 124.77 (*C*H) 128.55 (*C*), 135.66 (*C*), 136.82 (*C*), 150.29 (*C*), 168.67 (*C*), 171.90 (N*C*=O).

MS (CI) *m/z* 503 [MH$^+$, 100%]; (Found: MH$^+$, 503.1665. $C_{25}H_{31}N_2O_5S_2$ requires 503.1669).

tert-Butyl 2-(2-methoxy-2-oxoethyl)-4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(108a, b)**



tert-Butyl2-(2-(methoxycarbonyl)-1,3-dithiolan-2-yl)-4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(107a, b)** (0.06 g, 0.12 mmol) was dissolved in a 1:3 mixture of tetrahydrofuran: methanol (8 ml). The solution was cooled using an ice bath and nickel chloride hexahydrate (0.29 g, 1.20 mmol) was added. When this had completed dissolved, sodium borohydride (0.14 g, 3.60 mmol) was added cautiously. The reaction was then left to stir at room temperature for 4 hours.

After this time it was filtered through celite, concentrated, extracted into ethyl acetate (3 x 20 ml) and washed with brine (40 ml). It was then dried over anhydrous magnesium sulphate, which was removed by filtration and the solvents were removed under reduced pressure. The crude product was absorbed onto silica and purified by flash column chromatography over silica using 1:1 ethyl acetate: petroleum ether as the eluent. This yielded a white oil (0.03g, 61%).

v_{max} (cm^{-1}) 1644 NC=O.

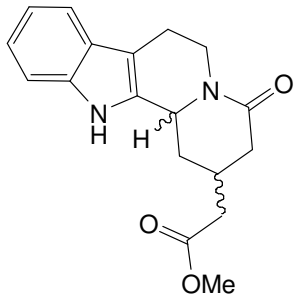
δ_H (300 MHz; $CDCl_3$) 1.17-1.29 (1H, m, C*H*(H)CHCH$_2$CO), 1.69 (9H, s, NCOOC(C*H*$_3$)$_3$), 2.08-2.18 (1H, m, CH$_2$CHC*H*(H)CO), 2.24-2.40 (2H, m, C*H*$_2$CO$_2$CH$_3$), 2.45-2.59 (1H, m, CH$_2$C*H*CH$_2$CO), 2.63-2.88 (1H, m, C*H*(H)CHCH$_2$CO), 2.63-2.88 (1H, m, CH$_2$CHC*H*(H)CO), 2.63-2.88 (2H, m, C=CC*H*$_2$), 2.63-2.88 (1H, m, C=CCH$_2$C*H*(H)), 3.69

(3H, s, OC*H*$_3$), 5.11-5.17 (1H, m, C=CCH$_2$C*H*(H)), 5.11-5.17 (1H, m, NC*H*C=C), 7.23-7.34 (2H, m, Ar*H*), 7.43-7.46 (1H, m, Ar*H*), 8.05 (1H, d, *J* 7.8, Ar*H*).

δ_C (300 MHz; $CDCl_3$) 21.70 (*C*H$_2$), 28.12 (*C*H$_3$)$_3$, 28.45 (*C*H), 35.97 (*C*H$_2$), 38.27 (*C*H$_2$), 39.00 (*C*H$_2$), 40.40 (*C*H$_2$), 51.77 (*C*H$_3$), 55.59 (*C*H), 84.55 (*C*), 115.53 (*C*H), 118.34 (*C*H), 118.57 (*C*), 123.07 (*C*H), 124.75 (*C*H), 128.58 (*C*), 134.78 (*C*), 136.92 (*C*), 150.21 (*C*) 168.52 (*C*), 171.95 (N*C*=O).

MS (CI) *m/z* 413 [MH^+, 100%]; (Found: MH^+, 413.2070. $C_{23}H_{29}N_2O_5$ requires 413.2071).

Methyl 2-(4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate **(109a, b)**



tert-Butyl 2-(2-methoxy-2-oxoethyl)-4-oxo-1,2,3,4,6,7-hexahydroindolo[2,3-a]quinolizine-12(12bH)-carboxylate **(108a, b)** (0.86 g, 2.08 mmol) was dissolved in formic acid (30.52 ml, 807.84 mmol) under a nitrogen atmosphere and stirred at room temperature for 24 hours.

After this time the solvent was evaporated and the remaining oil was shaken with 10% aqueous sodium carbonate solution, then extracted into dichloromethane, dried over anhydrous magnesium sulphate, which was then removed by filtration. The solvent was removed under reduced pressure to yield a brown oil (0.53g, 82%) which required no further purification.

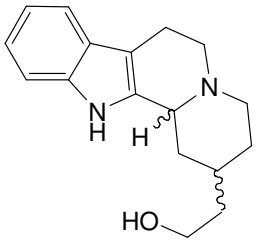
v_{max} (cm^{-1}) 1619 NC=O, 3249 NH.

