**School of Pharmacy**

# The Synthesis and Biological Evaluation of Novel Analogues of Isocryptolepine

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**Doctor of Philosophy**
**of**
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# Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

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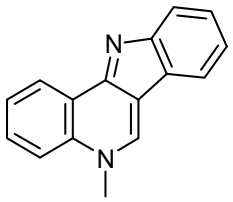
# Abstract

This thesis investigates the potential of the alkaloid isocryptolepine **16** as a lead compound in antimalarial drug development. Fifteen derivatives of the parent alkaloid were prepared and fully characterised, twelve of which were novel compounds. A select group of compounds were subsequently evaluated for both antimalarial activity and cytotoxicity.

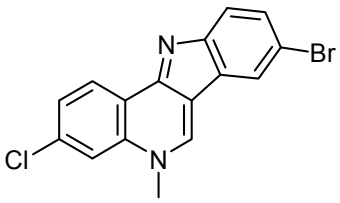


**16**

Three previously reported synthetic methodologies to the parent alkaloid were initially investigated; wherein two approaches were able to be reproduced or improved. These two synthetic methodologies were subsequently applied to the preparation of derivatives. The first of these methodologies, the Jonckers Method, involved two consecutive palladium catalysed coupling reactions. During the course of these investigations it was found that these two reactions could be combined into a single 'domino' reaction resulting in a reduction in the number of steps required to prepare the parent alkaloid. This methodology was then applied to the preparation of both ring-substituted and structural isomers. The second methodology, The Molina Method, involved a benzotriazole-mediated strategy and was applicable to preparing isocryptolepine derivatives with ring substituents on the quinoline ring. Finally a method for selective electrophilic aromatic substitution was developed and applied to the preparation of a further range of halogenated derivatives.

Eight of the prepared derivatives were selected for biological evaluation. Antimalarial activity was assessed against a chloroquine sensitive and resistant strain of *P. falciparum*, whilst cytotoxicity was evaluated against mouse embryonic fibroblasts (3T3 cells). All compounds were found to be more active compared to the parent alkaloid against the chloroquine resistant strain of *P. falciparum*; specifically 8-bromo-2-chloroisocryptolepine **107** ($IC_{50}$ = 85 nM) and 8-bromo-3-chloroiso-cryptolepine **105** ($IC_{50}$ = 100 nM) were the most potent. Cytotoxicity evaluations

revealed that ring substitution did not enhance cytotoxicity and the most potent antimalarial derivative, 8-bromo-2-chloroisocryptolepine **107** ($IC_{50}$ = 9.01 μM), displayed a 4-fold reduction in cytotoxicity.

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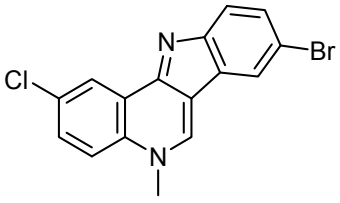


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In conclusion, isocryptolepine **16** and its derivatives have significant potential as antimalarial lead compounds, with many derivatives possessing enhanced bioactivity versus the parent. This study has also identified 8-bromo-2-chloroisocryptolepine **107** to be a very promising lead compound which warrants further biological or pharmaceutical investigation.

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# Acknowledgements

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# Glossary

| | |
|---|---|
| BINAP | 2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl) |
| Boc | *Tert*-butoxycarbonyl |
| COSY | Correlation Spectroscopy |
| DEMM | Diethyl ethoxymethylmalonate |
| DMAP | 4-(Dimethylamino)pyridine |
| DMF | *N,N*-dimethylformamide |
| DCM | Dichloromethane |
| DMSO | Dimethylsulfoxide |
| EI | Electron impact (ionisation) |
| Ether | Diethyl ether |
| FAB | Fast Atom Bombardment |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HPLC | High Pressure Liquid Chromatography |
| HRMS | High Resolution Mass Spectrometry |
| HSQC | Heteronuclear Single Quantum Correlation |
| MIQ | 6-Methyl-6*H*-indolo[3,2-*c*]isoquinoline |
| NOE | Nuclear Overhauser Effect |
| NBS | *N*-Bromosuccinimide |
| NCS | *N*-Chlorosuccinimide |
| $Pd_2(dba)_3$ | Tris(dibenzylideneacetone)dipalladium |
| $Pd(OAc)_2$ | Palladium acetate |
| $Pd(PPh_3)_4$ | Tetrakis(triphenylphosphine)palladium |
| *P. falciparum* | *Plasmodium falciparum* |
| PPA | Polyphosphoric acid |
| P(*t*-Bu)$_3$ | Tri-*tert*-butylphosphine |
| SEM | [2-(Trimethylsilyl)ethoxy]methyl |
| THF | Tetrahydrofuran |
| TLC | Thin Layer Chromatography |
| XANTPHOS | 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene |

# Chapter 1

## Introduction

## 1.1. Malaria

The parasitic disease malaria, whilst treatable, is still a major global health issue, especially in many tropical regions. Over 200 million cases of infection occur annually, leading to an estimated 800,000 deaths.[1] Some of the poorest nations in the world have the highest malaria burden, specifically Africa where approximately 89% of all cases occur. Children under five are the most vulnerable and an estimated 732,000 children die from malaria annually.[2]

Malaria is caused by the protozoan parasite of the genus *Plasmodium,* of which there are four strains that infect humans; *Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium malariae (P. malariae)* and *Plasmodium ovale (P. ovale)*. A fifth strain, the simian parasite *Plasmodium knowlesi (P. knowlesi),* has more recently also been found to infect humans.[3] *P. falciparum* and *P. vivax* are the most common strains, with *P. falciparum* generally resulting in more severe symptoms and causing 98% of malaria related deaths.[1] Although *P. vivax* is generally regarded as benign, it can still result in death and has the ability to remain dormant *in vivo* leading to relapse months or years post-infection.[4]

Malaria was once endemic in regions such as Central America, India and the Caribbean. However, the disease is no longer prevalent in these areas due to large scale health strategies: mass spraying with the insecticide DDT, distribution of insecticide treated nets and improved access to antimalarial drugs.[5] In contrast similar strategies employed in sub-Saharan Africa have not had the same outcome, with many of the countries still being classified as endemic. This is predominately because *P. falciparum* is the more prevalent strain in Africa and has developed resistance to many antimalarial drugs.[6] On the other hand, in Asia and the Americas the less severe form of the disease linked to *P. vivax* is more prevalent and drug-resistance to this strain is less widespread.[7]

### 1.1.1. The *Plasmodium* Life Cycle

The life cycle of the *Plasmodium* parasite is significantly more complicated than most other parasitic diseases as the parasite requires two hosts, a human and a female Anopheles mosquito. The life cycle has been comprehensively detailed[8-10] and will be briefly described here.

The disease begins when an infected mosquito feeds on a human, resulting in the simultaneous injection of immature parasites (sporozoites; ①; Figure 1.1). These sporozoites subsequently migrate towards the liver and here the parasite enters the 'Liver Stage' ②. Upon entry into the liver cells (hepatocytes) the sporozoites quickly mature into schizonts, which later rupture to release thousands of daughter cells (merozoites) into the blood stream. In the case of *P. vivax,* it is at this particular stage that dormant forms of the parasite (hypnozoites) can develop.[4]

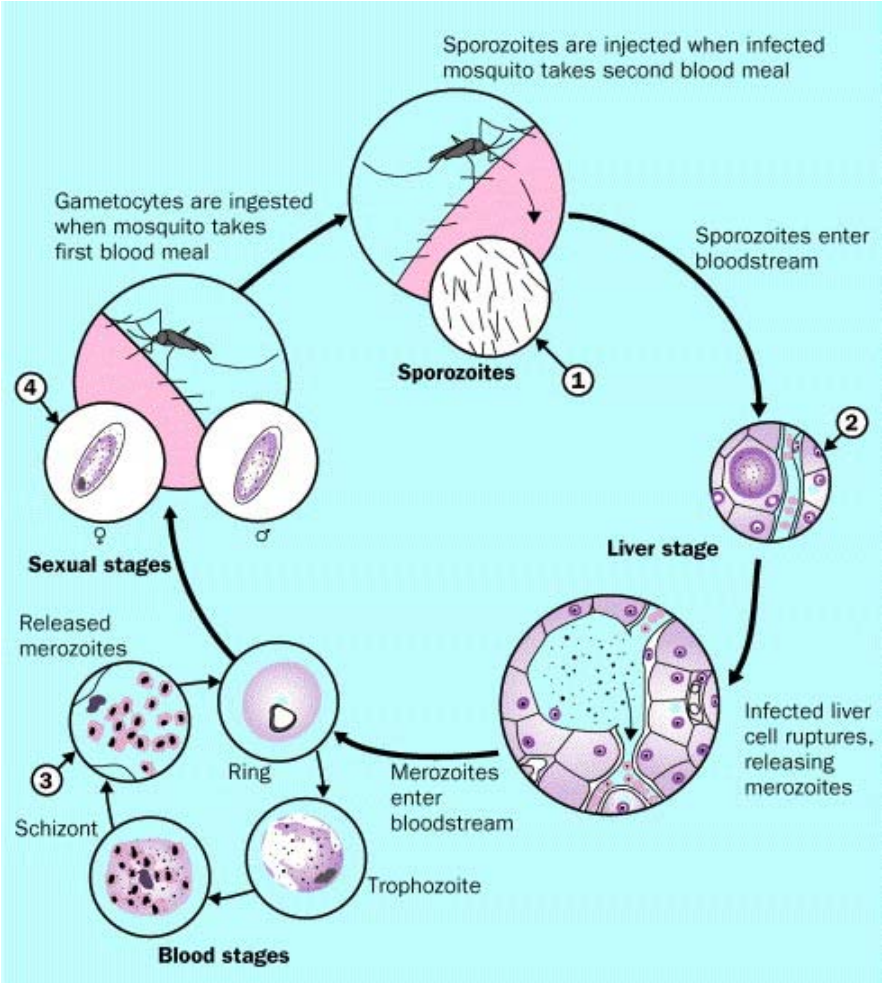



**Figure 1.1:** The life cycle of the *Plasmodium* parasite.[11]

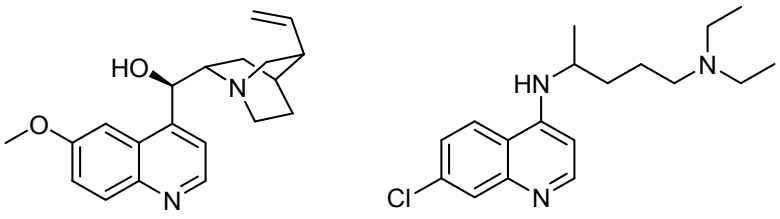
Once in the bloodstream the merozoites infect red blood cells (erythrocytes) and the parasite enters the 'Blood Stage'. Within erythrocytes the parasites develops into their feeding forms (trophozoites), which consume haemoglobin and mature into schizonts. The schizonts then rupture and daughter merozoites are released ③ which subsequently infect more erythrocytes and a blood-cycle, which lasts 48 hours (72 hours for *P. malariae*), recommences. The rupturing of schizonts during this stage

results in the release of cell debris and toxins into the blood, which is believed to be responsible for many of the symptoms associated with malaria (i.e. fever and chills).

After multiple cycles of the 'Blood Stage' the parasites enters their 'Sexual Stage', where merozoites can develop into the sexual form of the parasite (gametocytes; ④). When a gametocyte is ingested by a feeding mosquito it undergoes sexual reproduction within the insect's gut to form sporozoites, which migrate to the salivary glands of the mosquito thus reinitiating the parasitic life cycle.

### 1.1.2. Antimalarial Drugs

One of the first known antimalarial drugs was the natural product quinine **1** (Figure 1.2), which was originally isolated from the bark of the South American cinchona tree in 1820 by Pelletier and Caventou.[12] From a historical perspective bark extracts derived from the tree were used to treat malaria in Europe as early as the 1640s. The bark, and later the isolated alkaloid, was the most effective treatment for malaria in Europe for the next 300 years.[12] During World War II quinine **1** supply issues prompted the development of the synthetic derivative chloroquine **2** (a 4-aminoquinoline; Figure 1.2), which subsequently became widely used in malaria affected areas.[13] Quinine **1** is currently mainly used to treat the most severe case of malaria, principally acute cerebral malaria.[14]




**1** **2**

**Figure 1.2:** Quinine **1** and its synthetic derivative chloroquine **2**

Chloroquine **2**, and other related 4-aminoquinolines, act during the 'Blood Stage' of the parasite and accumulate in the parasitic food vacuole.[15] Within this particular organelle, host haemoglobin is digested by the parasite resulting in amassing of toxic free haem (ferriprotoporphyrin IX). The parasite is able to sequester haem by converting it into highly insoluble crystalline haemozoin. Haemozoin (termed β-haematin when synthetically prepared) is a cyclic dimer of ferriprotoporphyrin IX (FPP IX).[16]

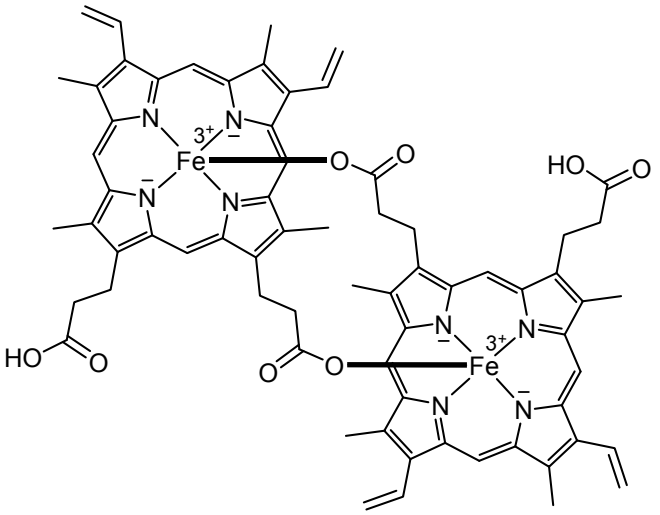
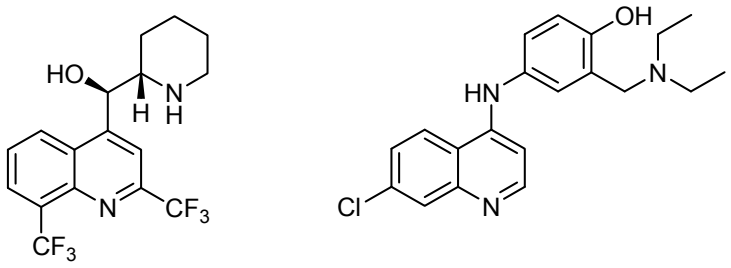


**Figure 1.3:** The structure of haemozoin; two FPP IX units linked *via* coordination of propionate groups and ferric ($Fe^{3+}$) centres

Chloroquine **2** disrupts the parasitic feeding process by interrupting haem detoxification. How this is accomplished is not completely understood, but there is strong evidence to suggest that the drug directly interacts with haemozoin.[17,18] It has been proposed that the quinoline ring of the drug may intercalate into the porphyrin rings on the surface of haemozoin to interrupt crystal formation and cause a build-up of toxic haem within the parasite.[19]

Since its development chloroquine **2** had been the cheapest and most effective antimalarial available until clinical reports began to emerge of resistance in the 1960s.[20] In the last 50 years chloroquine *P. falciparum* resistance has spread to most of Asia, South America and Africa and the drug is currently only effective in some areas of Central America.[6] Recent studies have found that chloroquine resistance is linked to gene mutations that effect proteins involved in the drug's transport into the food vacuole.[21] *P. falciparum* has similarly developed resistance to a number of other quinoline based drugs, e.g. mefloquine **3** (a quinoline methanol) and amodiaquine **4** (a 4-aminoquinoline; Figure 1.4).[6]

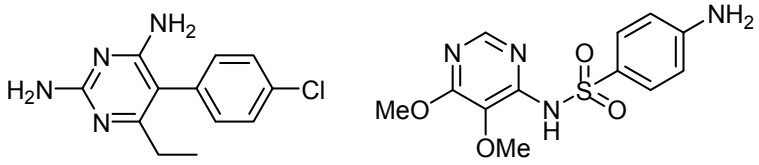
One of the first drug alternatives to chloroquine **2**, introduced following the discovery of resistance, was the combination of pyrimethamine **5** and sulfadoxine **6** (Figure 1.5). Implemented in the 1940s under the commercial name Fansidar, the pharmacological activity of these drugs is much better understood in comparison to the quinoline antimalarials.[22]



**3** **4**

**Figure 1.4:** The quinoline based drugs mefloquine **3** and amodiaquine **4**

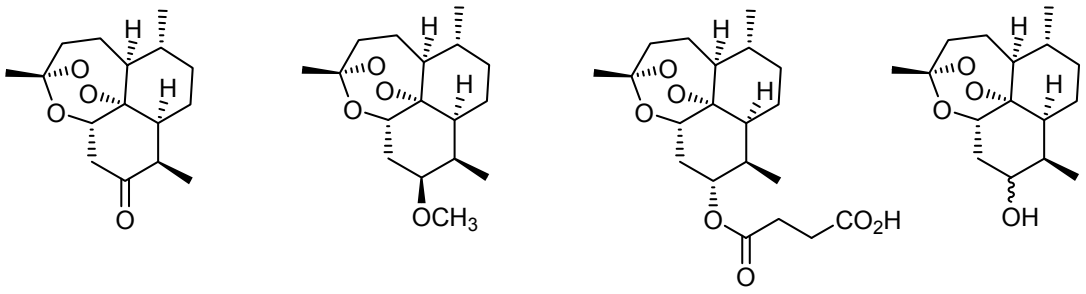
These drugs, often termed antifolate antimalarials, act during the blood and sexual stages of the parasite by inhibiting folate biosynthesis, a process that is essential to parasitic DNA synthesis. This is achieved *via* inhibition of essential enzymes in the folate cycle, pyrimethamine **5** inhibits dihydrofolate reductase whilst sulfadoxine **6** inhibits dihydropteroate synthetase. The emergence of resistance to these drugs was noted during the 1980s[23] and arose due to the development of gene mutation in the target enzymes.[22]



**5** **6**

**Figure 1.5:** The antifolate antimalarials pyrimethamine **5** and sulfadoxine **6**

The most recently introduced class of antimalarials are those based on the natural product artemisinin **7** (Figure 1.6), initially isolated from the Chinese plant *Artemisia annua* (also known as *qing hao* or sweet wormwood).[24]



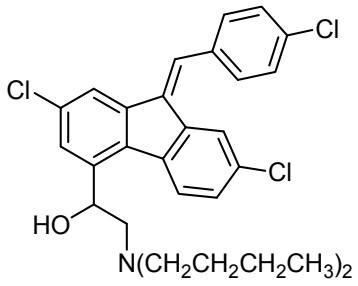
**7** **8** **9** **10**

**Figure 1.6:** Artemisinin **7** and some of its synthetic derivatives; artemether **8**, artesunate **9** and dihydroartemisinin **10**

Although a highly effective antimalarial agent, artemisinin **7** suffers from poor solubility in both water and oil, which prompted the development of various derivatives that could be administered intravenously or intramuscularly.[9] The oil soluble derivative artemether **8** and the water soluble artesunate **9** (Figure 1.6) are amongst the most potent antimalarial drugs currently available. The metabolite of both these drugs, dihydroartemisinin **10**, possesses enhanced antimalarial activity in comparison to the parent artemisinin **7** and is also a common antimalarial drug.[9]

The artemisinin based drugs act during both the blood and sexual stages of the parasite but, like the quinoline antimalarials, their pharmacological mode of action is not fully understood. The endoperoxide unit has been found to be essential for activity and one of the earlier theories suggested that this unit interacted with $Fe^{2+}$ ions, or haem, to form free radicals which inhibited the formation of essential proteins to ultimately cause parasite death.[25] More recent evidence suggests that artemisinins more likely interfere directly with essential proteins or transporters.[26] The calcium transporter sarcoplasmic endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA)[27,28] is one such proposed target, but is a source of much contention. Some studies have reported artemisinins to have an inhibitory affect on SERCA[27,28] whilst others found no such relationship.[29]

Recently there have been reports of increasing tolerance to the artemisinin based drugs, with failure rates rising in areas of Cambodia and French Guinea.[30,31] This may be the beginnings of artemisinin resistance and, in order to minimise further development, it is now recommended that these drugs be used in combination not as a monotherapy. Combination therapies reduce the possibility of resistant parasites surviving drug treatment as a fast acting artemisinin, which typically has a short half-life, is combined with a long half-life drug such as a quinoline. The World Health Organisation (WHO) recommends combinations such as artesunate **9** and mefloquine **3**, or artemether **8** and lumefantrine **11** (an aryl aminoalcohol; Figure 1.7), depending upon the inherent resistance present in the infection area.[14]




**11**

**Figure 1.7:** The common antimalarial combination partner lumefantrine **11**

### 1.1.3. Current Research in Antimalarial Therapy

A variety of therapeutic modalities are currently under investigation in order to combat malaria and tackle drug resistance. In the last ten years there has been an extensive amount of research into the development of a malaria vaccine; however this process must overcome many obstacles. Not only does the *Plasmodium* parasite have over 5,000 genes that could be targeted as potential antigens, but the complex life cycle of the parasite adds another level of difficulty to vaccine development.[32] Despite these issues there are a variety of vaccine candidates currently in clinical trials and many are based on the circumsporozoite protein (CSP), one of the earliest antigens identified from *Plasmodium*, which is found on the surface of sporozoites and infected liver cells.[33] RTS,S represents one such vaccine candidate based on CSP, which has been co-developed by Glaxo-Smith Kline (Belgium) and the Walter Reed Army Institute of Research (USA).[34] This vaccine has shown promising results in phase II clinical trials but does not provide complete protection against the parasite and its efficacy is reduced over time. Improved formulation methods are currently being investigated in attempts to address these issues. Thus it is generally believed that a viable vaccine is still many years away.[32]

In the meantime, novel antimalarial drugs are being explored by various drug research groups globally.[8,35] Ideally these new antimalarial drugs should function upon the parasite in a different manner to previous drugs to negate or delay the emergence of resistance.

## 1.2. Alkaloids in Drug Therapy

Natural products have historically played an important role in medicine and, in the last 25 years, over one thousand compounds based on natural products have been used as either drugs or vaccines to treat a variety of human diseases.[36] Due to their chemical diversity and range of therapeutic properties natural products are an attractive group of medicinal compounds, especially with respect to novel drug identification.[37,38]

An important sub-class of natural product are the alkaloids, which make up 18% of all characterised natural products.[39] Approximately 27,000 alkaloids have been identified and they possess a variety of biological and medicinal properties. These complex heterocyclic compounds are predominantly plant based, but are also found in bacteria, fungi and marine animals. Most alkaloids are bitter tasting and possess a degree of toxicity as they are believed, for the most part, to act as deterrents to animal predation.[40] Alkaloids have also found application in many areas of medicine; for example the analgesic drugs morphine **12** and codeine **13** (Figure 1.8) are both well known medicinal alkaloids that were originally derived from the opium poppy (*Papaver somniferum*).[41]

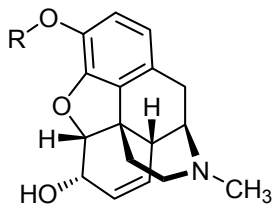



**Figure 1.8:** Alkaloids morphine **12** (R = H) and codeine **13** (R = $CH_3$)

Many alkaloids have also been found to possess significant antimalarial activity. These alkaloids have been reviewed recently,[42] and a notable example is the aforementioned quinine **1**. The attractiveness of alkaloids in the field of antimalarial drug development stems from the fact that novel alkaloids may have different antimalarial modes of action to previous drugs. In addition synthetic derivatives can often have superior biological activities in comparison to their parent alkaloid, as demonstrated by quinine **1** from which has stemmed a range of synthetic derivatives (i.e. chloroquine **2**, mefloquine **3** and amodiaquine **4**) that have been applied therapeutically with some success.

## 1.3. Antimalarial Alkaloids from *Cryptolepis sanguinolenta*

The West African climbing shrub *Cryptolepis sanguinolenta* (of the Periplocaceae family; Figure 1.9) represents an essential component in many traditional African herbal remedies. In areas of Ghana and Senegal root decoctions have been used to treat fevers, urinary infections, stomach disorders and malaria.[43,44] These medicinal properties have been mostly attributed to the various bioactive indoloquinoline alkaloids that are present in both the leaves and roots of this plant.

**The image of *Cryptolepis sanguinolenta* (Addae-Kyereme, J. *Cryptolepis sanguinolenta*. In *Traditional Medicinal Plants and Malaria,* 1st ed.; Wilcox, M.; Bodeker, G.; Rasoanaivo, P., Eds. CRC Press: Boca Raton, FL, 2004; pp 131-139.) is unable to be reproduced here due to copyright resirictions.**

**Figure 1.9:** The climbing shrub *Cryptolepis sanguinolenta*[44]

The major bioactive alkaloid, cryptolepine **14** (Figure 1.10), was isolated from the roots of *Cryptolepis sanguinolenta* in 1951.[45] In subsequent years a range of other alkaloids with a similar indoloquinoline skeleton have been isolated. These include quindoline **15**, isocryptolepine **16**,[46] hydroxycryptolepine **17**,[47] cryptoheptine **18**, and neocryptolepine **19**.[48,49] However, only cryptolepine **14**, isocryptolepine **16** and neocryptolepine **19** have been shown to possess any significant biological activity.