δ_H (300 MHz; $CDCl_3$) 1.34 (1H, q, *J* 12, C*H*(H)CHCH$_2$CO), 2.00 (1H, q, *J* 12, CH$_2$CHC*H*(H)CO), 2.14-2.30 (1H, m, CH$_2$C*H*CH$_2$CO), 2.14-2.30 (2H, m, C*H*$_2$CO$_2$Me), 2.50-2.79 (1H, m, C*H*(H)CHCH$_2$CO), 2.50-2.79 (1H, m, CH$_2$CHC*H*(H)CO), 2.50-2.79 (2H, m, C=CC*H*$_2$), 2.50-2.79 (1H, m, C=CCH$_2$C*H*(H)), 3.59 (3H, s, OCH$_3$), 4.67 (1H, d, *J* 8.4, NC*H*C=C), 5.04 (1H, d, *J* 7.8, C=CCH$_2$C*H*(H)), 7.04 (2H, qu, *J* 6.9, Ar*H*), 7.22 (1H, d, *J* 7.8, Ar*H*), 7.39 (1H, d, *J* 7.5, Ar*H*), 8.89 (1H, br s, N*H*).

δ_C (300 MHz; $CDCl_3$) 21.05 (*C*H_2), 28.20 (*C*H), 34.78 (*C*H_2), 38.29 (*C*H_2), 39.64 (*C*H_2), 40.08 (*C*H_2), 51.87 (*C*H_3), 53.96 (*C*H), 108.83 (*C*), 111.12 (*C*H), 118.35 (*C*H), 119.63 (*C*H), 122.03 (*C*H), 126.65 (*C*), 133.08 (*C*), 136.42 (*C*), 168.43 (*C*), 172.27 (N*C*=O).

MS (CI) *m/z* 312 [MH^+, 100%]; (Found: MH^+, 313.1551. $C_{18}H_{21}N_2O_3$ requires 313.1547).

(+/-) -12*b*-Epidevinylantirhine **(110a, b)**



Lithium aluminium hydride (0.44 g, 11.59 mmol) was weighed into a dry, three necked flask under a nitrogen atmosphere. Anhydrous tetrahydrofuran (20 ml) was added and cooled to 0 °C. Methyl 2-(4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate **(109a, b)** (0.46 g, 1.47 mmol) dissolved in anhydrous tetrahydrofuran (10 ml) was added dropwise *via* syringe. The experiment was heated under reflux for 3 hours and then stirred at room temperature for a further 12 hours.

The reaction was quenched with diethyl ether (20 ml) and the addition of a potassium tartrate solution (10 ml); it was then left stirring for one hour. The organic layer was separated and dried with anhydrous magnesium sulphate which was then removed by filtration and the solvents were removed under reduced pressure. The crude product was absorbed onto alumina and purified by flash column chromatography over alumina using 1% methanol into chloroform. This yielded a yellow solid (0.30 g, 76%).

Mp: 238-240 °C; v_{max} (cm^{-1}) 3181 NH, OH.

δ_H (300 MHz; MeOD) 1.17-1.29 (1H, m C*H*(H)CHCH$_2$CH$_2$N), 1.46 (1H, dd, *J* 12.3, 3.6, CHC*H*(H)CH$_2$N), 1.53-1.63 (2H, m, C*H*$_2$CH$_2$OH), 1.73-1.90 (1H, m, CHC*H*(H)CH$_2$N), 2.40-2.53 (1H, m, C*H*(H)CHCH$_2$CH$_2$N), 2.40-2.53 (1H, m, C*H*CH$_2$CH$_2$N), 2.60-2.77 (2H, m, C=CC*H*$_2$), 2.60-2.77 (1H, m, C=CCH$_2$C*H*(H)), 2.95-3.15 (1H, m, C=CCH$_2$C*H*(H)), 2.95-

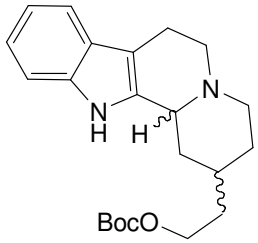
3.15 (1H, m, NC*H*C=C), 2.95-3.15 (2H, m, CHCH$_2$C*H*$_2$N), 3.71 (2H, t, *J* 6.6, C*H*$_2$OH), 7.02 (2H, si, *J* 7.2, Ar*H*), 7.30 (1H, d, *J* 7.8, Ar*H*), 7.39 (1H, d, *J* 7.5, Ar*H*).

NH and OH protons were not observed

δ_C (300 MHz; MeOD) 22.38 (*C*H$_2$), 32.88 (*C*H$_2$), 33.76 (*C*H), 36.69 (*C*H$_2$), 40.46 (*C*H$_2$), 54.43 (*C*H$_2$), 56.54 (*C*H$_2$), 60.36 (*C*H$_2$), 61.67 (*C*H), 107.66 (*C*), 111.95 (*C*H), 118.57 (*C*H), 119.74 (*C*H), 121.92 (*C*H), 128.35 (*C*), 135.81 (*C*), 138.05 (*C*).