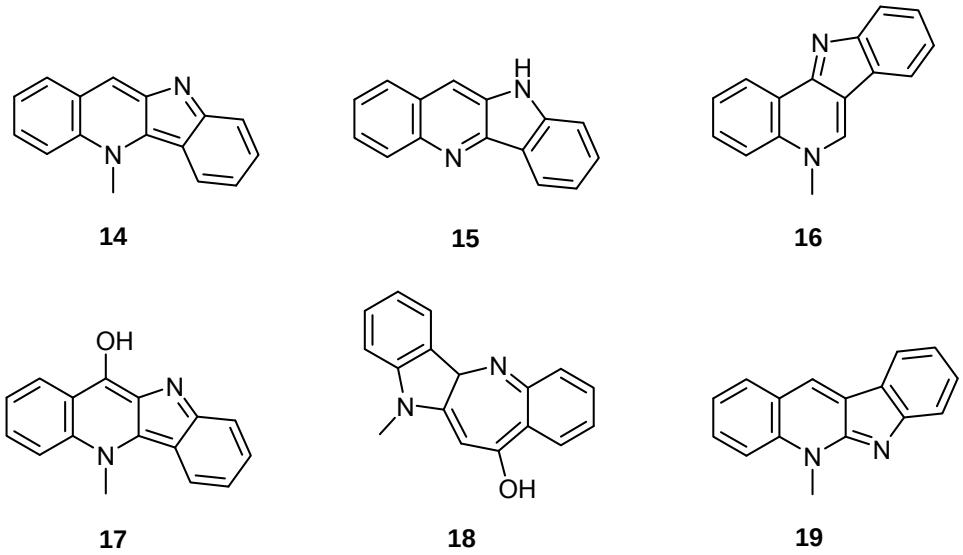



**Figure 1.10:** Alkaloids isolated from *Cryptolepis sanguinolenta;* cryptolepine **14**, quindoline **15**, isocryptolepine **16**, hydroxycryptolepine **17**, cryptoheptine **18** and neocryptolepine **19**

Cryptolepine **14** (5-methyl-5*H*-indolo[3,2-*b*]quinoline) has demonstrated antibacterial,[50,51] antimuscarinic,[52] antifungal[53] and antihyperglycemic[54] properties. It has also been found to possess *in vitro* activity against both sensitive and resistant strains of *P. falciparum.*[45,55-58] In one of the most recent studies, Van Miert *et al.*[59] reported cryptolepine **14** to have an $IC_{50}$ (the concentration at which 50% of parasites are killed *in vitro*) of 0.12 μM against the chloroquine resistant strain K1 (Table 1.1). Therefore in relative terms cryptolepine **14** is approximately three-fold less active compared to artemisinin **7** ($IC_{50}$ = 0.042 μM) and is similar in activity to chloroquine **2** ($IC_{50}$ = 0.17 μM). Its therapeutic application as a potential antimalarial drug, however, is impeded by its high cytotoxicity. Cryptolepine **14** has been found to be cytotoxic in non-cancerous cell lines such as L-6 cells (rat skeletal myoblast).[58,59] Based on recent *in vitro* cytotoxicity results for artemisinin **7**,[60] cryptolepine **14** is approximately four hundred times more cytotoxic. In addition a recent *in vivo* study, conducted on *P. berghei* infected mice, reported cryptolepine **14** to be toxic to the mice after two doses of 20 mg $kg^{-1}$.[57]

The isomeric analogue of cryptolepine **14**, isocryptolepine **16** (5-methyl-5*H*-indolo[3,2-*c*]quinoline), was first isolated from *Cryptolepine sanguinolenta* in 1995 by Pousset *et al.*[46] This alkaloid possesses antimalarial activity ($IC_{50}$ = 0.78 μM),[59] and is nearly seven-fold less active than cryptolepine **14**. In addition it displayed cytotoxicity at similar levels to cryptolepine **14**. Neocryptolepine **19** (5-methyl-5*H*-

indolo[2,3-*b*]quinoline), isolated independently by Cimanga *et al.*[61] and Sharaf *et al*.[48] in 1996, also possesses antimalarial[62] and antibacterial properties.[63] Against *P. falciparum* it displayed antimalarial activity ($IC_{50}$ = 2.61 μM)[59] at levels approximately 20-fold lower than cryptolepine **14**. In contrast it was nearly three-fold less cytotoxic against non-cancerous cells.

**Table 1.1:** Bioactivity of Cryptolepis Alkaloids **14**, **16** and **19**.

| Compound [a] | Antimalarial activity; $IC_{50}$ (μM) [b] | Cytotoxicity; $IC_{50}$ (μM) [c] | Selectivity Index (SI) |
|---|---|---|---|
| Cryptolepine **14** | 0.12 [59] | 1.12 [59] | 9.3 |
| Isocryptolepine **16** | 0.78 [59] | 1.19 [59] | 1.5 |
| Neocryptolepine **19** | 2.61 [59] | 3.24 [59] | 1.3 |
| Artemisinin **7** | 0.042 [59] | 450.5 [60] | 10,726 |
| Chloroquine **2** | 0.17 [59] | - | - |

[a] Tested in salt form. [b] *In vitro* activity against *P. falciparum* (K1). [c] *In vitro* cytotoxicity against L-6 cells.

The ratio of cytotoxicity to antimalarial activity, known as the selectivity index (SI), is a useful guide for assessing the potential of a compound for use as an antimalarial agent. A high SI value indicates a compound is more therapeutically viable; for example artemisinin **7** has an SI of over 10,000. In contrast cryptolepine **14**, isocryptolepine **16** and neocryptolepine **19** have SI values 9.3, 1.5 and 1.2 respectively (Table 1.1).[59] Despite these low SI values the cryptolepis alkaloids represent an interesting set of novel lead structures and have been studied over the past ten to fifteen years for their potential as antimalarial drugs.

### 1.3.1. Antimalarial Mode of Action of Cryptolepis Alkaloids

The antimalarial mechanism of cryptolepine **14**, isocryptolepine **16** and neocryptolepine **19** is not fully understood. At present there is evidence to suggest that at least two different modes of action may be occurring concurrently.[64]

These alkaloids have been found to inhibit the formation of β-haematin (synthetic haemozoin) and therefore are assumed to act upon the parasite in a similar manner to chloroquine **2**.[59] The most bioactive alkaloid, cryptolepine **14**, exhibits greater inhibition towards β-haematin than either neocryptolepine **19** or isocryptolepine **16**. In contrast neocryptolepine **19** inhibits β-haematin more

efficiently than isocryptolepine **16** despite being less bioactive. In addition Arzel *et al.*[65] found that cryptolepine **14** most likely accumulates in parasitic nuclei, indicating that cryptolepine **14** does not have sufficient affinity for haem to cause accumulation in the food vacuole.[66] Therefore β-haematin inhibition is not the primary mode of action and there must be another mechanism responsible for the antimalarial activity associated with some of these alkaloids.

DNA intercalation, the process whereby a molecule binds between the base pairs in DNA resulting in inhibition of DNA biosynthesis, has also been proposed as a possible mechanism for the antimalarial activity of these alkaloids.[59] Cryptolepine **14** has been shown to intercalate into DNA *via* binding to guanine-cytosine (GC) rich sequences containing non-alternative cytosine-cytosine (CC) sites (Figure 1.11).[67] Usually a compound binds into DNA at an alternating site (i.e. CG) and thus cryptolepine **14** represents the first compound to bind into DNA in this particular manner. This novel mode of DNA intercalation has been attributed to the asymmetry of cryptolepine **14** and the tight binding observed can be ascribed to its highly planar ionised character at physiological pH.

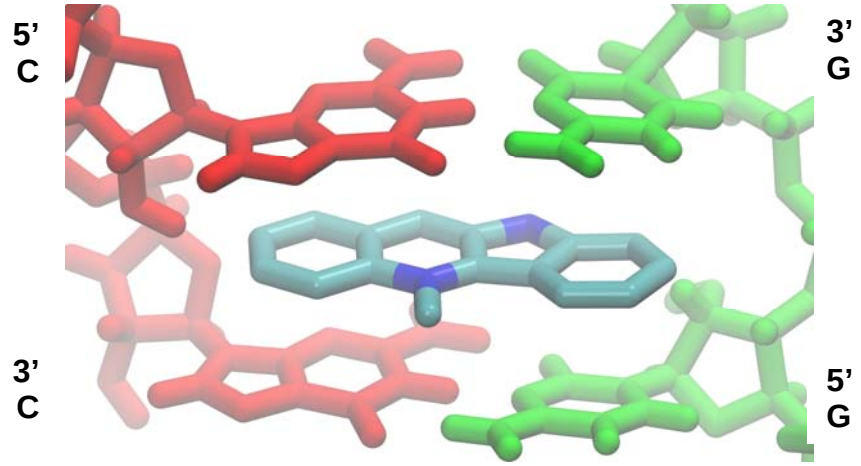



**Figure 1.11:** Cryptolepine **14** intercalating into DNA (image prepared using VMD Molecular Graphics Viewer)[68-69]

Whilst DNA intercalation into parasitic DNA may result in antimalarial activity, non-specific intercalation into human DNA is likely to cause the unfavourable cytotoxicity observed with these alkaloids. In addition cryptolepine **14** has been found to inhibit topoisomerase II, thus inducing DNA cleavage, a process that may play a minor role in the alkaloid's cytotoxicity.[70,71] Neocryptolepine **19** has also been shown to bind with DNA in a similar manner as cryptolepine **14**, preferring to

intercalate into GC rich sequences.[72] It has a lower affinity for DNA compared to cryptolepine **14**, and this may account for the lower cytotoxicity observed with this alkaloid. Whilst the ability of isocryptolepine **16** to intercalate into DNA has yet to be confirmed, its interaction with DNA in simple assays suggests that intercalation is likely.[59]

More recently an additional antimalarial mode of action of cryptolepine **14** has been proposed. Cryptolepine **14** has been found to inhibit NF-κB, a protein which controls DNA transcription.[73] Such a process has been linked with anti-inflammatory effects but NF-κB may also be important in the pathogenesis of malaria.[74]

### 1.3.2. Synthetic Derivatives of Cryptolepis Alkaloids

As demonstrated with quinine **1** and artemisinin **7**, synthetic derivatives of natural products can often have improved bioactivity compared to their parent compounds. In recent years numerous synthetic derivatives of both cryptolepine **14** and neocryptolepine **19** have been prepared, and biologically evaluated, in efforts to improve antimalarial activity and decrease cytotoxicity versus the parent form.

In relation to cryptolepine derivatives, the *N*-methyl group was found to be essential for activity as the desmethyl analogue quindoline **15** displayed significantly reduced antimalarial activity.[65] In addition halogen ring substituted derivatives were the most promising compounds, as determined by a comprehensive study of a range of mono and disubstituted cryptolepine derivatives.[57,75] Generally chloro and bromo compounds were more active than their methyl, methoxy or nitro counterparts. Activity was also strongly dependent on the position of the substituent; compounds with groups aligned with the long axis of the molecule (i.e. C2, C3, C7 and C8) were generally more active than those with groups orthogonal to the long axis of the molecule (i.e. C1, C4, C6 and C9; Figure 1.12).

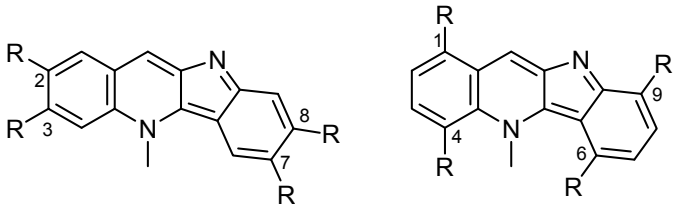



**Figure 1.12:** Cryptolepine **14** with R groups aligned with the long axis of the molecule (left) and groups orthogonal to the long axis of the molecule (right)

For example 2-chlorocryptolepine **20** (Figure 1.13) displayed a three-fold increase in antimalarial activity compared to the parent, 3-chlorocryptolepine **21** had similar activity and 4-chlorocryptolepine **22** was approximately 11-fold less active (Table 1.2).

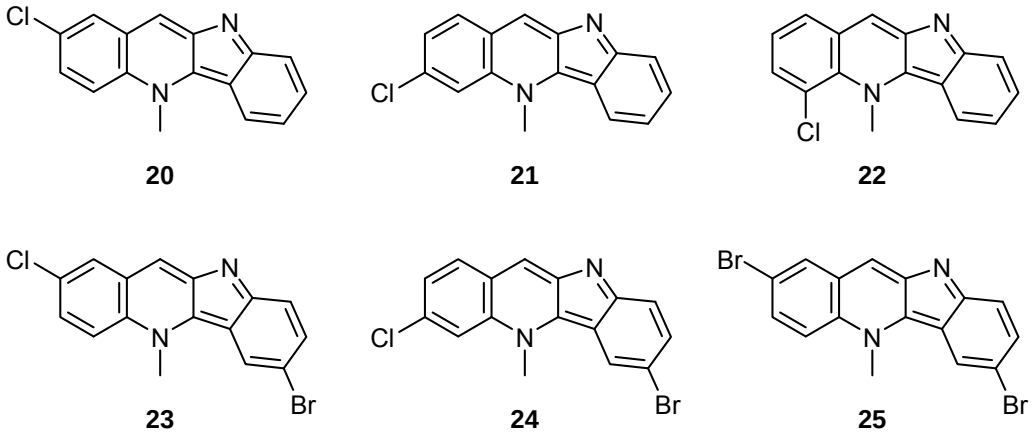



**Figure 1.13:** Various ring-substituted cryptolepine derivatives previously prepared and biologically evaluated by Wright *et al*.[57]

In addition dihalogenated derivatives displayed higher activity than their mono analogues; specifically 7-bromo-2-chlorocryptolepine **23**, 7-bromo-3-chlorocryptolepine **24** and 2,7-dibromocryptolepine **25** were the most active of all the derivatives assessed in this particular study.

**Table 1.2:** Bioactivity of cryptolepine derivatives **20 - 25**[57]

| Compound [a] | Antimalarial activity; $IC_{50}$ (μM) [b] | Cytotoxicity; $IC_{50}$ (μM) [c] | Selectivity Index (SI) |
|---|---|---|---|
| **20** | 0.17 | 2.24 | 13 |
| **21** | 0.49 | 1.75 | 3.6 |
| **22** | 4.69 | 3.54 | 0.8 |
| **23** | 0.03 | 1.73 | 58 |
| **24** | 0.037 | 1.14 | 31 |
| **25** | 0.049 | 6.04 | 123 |

[a] Tested in salt form. [b] *In vitro* activity against *P. falciparum* (K1). [c] *In vitro* cytotoxicity against MAC 15a cells (murine adenocarcinoma of the colon).

Both compounds **23** and **25** also suppressed parasitaemia in *P. berghei* infected mice by over 90% (25 mg $kg^{-1}$) with no observed toxicity, whilst cryptolepine **14** was toxic.[57] Unfortunately, the cytotoxicity of the most promising derivative **25** is still too high for drug applications (approximately 75-fold more cytotoxic than artemisinin **7**).

The latest study of cryptolepine analogues found that alkyl diamine chains at position C11 can result in enhancement of antimalarial activity.[76] However, the most active derivative (a piperidine analogue) was still too cytotoxic to be therapeutically applicable. The recurrent cytotoxicity issues with cryptolepine derivatives has led to a shift in focus in recent years and these compounds are now under investigation for their potential as anticancer agents.[77-79]

Various derivatives of neocryptolepine **19** have also been synthesised and investigated. Jonckers *et al.*[58] also found that halogenated derivatives of neocryptolepine **19** were the most promising derivatives and generally such derivatives had reduced cytotoxicity. In addition the position of the substituent greatly affected activity. For example 2-bromoneocryptolepine **26** (Figure 1.14) was approximately four-fold more active against *P. falciparum* (chloroquine resistant strain W2) compared to the parent alkaloid. 3-Bromoneocryptolepine **27** displayed a three-fold increase in activity and the derivative with an orthogonal group, 1-bromoneocryptolepine **28**, displayed no antimalarial activity (Table 1.3).

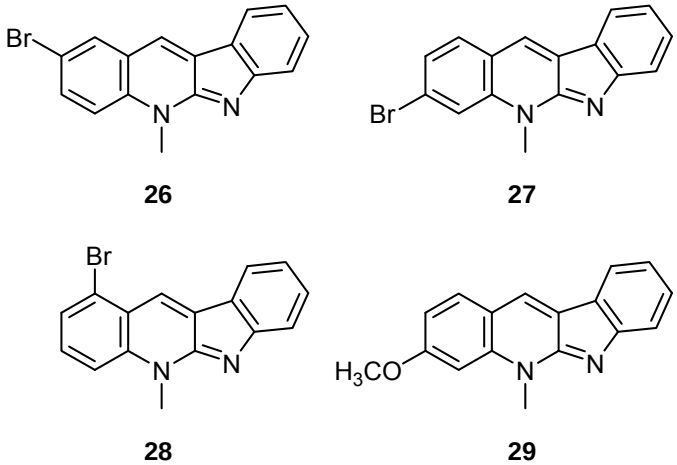



**Figure 1.14:** Various ring-substituted neocryptolepine derivatives previously prepared and biologically evaluated by Jonckers *et al.*[58]

This study also found 3-methoxyneocryptolepine **29** to possess the highest antimalarial activity of the tested derivatives, but unfortunately it also displayed

enhanced cytotoxicity compared to the parent alkaloid. Interestingly, compound **29** was found to have no inhibitory effect on β-haematin and less affinity for DNA in comparison to the parent alkaloid, which indicates that a hitherto unknown antimalarial mechanism may be active with this particular alkaloidal derivative.

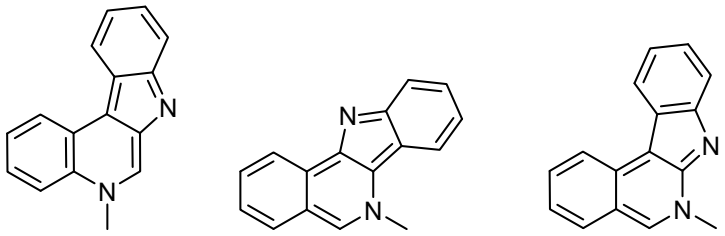
**Table 1.3:** Bioactivity of neocryptolepine derivatives **26 - 29**[58]

| Compound [a] | Antimalarial activity; $IC_{50}$ (μM) [b] | Cytotoxicity; $IC_{50}$ (μM) [c] | Selectivity Index (SI) |
|---|---|---|---|
| **26** | 4.0 | >32 | >8 |
| **27** | >32 | >32 | - |
| **28** | 4.7 | 18.5 | 4 |
| **29** | 1.7 | 3.5 | 2 |
| **19** | 14 | 11 | 0.8 |

[a] Tested in salt form. [b] *In vitro* activity against *P. falciparum* (W2). [c] *In vitro* cytotoxicity against MRC-5 cells (human diploid embryonic lung).

A recent study relating to neocryptolepine derivatives found that alkyl-amino substituents (e.g. (4-(diethylamino)-1-methylbutyl)amino) significantly enhanced antimalarial activity and SI values, but these compounds were only evaluated against a chloroquine sensitive strain of *P. falciparum*.[80] In addition when selected derivatives were evaluated *in vivo*, using *P. berghei* infected mice, they were either toxic or did not sufficiently suppress parasitaemia to be of apparent therapeutic benefit.

Synthetic structural isomers of the cryptolepis alkaloids have also been prepared and assessed for their antimalarial activity. Isoneocryptolepine **30** (5-methyl-5*H*-indolo[2,3-*c*]quinoline; Figure 1.15)[81] is one such compound which possesses *in vitro* bioactivity against *P. falciparum* ($IC_{50}$ = 0.23 μM; Table 1.4).[59] This non-natural compound was also found to be less cytotoxic against non-cancerous cells, but did not sufficiently suppress parasitaemia *in vivo*.[59] The synthesis and biological evaluation of the isoquinoline analogues 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **31** and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline **32** was also recently reported (Table 1.4).[82]



**30** **31** **32**

**Figure 1.15:** Synthetic cryptolepis alkaloid analogues; isoneocryptolepine **30**, 6-Methyl-6*H*-indolo[3,2-*c*]isoquinoline **31** and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline **32**

Both compounds were active against *P. falciparum in vitro*, with compound **31** found to be appreciably more active than cryptolepine **14**. Both were also evaluated for cytotoxicity against L-6 cells and were slightly less cytotoxic than cryptolepine **14**. Of these non-natural heterocycles, compound **31** had the highest SI value of approximately 33 and represents a potential lead compound.

**Table 1.4:** Bioactivity of synthetic cryptolepis alkaloid analogues **30**, **31** and **32**

| Compound [a] | Antimalarial activity; $IC_{50}$ (μM) [b] | Cytotoxicity; $IC_{50}$ (μM) [c] | Selectivity Index (SI) |
|---|---|---|---|
| **30** | 0.23[59] | 1.32[59] | 19 |
| **31** | 0.04[82] | 1.31[82] | 33 |
| **32** | 0.68[82] | 1.48[82] | 2 |

[a] Tested in salt form. [b] *In vitro* activity against *P. falciparum* (K1). [c] *In vitro* cytotoxicity against L6 cells.

## 1.4. Project Aims

Although isocryptolepine **16** has a similar SI to neocryptolepine **19**, no ring-substituted derivatives have been investigated with the aim of improving bioactivity. The reasoning for the neglect of this potential lead compound is unclear. Isocryptolepine **16** may possess similar structure activity relationships as neocryptolepine **19**, which may result in substituted derivatives with improved antimalarial activity without enhanced cytotoxicity. Given that isocryptolepine **16** possesses higher antimalarial activity than neocryptolepine **19**, derivatives may also be superior in this respect. Needless to say there is the possibility that derivatives will behave more like cryptolepine derivatives and possess enhanced cytotoxicity. The primary aim of this research project was thus to investigate the potential of the naturally occurring indoloquinoline alkaloid isocryptolepine **16** as a lead compound for future antimalarial drugs. In order to accomplish this aim a range of synthetic derivatives of isocryptolepine **14** were prepared and biologically evaluated for both antimalarial activity and cytotoxicity.

Previous studies of cryptolepine **14** and neocryptolepine **19** have established that halogenated derivatives, particularly chloro and bromo, showed the most improvement in biological activity in comparison to the parent alkaloid. In addition derivatives with substituents aligned with the long axis of the molecule were often more active than compounds with groups orthogonal to the long axis of the molecule. Routes to corresponding isocryptolepine derivatives were a priority (i.e. halogen ring substituents at positions C2, C3, C8 and C9; Figure 1.16) in an effort to ascertain if similar structure activity relationships existed.

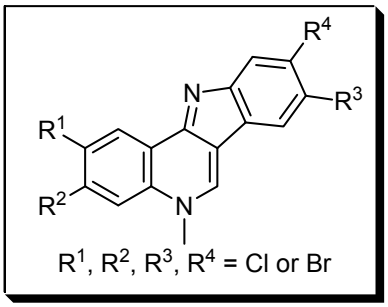



**Figure 1.16:** Proposed isocryptolepine derivatives

The previously published synthetic methodologies to isocryptolepine **16** were thoroughly assessed and a selection chosen for further investigation based on

numerous factors: compound yield (intermediates and total), the number of steps, reproducibility and the ease of compound isolation and purification. The chosen methodologies were accordingly optimised (Chapter 2) and a selection were applied to the preparation of the proposed derivatives in Figure 1.16 (Chapter 3).

At the commencement of this research project the structural isomer of isocryptolepine **16**, 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **31**, had yet to be reported. Developing a synthetic route to this compound was initially a secondary aim. However, following the report of its synthesis, and potent biological activity, the focus shifted to improving the published synthetic method with the aim to confirm its antimalarial activity.

The final step of the research project involved the biological evaluation of a selection of the prepared derivatives (Chapter 4). Compounds were assessed for antimalarial activity against a chloroquine sensitive and chloroquine resistant strain of *P. falciparum*. Additionally the same set of derivatives was assessed for cytotoxicity.

The data obtained from these latter studies has facilitated the elucidation of certain structure activity relationships and allowed the identification of isocryptolepine derivatives with potential for future investigation. This research represents the first analysis of isocryptolepine derivatives in relation to their prospects as lead compounds in antimalarial drug development.

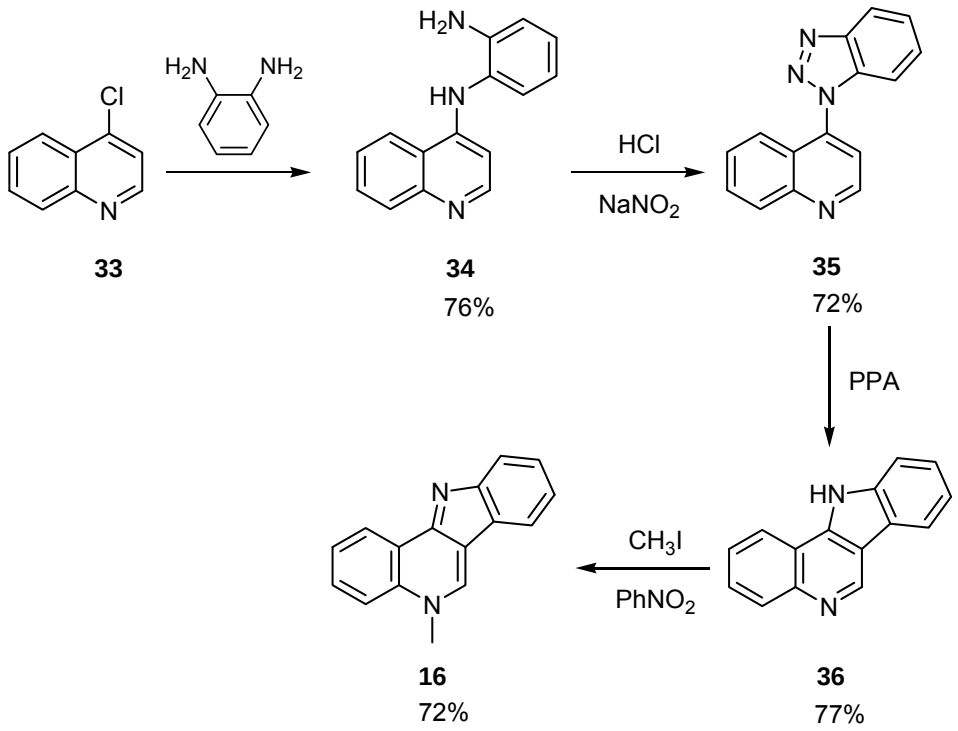
# Chapter 2

## Synthesis of Isocryptolepine

## 2.1. Introduction

Numerous synthetic routes to isocryptolepine **16** have previously been reported and most are accomplished by means of either indole or quinoline containing compounds. These synthetic methods to the parent alkaloid are described and evaluated below. Based on the number of synthetic steps involved, reported yields and the ease with which synthetic routes may be modified, various published synthetic procedures were considered for application with respect to future synthesis of isocryptolepine derivatives. However, prior to the application of these approaches to the preparation of analogues efforts were made to reproduce, and if possible optimise, these methodologies.

The first reported synthesis of isocryptolepine **16** was conceived by Kermack and Storey[83] in 1950, long before the compound was isolated from *Cryptolepis sanguinolenta* (Scheme 2.1). This four step synthesis proceeds from 4-chloroquinoline **33**, which was initially coupled to *o*-phenylenediamine to give 4-(2-aminoanilino)quinoline **34**.



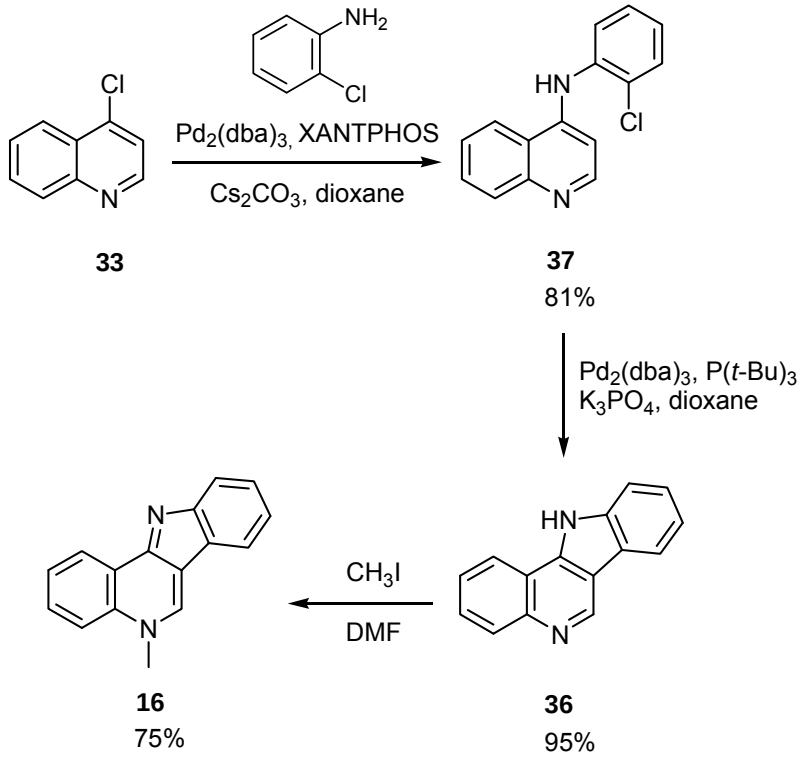

**Scheme 2.1:** Kermack and Storey[83] synthetic route to isocryptolepine **16**

Subsequent treatment of intermediate **34** with nitrous acid (formed *in situ*) resulted in diazotisation and cyclisation to yield 4-(1-benzotriazolyl)quinoline **35**. Acid

catalysed cyclisation of the later intermediate in polyphosphoric acid (PPA), *via* a modified Graebe-Ullmann mechanism, afforded 11*H*-Indolo[3,2-*c*]quinoline **36**. Finally *N*-methylation of the cyclic intermediate **36**, with iodomethane, gave isocryptolepine **16** in an overall yield of 30% from 4-chloroquinoline **33**.

Molina *et al.*[84] adapted the above synthetic route by directly coupling benzotriazole to 4-chloroquinoline **33**, to generate the benzotriazole intermediate **35** in high yield (96%). The cyclic intermediate **36** was obtained in 83% yield, using the same reaction conditions as applied by Kermack and Storey,[83] but the yields of *N*-methylation were improved by using acetonitrile as the solvent (92%). Consequently the overall yield of isocryptolepine **16** from 4-chloroquinoline **33** was enhanced to 73%, an increase of 44% from the original synthesis.

Jonckers *et al.*[58] developed an alternative synthetic route towards isocryptolepine **16**, which also applied 4-chloroquinoline **33** as the primary starting material (Scheme 2.2).



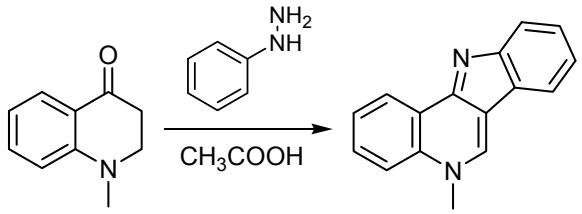

**Scheme 2.2:** Jonckers *et al.*[58] synthetic route to isocryptolepine **16**

Initially compound **33** was coupled to 2-chloroaniline *via* a Buchwald-Hartwig reaction, catalysed by tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$) and the heterocyclic xanthene bidentate ligand XANTPHOS. The resulting intermediate 4-

(2-chlorophenylamino)quinoline **37** was subsequently cyclised to give **36** *via* an intramolecular Heck-type reaction, catalysed by $Pd_2(dba)_3$ and the phosphate ligand tri-*tert*-butylphosphine (P(*t*-Bu)$_3$). Finally, the cyclised intermediate **36** was *N*-methylated, using iodomethane, to afford isocryptolepine **16** in moderate overall yield (58%).

Dhanabal *et al.*[85] modified this synthesis by removing the palladium based catalysts and conducting the coupling reaction at high temperatures (200 °C). Similarly the cyclisation was achieved by photochemical irradiation, negating the need for a catalyst. Whilst simplifying the reaction processes these modifications also resulted in a slight reduction of the overall synthetic yield (48%).

Grellier *et al.*[45] reported a single step synthesis of isocryptolepine **16** from *N*-methyl-2,3-dihydro-4-quinolone **38** (Scheme 2.3), which was itself prepared from propiolactone and *N*-methyl aniline. Phenylhydrazine and **38** undergo a Fischer indole synthesis to provide isocryptolepine **16** in fairly low yield (15%).

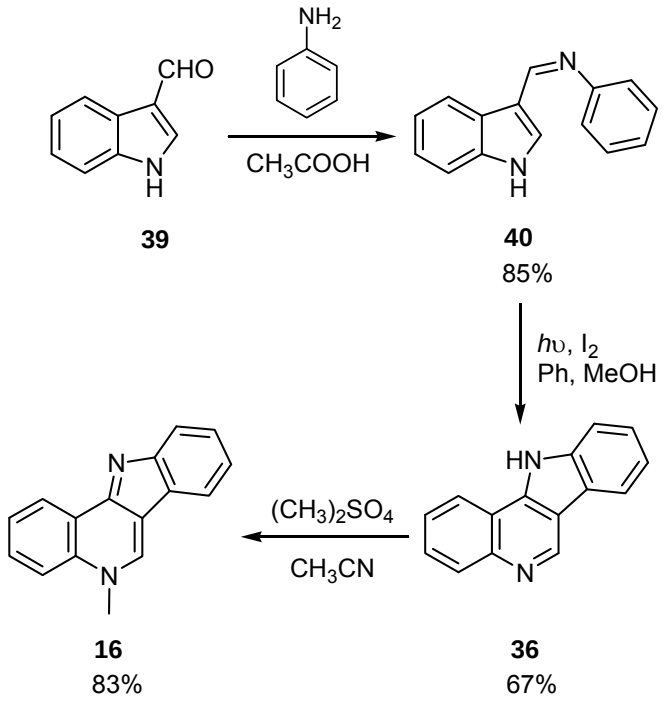



**38** **16**
15%

**Scheme 2.3:** Grellier *et al.*[45] synthetic route to isocryptolepine **16**

Timári *et al.*[86] reported a synthetic route to the parent alkaloid which proceeded from 3-bromoquinoline that was initially coupled to a phenylboronic acid *via* a Suzuki reaction. Isocryptolepine **16** was obtained in five steps in an overall yield of 47% - lower than some of the previously described quinoline based methodologies.

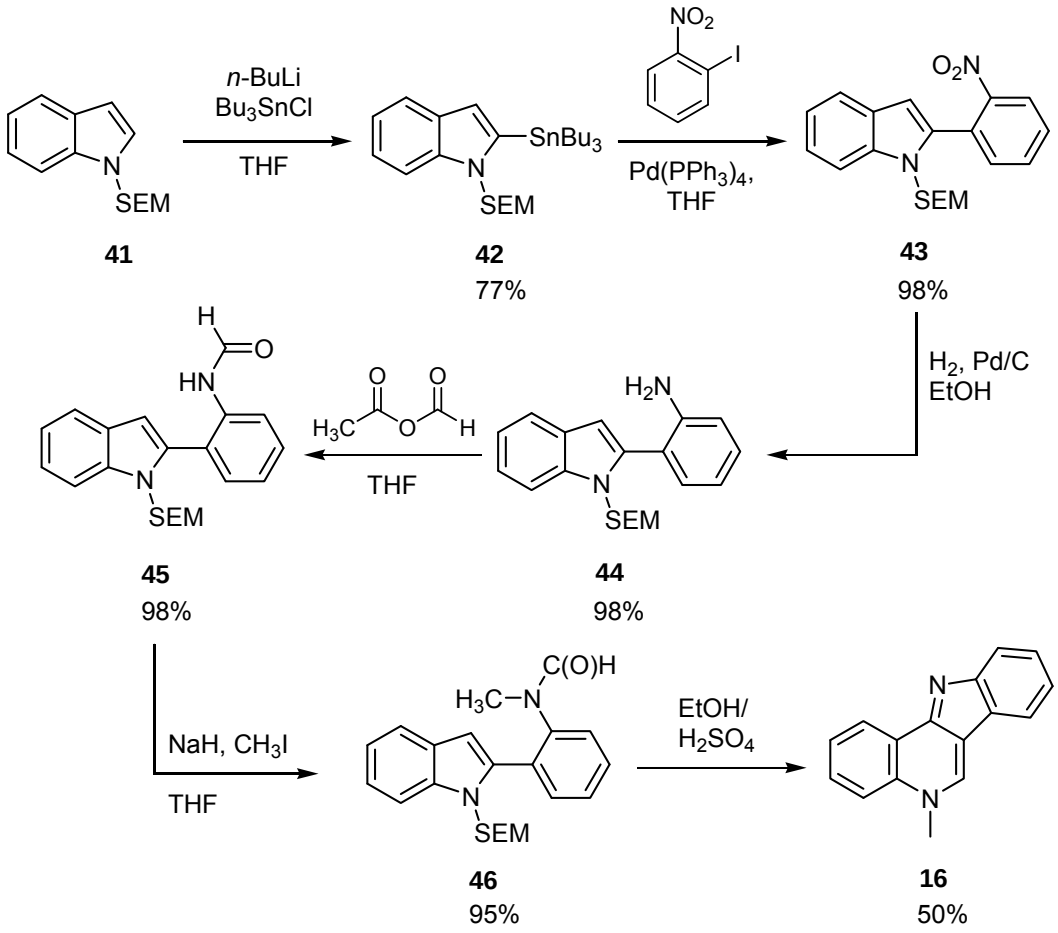
In comparison to the quinoline mediated routes there are fewer synthetic methods that initially progress *via* indole containing moieties. The three step procedure reported by Kumar *et al.*[87] proceeded from commercially available indole-3-carboxaldehyde **39** (Scheme 2.4). Compound **39** was initially coupled to aniline, in glacial acetic acid, thus forming the Schiff base **40** which underwent photo-induced cyclisation to provide indoloquinoline **36**. Final *N*-methylation of **36** was achieved with dimethyl sulfate to give isocryptolepine **16** in moderate overall yield (47%).




**Scheme 2.4:** Kumar *et al.*[87] synthetic route to isocryptolepine **16**

Murray *et al.*[88] described a six step synthetic procedure to isocryptolepine **16** starting from [2-(trimethylsilyl)ethoxy]methyl (SEM) protected indole **41** (Scheme 2.5). Treatment of *N*-SEM-indole **41** with *n*-butyllithium and subsequent quenching with tributyltin chloride afforded the stannane **42**. Stille coupling of intermediate **42** with 2-iodonitrobenzene, catalysed with tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$), gave the nitro intermediate **43**, which was reduced, formylated and methylated to give isocryptolepine **16** in moderate overall yield (34%).

Of the remaining synthetic methodologies to isocryptolepine **16** not yet discussed, the 11 step procedure reported by Fresneda *et al.*[89] was considered too long. Similarly the three step procedure described by Dhanabal *et al.*[90] produced isocryptolepine **16** in low overall yield (28%). In addition a number of alternative synthetic methodologies have been described following the commencement of the present research project and could not be considered.[91,92] Some may be applied to the preparation of further derivatives at a later stage, notably the method reported by Kumar *et al.*[93] This synthetic route to isocryptolepine **16** was an adaption of the single step method developed by Grellier *et al.* (Scheme 2.3)[45] and the final yield of isocryptolepine **16** was improved to 83% using *p*-toluenesulfonic acid as the catalyst instead of glacial acetic acid.




**Scheme 2.5:** Murray *et al.*[88] synthetic route to isocryptolepine **16**

## 2.2. Approaches to an Improved Synthesis of Isocryptolepine 16

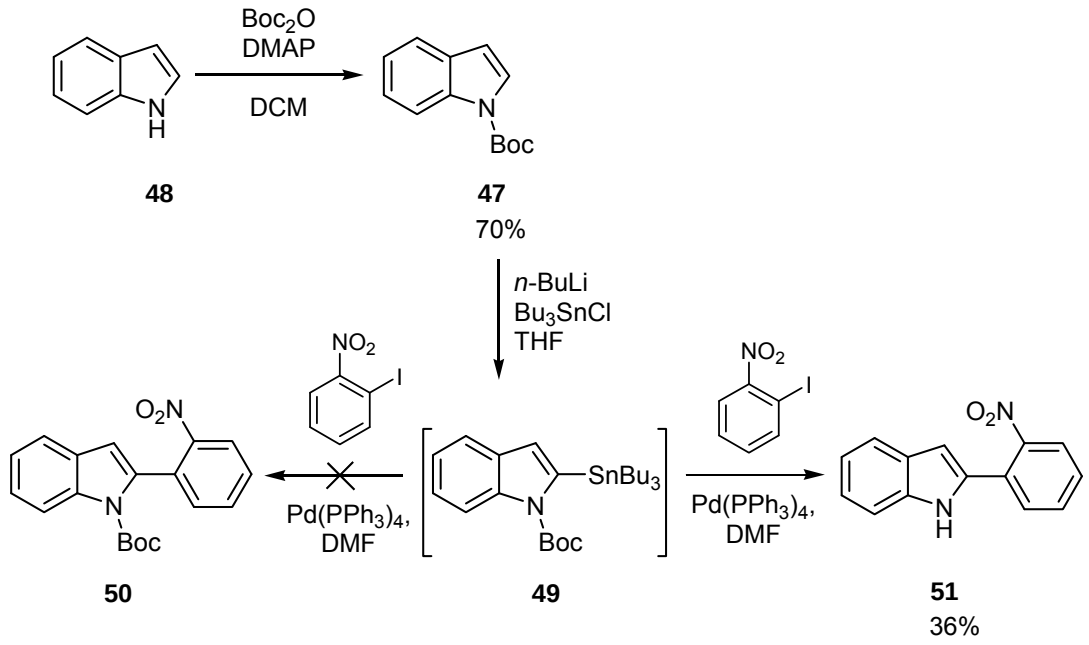
The three step synthetic procedures described by Molina *et al.*,[84] Jonckers *et al.*[94] and Kumar *et al.*[87] provide isocryptolepine **16** in moderate overall yields (47-73%). The method reported by Molina *et al.*, henceforth referred to as the Molina Method, was the highest yielding and also involved relatively straightforward synthetic steps, of which the first two proceeded without the need for a solvent. The reproduction of this particular procedure is outlined in Section 2.5.

The synthesis reported by Jonckers *et al.,* henceforth referred to as the Jonckers Method, requires expensive palladium catalysts. It was hypothesized that the relatively exotic and expensive palladium catalyst $Pd_2(dba)_3$ could be substituted for more readily available and less costly catalysts, such as palladium acetate ($Pd(OAc)_2$) or $Pd(PPh_3)_4$. In addition, with the substitution of 4-chloroquinoline **33** for its bromo analogue, 4-bromoquinoline, the reaction rates and yields of the palladium catalysed reactions may be improved. Optimisation of both palladium catalysed coupling reactions was thoroughly investigated and the results obtained are discussed in Section 2.4.

The highest yielding indole based method, described by Kumar *et al.*, could not be reproduced owing to the lack of photochemical reaction facilities at our laboratories. The procedure outlined by Murray *et al.*, henceforth referred to as the Murray Method, was instead investigated at the commencement of the project. Although this was a six step procedure, it represents the next highest yielding indole based method and its application may afford an alternative route to isocryptolepine derivatives. It was postulated that the overall reaction yield could be improved with a more easily removable indole protecting group, as Murray *et al.* reported that the removal of the SEM group was problematic and resulted in a low yield of the alkaloid during the final step of the synthesis. During the early stages of research a variety of *N*-protecting groups were trialled and the outcomes of this particular trial process are discussed in the following section.

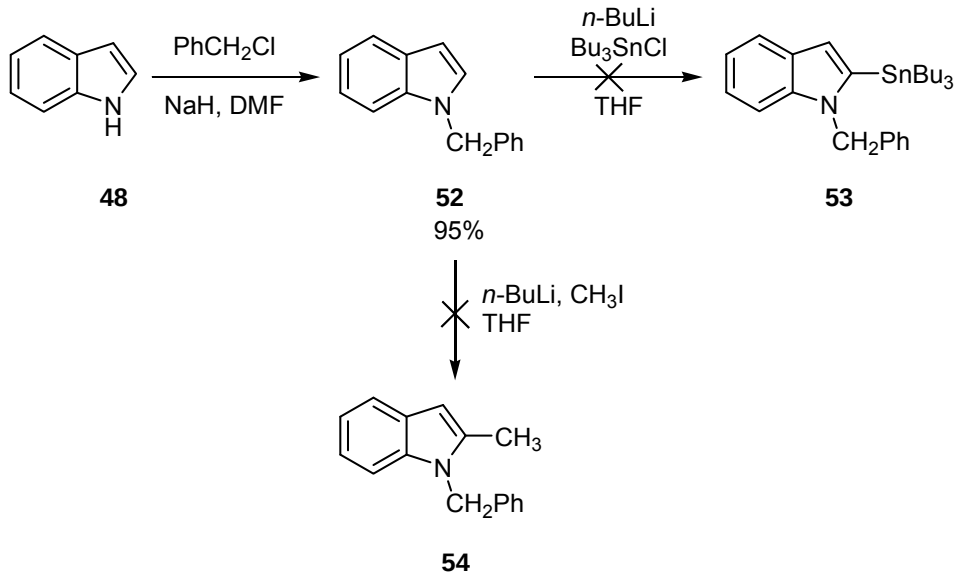
## 2.3. Isocryptolepine 16 *via* the Murray Method

There are a wide variety of *N*-protecting groups available that are compatible with indoles. The *tert*-butoxycarbonyl (Boc) group is widely used, as it can be easily added and its removal is relatively straightforward; generally treatment with trifluoroacetic acid is sufficient.[95] The Boc protected indole **47** was synthesised in 70% yield from indole **48**, as previously reported,[96-98] by reaction with 4-dimethylaminopyridine (DMAP) and di-*tert*-butyl dicarbonate (Scheme 2.6). Subsequent lithiation of compound **47**, with *n*-butyllithium and quenching with tributyltin chloride gave the desired stannane **49** (as confirmed by TLC initially). Attempts were made to purify the stannane **49**, but it was found to be unstable and degraded on the silica. The stannane **49** was thus used without further purification in the subsequent Stille cross-coupling with 2-iodonitrobenzene, catalysed by $Pd(PPh_3)_4$. Unfortunately the coupling reaction did not produce the desired product **50** and for the most part resulted in premature indole deprotection to produce 2-(2-nitrophenyl)indole **51** in low yield (36%), which was confirmed by NMR spectroscopy.[99] Consequently the Boc protecting group was deemed too labile and unsuitable for this synthetic route resulting in a discontinuation of its further use.




**Scheme 2.6:** Attempted synthesis of intermediate **50**

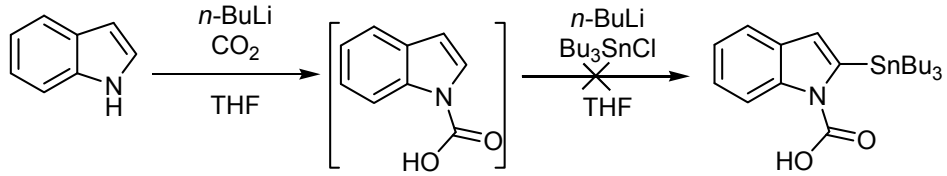
The benzyl group has been infrequently used as an *N*-indole protecting group in comparison to Boc and SEM. However, it has still proven useful in the synthesis of a range of heterocycles and is well tolerated in palladium-catalysed reactions.[100,101] *N*-benzylindole **52** was synthesised *via* an adaptation of a previously reported procedure used to prepare similar compounds (Scheme 2.7).[102] Deprotonation of indole **48** with sodium hydride and subsequent reaction with benzyl chloride gave *N*-benzylindole **52** in high yield (95%). Lithiation of compound **52** and subsequent quenching with tributyltin chloride did not produce the desired stannane intermediate **53** as only the deprotected indole **48** was isolated. It was postulated that the lithiation process was facilitating debenzylation and in order to confirm this, quenching of the lithiated species with iodomethane was attempted. However, this reaction did not form 2-methyl-*N*-benzylindole **54** but again resulted in the isolation of deprotected indole **48**, thus confirming that the 2-lithiated species was not forming. This finding is in agreement with the literature report by Suzuki *et al.*[103] that linked the use of lithium bases, such as lithium diisopropylamine and methyllithium, to the debenzylation of *N*-benzylindoles. As a result this particular protecting group was also deemed unsuitable and its further use was not pursued.




**Scheme 2.7:** Attempted synthesis of intermediate **53**

As a final resort the carboxyl protecting group was investigated as it had previously been successfully used for *N*-indole protection in a report by Hudkins *et al.*[104] Indole **48** was initially reacted with *n*-butyllithium and quenched with carbon dioxide, which reportedly forms *N*-carboxylindole **55** *in situ* (Scheme 2.8). However

subsequent lithiation and quenching with tributyltin chloride did not form the desired stannane **56**. Preliminary TLC analysis indicated the presence of mainly indole **48**, implying that the stannane intermediate may have decomposed. Similarly it is possible that the *N*-protected indole **55** may not have been formed in the first instance. The instability of the carboxy intermediate **55** was also reported by Hudkins *et al.*[104] as it was found to degrade in acid and base or at high temperature. Subsequent attempts to generate intermediate **55** under various reaction conditions were unsuccessful and formation of the stannane **56** was not achieved *via* this route. Hence this synthetic pathway was abandoned.

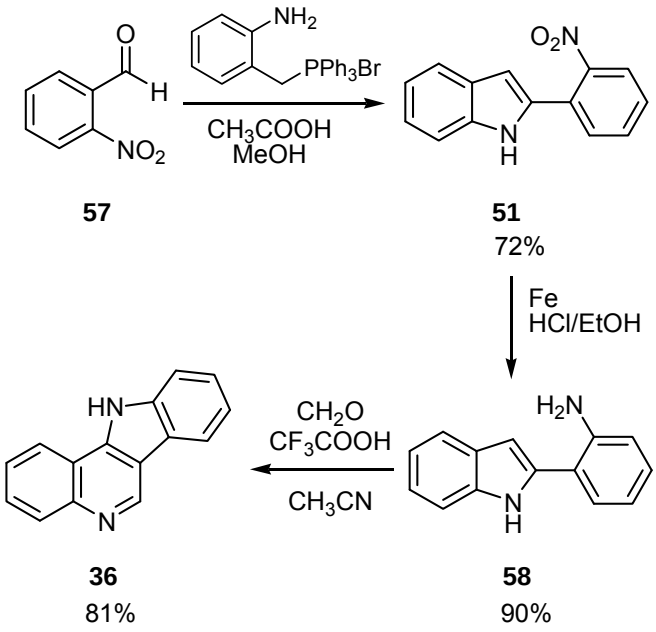



**48** **55** **56**

**Scheme 2.8:** Attempted synthesis of intermediate **56**

Because attempts thus far to find an alternative protecting group to SEM were unsuccessful, efforts were made to reproduce the Murray Method (Scheme 2.5) and optimise the later removal of the SEM protecting group. *N*-SEM-indole **41** was synthesised *via* previously reported procedures[105,106] (in 84% yield), lithiated, quenched with tributyltin chloride and coupled to give 2-(2-nitrophenyl)-*N*-SEM-indole **43** in 63% yield. The high yields reported by Murray (98%) were not reproduced despite multiple attempts. Tributyltin residues were also detected by NMR analysis but removal attempts, by chromatography on neutral alumina, were unsuccessful and the unpurified product was used. Subsequent reduction, formylation and methylation of the nitro intermediate **43** resulting in low overall yield (48%) of 2-[2-(*N*-methyl)formylaminophenyl]-*N*-SEM-indole **46**. The low yield of intermediate **46** was attributed to inadequate hydrogenation apparatus on site which impeded the stoichiometric reduction of the nitro intermediate **43**. The concluding acid catalysed cyclisation and *N*-deprotection step produced the desired isocryptolepine **16** in only 23% yield. The overall yield of isocryptolepine **16** from *N*-SEM-indole **41** was significantly lower (7%) than reported by Murray (34%) and further efforts were unable to improve reaction yields. Thus this synthetic route to isocryptolepine **16** was judged unsuitable for our purposes.