MS (CI) *m/z* 270 [MH$^+$, 100%]; (Found: MH$^+$, 271.1809. $C_{17}H_{23}N_2O$ requires 271.1805).

tert-Butyl 2-(1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)ethyl carbonate **(112a, b)**



(+/-) -12*b*-Epidevinylantirhine **(110a, b)** (0.05 g, 0.176 mmol) was dissolved in anhydrous tetrahydrofuran (20 ml), triethylamine (0.037 ml, 0.264 mmol), 4-dimethylaminopyridine (0.011 g, 0.088 mmol) and di-*tert*-butyl dicarbonate (0.06 g, 0.264 mmol) were subsequently added. The reaction was left stirring for one hour after which thin layer chromatography was used to determine the reaction was complete.

The solvents were removed under reduced pressure and the crude oil was dissolved in ethyl acetate, and washed with saturated ammonium chloride solution (2 x 50 ml), saturated sodium carbonate solution (2 x 50 ml) and brine (100 ml). The organic layer was dried using anhydrous magnesium sulphate which was then removed by filtration and the solvents were removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with 1:1 ethyl acetate: methanol as the eluent. This yielded a yellow oil (0.05 g, 77%).

ν_{max} (cm^{-1}) 1639 NC=O.

δ_H (300 MHz; MeOD) 1.07-1.17 (1H, m, C*H*(H)CHCH$_2$CH$_2$N), 1.07-1.17 (1H, m, CHC*H*(H)CH$_2$N), 1.37 (9H, s, CH$_2$OCOOC(C*H*$_3$)$_3$), 1.52-1.58 (2H, m, C*H*$_2$CH$_2$OH), 1.68 (1H, d, *J* 13.2, CHC*H*(H)CH$_2$N), 2.25-2.34 (1H, m, C*H*(H)CHCH$_2$CH$_2$N), 2.25-2.34 (1H, m,

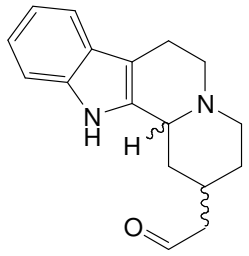
C*H*CH_2CH_2N), 2.50 (1H, td, *J* 10.8, 4.02, C=CC*H*(H)), 2.60 (1H, d, *J* 15.3, C=CC*H*(H)), 2.82-3.01 (2H, m, C=CCH_2CH_2), 2.82-3.01 (1H, m, CHCH_2C*H*(H)N), 3.14 (1H, d, *J* 10.8, CHCH_2C*H*(H)N), 3.14 (1H, d, *J* 10.8, NC*H*C=C), 4.05 (2H, t, *J* 6, CH_2OH), 6.82-6.92 (2H, m, Ar*H*), 7.19 (1H, d *J* 7.8, Ar*H*), 7.27 (1H, d, *J* 7.5, Ar*H*).

NH proton not observed.

δ_C (300 MHz; MeOD) 20.71 (*C*H_2), 26.49 (*C*H_3)$_3$, 30.99 (*C*H_2), 32.15 (*C*H), 34.76 (*C*H_2), 34.92 (*C*H_2), 52.70 (*C*H_2), 54.71 (*C*H_2), 59.87 (*C*H), 64.09 (*C*H_2), 81.12 (*C*), 106.06 (*C*), 110.47 (*C*H), 117.06 (*C*H), 118.23 (*C*H), 120.44 (*C*H), 126.72 (*C*), 133.90 (*C*), 136.46 (*C*), 153.67 (*C*).

MS (CI) *m/z* 370 [MH^+, 100%]; (Found: MH^+, 371.2332. $C_{22}H_{31}N_2O_3$ requires 371.2332).

2-(1,2,3,4,6,7,12,12b-Octahydroindolo[2,3-a]quinolizin-2-yl)acetaldehyde **(111a, b)**



Methyl 2-(4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate **(109a, b)** (0.032 g, 0.102 mmol) was dissolved in anhydrous dichloromethane (6 ml) under a nitrogen atmosphere. The reaction was cooled to -100 °C using liquid nitrogen and methanol. Di*iso*butylaluminium hydride (1M in hexanes, 0.31 ml, 0.305 mmol) was added and the reaction stirred at -100 °C for 3 hours. Thin layer chromatography showed formation of new compounds, and no remaining starting material.

The reaction was quenched by the slow addition of methanol (20 ml), and the solvents removed under reduced pressure. The crude product was absorbed onto silica and purified using flash column chromatography over silica with ethyl acetate as the eluent. This yielded a brown oil (0.0102 g, 35%).

ν_{max} (cm^{-1}) 3215 NH, 2852 and 2923 *CH*O.

δ_H (300 MHz; $CDCl_3$) 1.26-1.47 (1H, m, C*H*(H)CHCH$_2$CH$_2$N), 2.03-2.11 (1H, m, CH$_2$C*H*CH$_2$CH$_2$N), 2.34-2.58 (2H, m, CH$_2$CHC*H*$_2$CH$_2$N), 2.34-2.58 (2H, m, C*H*$_2$CHO), 2.34-2.58 (1H, m, C*H*(H)CHCH$_2$CH$_2$N), 2.64-2.86 (2H, m, CH$_2$CHCH$_2$C*H*$_2$N), 2.64-2.86 (2H, m, C=CC*H*$_2$CH$_2$), 2.64-2.86 (1H, m, C=CCH$_2$C*H*(H)), 4.76-4.80 (1H, m, NC*H*C=C), 5.05-5.11 (1H, m, C=CCH$_2$C*H*(H)), 7.02-7.15 (2H, m, Ar*H*), 7.27 (1H, d, *J* 7.5, Ar*H*), 7.44 (1H, d, *J* 7.5, Ar*H*), 7.97 (1H, br s, N*H*), 9.76 (1H, s, C*H*O).

δ_c (300 MHz; $CDCl_3$) 20.98 (*C*H_2), 30.30 (*C*H), 31.95 (*C*H_2), 35.92 (*C*H_2), 38.34 (*C*H_2), 53.03 (*C*H_2), 55.15 (*C*H_2), 59.56 (*C*H), 108.10 (*C*), 110.87 (*C*H), 118.16 (*C*H), 119.43 (*C*H), 121.46 (*C*H), 127.26 (*C*), 134.19 (*C*), 136.03 (*C*), 201.80 (*C*HO).

MS (CI) *m/z* 268 [MH^+, 100%]; (Found: MH^+, 269.1649. $C_{17}H_{21}N_2O$ requires 269.1648).

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Chapter 4

Appendix

4.1 Publication

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Synthesis and evaluation of a novel series of indoloisoquinolines as small molecule anti-malarial leads

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ABSTRACT

A group of novel synthetic indoloisoquinolines was prepared and its potential as a novel series o molecule anti-malarial leads was assessed. The structure–activity relationship on variation of th tinct regions of chemical space was investigated. A lead compound was generated with an activi to that observed for a known anti-malarial natural product, dihydrousambarensine, that shares th loisoquinoline template structure.

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Malaria continues to pose a significant global health and socio-economic burden on those regions where it is endemic. Whilst substantial investment in the delivery of front-line artemisinin-based combination therapies and use of insecticide impregnated bednets have seen a fall in the mortality attributed to malaria over recent years, data from 2009 show that this disease still imposes a significant impact both in terms of morbidity (~255 million cases) and mortality (~781,000 deaths) annually.[1] A major challenge is the narrow drug discovery pipeline, a problem exacerbated by recent reports of artemisinin treatment failure in South East Asia.[2,3] In recent years, however, high throughput screening of small-molecule libraries as well as natural products derived from plants and marine organisms have sought to seed this pipeline with diverse and novel chemical entities.[4–6] Indole alkaloids isolated from various *Strychnos* species of plants have been demonstrated to show in vitro selectivity and activity against both chloroquine resistant (CQR) and sensitive (CQS) isolates of *Plasmodium falciparum*, the aetiological agent of the most severe form of human malaria.[7,8] Of these compounds characterized, dihydrousambarensine, **1**, emerged as an interesting candidate showing altered levels of activity between CQR and CQS isolates, with a clear preferred activity against CQR isolates (0.85 μM vs 0.03 μM for CQS and CQR, respectively) recent years our research group has become expert in the ste lective synthesis of indole alkaloid targets and we have recer ported the asymmetric synthesis of alkaloids including harm deplancheine **3** and 12*b*-epidevinylantirhine **4** (Fig. 1).[9] Giv structural similarity of dihydrousambarensine, **1**, to these pounds we have initiated a research program to investig structure–activity relationship (SAR) on the indoloisoqu template **5**. Examination of a collection of heterocyclic comp originating from our historical research efforts identified 1(pounds that shared some structural similarity to dihydrous rensine. Of these, the indoloisoquinoline derivative **6a** (Fig. identified as a potential lead compound for this invest (IC_{50} of 20 μM, Table 1). Based on this structure we deci undertake an initial investigation of three areas of chemica (Fig. 2) with the aim of gaining an increased appreciation resulting activity that could be realised by simple chemical r cation of the readily available indoloisoquinoline core **5a**, n (i) the indole N-substituent (R); (ii) the hydroxymethyl O-su ent (R^1); (iii) alkenic substitution on or around the lactam rir

Compound **5a** was prepared by our previously reported from L-tryptophan,[10] and utilized as a starting point for structural diversity in our investigation. Symmetrical alk of the *N*- and *O*-groups was readily achieved by the general dure outlined in Scheme 1 to yield compounds **7a–c**.