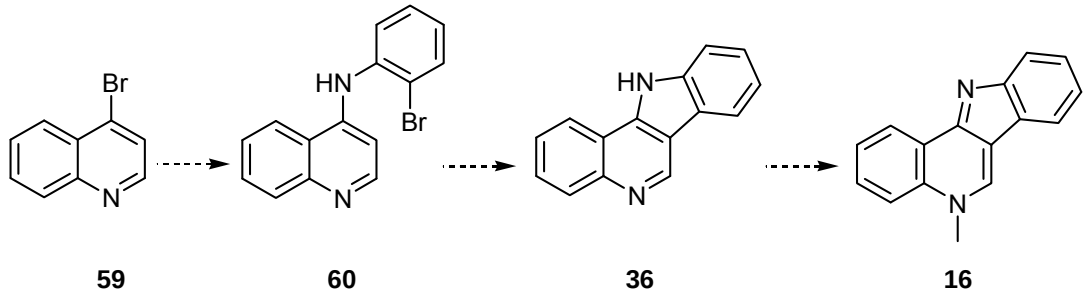
Recently Kraus *et al.*[99] reported a novel synthesis of 2-(2-nitrophenyl)indole **51** from *o*-nitrobenzaldehyde **57** and a phosphonium salt in moderate yield (72%; Scheme 2.9). The authors subsequently demonstrated that compound **51** could be reduced to 2-(2-aminophenyl)indole **58** and finally cyclised to 11*H*-indolo[3,2-*c*]quinoline **36** without the need of an *N*-protecting group. Although there are some inconsistencies in this paper (i.e. the intermediate *via* which **58** cyclises to **36** cannot be a 3-formylindole as this would result in a dihydro product) this synthetic methodology represents a possible novel high yielding route to isocryptolepine **16**. This synthetic methodology could not be investigated in the present project but it may be later applied to the preparation of additional derivatives.




**Scheme 2.9:** Kraus *et al.*[99] synthetic route to 11*H*-indolo[3,2-*c*]quinoline **36**

## 2.4. Isocryptolepine 16 *via* the Jonckers Method

The application of 2-bromoaniline and 4-bromoquinoline **59** as starting materials to the previously described Jonckers Method was initially investigated (Scheme 2.10).




**59** **60** **36** **16**

**Scheme 2.10:** Proposed adaptation to the Jonckers synthetic route to isocryptolepine **16**

The formation of 4-(2-bromophenylamino)quinoline **60** (*via* a Buchwald-Hartwig reaction) and the subsequent intramolecular Heck-type reaction to give 11*H*-indolo[3,2-*c*]quinoline **36** were each optimised by assessing a range of different catalysts and reaction conditions. Final *N*-methylation of intermediate **36**, to give the parent alkaloid isocryptolepine **16**, was investigated using different solvents in an effort to improve the yield of this last synthetic step.

### 2.4.1. Synthesis of 4-(2-Bromophenylamino)quinoline 60

The Buchwald-Hartwig reaction, developed independently by two research groups, allows the amination of aryl halides to be achieved under mild conditions with the assistance of a palladium based catalyst.[107-110] This reaction has been extensively studied and utilised synthetically since its development, and been applied to a wide range of aryl halides in addition to various amines.[111] Similarly a range of both palladium based catalysts and associated ligands have been investigated. The main role of the ligand is to stabilise the catalytic intermediates during the coupling process and quite often the choice of ligand is fine-tuned to a particular reaction in order to improve its efficiency and scope. Notable ligands that have proven highly effective in Buchwald-Hartwig aminations include bidentate phosphines (i.e. BINAP; 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl),[112] chelating alkylphosphines (i.e. DB$^t$PF; 1,1′-bis(di-*tert*-butylphosphino)ferrocene)[113] and monophosphinobiaryl

ligands (i.e. XPhos; 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl) (Figure 2.1).[114]

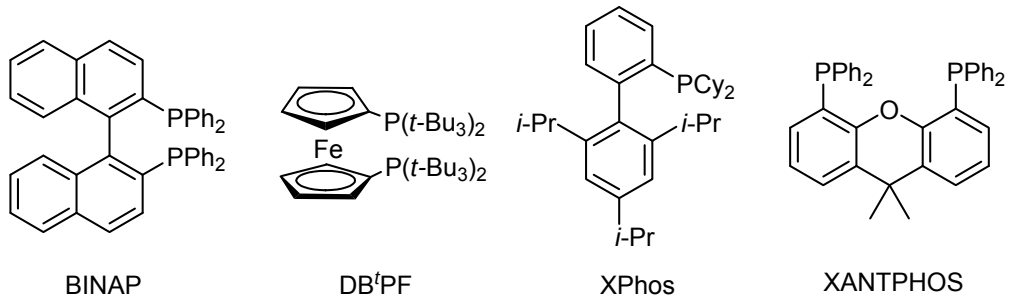



**Figure 2.1:** Common ligands utilised in Buchwald-Hartwig reactions

The palladium catalyst $Pd(OAc)_2$ is commonly combined with BINAP and its intermediates in the Buchwald-Hartwig catalytic cycle have been extensively investigated.[112] The three main chemical steps within the catalytic cycle include; (i) oxidative addition by coordination to an aryl halide (Ar-X), (ii) coordination to the amine ($NH_2$-R) facilitated by a base (BA) and finally (iii) reductive elimination to give the coupled product (R-NH-Ar) and regeneration of the catalyst (Figure 2.2).

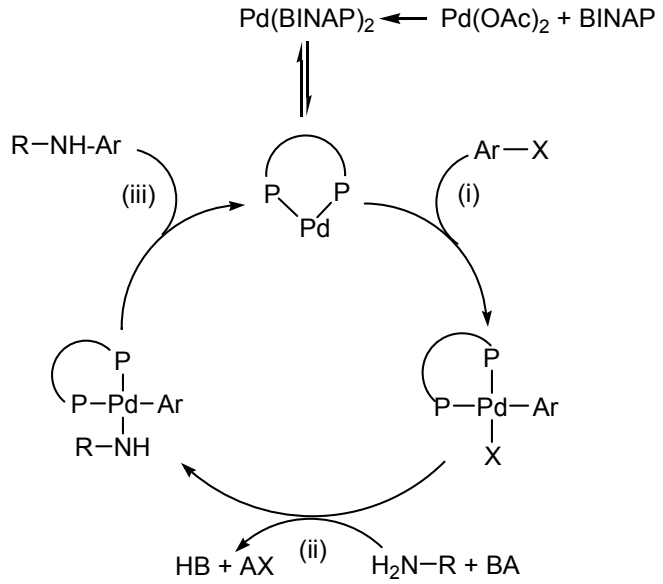
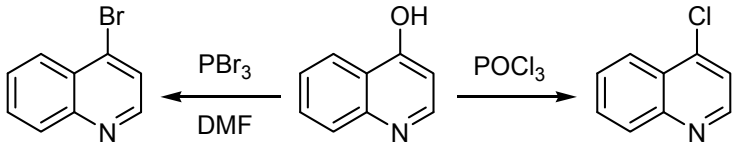



**Figure 2.2:** General catalytic cycle of a Buchwald-Hartwig reaction. $Pd(OAc)_2$ catalyses the coupling of an aryl halide (Ar-X) to an amine ($NH_2$-R)

Aryl halide reduction and homocoupling can produce various side-products during a Buchwald-Hartwig reaction that reduces the efficiency of the catalytic cycle. It has been postulated that aryl halide reduction proceeds *via* a β-hydride elimination pathway[115] and may be suppressed by the presence of bidentate

phosphine ligands.[112,116] These ligands may block the vacant coordination site on the palladium metal centre that is required for β-hydride elimination to occur. Another ligand commonly applied to the amination of aryl halides is XANTPHOS (Figure 2.1).[117-119] Only two bidentate phosphine ligands (i.e. BINAP and XANTPHOS) were investigated in the current study.

The starting material for the Buchwald-Hartwig reaction, 4-chloroquinoline **33**, was prepared from commercially available 4-quinolinol **61** *via* chlorination with phosphorus oxychloride ($POCl_3$) in good yield (79%) using a previously reported procedure (Scheme 2.11).[120] Similarly 4-bromoquinoline **59** was prepared, in high yield (80%), by bromination of 4-quinolinol **61** with phosphorus tribromide ($PBr_3$) again *via* a previously reported procedure.[121]




**59** 80% **61** **33** 79%

**Scheme 2.11:** Synthesis of 4-chloroquinoline **33** and 4-bromoquinoline **59**

The Buchwald-Hartwig coupling of 2-chloroaniline to 4-chloroquinoline **33**, catalysed by $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%) in refluxing dioxane, gave the intermediate 4-(2-chlorophenylamino)quinoline **37** in comparable yields (62%) to those reported by Jonckers *et al.*[94] under the same conditions (60%).

The coupling of 2-bromoaniline to 4-bromoquinoline **59**, using the above reaction conditions, resulted in the formation of the novel compound 4-(2-bromophenylamino)quinoline **60** in slightly lower yield (55%). The structure of the product was confirmed by proton and carbon NMR spectroscopy and spectral signals were fully assigned with the assistance of 2D-NMR spectroscopy (notably COSY, HSQC and HMBC).

Aryl bromides are generally considered more reactive than aryl chlorides in palladium coupling reactions under the same conditions,[122] and it was initially predicted that the bromo coupled intermediate **60** would be formed in higher yields than the chloro coupled intermediate **37**. However, this was not the case and it was hypothesized that the bromo based reactants were also increasing the rates of side-product formation resulting in the observed reduction in product yield.

In an effort to optimise the synthesis of the coupled intermediate **60**, a series of small scale (50 mg of 4-haloquinoline) reactions were conducted using a variety of reaction conditions. Reaction mixtures were analysed by HPLC, whereby starting materials and products were identified *via* spectrophotometric detection. The percentage of the coupled product formed was determined by calculating the ratio of products to starting material, after correcting for the different extinction coefficients of the compounds with a set of standards. For example, assessment of the standards determined that quinoline **59** was generally 1.8-fold more UV absorbing than intermediate **60**. This method of reaction evaluation has previously been used to monitor Buchwald-Hartwig reactions for similar quinolines and anilines[117,123] and the various results of the present HPLC study are summarised in Table 2.1.

**Table 2.1:** Optimisation of the Buchwald-Hartwig coupling of quinoline **59** and 2-bromoaniline [a]

| Catalyst | Ligand | Base | Solvent | % Conversion [b] |
|---|---|---|---|---|
| $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | Dioxane | 87 (55) |
| $Pd(OAc)_2$ | XANTPHOS | $K_2CO_3$ | Dioxane | 96 (65) |
| $Pd_2(dba)_3$ | XANTPHOS | $Cs_2CO_3$ | Dioxane | 99 (72) |
| $Pd(PPh_3)_4$ | - | $K_2CO_3$ | Dioxane | 93 |
| $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | DMF | 96 (38) |

[a] Reagents and Conditions: 24hr; reflux or 110 °C; $Pd_2(dba)_3$ (1 mol%), $Pd(OAc)_2$ (2 mol%) or $Pd(PPh_3)_4$ (10 mol%); XANTPHOS (2.2 mol%) or BINAP (2 mol%); $K_2CO_3$ (20 mol eq.) or $Cs_2CO_3$ (1.3 mol eq.). [b] Conversion **60**/(**59**+**60**) by HPLC-UV; isolated yield of **60** in parenthesis.

The above HPLC investigation found that all the reaction conditions trialled resulted in approximately 90% conversion of quinoline **59** to intermediate **60**, indicating that the majority of the starting material **59** had been consumed. However, when these reaction conditions were applied on a larger scale, the isolated yields did not correlate to the percentage conversions. For example, reaction with the catalytic combination $Pd(OAc)_2$ (2 mol%) and XANTPHOS (1.1 mol%) resulted in the coupled intermediate **60** being isolated in 65% yield whilst HPLC indicated 96% conversion. Possibly side-product formation was resulting in the consumption of quinoline **59**, giving a percentage conversion that did not accurately represent the reaction yield. In hindsight the use of an inert internal standard would have provided

clearer results and may be applied at a later stage to more thoroughly investigate this particular reaction.

The Buchwald-Hartwig reaction of 2-bromoaniline and 4-bromoquinoline **59** with $Pd(OAc)_2$ (2 mol%) gave the coupled intermediate **60** in higher isolated yields with XANTPHOS (65%) compared to BINAP (55%). It is conceivable that XANTPHOS may be more effective at suppressing side-product formation than BINAP. The palladium catalyst $Pd(PPh_3)_4$ is not commonly applied to the Buchwald-Hartwig reaction and as expected the use of $Pd(PPh_3)_4$ (10 mol%) resulted in a mixture of products. Therefore this catalyst was unsuitable for this particular reaction.

The preparation of the coupled intermediate **60** using $Pd_2(dba)_3$ (1 mol%) and XANTPHOS (2.2 mol%) with caesium carbonate in refluxing dioxane gave much improved yields (72%). Similarly Jonckers *et al.*[94] found that the catalytic combination of $Pd_2(dba)_3$ with XANTPHOS was superior to $Pd(OAc)_2$ with BINAP. However, both methods used different palladium catalysts and different bases and thus are not directly comparable.

When the reaction was conducted in DMF using $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%) at 120 °C the yield of the coupled product **60** was significantly reduced (38%). Both TLC and HPLC analysis indicated that 11*H*-indolo[3,2-*c*]quinoline **36** was forming, but it was unclear if this was due to the solvent or the slightly elevated reaction temperature. Thus attempts were made to determine if the Buchwald-Hartwig reaction and intramolecular cyclisation could be undertaken as a single step reaction process (i.e. the coupled product **60** cyclising to **36** *in situ* without the addition of extra reagents).

### 2.4.2. Synthesis of 11*H*-Indolo[3,2-*c*]quinoline 36

The palladium-catalysed intramolecular Heck-type cyclisation of the coupled product **60** proceeds *via* a slightly different mechanism to that of a traditional Heck reaction, which is normally applied to the coupling of unsaturated halides and alkenes.[124] The cross-coupling of an electron-rich heterocycle with an aryl halide is more accurately termed a palladium-catalysed direct C-H arylation. The catalytic cycle for this reaction involves three general steps; (i) oxidative addition of an aryl halide (Ar-X),

(ii) electrophilic metallation of a heterocycle (Het-H) and (iii) reductive elimination to afford the coupled product (Het-Ar) and regenerate the catalyst.[125]

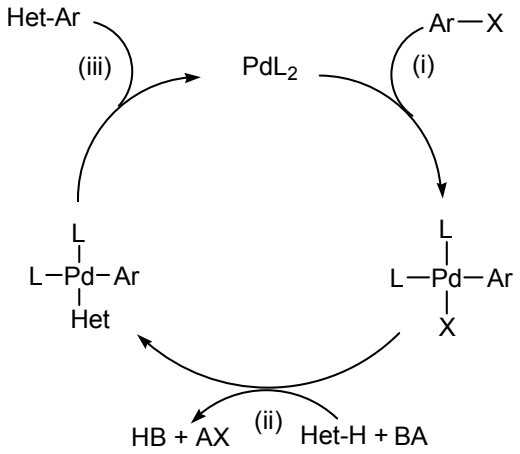



**Figure 2.3:** General catalytic cycle of a direct C-H arylation. $PdL_2$ catalyses the coupling of an aryl halide (Ar-X) to a heterocycle (Het-H)

Palladium-catalysed direct C-H arylation has been successfully applied to a wide range of substrates; aryl iodides, bromides and chlorides are all applicable.[126] Aryl bromides and chlorides, however, are less susceptible to oxidative addition and often require more electron-rich and sterically hindered phosphine ligands (such as P(*t*-Bu)$_3$).[125]

The catalyst $PdCl_2(PPh_3)_2$ and also the catalytic combination of $Pd_2(dba)_3$ and P(*t*-Bu)$_3$ have previously been used with great success for the intramolecular direct C-H arylation of heterocyclic bromides and chlorides.[58,81,82] However, given the aim of attempting to prepare compound **36** in a single synthetic step from 4-bromoquinoline **59**, whereby the cyclisation of intermediate **60** occurs *in situ*, the catalysts applied to the direct C-H arylation must also be applicable to the Buchwald-Hartwig reaction. Therefore only the catalytic combinations discussed in Section 2.4.1 were investigated in attempts to optimise the cyclisation of the coupled intermediate **60** to the cyclised intermediate **36**.

Cyclisation of the coupled intermediate **60** with $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%), in refluxing dioxane, resulted in no product. However, when the reaction was conducting in DMF at 150 °C the cyclised product **36** was isolated in moderate yield (71%). Other catalytic combinations were also investigated and a series of small scale reactions were conducted. The reaction mixtures obtained were analysed by the HPLC method previously described (Section 2.4.1) wherein compound **36** was

generally 3.5-fold and 5-fold more UV absorbing than intermediates **37** and **60** respectively. Unlike the Buchwald-Hartwig reaction, the intramolecular nature of this reaction prevents the formation of side-products and HPLC analysis gives a much clearer indication of reaction efficiency. The results of this particular study are summarised in Table 2.2.

**Table 2.2:** Optimisation of the intramolecular direct C-H arylation of the coupled intermediates **37** and **60** [a]

| Reactant | Catalyst | Ligand | Base | Solvent | % Conversion [b] |
|---|---|---|---|---|---|
| **37** | $Pd_2(dba)_3$ | XANTPHOS | $Cs_2CO_3$ | Dioxane | 3 |
| **37** | $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | Dioxane | 4 |
| **37** | $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | DMF (150°C) | 9 |
| **60** | $Pd_2(dba)_3$ | XANTPHOS | $Cs_2CO_3$ | Dioxane | 7 |
| **60** | $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | Dioxane | 8 |
| **60** | $Pd(OAc)_2$ | BINAP | $K_2CO_3$ | DMF (150°C) | 93 (71) |
| **60** | $Pd_2(dba)_3$ | XANTPHOS | $Cs_2CO_3$ | DMF (150°C) | 7 |
| **60** | $Pd(PPh_3)_4$ | - | $K_2CO_3$ | DMF (150°C) | 98 (68) |

[a] Reagents and Conditions: 24 hr; reflux or 150°C; $Pd_2(dba)_3$ (1 mol%), $Pd(OAc)_2$ (2 mol%) or $Pd(PPh_3)_4$ (10 mol%); XANTPHOS (2.2 mol%) or BINAP (2 mol%); $K_2CO_3$ (20 mol eq.) or $Cs_2CO_3$ (1.3 mol eq.). [b] Conversion: **36**/(**37 (or 60)**+**36**) by HPLC-UV; isolated yield of **36** in parenthesis.

From these results it is evident that the cyclisation of the chloro coupled intermediate **37** does not occur with the catalytic combinations $Pd_2(dba)_3$ (1 mol%) and XANTPHOS (2.2 mol%) or $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%) in refluxing dioxane. Similarly when the reaction was conducted in DMF at elevated temperature (150 °C) cyclisation was not observed. Also the cyclisation of the bromo coupled intermediate **60** does not occur with either catalyst in refluxing dioxane. However, when the reaction was conducted with $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%), or $Pd(PPh_3)_4$ (10 mol%), in DMF at elevated temperatures (150 °C) cyclisation was observed.

As a consequence of previous findings, larger scale coupling reactions with $Pd(PPh_3)_4$ (10 mol%) were conducted and the cyclised product **36** was isolated in moderate yield (68%). In contrast, Jonckers *et al.*[94] were able to obtain compound **36** from the chloro coupled intermediate **37** using $Pd_2(dba)_3$ and P(*t*-Bu)$_3$ in high yield

(95%) after a period of only 3 hours. By using the more reactive bromo coupled intermediate **60**, cyclisation could be conducted with the less costly catalytic combinations ($Pd(OAc)_2$ and BINAP or $Pd(PPh_3)_4$), albeit in lower yield (reduced by 26%) and with an extended reaction time (24 hours).

The two optimum catalytic combinations described above, were subsequently applied to the coupling of 2-bromoaniline and 4-bromoquinoline **59**. By both HPLC and TLC analysis, the cyclised product **36** was detected within 2 hours. However, the reaction with $Pd(PPh_3)_4$ (10 mol%) appeared to have formed multiple products, presumably due to side-products being produced during the Buchwald-Hartwig reaction, and therefore was not pursued further. In contrast application of $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%) resulted in the isolation of the cyclised product **36** in moderate yield (60%) and if the reaction temperature was increased, such that the solution was refluxing, the yield could be improved further to 82%. At high temperatures DMF is known to decompose and can result in the formation of undesirable side-products to reduce reaction yields.[127] Similarly the breakdown of catalytic intermediates can be an issue but if these processes are occurring they are not at sufficient levels to significantly impede this particular reaction.

A detailed investigation of the combined coupling and cyclisation reaction was later undertaken *via* monitoring of the reaction mixture over a 24 hour period by HPLC analysis. It was found that the coupled intermediate **60** formed within 30 minutes; presumably the coupling reaction occurs at a sufficiently rapid rate to prevent excessive side-product formation. The cyclised product **36** was formed after 20 hours of reaction and hence it was surmised that the intramolecular direct C-H arylation was the rate limiting process. Based on recent reports[82,117] it was proposed that future investigations of the synthesis of compound **36** under microwave conditions may permit a reduction in both reaction time and also the catalytic loading.

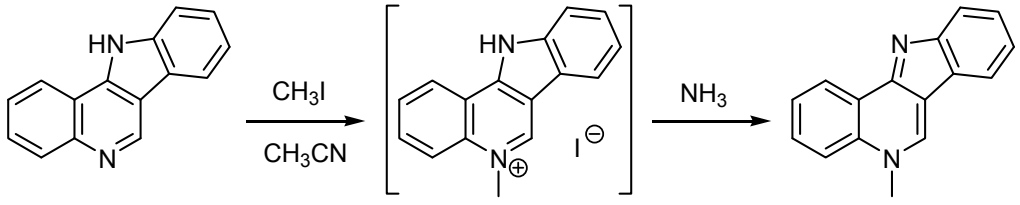
During these investigations of the preparation of the cyclised intermediate **36** in a single step from 4-bromoquinoline **59**, a similar report appeared in the literature. Meyers *et al.*[128] described the preparation of the cyclised intermediate **36** in 82% from 4-chloroquinoline **33** and 2-chloroaniline using $Pd_2(dba)_3$ (5 mol%) and P(*t*-Bu)$_3$ (20 mol%) in dioxane (125 °C). Performing both the Buchwald-Hartwig and intramolecular direct C-H arylation reactions in one pot represents an example of a 'tandem' or 'domino' reaction, where one catalyst activates multiple reaction

processes.[129,130] By combining the Buchwald-Hartwig and C-H arylation reactions into a single synthetic step the original Jonckers Method to isocryptolepine **16** has been reduced to two steps. Although previously reported by Meyers *et al.*, the present optimised 'domino' Jonckers Method allows the use of less costly and more readily available palladium catalysts.

### 2.4.3. Synthesis of Isocryptolepine 16 from Intermediate 36

The *N*-methylation of the cyclised intermediate **36** can be achieved *via* reaction with methylating agents such as dimethyl sulfate or iodomethane.[84,87,92,94,128] This particular *N*-methylation is an example of a Menshutkin reaction, where an alkylated quaternary salt is formed from the reaction of tertiary amines and an alkyl halides (proceeding *via* a $S_N2$ based mechanism).[131] The use of aprotic solvents (e.g. acetonitrile and toluene) generally enhance $S_N2$ reaction rates in comparison to protic solvents (e.g. methanol and water) but other factors, including nucleophilicity of the reactant, can also affect the final product yield.

Iodomethane was chosen as the methylating reagent due to its lower toxicity in comparison to dimethyl sulfate. The cyclised intermediate **36** was reacted with a large excess of iodomethane (100 molar equivalents) in refluxing acetonitrile for 20 hours and the resulting methiodide salt of isocryptolepine **16** isolated (Scheme 2.12). The free base was liberated on treatment with ammonia and purification by flash column chromatography gave isocryptolepine **16** in high yield (94%).