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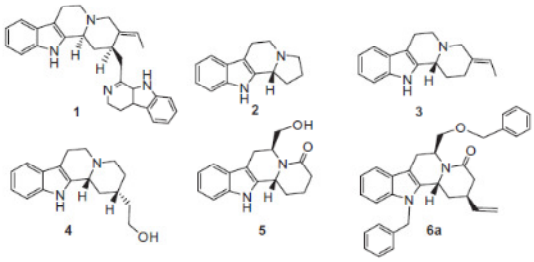
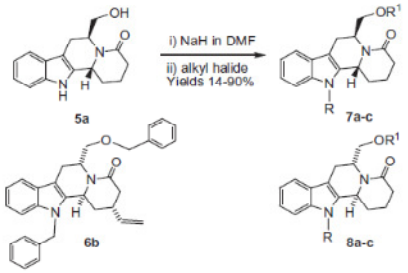


Figure 1. The indoloisoquinoline template and anti-malarial leads.



Scheme 1. Synthesis of indoloisoquinoline targets.

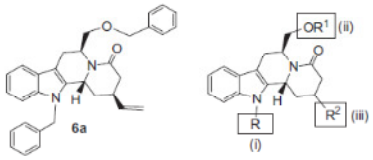


Figure 2. Initial lead compound and proposed sites of chemical modification on the indoloisoquinoline template.

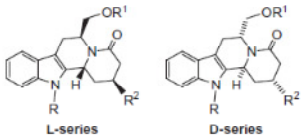


Figure 3. Alternative template structures of the lead indoloisoquinoline compounds

We were mindful of the fact that the absolute stereochemistry present within the indoloisoquinoline core of the anti-malarial natural product dihydrousambarensine, **1**, did not match that of our initial series of compounds and, due to the known potential differences in activity of enantiomeric series of biologically active compounds, we also prepared the analogous series of diastereoisomeric compounds, **8a–c**, by following the same series of alkylation reactions from the diastereoisomeric template **5b**, itself produced from the D-enantiomer of the original tryptophan precursor (Fig. 3).[10] Unsymmetrically substituted compounds **9–17** (Table 1) were prepared by a modified procedure in which the appropriate precursor (**5a** or **5b**) underwent stepwise derivatization. Initial alkylation using NaH in DMF with 1.5 equiv of the alkyl halide gave the *N*-alkyl product followed (if required) by a second alkylation on the hydroxymethyl oxygen atom (using NaH in DMF with 2 equiv of the appropriate alkyl halide). We have also prepared and investigated the enantiomer **6b** of our lead compound from the initial screen, **6a**. The synthesis of **6b** followed the previously reported synthesis of **6a** albeit starting from the D-enantiomer of the original tryptophan precursor.[11] It is worth noting at this point that compounds **5**, **7–17** lacked the presence of the vinyl substituent (R^2) on the lactam ring.

Anti-malarial activity was determined using an adaptation o the lactate dehydrogenase (LDH) assay.[12,13] Assays to determin the 50% inhibitory concentration (IC_{50}) were carried out using intraerythrocytic cultures of *P. falciparum* lines Dd2 (chloroquine resistant) and 3D7 (chloroquine sensitive). It is immediately apparent from Table 1 that the compounds tested in this screen show a significant increase in antiplasmodial activity ($p = 0.015$) on moving from the L-tryptophan to the D-tryptophan series, highlighting the importance of absolute stereochemistry in this indoloisoquinoline template. Indeed the more active D-series of compounds has the same absolute stereochemistry at the ring junction within the heterocyclic core as the natural product that formed the basis of this study (dihydrousambarensine, **1**) and also shares the relationship between stereochemistry and activity with other natural products from the Frédérich study.[8] As a result we therefore decided to continue our own study with compounds derived from the D-tryptophan enantiomer.

Themes that emerge from this study include the apparent preference for a benzyl substituent on the indole nitrogen atom (R) with a methyl (as in **11b**) or allyl (as in **12b**) substituent at R^1 leading to the highest levels of activity in this screen (both have an IC_{50} value of 1.3 μM, Table 1). An increase in steric bulk at R^1 on moving

Table 1
Structure–activity relationships of indoloisoquinoline derivatives[14]

Compound L-series	R	R^1	R^2	IC_{50} (μM) Dd2	Compound D-series	R	R^1	R^2	IC_{50} (μM)Dd2
5a	H	H	H	71	**5b**	H	H	H	56
6a	Benzyl	Benzyl	vinyl	20	**6b**	Benzyl	Benzyl	Vinyl	13
7a	Methyl	Methyl	H	30	**8a**	Methyl	Methyl	H	29
7b	Allyl	Allyl	H	16	**8b**	Allyl	Allyl	H	13
7c	Benzyl	Benzyl	H	12	**8c**	Benzyl	Benzyl	H	3.5
					9	Benzyl	H	H	5
					10	Allyl	H	H	41
11a	Benzyl	Methyl	H	32	**11b**	Benzyl	Methyl	H	1.3
12a	Benzyl	Allyl	H	35	**12b**	Benzyl	Allyl	H	1.3
					13	Benzyl	Propyl	H	2.8
					14	Benzyl	Cyclohexylmethyl	H	19
					15	Cyclohexylmethyl	Allyl	H	9
					16	Cyclobutylmethyl	Allyl	H	7
					17	4-Pyridyl methyl	Allyl	H	2.1