**36** **16.HI** **16** 94%

**Scheme 2.12:** Synthesis of isocryptolepine **16** from intermediate **36**

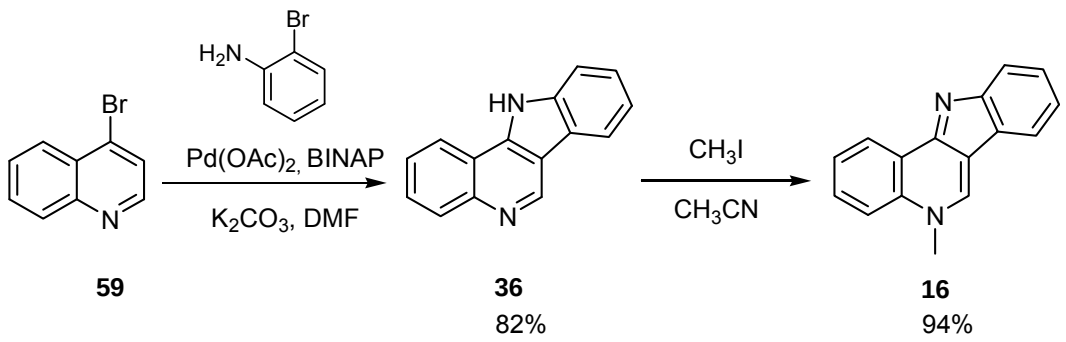
When *N*-methylation was conducted in toluene, isocryptolepine **16** was isolated in only moderate yield (61%). It was observed that the cyclised intermediate **36** was less soluble in toluene, compared to acetonitrile, and reaction in the former solvent may be impeded by lower dissolution of the reactant. Previous literature reports have reported that toluene and acetonitrile both give high yield of isocryptolepine **16** (91-

92%).[84,92] However, Agarwal *et al.*[92] employed toluene as the solvent with a very large excess of iodomethane (200 molar equivalents); i.e. there was approximately a 1:1 ratio of solvent to methylating agent. Thus the methylating agent may also have affected solubility and direct comparison with this report is unfeasible.

The polar aprotic solvent DMF is also applicable as a solvent in *N*-methylation reactions. However, the literature indicates that the high solubility of DMF may encourage dimethylation,[117] which could account for the slightly lower yield (75%) of isocryptolepine **16** obtained by Jonckers *et al.*[94] Consequently DMF was not utilised as a solvent.

### 2.4.4. Optimised 'domino' Jonckers Method

The optimum conditions for the synthesis of isocryptolepine **16** *via* the Jonckers Method are depicted in Scheme 2.13. The original method has been reduced from a three step to a two step synthesis by combining the Buchwald-Hartwig and C-H arylation reactions into a single 'domino' reaction. This was possible through the substitution of 4-chloroquinoline **33** and 2-chloroaniline for their more reactive analogues, 4-bromoquinoline **59** and 2-bromoaniline. The cyclised intermediate **36** was prepared in comparable high yield (82%) to Meyers *et al.*[128] but utilised more accessible palladium catalysts. *N*-methylation was also achieved in higher yield than Jonckers *et al.*[94] (75%), but in comparative yields to Molina *et al.*[84] (92%). Although this procedure has previously been reported, the present method is able to produce isocryptolepine **16** in higher overall yield (77%) than Meyers *et al.*[128] (61%). Similarly the overall yield is higher than reported by Molina *et al.* (73%),[84] which was previously the highest yielding literature method to isocryptolepine **16**.




**59** **36** 82% **16** 94%

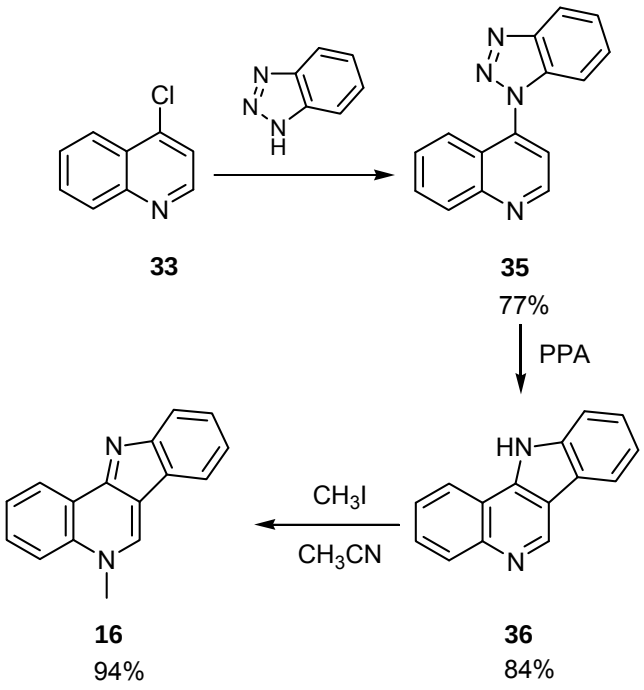
**Scheme 2.13:** Optimised 'domino' Jonckers Method to isocryptolepine **16**

## 2.5. Isocryptolepine 16 *via* the Molina Method

To conclude, the final synthetic route to isocryptolepine **16** that was investigated was the three step Molina Method. 4-(1-Benzotriazolyl)quinoline **35** was synthesised by reaction of 4-chloroquinoline **33** with benzotriazole at 110 - 120 °C in the absence of a solvent (Scheme 2.14). The resulting solid was recrystallised from ethanol to give the desired benzotriazole intermediate **35** in lower yields (77%) than those previously reported by Molina (92%).

The cyclised intermediate **36** was prepared by reaction of the benzotriazole intermediate **35** with polyphosphoric acid at 150 °C (1 hour). On quenching with water a precipitate formed, most likely a water insoluble phosphate salt, which was collected and re-suspended in water before conversion to the free base. Purification was achieved by washing with an organic solvent (e.g. dichloromethane) to give the cyclised product **36** in high yield (84%).

Finally, the cyclised intermediate **36** was *N*-methylated as previously described (Scheme 2.12) using iodomethane in acetonitrile. The optimum conditions for the synthesis of isocryptolepine **16** *via* the Molina Method are shown in Scheme 2.14. An overall yield of 61% is comparable to that reported by Molina *et al.*[84] (73%).




**Scheme 2.14:** Optimised Molina Method to isocryptolepine **16**

# Chapter 3

## Synthesis of Isocryptolepine Derivatives

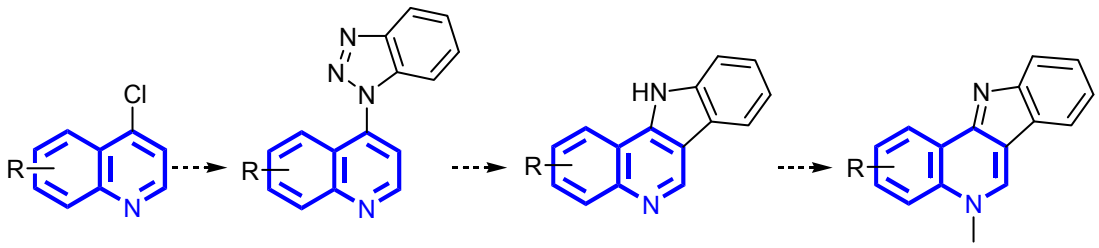
## 3.1. Introduction

As outlined in Section 1.3.2, certain substituted derivatives of cryptolepine **14** and neocryptolepine **19** have displayed improved biological activities, in comparison to their parent alkaloid. Halogenated derivatives, specifically dihalogenated bromo or chloro compounds, have shown the most promise with respect to their potential as antimalarial agents. Whilst the synthesis of several isocryptolepine derivatives has previously been described,[85,132] there have been no reports of their antimalarial activity. The previous synthetic methods to derivatives were assessed and as many involved photochemical routes they could not be applied in this project, due to the lack of necessary facilities (previously outlined in Section 2.1). In addition the single step procedure to isocryptolepine **16** reported after the commencement of the present project by Kumar *et al.*[93] could not be considered. Therefore the optimised routes to isocryptolepine **16**, discussed in Chapter 2, were more attractive synthetic methods to prepare derivatives.

The Molina Method (Section 2.5) was first employed to prepare ring-substituted isocryptolepine derivatives and this synthetic strategy is examined in the following section. The optimised 'domino' Jonckers Method (Section 2.4.4) was also applied and this strategy is discussed in Section 3.3. A synthetic procedure involving the electrophilic aromatic substitution of the parent alkaloid, and a selection of the previously prepared derivatives, was developed and is described in Section 3.4. The synthesis of the isomeric analogue 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **31** was briefly investigated and attempts were made to apply both the aforementioned synthetic methods in the preparation of this compound, which is described in Section 3.5.

## 3.2. Ring-Substituted Derivatives *via* the Molina Method

The Molina Method was first applied to the preparation of isocryptolepine derivatives due to its simplicity; it does not require the use of palladium catalysts and purification is readily achieved without chromatography. Also a similar benzotriazole strategy has previously been applied in the preparation of methyl substituted neocryptolepine derivatives.[133] This particular study found that substituents on the quinoline ring did not negatively affect the yields of both benzotriazole coupling and acid catalysed cyclisation. This finding encouraged the application of the Molina Method to various substituted 4-chloroquinolines (Scheme 3.1) and each step in this methodology will be described.

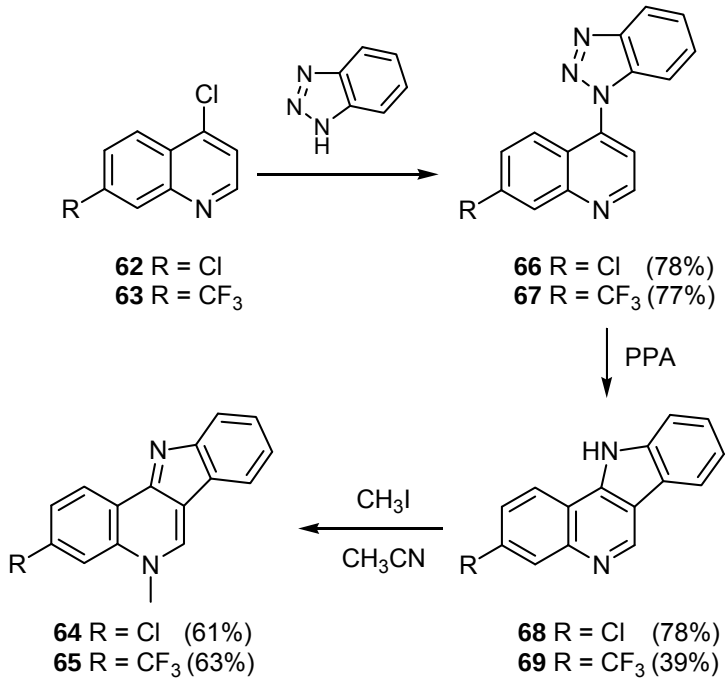



**Scheme 3.1**: Proposed route to isocryptolepine derivatives *via* the Molina Method

Substituted benzotriazoles were not applied to the Molina Method as it was predicted that the coupling would be non-specific and a mixture of products would be formed. This hypothesis was confirmed in a recent report by El Sayed *et al.*[80] where it was observed that reaction of 2-chloroquinoline with 5-chlorobenzotriazole produced a 1:1 mixture of the two inseparable regioisomers.

### 3.2.1. Synthesis of C3 Substituted Isocryptolepines

Isocryptolepine **16** with substituents at positions C2 and C3 were a priority, and required 4-chloroquinolines substituted at positions C6 or C7. 4-Chloroquinolines substituted at position C7 were available commercially and the most readily available of these, 4,7-dichloroquinoline **62** and 4-chloro-7-trifluoromethylquinoline **63**, were applied to the preparation of 3-chloroisocryptolepine **64** and 3-trifluoromethyl-isocryptolepine **65** (Scheme 3.2).




**Scheme 3.2:** Synthesis of the isocryptolepine derivatives **64** and **65**

Initially the above 4-chloroquinolines **62** and **63** were thermally coupled to benzotriazole and the novel benzotriazole intermediates 4-(1-benzotriazolyl)-7-chloroquinoline **66** and 4-(1-benzotriazolyl)-7-trifluoromethylquinoline **67** obtained in moderate yields (77 – 78%).

The following step in the Molina Method required the acid catalysed cyclisation of the benzotriazole intermediates **66** and **67**. 4-(1-Benzotriazolyl)-7-chloroquinoline **66** was initially cyclised under the same reaction conditions as 4-(1-benzotriazolyl)quinoline **35**, in polyphosphoric acid at 150 °C until the evolution of nitrogen ceased. Whilst this appeared to occur after 1 hour, the reaction was allowed to continue for an additional hour to ensure complete reaction. The product 3-chloro-11*H*-indolo[3,2-*c*]quinoline **68** was isolated in 77% yield and could be purified by washing the solid with an organic solvent (i.e. dichloromethane). The reaction was also attempted at a slightly lower temperature (140 °C) for three hours and found to have little effect on the yield; with **68** being isolated in 78% yield. The benzotriazole intermediate **67** was subsequently cyclised at 140 °C (3 hours) and 3-trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline **69** obtained in 39% yield.

The *N*-methylation of the above 11*H*-indolo[3,2-*c*]quinolines **68** and **69** was conducted using the same method applied to prepare isocryptolepine **16** from 11*H*-indolo[3,2-*c*]quinoline **36** (Section 2.4.3). Reaction of the chloro intermediate **68**

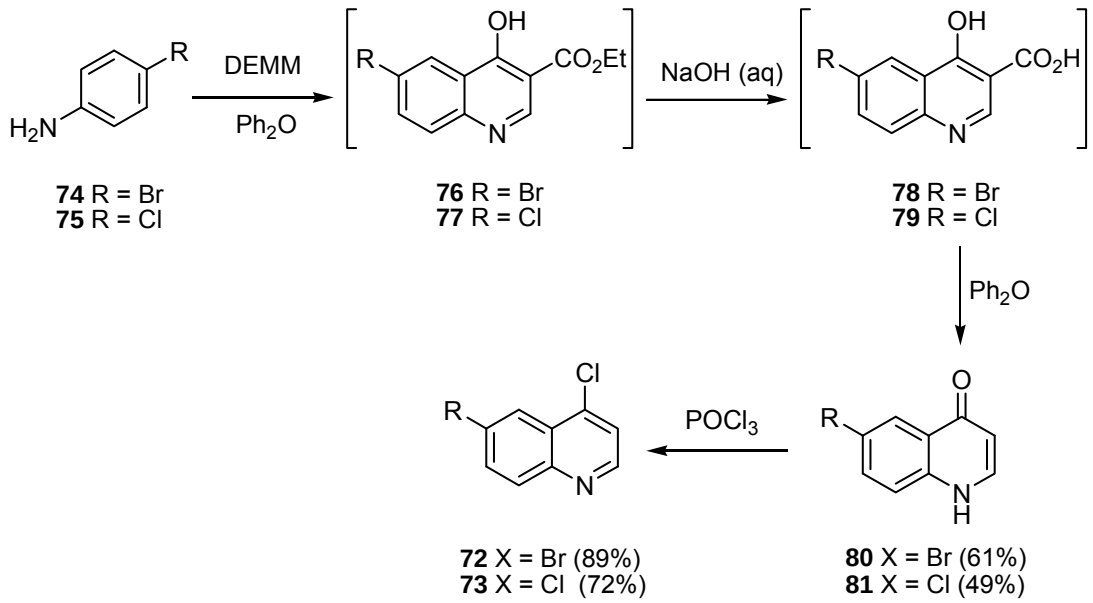
with iodomethane in refluxing acetonitrile (20 hours), followed by conversion to the free base and subsequent purification *via* flash column chromatography, resulted in the isolation of 3-chloroisocryptolepine **64** in moderate yield (61%; Scheme 3.2). Reaction of the trifluoromethyl cyclised intermediate **69** was conducted in the same manner except that 3-trifluoromethylisocryptolepine **65** was purified, by column chromatography, as its methiodide salt because the free base was found to be unstable on silica.

The structures of the novel products **64**, **65**, **66**, **67** and **69** were confirmed by proton and carbon NMR spectroscopy by comparison with the spectra of 4-(1-benzotriazolyl)quinoline **35**, 11*H*-indolo[3,2-*c*]quinoline **36** or isocryptolepine **16**. 4-(1-Benzotriazolyl)-7-chloroquinoline **66** and 3-chloroisocryptolepine **64** possessed a number of signals in their carbon NMR spectra that were close together and both HMBC and HSQC experiments were required to unequivocally assign the peaks. The synthesis of 3-chloro-11*H*-indolo[3,2-*c*]quinoline **68** has previously been reported, *via* a Fisher indolisation of a chlorotetrahydroquinoline, but the compound was not previously fully characterised nor used to prepare its isocryptolepine analogue.[134]

### 3.2.2. Synthesis of C2 Substituted Isocryptolepines

4-Chloroquinolines with a halogen substituent at position C6, the necessary starting materials required for the preparation of C2 substituted isocryptolepines, were not commercially available but could be prepared *via* literature methods. The quinolines required to synthesise 2-bromoisocryptolepine **70** and 2-chloroisocryptolepine **71**, 6-bromo-4-chloroquinoline **72** and 4,6-dichloroquinoline **73** respectively, were thus prepared from readily available anilines (Scheme 3.3).[135]

Initially 4-bromoaniline **74** and 4-chloroaniline **75** were condensed with diethyl-ethoxymethylmalonate (DEMM), followed by cyclisation in diphenyl ether ($Ph_2O$) to give 6-halo-3-carbethoxy-4-hydroxyquinolines **76** and **77**. The esters **76** and **77** were hydrolysed in aqueous sodium hydroxide solution to give 6-halo-3-carboxy-4-hydroxyquinolines **78** and **79** and subsequently decarboxylated upon boiling in diphenyl ether to generate the 4-quinolones **80** and **81** in moderate yield (61% and 49% respectively from the 4-haloanilines **74** and **75**). The 3-carbethoxy-4-hydroxyquinoline and 4-quinolone intermediates were identified by melting point and infrared spectroscopy, to ensure that decarboxylation had occurred.

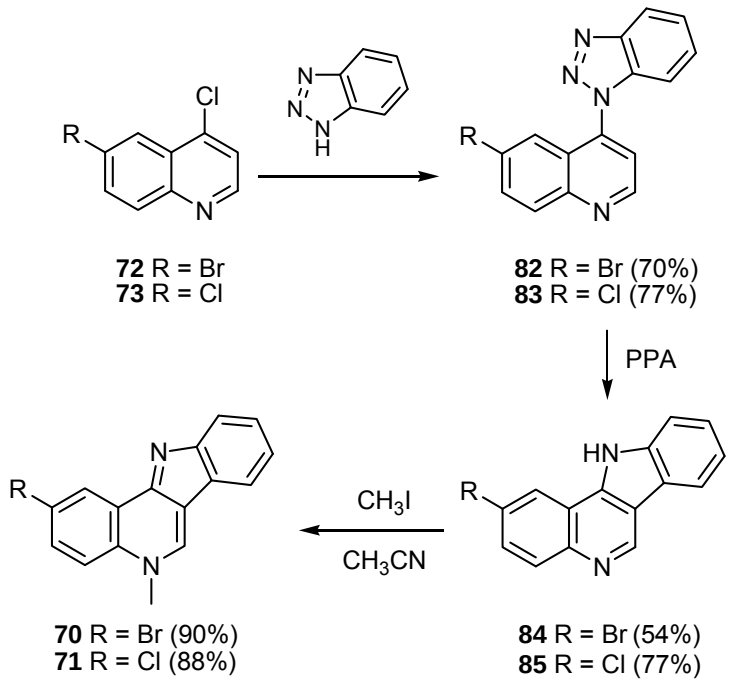



**Scheme 3.3:** Synthesis of 4-chloroquinoline **72** and **73**

Finally 6-bromo-4-quinolone **80** and 6-chloro-4-quinolone **81** were chlorinated with phosphorus oxychloride ($POCl_3$) to afford **72** and **73** in high yield (89% and 72% respectively). Although compounds **80**, **72** and **73** were known compounds, they had not previously been fully characterised in the literature and both NMR and mass spectra were acquired for each.

As per the preparation of novel benzotriazole intermediates **66** and **67** (Section 3.2.1), the 4-chloroquinolines **72** and **73** were coupled with benzotriazole to produce 4-(1-benzotriazolyl)-6-bromoquinoline **82** and 4-(1-benzotriazolyl)-6-chloromethyl-quinoline **83** in moderate yields (70 – 77%; Scheme 3.4).

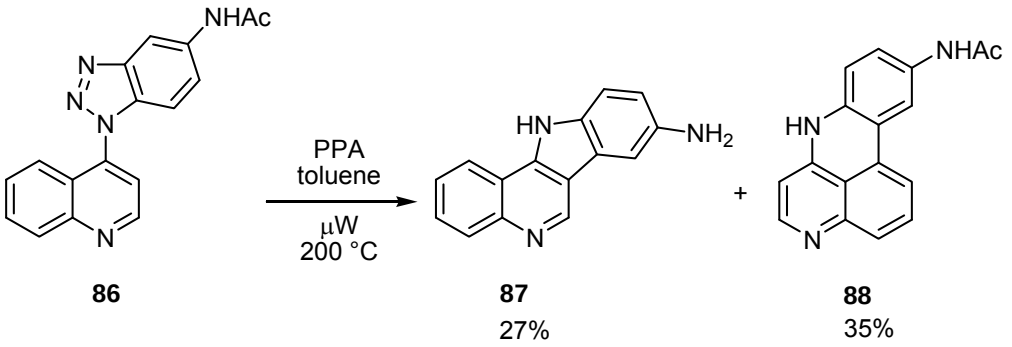
The benzotriazole intermediates **82** and **83** were subsequently cyclised at 140 °C (3 hours). 2-Chloro-11*H*-indolo[3,2-*c*]quinoline **85** was obtained in moderate yield (77%) and could be purified by washing the product obtained with an organic solvent. In contrast the cyclisation of 4-(1-benzotriazolyl)-6-bromoquinoline **82** to 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84**, and subsequent washing with dichloromethane, did not produce a pure compound. Chromatography was not possible as the product **84** possessed very low solubility in organic solvents. After several attempts it was found that washing the solid with methanol produced relatively pure **84** in 54% yield. During this process a by-product was observed by TLC analysis and this compound was significantly more polar and UV absorbing than **84**. Attempts were made to isolate this compound but its high polarity made chromatography difficult.




**Scheme 3.4:** Synthesis of isocryptolepine derivatives **70** and **71**

It was postulated that a lower reaction temperature may negate the formation of this secondary compound. However, reaction at 130 °C resulted in similar yields of **84** and the secondary product was still observed by TLC analysis.

A re-examination of the cyclisation of intermediates **66** and **67** (Scheme 3.2) also revealed the presence of a secondary product in the preparation of the trifluoromethyl cyclised product **67** but not the chloro **66**. The cyclisation of a similar benzotriazole coupled quinoline **86** (Scheme 3.5) was recently reported, and it was found that reaction at high temperature, under microwave irradiation, produced both **87** (27%) and **88** (35%).[136]




**Scheme 3.5:** Beauchard *et al.*[136] synthetic route to **87** and **88**

Therefore there appears to be a secondary site of cyclisation and the formation of similar by-products during the cyclisation of certain benzotriazole intermediates (i.e. **67** and **82**) may have caused the reduced yields observed. Such a by-product would need to be isolated for confirmation but the major aim at this stage of the project was to prepare isocryptolepine derivatives for biological evaluation and improving the yields was a secondary priority. If these compounds prove particularly active, a re-investigation of the synthetic method to improve yields would be warranted.

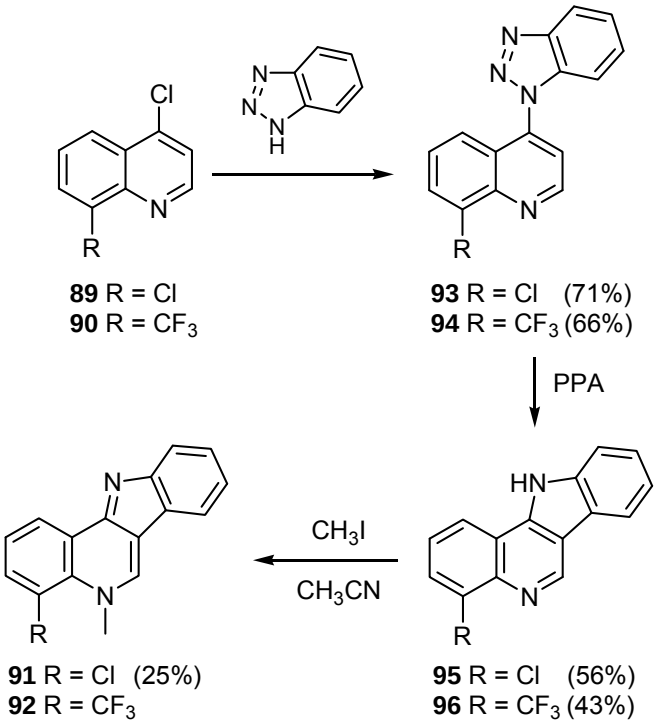
The 11*H*-indolo[3,2-*c*]quinolines **84** and **85** were *N*-methylated as per **68** and **69** and the derivatives 2-bromoisocryptolepine **70** and 2-chloroisocryptolepine **71** isolated in yields of 90% and 88% respectively.

The structures of all novel compounds were confirmed by proton and carbon NMR spectroscopy by comparison with the spectra of their parent compounds. The exception was 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84**, wherein the peaks in the carbon spectrum due to C2 and C11a were close together and a HMBC experiment was needed to definitively assign the signals.

### 3.2.3. Synthesis of C4 Substituted Isocryptolepines

4-Chloroquinolines substituted at positions C8 were also available commercially and although initial aims did not include preparing isocryptolepine derivatives substituted at position C4, the accessibility of the starting materials prompted an investigation. 4,8-Dichloroquinoline **89** and 4-chloro-8-trifluoromethylquinoline **90** were thus applied to the preparation of 4-chloroisocryptolepine **91** and 4-trifluoromethyl-isocryptolepine **92** respectively.

The above 4-chloroquinolines **89** and **90** were thermally coupled to benzotriazole and the novel benzotriazole intermediates 4-(1-benzotriazolyl)-8-chloroquinoline **93** and 4-(1-benzotriazolyl)-8-trifluoromethylquinoline **94** were obtained in moderate yield (66-71%; Scheme 3.6). Again substituents did not greatly affect yields of this reaction, such that the intermediates **89** and **90** were isolated in comparable yields to unsubstituted 4-(1-benzotriazolyl)quinoline **35** (77%; Scheme 2.14). This observation is in agreement with previous studies, which have reported that both methyl and chloro quinoline ring substituents had little impact on yields of benzotriazole coupling.[80,84,133]




**Scheme 3.6:** Synthesis of isocryptolepine derivatives **91** and **92**

Acid catalysed cyclisation in polyphosphoric acid, at 140 °C, of intermediates **93** and **94** produced 4-chloro-11*H*-indolo[3,2-*c*]quinoline **95** and 4-trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline **96** in low yields (33 - 43%). It was found that if the cyclisation of **93** was conducted at a lower temperature of 130 °C yields were much improved (56%). However neither this result or the additional product observed during the cyclisation of the trifluoromethyl intermediate **94** was further investigated.

*N*-Methylation of 4-chloro-11*H*-indolo[3,2-*c*]quinoline **95** in acetonitrile resulted in low yield (25%) of the product 4-chloroisocryptolepine **91** and can partly be attributed to steric affects. Peczyńska-Czoch *et al.*[133] reported the *N*-methylation of a variety of substituted neocryptolepine derivatives and found that a substituent at position C4 resulted in reduced yields. This theory is further confirmed by the lack of reactivity of 4-trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline **96**. Attempts at *N*-methylation in acetonitrile, or on more extreme heating in DMF, gave no product. Hence the bulky trifluoromethyl group may be blocking the reaction site to a larger extent than the chloro group.

Toluene was also trialled as a reaction solvent as Meyers *et al.*[128] reported its success in the *N*-methylation of some 11*H*-indolo[3,2-*c*]quinolines and it was envisioned that the higher reaction temperature may improve the yield of compound **91**. The *N*-methylation of all 11*H*-indolo[3,2-*c*]quinolines, however, gave lower

yields of the corresponding isocryptolepines (56-68%) compared to when the reaction was conducted in acetonitrile (Table 3.1). This observation is in agreement with our early findings for the *N*-methylation of 11*H*-indolo[3,2-*c*]quinoline **36** (Section 2.4.3).

**Table 3.1:** Yields of isocryptolepines **64**, **65**, **70**, **71**, **91** and **92** [a]

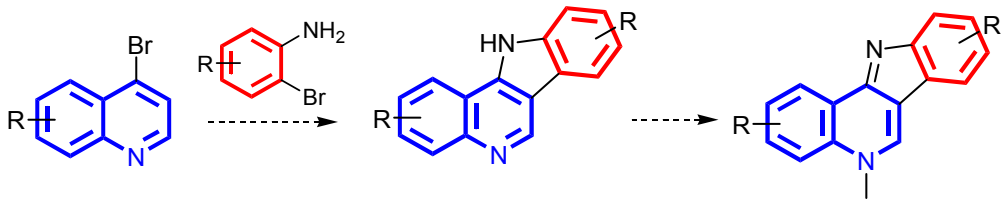
| Products | Isolated yield (%) [a] | |
|---|---|---|
| | acetonitrile | toluene |
| 3-Chloroisocryptolepine **64** | 61 | 56 |
| 3-Trifluoromethylisocryptolepine **65** | 63 | 59 |
| 2-Bromoisocryptolepine **70** | 90 | 68 |
| 2-Chloroisocryptolepine **71** | 88 | 68 |
| 4-Chloroisocryptolepine **91** | 25 | no rxn |
| 4-Trifluoromethylsocryptolepine **92** | no rxn | - |

[a] Reaction conditions: i) $CH_3I$ (100 mol eq.), ii) $NH_3$(aq)

Another effect governing the reactivity of $S_N2$ reactions is the nucleophilicity of the reactants. Increased basicity often corresponds to an increased nucleophilicity of a reactant and consequently improved reaction yields.[131] Previously it has been noted that *N*-methylation of various indoloquinolines proceeds in reduced yield in the presence of electron-withdrawing groups (which reduce basicity and nucleophilicity of a reactant).[117,128] Thus the $pK_a$ values of the 11*H*-indolo[3,2-*c*]quinolines were predicted, using the Advanced Chemistry Development Interactive Laboratory (ACD/I-Lab) web service,[137] and a similar observation was made. The most basic compounds were predicted to be 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84** and 2-chloro-11*H*-indolo[3,2-*c*]quinoline **85** ($pK_a$ values of 6.45 and 6.52 respectively) and these compounds were those *N*-methylated in highest yields. 4-Chloro-11*H*-indolo[3,2-*c*]quinoline **95** and 4-trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline **96** were the least basic ($pK_a$ values of 5.62 and 5.27 respectively) and also the least reactive. Consequently there are two different effects impeding the formation of 4-chloroisocryptolepine **91** and 4-trifluoromethylisocryptolepine **92**, both the position of the substituent and the electronic nature of the starting material.

## 3.3. Ring-Substituted Derivatives *via* the Jonckers Method

A similar synthetic methodology to the Jonckers Method has previously been applied to the synthesis of isoneocryptolepine **30** and substituted derivatives.[117] The authors successfully applied substituted anilines and this report prompted a similar investigation of the optimised 'domino' Jonckers Method in efforts to develop routes to derivatives with substituents at positions C8 or C9 (Scheme 3.7).



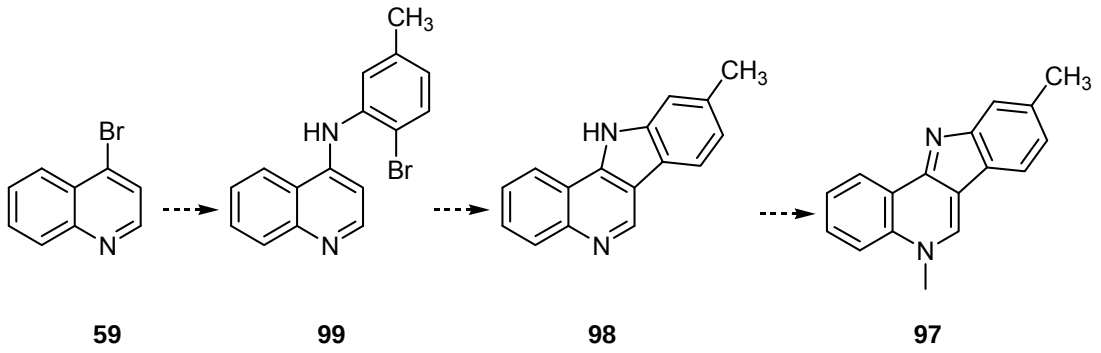

**Scheme 3.7:** Proposed synthetic route to isocryptolepine derivatives *via* the Jonckers Method

During the course of the present project, Meyers *et al.*[128] reported the application of substituted anilines to the Jonckers Method, further confirming that this synthetic strategy should allow the preparation of the desired derivatives. Although a method to prepare isocryptolepine derivatives with ring substituents on the quinoline ring (Molina Method) has already been developed, attempts were also briefly made to assess if substituted quinolines could be applied to the Jonckers Method.

### 3.3.1. Synthesis of 9-Methylisocryptolepine 97

The preparation of isocryptolepine derivatives substituted at positions C8 or C9, *via* the optimised 'domino' Jonckers Method, required the application of 2-bromoanilines substituted at positions C4 or C5. 5-Methyl-2-bromoaniline was readily available commercially, the starting material required to form 9-methylisocryptolepine **97** (Scheme 3.8). Although methyl derivatives were not target compounds this species was used to investigate the application of substituted anilines to this synthetic methodology.

Initially the optimum conditions determined for the 'domino' preparation of 11*H*-indolo[3,2-*c*]quinoline **36** from 4-bromoquinoline **59** were applied. Reaction of 5-methyl-2-bromoaniline and 4-bromoquinoline **59** with $Pd(OAc)_2$ (2 mol%), BINAP (2 mol%) and potassium carbonate in refluxing DMF resulted in the isolation of the cyclised product 9-methyl-11*H*-indolo[3,2-*c*]quinoline **98** in good yield (74%).




**59** **99** **98** **97**

**Scheme 3.8:** Proposed synthetic route to 9-methylisocryptolepine **97**

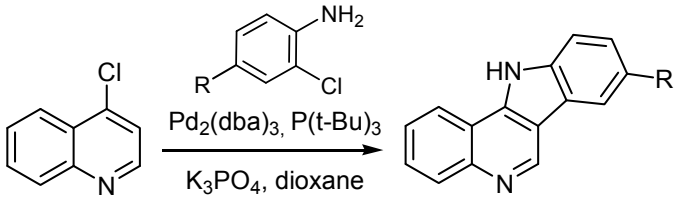
The novel product **98** was confirmed by NMR spectroscopy and the proton spectrum could be fully assigned with reference to the spectrum of 11*H*-indolo[3,2-*c*]quinoline **36**, but 2D-NMR correlation spectroscopy was needed for the assignment of the carbon spectrum.

When the reaction was conducted in refluxing dioxane the coupled compound 4-(2-bromo-5-methylphenylamino)quinoline **99** was the major product (58%), as was the case on reaction of 4-bromoquinoline **59** and 2-bromoaniline under the same conditions (Section 2.4.1). When the catalytic combination $Pd_2(dba)_3$ (1 mol%), XANTPHOS (2.2 mol%) and caesium carbonate were employed (refluxing dioxane) the yield of the coupled product **99** was much improved (76%). The coupled product **69** was similarly confirmed by NMR spectroscopy and could be fully assigned with reference to the spectrum of 4-(2-bromophenylamino)quinoline **60**.

The differences in reaction yields of compounds **99** and **60** can, to some extent, be explained due to the differences in nucleophilicity of the amines. The amine coordinated to the metal centre in a Buchwald-Hartwig reaction is essentially acting as a nucleophile and an electron-donating group would increase nucleophilicity to possibly improve yields. In contrast an electron-withdrawing group may have the opposite effect. In line with these predictions Hostyn *et al.*[117] found that the Buchwald-Hartwig coupling of 3-bromoquinoline to substituted 2-bromoanilines resulted in higher yields of the coupled products when anilines with electron-donating groups (i.e. Me) were employed compared to electron-withdrawing groups (i.e. Cl). Therefore, in the case of the reaction of 5-methyl-2-bromoaniline and 4-bromoquinioline **59** it was expected that the Buchwald-Hartwig coupling would occur in higher yields than in the coupling of the unsubstituted aniline. This was found to be the case, 4-(2-bromo-5-methylphenylamino)quinoline **99** was formed in

higher yield (76%) than 4-(2-bromophenylamino)quinoline **60** (72%) with $Pd_2(dba)_3$ (1 mol%) and XANTPHOS (2.2 mol%); albeit only slightly.

In relation to the intramolecular C-H arylation reaction, Hostyn *et al.* observed that this reaction proceeded in higher yield with an electron-withdrawing group compared to an electron-donating group. This observation may be explained by the increased electron density on the aniline when an electron-donating group was used, which would increase electron density within the C-Br bond and possibly reduce susceptibility to oxidative addition. It was expected that the intramolecular C-H arylation of 4-(2-bromo-5-methylphenylamino)quinoline **99** would proceed in reduced yield in comparison to 4-(2-bromophenylamino)quinoline **60**. This was demonstrated by the lower yields of 9-methyl-11*H*-indolo[3,2-*c*]quinoline **98** isolated after Buchwald-Hartwig coupling and *in situ* cyclisation (74%) in comparison to 11*H*-indolo[3,2-c]quinoline **36** (82%). Meyers *et al.*[128] also found that anilines bearing electron-donating groups (i.e. OMe and $^t$Bu) gave reduced yields of the substituted 11*H*-indolo[3,2-*c*]quinolines **100** and **101**, following application of a similar 'domino' Jonckers Method (Scheme 3.9).



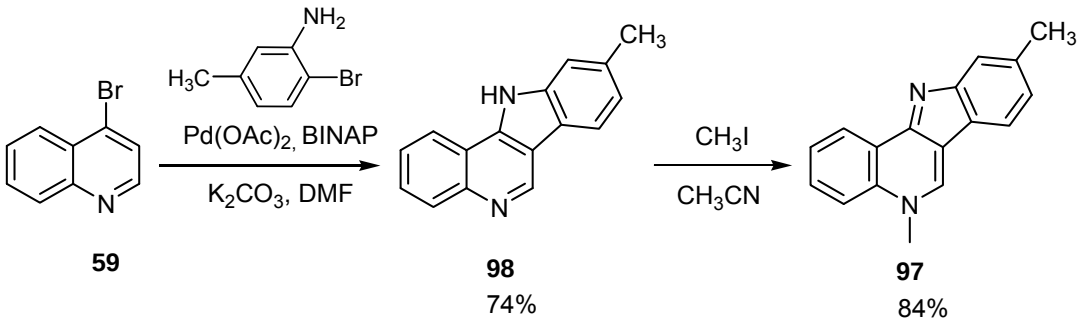

**33**

**36** R = H (82%)
**100** R = $^t$Bu (76%)
**101** R = MeO (52%)

**Scheme 3.9:** Meyers *et al.*[128] synthetic route to **36**, **100** and **101**

*N*-Methylation of 9-methyl-11*H*-indolo[3,2-*c*]quinoline **98** was conducted as for the preparation of the parent alkaloid, by reaction with iodomethane in refluxing acetonitrile. Following conversion to the free base and chromatography, 9-methylisocryptolepine **97** was isolated in high yield (84%). Reaction in non-polar aprotic toluene gave reduced yield (61%), as was observed during the *N*-methylation of **36**. The structure of the novel methyl isocryptolepine derivative **97** was confirmed by NMR spectroscopy and the spectra could be assigned with reference to the previously reported assigned spectrum of isocryptolepine **16**.[48]

The optimum conditions for the preparation of 9-methylisocryptolepine **97** are shown in Scheme 3.10. As originally predicted substituted anilines are applicable to the optimised 'domino' Jonckers Method, although this was only assessed using one such derivative. This synthetic method provided a route to an isocryptolepine derivative substituted at position C9 but was not further pursued due to time constraints, and the Molina Method had already supplied a range of novel derivatives.

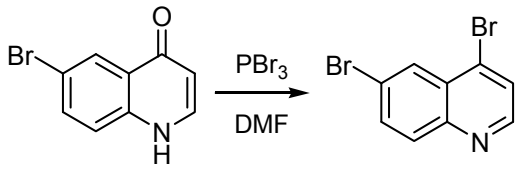



**59** **98** 74% **97** 84%

**Scheme 3.10:** Optimised synthetic route to 9-methylisocryptolepine **97**

### 3.3.2. Attempts to Prepare 2-Bromoisocryptolepine 70

Although the Molina Method allowed the preparation of 2-bromoisocryptolepine **70** in good yield, efforts were made to quickly evaluate if substituted quinolines were applicable to the optimised 'domino' Jonckers Method. The required starting material 4,6-dibromoquinoline **102** was prepared *via* bromination of 6-bromo-4-hydroxyquinoline **80** with phosphorus tribromide (82%; Scheme 3.11).

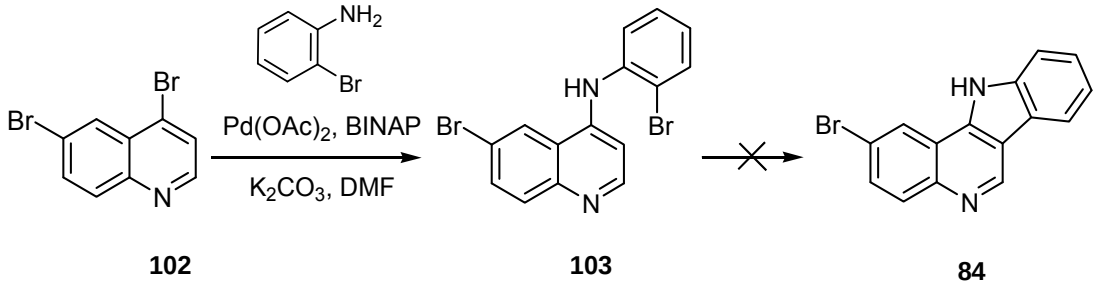



**80** **102** 82%

**Scheme 3.11:** Synthesis of 4,6-dibromoquinoline **102**

The Buchwald-Hartwig coupling and *in situ* cyclisation of **102** to 2-bromoaniline, using the optimum catalytic conditions used to prepare 9-methyl-11*H*-indolo[3,2-*c*]quinoline **98**, was attempted. However, the major product was 4-(2-bromophenylamino)-6-bromoquinoline **103**, not the desired 2-bromo-11*H*-

indolo[3,2-*c*]quinoline **84** (Scheme 3.12). The structure of the impure product was confirmed by NMR spectroscopy but no further attempts were made to improve this method. This preliminary investigation suggests that the optimised 'domino' Jonckers Method is applicable to substituted anilines, over quinolines, but a more thorough investigation of the effects of ring substitution is warranted.

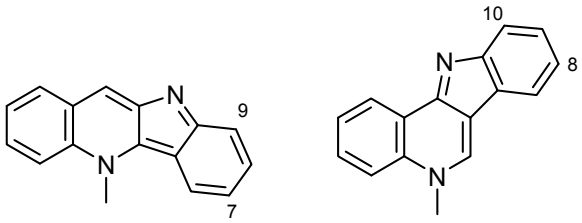



**102** **103** **84**

**Scheme 3.12:** Attempted synthesis of 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84**

## 3.4. Ring-Substituted Derivatives *via* Electrophilic Aromatic Substitution

Electrophilic aromatic substitution was investigated as an additional method for preparing isocryptolepine derivatives. Previously cryptolepine **14** had been nitrated by reaction of the parent compound with concentrated nitric acid and glacial acetic acid.[57] Substitution of this species occurred exclusively at positions C7 and C9 and this result prompted an investigation of similar reactions with isocryptolepine **16**. Presumably corresponding positions on isocryptolepine **16** would also readily undergo electrophilic aromatic substitution and may provide an additional synthetic method to prepare derivatives with ring substituents on the indole ring. Figure 3.1 illustrates the most susceptible positions of aromatic substitution on cryptolepine **14** and the predicted positions on isocryptolepine **16**.




**14** **16**

**Figure 3.1:** Most susceptible sites of electrophilic aromatic substitution

### 3.4.1. Synthesis of Halogenated Derivatives

Initially bromination of isocryptolepine **16** was attempted using *N*-bromosuccinimide (NBS) as this brominating agent is generally one of the more selective reagents applied to the bromination of carbazoles.[138-140] In addition related compounds to isocryptolepine **16** have previously been brominated with *N*-bromosuccinimide.[141]

Reaction of isocryptolepine **16** with one molar equivalent of *N*-bromosuccinimide in DMF (150 $^{0}$C) produced a single brominated product in 74% yield, which was easily purified *via* recrystallisation. The reaction was also monitored by HPLC analysis and it was found that bromination was completed within 30 minutes. The proton NMR spectra of the product and isocryptolepine **16** (Figure 3.2) were compared in an effort to identify the product but the results were inconclusive.

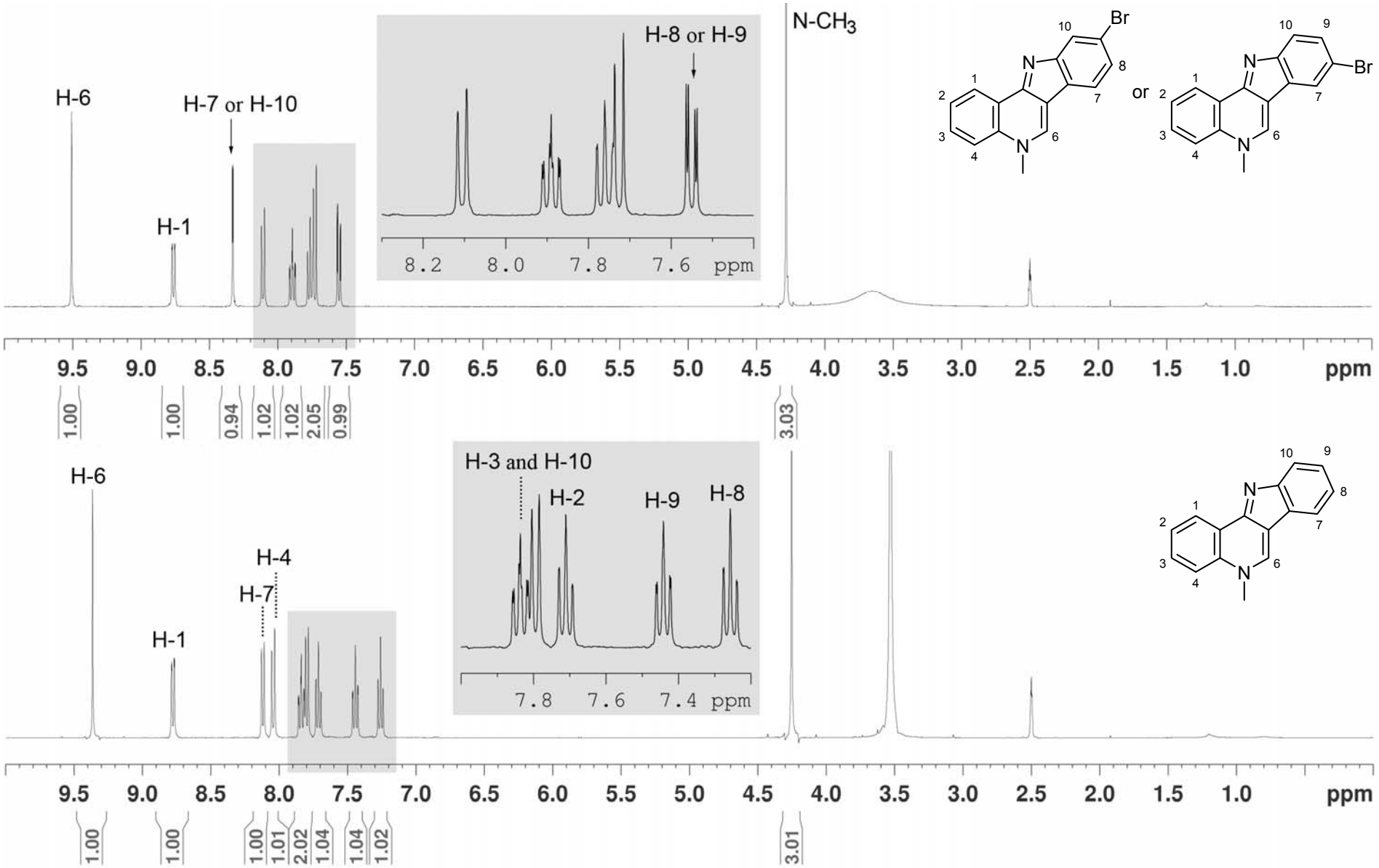



**Figure 3.2:** $^{1}$H NMR spectra of isocryptolepine **16** and the brominated product (400 MHz, $d_6$-DMSO).

Whilst the proton spectrum of the product indicated the presence of eight aromatic protons, confirming monobromination, it was unclear if this has occurred at positions C8 or C9 as the proton spectra of both 8-bromoisocryptolepine and 9-bromoisocryptolepine would appear similar. Two triplets at 7.26 and 7.44 ppm (due to H-8 and H-9 respectively) were observed in the proton spectrum of isocryptolepine **16**, but only a single doublet of doublets at 7.54 ppm was observed in the proton spectrum of the brominated product (either H-8 or H-9). In addition the proton spectrum of isocryptolepine **16** displayed a doublet at 8.12 ppm ($^{3}J_{H,H}$= 7.6 MHz) due to H-7 while the spectrum of the product showed a doublet at 8.30 ppm with a lower coupling constant ($^{4}J_{H,H}$ = 2.4 Hz) to indicate this proton is adjacent to the bromo group and could be either H-7 or H-10.

In an effort to unequivocally determine the product, 1D NOE difference spectrometry was utilised. This common spectroscopic technique takes advantage of the nuclear Overhauser effect (the transfer of polarisation between nuclear spins) and is able to identify which protons are close through space. A single resonance (or peak) is irradiated at its resonance frequency (or saturated) and protons that are spatially close (can transfer polarisation) are enhanced. The NOE difference spectrum is then subtracted from the original proton spectrum, such that protons that are enhanced by NOE are observed as positive peaks and the irradiated proton as a negative peak. Patterns of positive and negative peaks are also often observed for protons that were coupled to the irradiated proton.

The results of the 1D NOE difference experiments on the brominated product are shown in Figure 3.3. The saturation of the peak at 8.30 ppm results in a positive NOE peak at 9.39 ppm (H-6 based on our knowledge of the spectrum of the parent). As the peak at 8.30 ppm has arisen from a proton that is spatially close to H-6, it must be H-7 and not H-10. A pattern of positive and negative peaks at 7.54 and 7.71 ppm was also observed and have consequently arisen from H-7 coupling to two other protons. The positive and negative peak at 4.25 ppm was a residual signal from $NCH_3$, where it is well known that strong signals cannot be fully eliminated in a difference spectrum.[142] A second NOE experiment was conducted where the peak at 9.39 ppm (H-6) was saturated and this resulted in positive NOE peaks at 8.30 (H-7) and 4.25 ppm (N-$CH_3$).