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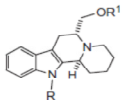

Figure 4. Tertiary amine derivatives **18** and **19**.

from an allyl (in **12b**) to either a propyl group, as in **13**, or to a cyclohexyl ring, **14**, gave a fall in activity. The propyl moiety may be viewed as being less planar, and consequently more degrees of freedom, than allyl as a result of the introduction of the double bond in the latter. The fully saturated cyclohexyl moiety would be expected to show conformational preference for a chair rather than the planarity present in the original aromatic ring of **12b**. The difference in activity between **8c** (R^1 = benzyl) and **14** (R^1 = cyclohexylmethyl) is significant and again points to the preference for some degree of planarity in the ring component, and also possibly an increase in electron density, within the R^1 substituent.

Variation of the ring moiety within the R grouping in **12b** leads to significant falls in activity with compounds **15** and **16**. Each of these analogues contains an all-carbon ring structure at R, but neither alternative group (cyclohexylmethyl nor cyclobutylmethyl) shares the electronic or steric properties of the aromatic ring contained within the original benzyl substituent at R in **12b**.

Compound **17** retains almost the same level of activity as **12b** on introduction of an alternative nitrogen-bearing pyridylmethyl substituent at R. Indeed, one would expect that the steric and electronic properties of the pyridylmethyl group would closely mimic that of the benzyl substituent at R in **12b**, albeit with an increase in the overall basicity of pyridyl analogue, **17**. In this current series of compounds an increase in the basic nature of the compounds does not seem to lead to a corresponding increase in activity, a fact supported by the work on compounds **18** and **19** (Fig. 4), discussed below.

With the results of the pyridyl analogue **17** in hand we decided to explore an alternative mode to introduce a higher level of basicity to the series. Tertiary amine analogues **18** and **19** (Table 2) were prepared by amide group reduction of compounds **11b** and **12b** respectively (using lithium aluminium hydride in dry THF).

Table 2
Activity of tertiary amine analogues

Compound Yield (%)	R	R^1	IC_{50} (μM) Dd2
18 (45%)	Benzyl	Me	3.3
19 (12)	Benzyl	Allyl	1.5

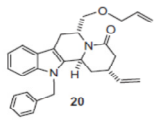

Figure 5. Most active synthetic indoloisoquinoline.

Comparing compound **18** with its amide analogue **11b** (Table 1) we observe a slight decrease in activity on removal of the lactam functionality. Compound **19**, bearing an allyl substituent at R^1, shows comparable activity (IC_{50} 1.5 μM) to its amide parent **12b** (IC_{50} 1.3 μM). Removal of the lactam carbonyl group to generate tertiary amines does not therefore lead to an apparent increase in antiplasmodial activity in this series.

Based on the observed increase in activity of **12b** over **8c** we decided to prepare compound **20** (Fig. 5), incorporating the *O*-allyl, *N*-benzyl substitution pattern of **12b** but now introducing the vinyl group present at R^2 in the original lead compound **6a**. Compound **20** was prepared by a route that is analogous to the preparation of compound **6b**, albeit starting from the *N*-benzyl, *O*-allyl compound, **12b**. Compound **20** was found to have an IC_{50} of 1.1 μM, and is our most active compound to date. It is interesting to compare this activity with that of compounds **6a** and **6b** (Table 1) which share the presence of the vinyl substituent at R^2. Simply replacing the *O*-benzyl substituent of **6b** by *O*-allyl (in **20**) leads to a considerable increase in activity. Although close to the margin for error, compound **20**, containing the vinyl substituent at R^2, *is* more active than its precursor **12b** and the original vinyl-containing leads **6a** and **6b**.

Finally, compounds **21** and **22**(Fig. 6) were prepared by typical selenoxide-induced unsaturation chemistry in order to investigate an alternative modification of the lactam ring, effecting a change in ring conformation on introduction of a conjugated double bond into this ring.

On comparing these results to those of the 'parent' compounds **12b** and **8c** there is no significant change in activity of their unsaturated analogues, **21** and **22** respectively (Table 3).

In this study we set out to investigate the structure–activity relationships of a range of synthetic indoloisoquinolines sharing some structural similarities to the known antiplasmodial natural product dihydrousambarensine, **1**. Through a series of rational structural modifications we have developed our compound series from initial activities of around 70 μM to our current lead compound, **20**, with an IC_{50} of 1.1 μM. This level of inhibition is comparable, if not better, than those previously reported for monoindole alkaloid moieties isolated from *Strychnos* spp.[8] Of note is the fact that, like most indole alkaloids, the inhibitory effects reported here for the CQR isolate Dd2 were not significantly different from those determined from the CQS isolate 3D7 (see Supplementary data).