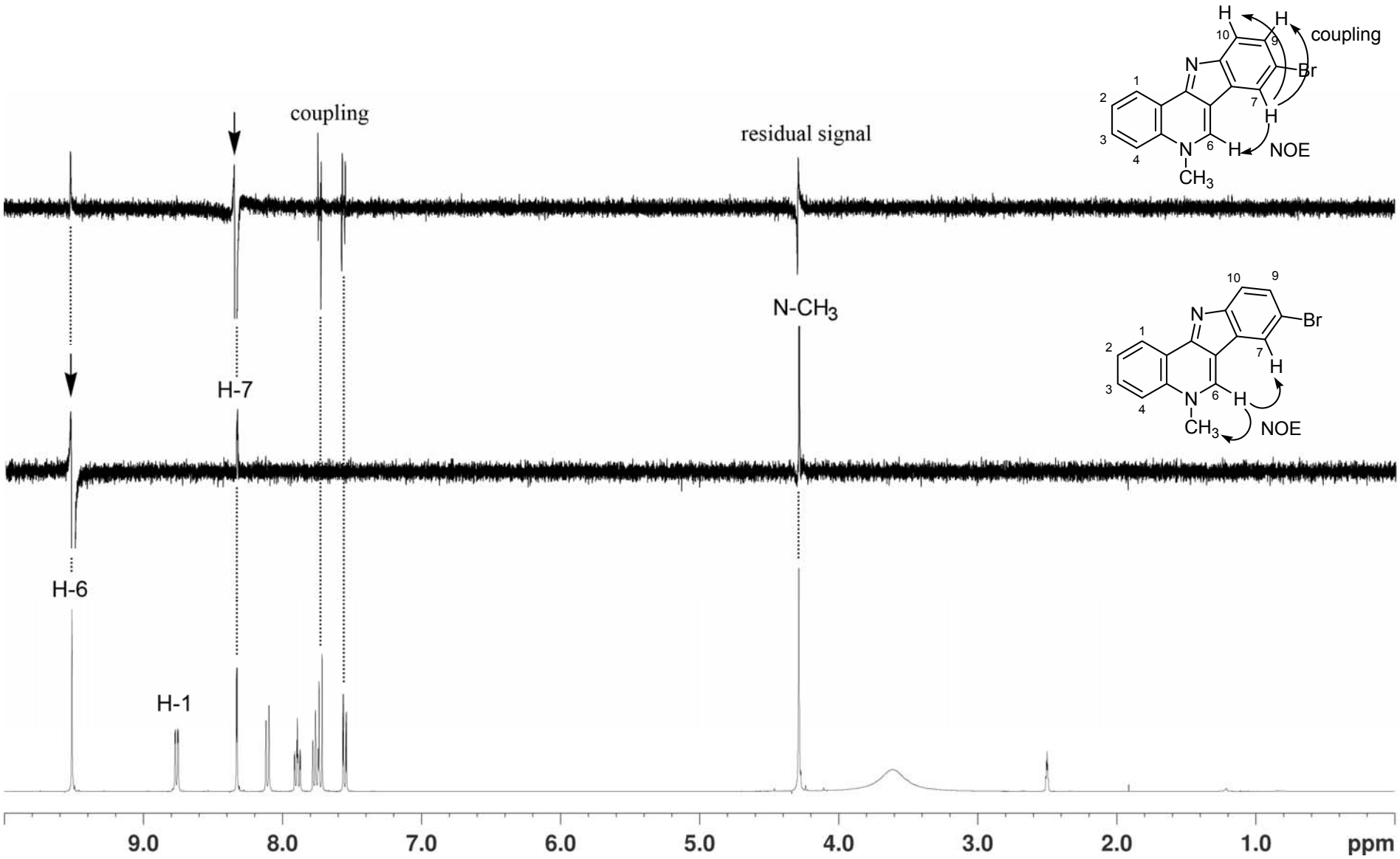
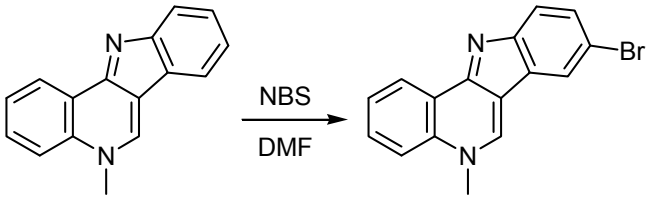



**Figure 3.3:** 1D NOE difference spectra of compound **104**. The arrow indicates the point of saturation (400 MHz, $d_6$-DMSO).

This observation further confirms the peak at 8.30 ppm is due to H-7, therefore its low coupling constant ($^4J_{H,H}$ = 2.4 Hz) indicates it has no protons adjacent and that bromination has occurred at position C8. Therefore it was concluded that 8-bromoisocryptolepine **104** was the product formed (Scheme 3.13).




**16** **104**
74%

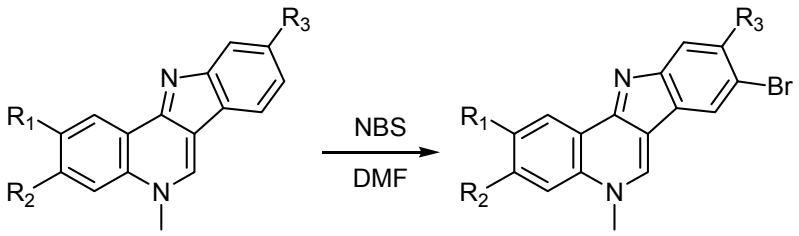
**Scheme 3.13:** Synthesis of 8-bromoisocryptolepine **104**

In order to prepare a dibrominated compound, isocryptolepine **16** was reacted with an excess of *N*-bromosuccinimide at 150°C in DMF. Analysis of the mixture by HPLC did not show any evidence of another compound peak (apart from the one due to 8-bromoisocryptolepine **104**) and therefore a second brominated product had not formed. As previous reports have shown that reaction of bromine with carbazoles often gives mono and disubstituted products,[138,139] this reagent was trialled as a brominating agent. A series of small scale bromination reactions were conducted with bromine in glacial acetic acid and the reaction mixtures analysed by HPLC, using the previously established methods for monitoring the palladium catalysed reactions in Chapter 2.

Reaction of isocryptolepine **16** with bromine (1 mol eq.) in glacial acetic acid at room temperature (25 °C) did not result in the formation of another product. Increasing the molar equivalents of bromine also did not result in a second brominated compound. When the reaction was conducted at 60 °C using one molar equivalent of bromine a secondary peak was observed, albeit with a very low peak area. Increasing the molar equivalents of bromine, or increasing reaction temperature further, did not stimulate the formation of this compound. Consequently the formation of a dibrominated species *via* electrophilic aromatic substitution was deemed unattainable. Unlike cryptolepine **14** position C8 on the isocryptolepine **16** ring is significantly more susceptible to electrophilic attack than position C10.

The method used to synthesise the bromo derivative **104** was subsequently applied to the bromination of other monosubstituted derivatives of isocryptolepine

previously prepared (Scheme 3.14). Reaction of 3-chloroisocryptolepine **64**, 2-bromoisocryptolepine **70** and 2-chloroisocryptolepine **71** with *N*-bromosuccinimide produced 8-bromo-3-chloroisocryptolepine **105** (71%), 2,8-dibromoisocryptolepine **106** (71%) and 8-bromo-2-chloroisocryptolepine **107** (77%). Bromination of the methyl derivative **97** produced 8-bromo-9-methylisocryptolepine **108** in slightly improved yield (80%) most likely due to the weakly activating nature of the methyl group. As for **104**, the position of substitution was confirmed with 1D NOE difference spectrometry and all derivatives were also fully characterised *via* NMR and mass spectroscopy.

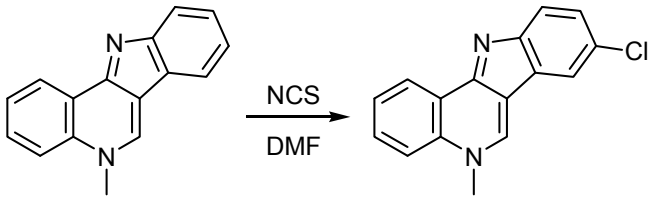



**64** $R_1$ = H $R_2$ = Cl $R_3$ = H
**70** $R_1$ = Br $R_2$ = H $R_3$ = H
**71** $R_1$ = Cl $R_2$ = H $R_3$ = H
**97** $R_1$ = H $R_2$ = H $R_3$ = $CH_3$

**105** $R_1$ = H $R_2$ = Cl $R_3$ = H
**106** $R_1$ = Br $R_2$ = H $R_3$ = H
**107** $R_1$ = Cl $R_2$ = H $R_3$ = H
**108** $R_1$ = H $R_2$ = H $R_3$ = $CH_3$

**Scheme 3.14:** Synthesis of 8-bromoisocryptolepines **105-108**

Chlorination of heterocyclic compounds can be selectively conducted by reaction with *N*-chlorosuccinimide (NCS).[143,144] However, *N*-chlorosuccinimide has been noted to be less reactive compared to *N*-bromosuccinimide and this has been attributed to the stronger N-Cl bond.[145] Reaction of isocryptolepine **16** with *N*-chlorosuccinimide in DMF (150 °C) produced 8-chloroisocryptolepine **109** in low yield (41%) in line with the literature (Scheme 3.15).

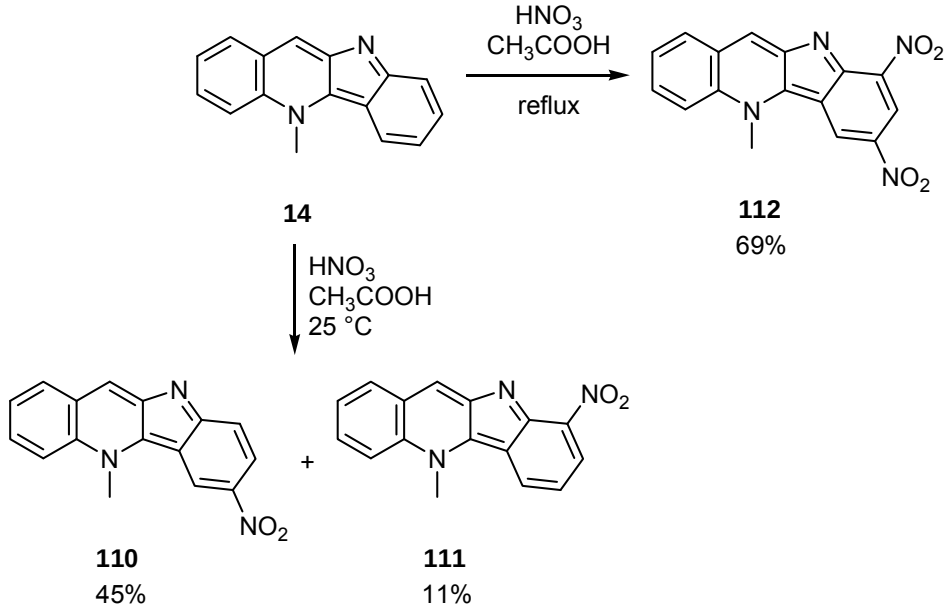



**16** **109** 41%

**Scheme 3.15:** Synthesis of 8-chloroisocryptolepine **109**

### 3.4.2. Attempts to Prepare Nitrated Derivatives

Nitration of cryptolepine **14**, in mixtures of concentrated nitric acid and glacial acetic acid, has previously been reported to exclusively occur at positions C7 and C9.[57] It was found that reaction at room temperature produces mixtures of 7-nitrocryptolepine **110** and 9-nitrocryptolepine **111**, whilst reflux produces solely the disubstituted 7,9-dinitrocryptolepine **112** (Scheme 3.16). Given that bromination only occurs at position C8 on the isocryptolepine ring it was also predicted that nitration would occur here. However, the harsher conditions of nitration may force disubstitution.




**Scheme 3.16:** Wright *et al.*[57] synthetic route to nitrated cryptolepine derivatives

Nitration of isocryptolepine **16** was attempted using the methiodide salt of the parent compound (**16.HI**), as Wright *et al.* had nitrated cryptolepine **14** in its methchloride salt form. Reaction in a 1:1 mixture of concentrated nitric acid (69%) and glacial acetic acid produced a single product that could be easily purified by recrystallisation. The proton NMR of the product (Figure 3.4) indicated the presence of 8 protons, confirming monosubstitution, and 1D NOE difference spectroscopy confirmed that substitution had occurred at position C8. The carbon NMR spectrum (Figure 3.4) showed 16 carbon signals, as expected, but a peak at 83.2 ppm was inconsistent with a nitro product.

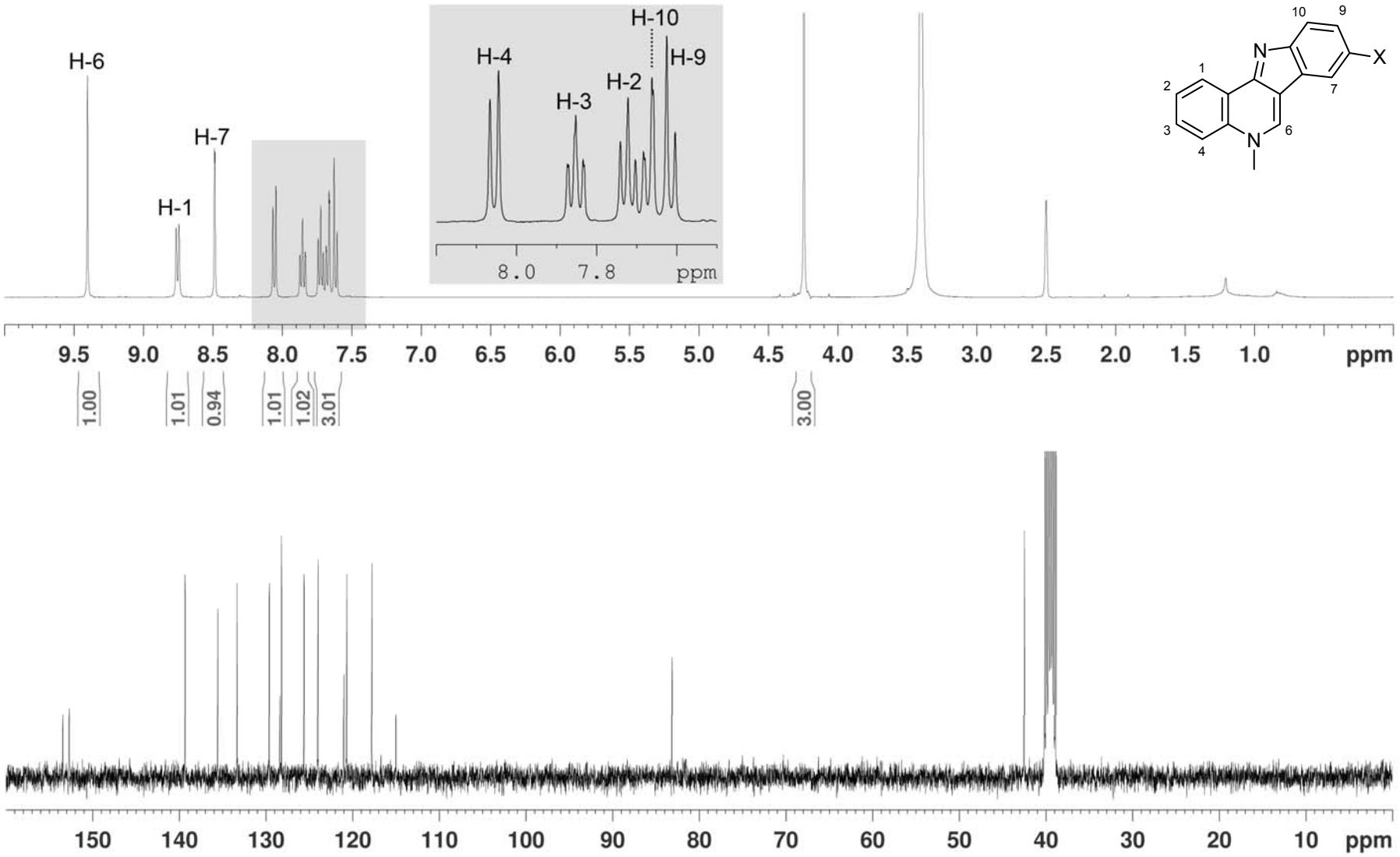
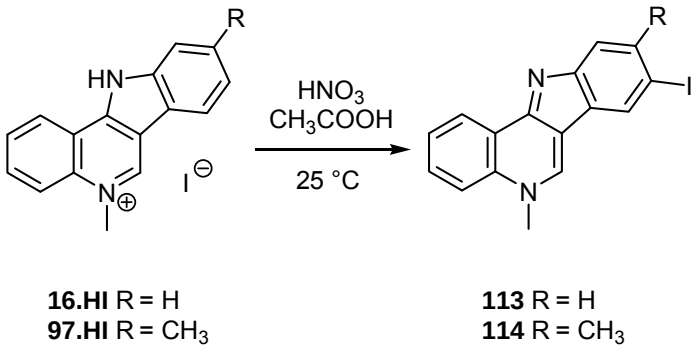



**Figure 3.4:** $^{1}$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra of the assumed nitro product ($d_6$-DMSO).

Mass spectrometry was conducted in attempts to identify the product and the EI mass spectrum showed an intense fragment peak at 358, which was also inconsistent with nitro substituted isocryptolepine. A high resolution mass spectrum was subsequently obtained and an accurate mass of 357.997044 found, which indicated the product has a molecular formula of $C_{16}H_{11}N_2I$ and that the sample was iodo substituted isocryptolepine. On re-examination of the carbon spectrum the peak at 83.2 ppm is consistent with an iodo substituted carbon atom to further confirm that the product is 8-iodoisocryptolepine **113** (Scheme 3.17), which has been formed in moderate yield (57%). Therefore the methiodide salt has most likely reacted with the nitric acid to form an iodo based electrophile.[131] Reaction of 9-methylisocryptolepine **97**, in its methiodide salt form, produced 8-iodo-9-methylisocryptolepine **114** in 71% yield (Scheme 3.17).




**16.HI** R = H
**97.HI** R = $CH_3$

**113** R = H
**114** R = $CH_3$

**Scheme 3.17:** Synthesis of 8-iodoisocryptolepines **113** and **114**

If the source of iodide was removed, by using isocryptolepine **16** in its free base form, it was envisioned that nitration could be achieved. Reaction of isocryptolepine **16** with a 1:1 mixture of concentrated nitric acid and glacial acetic acid was subsequently conducted. However, in 69% concentrated nitric acid at room temperature overnight no reaction was observed. In 90% concentrated nitric acid a mixture of products was detected by both TLC and HPLC analysis. Attempts to separate these species by chromatography were unsuccessful as they had very similar retention times.

Examination of the proton NMR spectrum of the mixture (Figure 3.5) revealed two distinct singlets at 9.76 and 9.66 ppm, which suggested there were two different H-6 peaks and thus two products have been formed. As the 9.76 ppm peak has an integral of 1.05 and the 9.66 ppm peak an integral of 0.77 it was inferred that the mixture contained approximately 60% of one species and 40% of another.

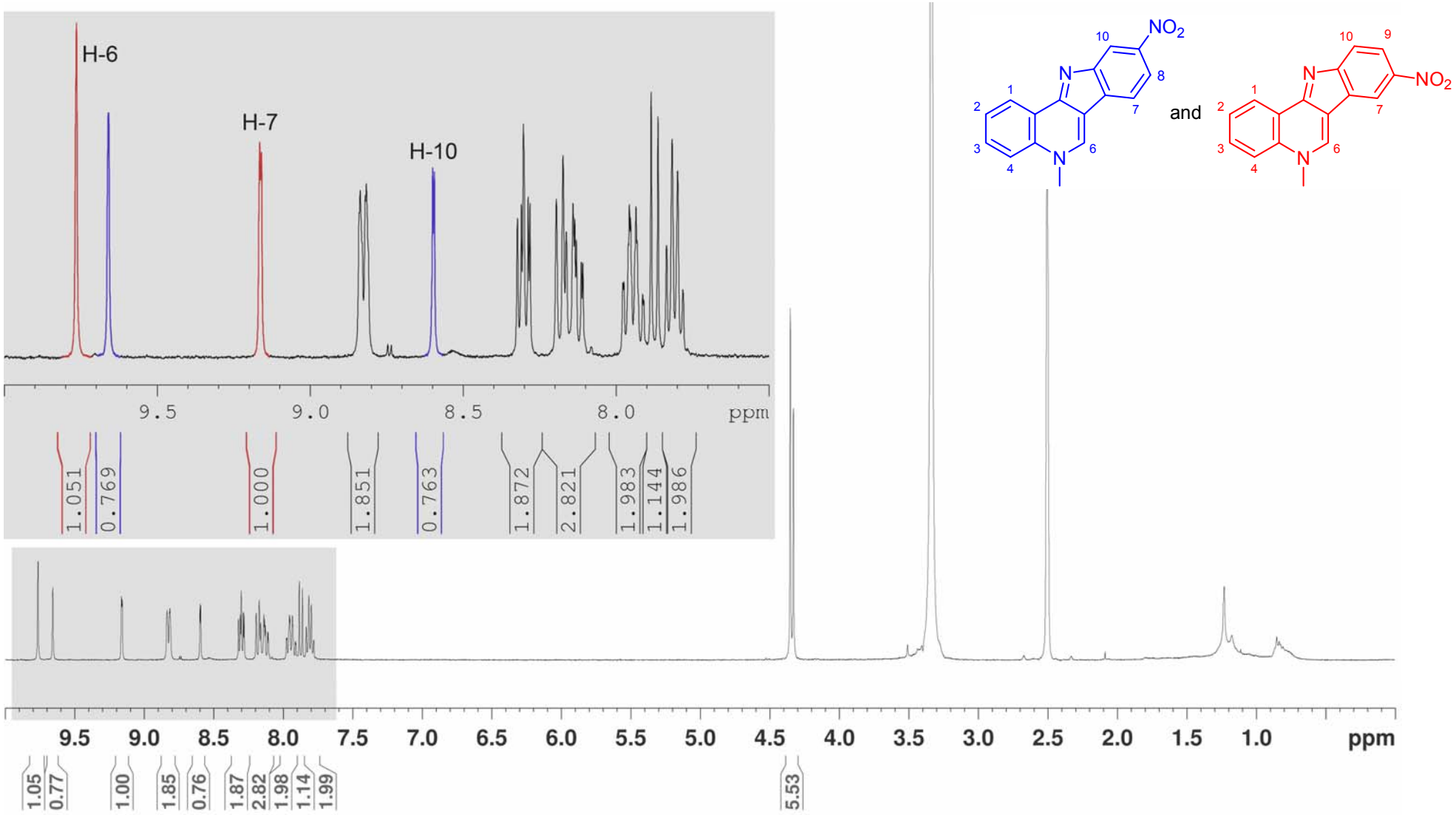
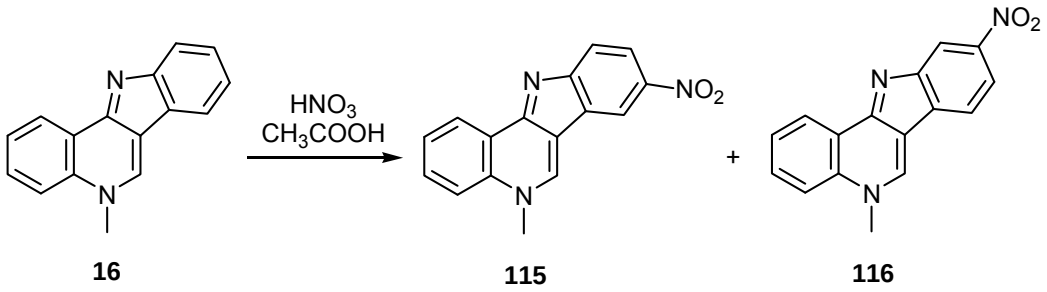



**Figure 3.5:** $^{1}$H NMR spectrum of the mixed nitrated product (400 MHz, $d_6$-DMSO).

Similarly the low coupling constants associated with the doublets at 9.16 ppm ($^4J_{H,H}$ = 2.4 Hz) and 8.60 ppm ($^4J_{H,H}$ = 2.0 Hz) indicate the presence of two different protons adjacent to a nitro group. Thus the two products were predicted to be 8-nitroisocryptolepine **115** and 9-nitroisocryptolepine **116** (Scheme 3.18).




**16** **115** **116**

**Scheme 3.18:** Synthesis of nitrated isocryptolepine derivatives

It was subsequently postulated that increasing the reaction temperature would result in the isolation of a single dinitrated product, as was the case in the nitration of cryptolepine **14**. A series of small scale reactions, in 69% or 90% nitric acid, were conducted and the reaction mixtures analysed by HPLC, as for the bromination of isocryptolepine **16**. Reaction at either 60 °C or 100 °C resulted in the formation of multiple products. As nitro compounds were not priority targets no further effort was made to separate and identify these compounds, most likely a mixture of mono and dinitrated isocryptolepines.

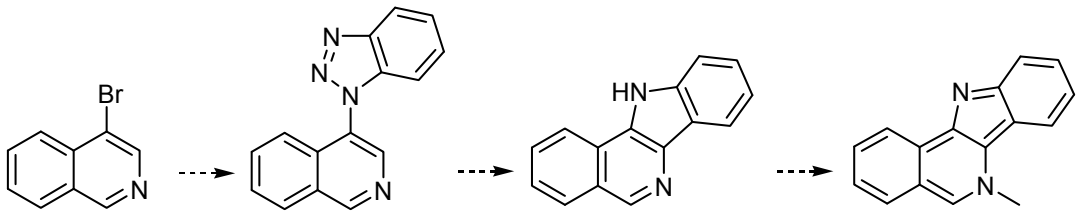
## 3.5. Isomeric Derivatives of Isocryptolepine

The natural isomeric analogue of isocryptolepine **16,** neocryptolepine **19**, was prepared for reference purposes and its synthesis is briefly described in Section 3.5.3. The preparation of the synthetic isoquinoline analogue 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **31** (henceforth referred to as MIQ) was attempted *via* application of both the Molina and Jonckers Methods and these attempts are described in the following section.