Table 3
Activity of unsaturated lactam derivatives

Compound Yield (%)	R	R^1	IC_{50} (μM) Dd2
21 (25%)	Benzyl	Allyl	1.7
22 (40)	Benzyl	Benzyl	3.6

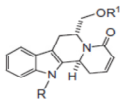

Figure 6. Unsaturated indoloisoquinolines **21** and **22**.

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We have identified the following general structural properties as having an effect on antiplasmodial activity in the synthetic indoloisoquinolines:

(i) absolute chirality of the template: compounds derived from D-tryptophan are preferred;
(ii) the steric bulk and a degree of planarity within R is important, with the presence of an aromatic ring being preferred;
(iii) introduction of allyl at R^1 can lead to an increase in activity;
(iv) an increase in basicity at R, or within the lactam ring, does not lead to an increase in activity;
(v) introducing unsaturation to the lactam ring does not lead to an increase in activity;
(vi) introduction of substitution, currently a vinyl group, at R^2 can lead to an increase in activity.

That chirality of the template, and the size and nature of substitutions at the R and R^1 position, correlates with the antiplasmodial activity in this compound series suggesting an interaction with a specific parasite target. Compounds **8c** and **20** when added to *P. falciparum* culture at 5 μM appear to induce major morphological alterations in the trophozoite stage of parasite development (data not shown), although this provides only very provisional insights to a potential target for the indoloisoquinoline target. Introduction of a vinyl substituent and the resulting levels of biological activity in compound **20** tantalizingly suggest that additional exploration of the R^2 position is desirable, with the aim of extending the indoloisoquinoline core to produce analogues that more closely mimic the structural properties found in this region of chemical space within the bisindole alkaloid, dihydrousambarensine, **1**. Such modifications, and more detailed analysis of the temporal and spatial effect of these compounds on parasite growth and development, will form the basis of future studies in our group and our results will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data (representative experimental conditio and characterization data for the synthesis of compounds **8a**, **11b**, **12b**, **19**,**20**, and **21**) associated with this article can be foun in the online version, at doi:10.1016/j.bmcl.2011.12.071.

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4.2 **Toxicity Report**

Blind Toxicity Assesment of 11 anti-malarial compounds

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Jan 2013

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Basic Experimental Protocol

- Cell Line: HepG2 human Caucasian hepatocyte carcinoma; original source: ECACC 85011430
- Media: MEM - 10% FBS, w. Glutamax, NEAA
- Seed HepG2 cells into columns 1-24 of a 384 well plate at a density of 1.00×10^5/ml. 25µl per well.
- Incubate at 37°C / 5% CO_2 for 24 hours.
- Add 125nl of all test compounds, and 100% inhibition control (10mM Doxorubicin), to assay plate using Labcyte Echo.
- Incubate for further 69 hrs at 37°C in an atmosphere of 5% CO_2
- Add 5µl of 250µM resazurin to each well on the assay plates and incubate for a further 1- 1.5 hrs
- Read plates at excitation 528nm, emission 590nm

% Inhibition for each test compound is calculated using the following equation:
% Inhibition = 100 – (((TEST COMPOUND – BLANK) / (NO INHIBITION – BLANK)) *100)

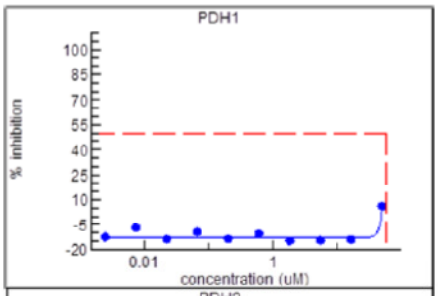
EC50 values are calculated using IDBS Activity Base XLFit version Model 205

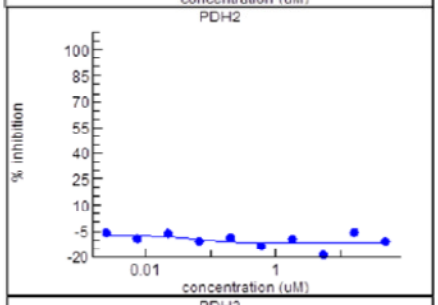
Each compound was run in duplicate with standard compound curve (doxorubicin) on each plate.

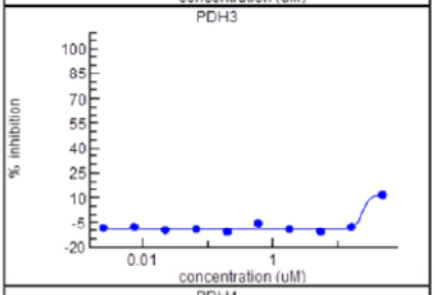
Data Summary

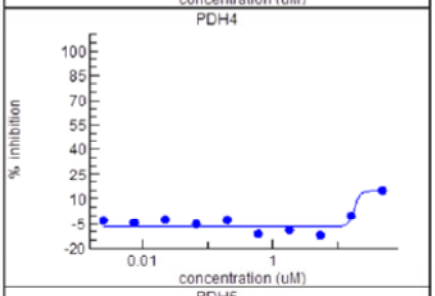
Compound Identifier	EC50 (µM)
PDH1	>50
PDH2	>50
PDH3	>50
PDH4	>50
PDH5	>50
PDH6	>50
PDH7	>50
PDH8	>50
PDH9	>50
PDH10	>50
PDH11	>50

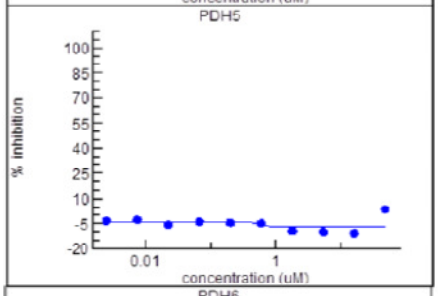
Compound Curves

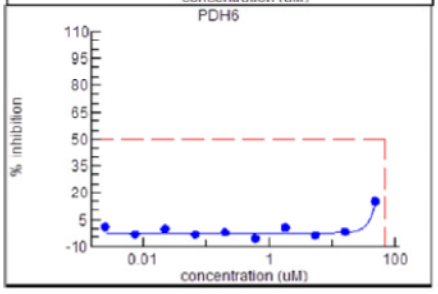


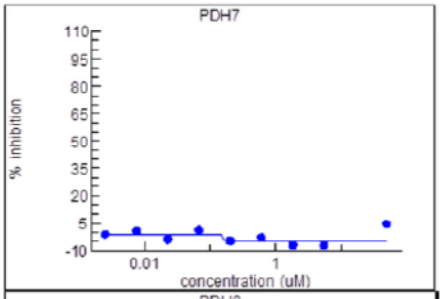


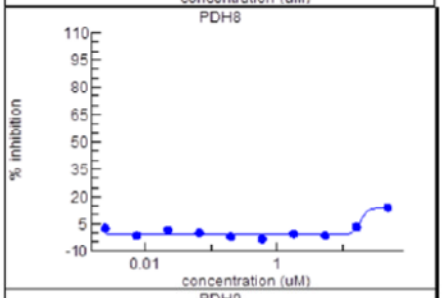


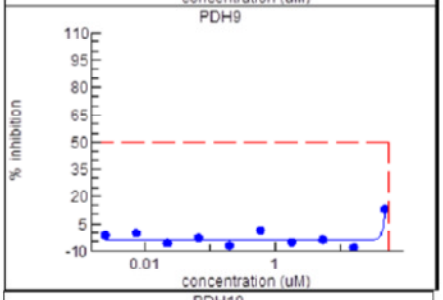


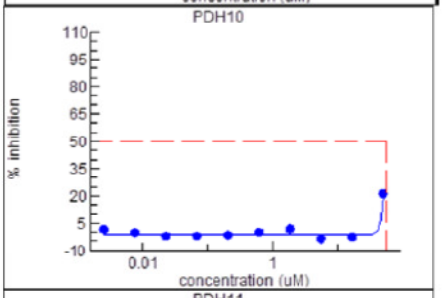


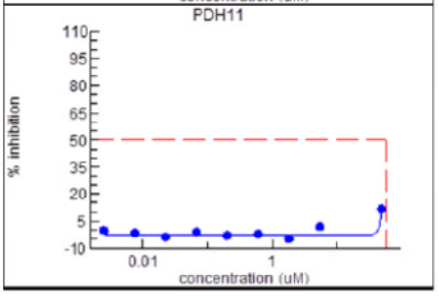








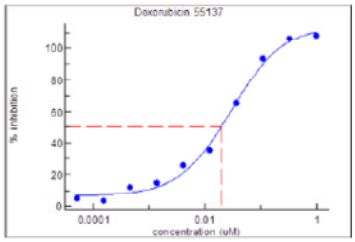


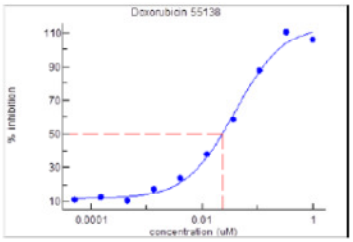


Assay performance and Plate statistics

Plate	Av 0% Inhib.	stdev	Av 100% Inhib.	stdev	S:B	Z'
55137	1298285.125	67315.836	234588.063	31262.419	5.534	0.722
55138	1150063.000	57649.646	210820.375	30620.226	5.455	0.718

Standard Compound





Doxorubicin EC50

Plate 55137 – 19.3nm

Plare 55138 – 23.3nm