### 3.5.1. Synthesis of MIQ 31

The structural isomer of isocryptolepine **16**, MIQ **31**, had not been reported at the commencement of the present project and it was envisioned that this compound would also possess antimalarial activity. Although there had been previous reports of the synthesis of the desmethyl intermediate 11*H*-indolo[3,2-*c*]isoquinoline **117**[146-148] it was decided that the application of either the Molina and Jonckers Methods would be the best approach to prepare the desired compound.

Initially attempts were made to substitute 4-bromoisoquinoline **118** into the Molina Method (Scheme 3.19). However it was found that **118** did not react with benzotriazole to form the desired intermediate **119**.



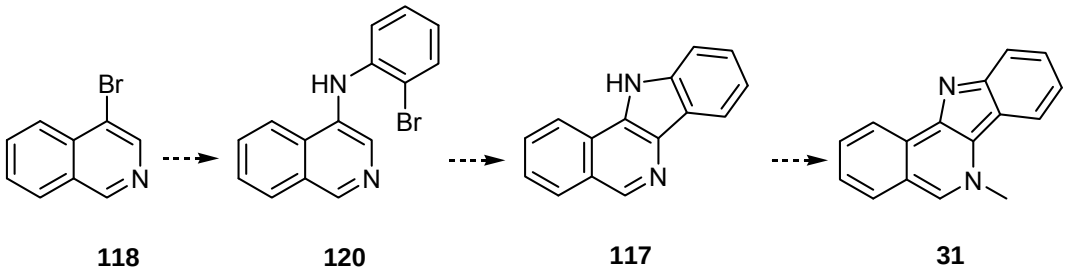

**118** **119** **117** **31**

**Scheme 3.19:** Proposed route to MIQ **31** *via* the Molina Method

This observation can be attributed to the different nucleophilicity of the quinoline ring compared to the isoquinoline ring. Position C4 is the most susceptible position to nucleophilic substitution on a quinoline ring and accounts for the high reactivity of 4-chloroquinolines in this particular reaction. However, on an isoquinoline ring position C1 is the most susceptible to nucleophilic substitution such that 1-(1-benzotriazolyl)-isoquinoline can be readily synthesised from 1-bromoisoquinoline.[149] The application of

the Molina method was thus not further explored for the preparation of MIQ **31** and it was envisioned that the Jonckers Method would provide a superior synthetic route.

During investigation into the application of the Jonckers Method to the preparation of MIQ **31** (Scheme 3.20), Van Baelen *et al.*[82] reported the synthesis of this compound also *via* an adaption of the Jonckers Method. The focus in the present project subsequently shifted to improving the reported synthetic method and confirming the potent antimalarial activity also reported by Van Baelen *et al.*




**118** **120** **117** **31**

**Scheme 3.20:** Proposed synthetic route to MIQ **31** *via* the Jonckers Method

Prior to applying the optimised 'domino' Jonckers Method, the preparation of the coupled intermediate **120** *via* the Buchwald-Hartwig reaction was briefly investigated. 4-Bromoisoquinoline **118** was coupled to 2-bromoaniline using $Pd(OAc)_2$ (2 mol%) and BINAP (2 mol%) with potassium carbonate in refluxing dioxane for 96 hours. Unfortunately, this resulted in the isolation of 4-(2-bromophenylamino)isoquinoline **120** in low yield (17%). The reaction was repeated with a 5-fold increase in catalytic loading and the coupled intermediate **120** was subsequently isolated in moderate yield (50%). When the reaction was conducted using $Pd_2(dba)_3$ (5 mol%) and XANTPHOS (11 mol%) with caesium carbonate the coupled intermediate **120** was obtained in improved yield (67%); comparable to the yield reported by Van Baelen *et al.* (74%) under the same reaction conditions.

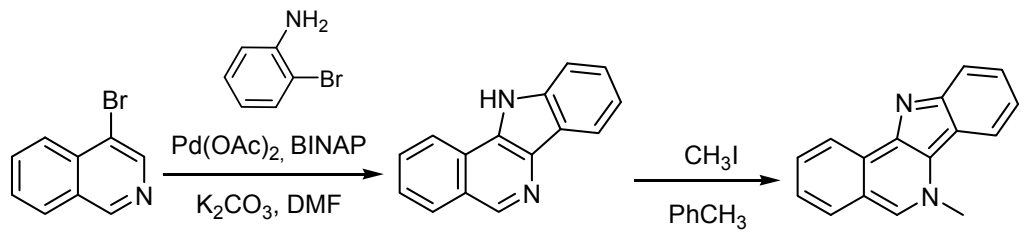
The coupled intermediate **120** was cyclised *via* the intramolecular C-H arylation reaction to form the cyclic intermediate 11*H*-indolo[3,2-*c*]isoquinoline **117** in moderate yield (58%) using $Pd(OAc)_2$ (10 mol%), BINAP (10 mol%) and potassium carbonate in refluxing DMF. Van Baelen *et al.* were able to obtain the cyclised intermediate **117** in higher yield (78%), using a larger catalytic loading of $PdCl_2(PPh_3)_2$ (20 mol%) in dimethylamine at 130 °C.

No further attempts were made to improve the cyclisation of intermediate **120** as the priority was to conduct the cyclisation *in situ* in a 'domino' type reaction as for

intermediate **60**. Attempts were made to monitor this reaction by HPLC but both the coupled intermediate **120** and cyclised intermediate **117** did not absorb well in the same areas of the UV spectrum. As a result, the catalytic conditions successfully used for the preparation of the methyl cyclised intermediate **98** (Scheme 3.10) were applied. 4-Bromoisoquinoline **118** was reacted with 2-bromoaniline using $Pd(OAc)_2$ (10 mol%), BINAP (10 mol%) with potassium carbonate in refluxing DMF. The cyclised intermediate **117** was isolated in 57% yield, comparable to the overall yield reported by Van Baelen *et al.* (58%) to give **117** in two steps.

The *N*-methylation of the cyclised product **117** was achieved as for isocryptolepine **16** (Scheme 2.12), by reaction in acetonitrile with a large molar excess of iodomethane. Purification of the product was achieved by flash column chromatography of the methiodide salt, as the free base was unstable on silica, and MIQ **31** was isolated in 66% yield. When the reaction was conducted in non-polar aprotic toluene, the yield of MIQ **31** was improved to 89%. Van Baelen *et al.* isolated MIQ **31** in slightly reduced yield (76%) using polar aprotic THF as the solvent. Given that the isocryptolepine derivatives were isolated in higher yields when acetonitrile was used as the solvent, compared to reaction in toluene, the opposite result had been expected. It was postulated that the cyclised intermediate **117** was more soluble in toluene than the 11*H*-indolo[3,2-*c*]quinolines, such that the higher yield obtained in toluene was simply due to the increase in reaction temperature. Assignment of the proton and carbon NMR spectra of MIQ **31** was achieved *via* 2D-NMR spectroscopy (COSY, HSQC and HMBC) and was in agreement with the data later published.[82]

The optimum conditions for the preparation of MIQ **31** are shown in Scheme 3.21. This method allows the isolation of MIQ **31** in two steps in comparable yield (51%) to the three step procedure (44%) reported by Van Baelen *et al.*[82].




**118** **117** 57% **31** 89%

**Scheme 3.21:** Optimised synthetic route to MIQ **31**

Given a viable method to MIQ **31**, and the promising biological activity reported for the compound, the preparation of some derivatives became an additional focus and this will be discussed in the following section.

### 3.5.2. Attempts to Prepare Brominated MIQ Derivatives

Given the ease with which both isocryptolepine **16** and its derivatives were brominated, attempts were made to brominate MIQ **31**. However, reaction of MIQ **31** with *N*-bromosuccinimide in DMF at 150 °C resulted in no reaction and stronger brominating conditions were sought. Subsequent attempts at bromination with bromine in glacial acetic acid, at room temperature, again resulted in no reaction. Reaction at 60 °C with one equivalent of bromine, for three days, resulted in some brominated product being detected by TLC analysis. Chromatography was conducted but a small amount of compound (9 mg; 4% yield) was isolated and the product was contaminated with starting material; clean separation could not be achieved owing to both the product and starting material being unstable on silica. Future chromatography could be conducted on neutral alumina or triethylamine treated silica. Proton NMR spectroscopy of the product (Figure 3.6) showed 8 protons, to indicate that monosubstitution had occurred, and the emergence of a peak at 111.8 ppm in the carbon spectrum was consistent with a bromo substituted carbon.

On closer examination of the aromatic regions in the proton spectra, it was observed that the multiplet at 8.30 ppm due to H-7 and H-4 in the spectrum of MIQ **31** had separated into two doublets in the spectrum of the product. In addition the doublet at 8.30 ppm ($^4J_{H,H}$ = 2 MHz) in the proton spectrum was most likely due to H-7 and indicates that the bromo group is adjacent (at position 8). Similarly the two triplets at 7.42 ppm (H-8) and 7.76 ppm (H-9) in the spectrum of MIQ **31** have become one doublet of doublets at 7.52 ppm in the spectrum of the product, also indicating substitution has occurred at position C8. Based on these observations the product was predicted to be 8-bromo-6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **121** (Scheme 3.22) but 1D NOE difference spectrometry was not conducted to confirm this in the absence of a pure product. Instead efforts were made to improve the efficiency of the synthesis.

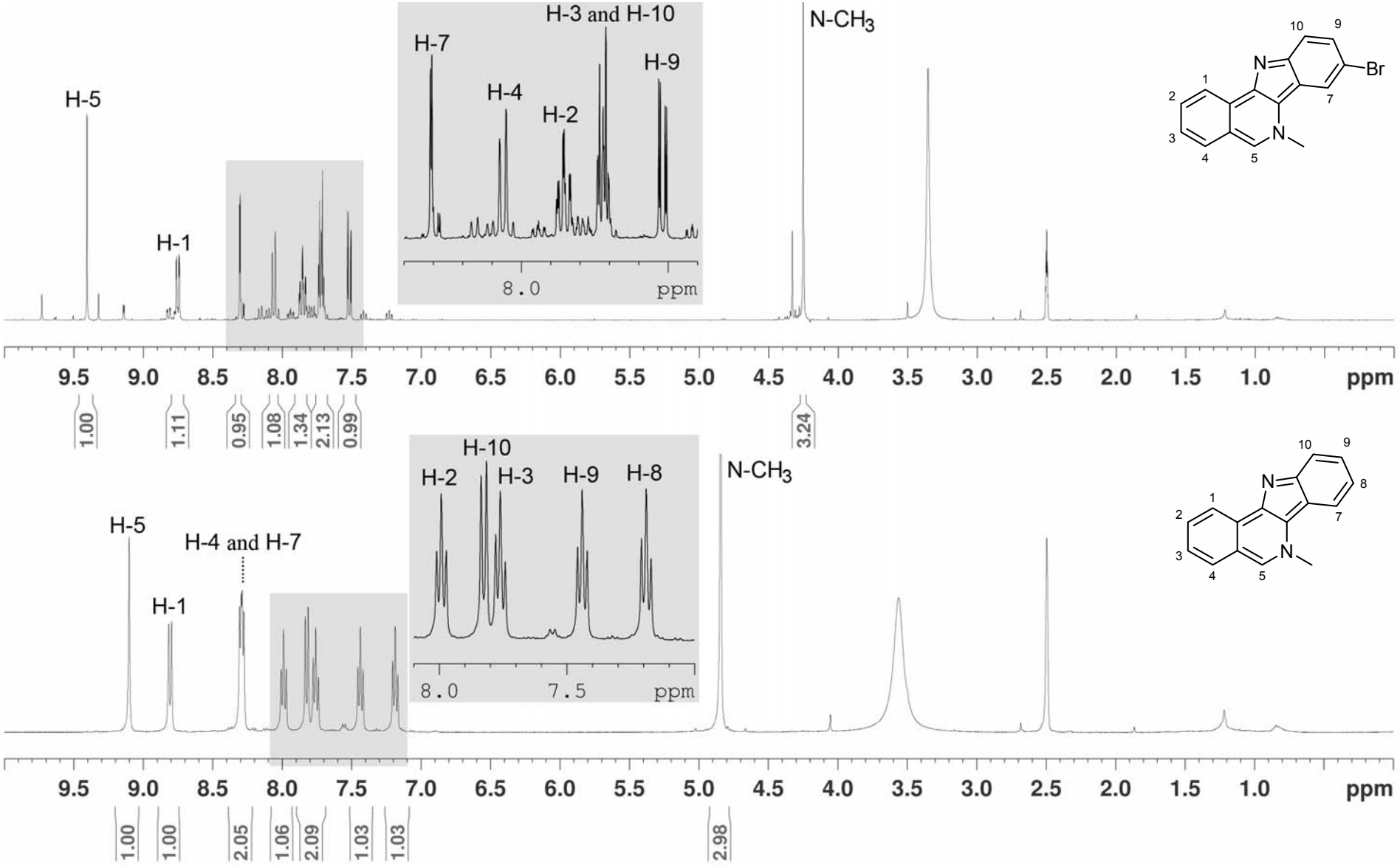
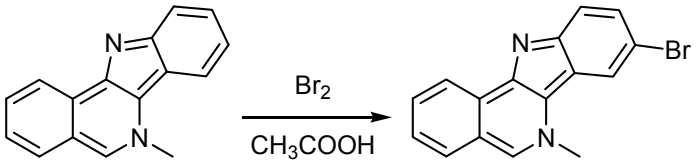



**Figure 3.6:** $^{1}$H NMR spectra of MIQ **31** and the impure brominated product (400 MHz, $d_6$-DMSO).




**31** **121**

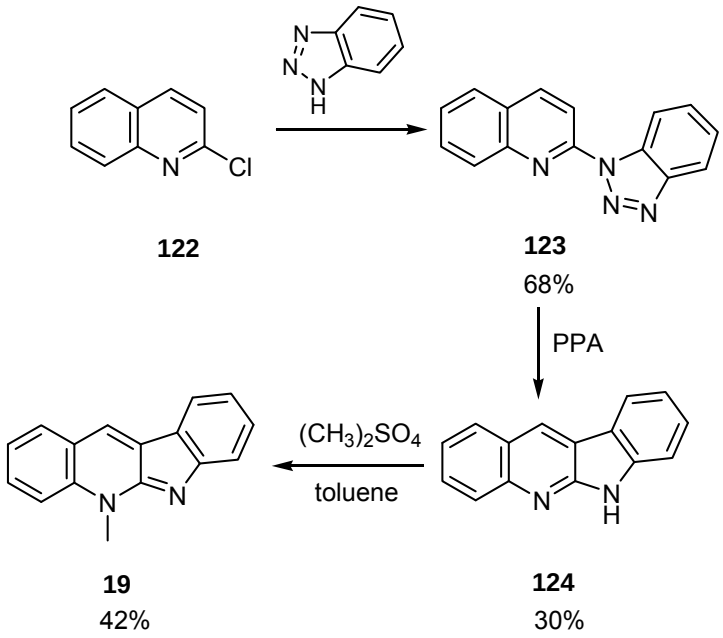
**Scheme 3.22:** Synthesis of 8-bromo-6-methyl-6*H*-indolo[3,2-*c*]isoquinoline **121**

A series of small scale bromination reactions were subsequently conducted and the reaction mixtures analysed by HPLC, as for the bromination of isocryptolepine **16** (Section 3.4.1). On reaction of MIQ **31** with one molar equivalent of bromine in glacial acetic acid (at 60 °C) a product peak was observed, but a significant portion of unreacted MIQ **31** was also present after 24 hours of reaction. Increasing the reaction temperature (to 100 °C) or increasing the molar equivalents of bromine (to three) did not result in complete bromination of MIQ **31**. Whilst electrophilic aromatic substitution of MIQ **31** appeared possible, it was extremely inefficient. Given that the parent **31** was formed in reduced yield, in comparison to isocryptolepine **16**, and bromination was also low yielding it was judged that a better method to synthesise derivatives was necessary. However, at this late stage in the project further synthetic experiments were not pursued. Nevertheless, if the high biological activity of MIQ **31** is confirmed a more thorough investigation may be warranted.

### 3.5.3. Synthesis of Neocryptolepine 19

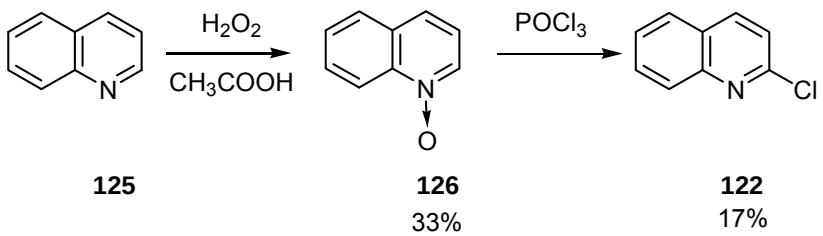
Neocryptolepine **19** also represents an isomeric derivative of isocryptolepine **16**, which has previously been prepared by Peczyńska-Czoch *et al.*[133] *via* a similar methodology to the Molina Method (Scheme 3.23). Initially 2-chloroquinoline **122** was coupled to benzotriazole, followed by cyclisation to form 6*H*-Indolo[2,3-*b*]quinoline **124** and finally *N*-methylation to give neocryptolepine **19** in an overall yield of 9%. Neocryptolepine **19** has been more extensively studied in comparison to isocryptolepine **16** and it was envisioned that this compound could be used as a reference compound during the biological evaluations. However, in the event, only a limited number of compounds were able to be biologically evaluated and as isocryptolepine **16** was available the examination of neocryptolepine **19** was not

considered necessary. Nevertheless this alkaloid was utilised in a later $pK_a$ experimental investigation.




**Scheme 3.23:** Peczyńska-Czoch *et al*.[133] synthetic route to neocryptolepine **19**

The necessary starting material, 2-chloroquinoline **122**, was prepared in two steps from quinoline **125** *via* a previously reported method (Scheme 3.24).[150] Reaction of **125** with glacial acetic acid and hydrogen peroxide gave quinoline-*N*-oxide **126**, which was subsequently reacted with phosphorous oxychloride. This produced a mixture of 4-chloroquinoline **33** and 2-chloroquinoline **122**, which were separated *via* chromatography. 2-Chloroquinoline **122** was isolated in lower yield (17%) from compound **125** than Rodríguez *et al.* (47%)[151] but no efforts were made at this stage to optimise this reaction.




**Scheme 3.24:** Synthesis of 2-chloroquinoline **85**

2-Chloroquinoline **125** was reacted with benzotriazole, applying the same reaction conditions used to prepare **35** (Scheme 2.14) and 2-(1-benzotriazolyl)-quinoline **123** was isolated in 75% yield. Subsequent reaction in polyphosphoric acid

resulted in the isolation of 6*H*-indolo[2,3-*b*]quinoline **124** in 33% yield. Similarly Peczyńska-Czoch *et al*.[133] were only able to isolate **124** in low yield, presumable due to the formation of a secondary cyclisation product.[152]

The cyclised intermediate **124** was *N*-methylated under the same reaction conditions applied in the preparation of isocryptolepine **16** (Scheme 2.12). Reaction with iodomethane in acetonitrile, followed by chromatography of the methiodide salt, resulted in the isolation of neocryptolepine **19** in 37% yield. Again the yield was lower than previously reported, but no efforts were made to optimise the reaction as the principle aim was to prepare sufficient compound for use as a reference in the biological evaluations.

## 3.6. Summary

The Molina Method allowed the preparation of five derivatives, **64**, **65**, **70**, **71** and **91**, from substituted 4-chloroquinolines. This method is most suitable to the application of 4-chloroquinolines with ring substituents at positions C6 and C7 and further application could allow the synthesis of a wider range of 2 and 3-substituted isocryptolepines. In general this method is only limited by the availability of the necessary 4-chloroquinolines.

The Jonckers Method allowed the preparation of the ring substituted derivative 9-methylisocryptolepine **97**. Initial investigations indicated that substituted anilines are more applicable to this method than substituted quinolines, but further examination of this approach is warranted.

Electrophilic aromatic substitution was found to favour substitution at position C8 and bromination using this method was thoroughly investigated and allowed the preparation of five novel derivatives (**104 - 108**). The method was further applied to the preparation of chloro and iodo derivatives and three additional derivatives (**109**, **113** and **114**) were prepared. Although a method for selective mononitration was not perfected, the initial investigation undertaken here is a useful foundation for future studies.

During the course of this work an alternative synthetic route to the chloro isocryptolepine derivatives **64** and **109** was reported by Kumar *et al.*[93] The products were isolated in a single step from substituted 2,3-dihydro-4-quinolones and accordingly this synthetic method may be worthy of further investigation at a later stage.

The isomeric derivative of isocryptolepine **16**, MIQ **31**, could be prepared *via* employment of the Jonckers Method, but the Molina Method was not applicable. Bromination of MIQ **31** was unsuccessful and a re-investigation of the synthetic procedures to the desmethyl intermediate **117** may be required in order to develop routes to substituted derivatives of this particular ring system.

# Chapter 4

## Biological Evaluation of Isocryptolepine Derivatives

## 4.1. Introduction

Eight of the fifteen synthesised isocryptolepine derivatives were selected for biological testing based on efficacy of related cryptolepine analogues and the diversity and number of substituent groups present; monosubstituted, disubstituted, halogenated and alkyl substituted derivatives (Figure 4.1). The isomer of isocryptolepine **16**, MIQ **31**, was also evaluated to confirm its previously reported potent antimalarial activity.[82]

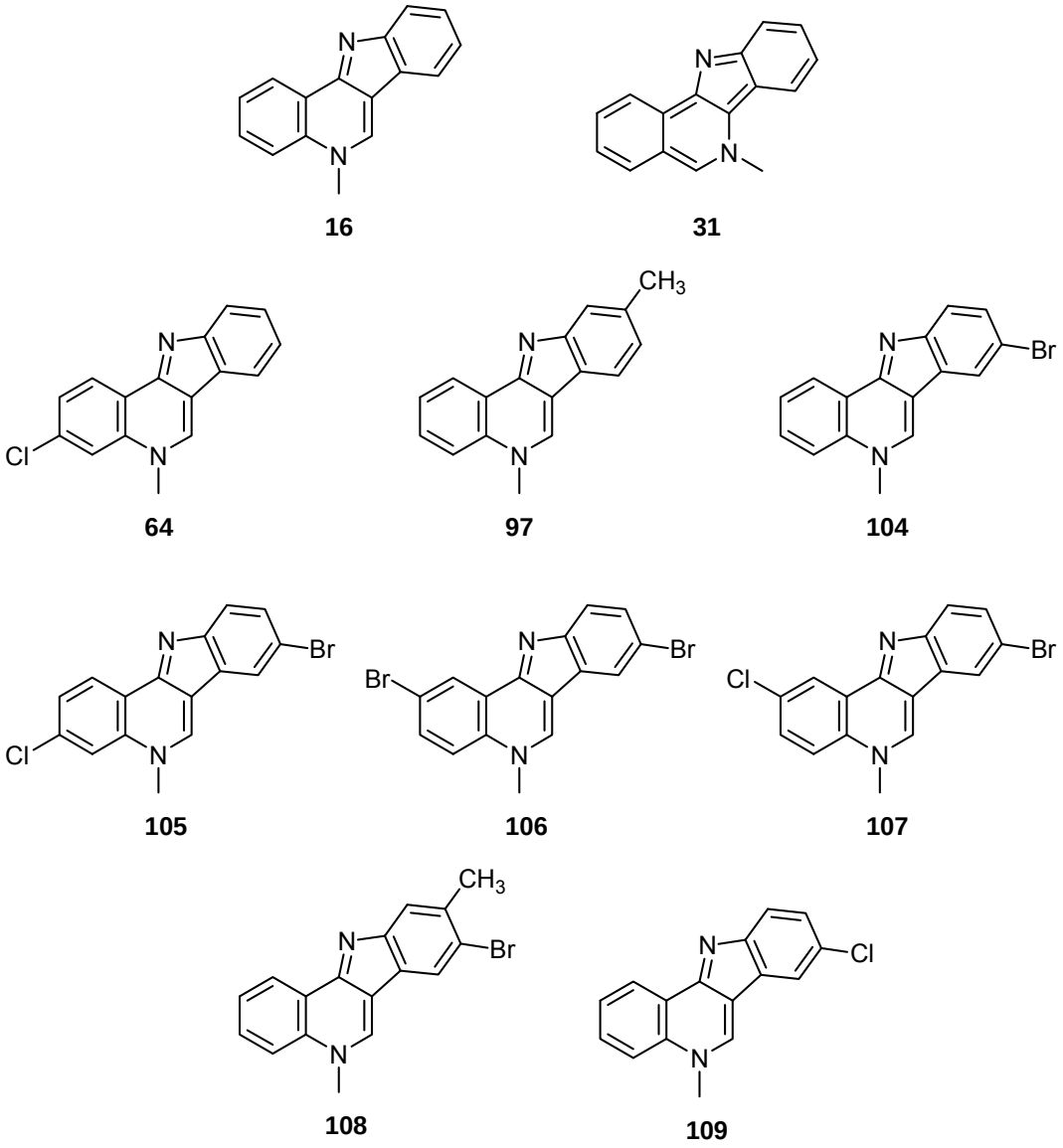



**Figure 4.1:** Isocryptolepine **16**, MIQ **31** and the isocryptolepine derivatives selected for biological evaluation

The biological evaluation of derivatives for antimalarial activity and cytotoxicity was conducted in collaboration with Professor Tim Davis (School of Medicine and Pharmacology; University of Western Australia) and Dr Simon Fox (School of Pharmacy; Curtin University) respectively. Compounds were assessed for antimalarial activity as their hydrochloride salts against two strains of *Plasmodium falciparum* (chloroquine sensitive 3D7 and chloroquine resistant W2mef) and for cytotoxicity against the 3T3 cell-line (mouse embryonic fibroblasts).

## 4.2. Determination of Physicochemical Properties

A compound which displays high or adequate bioactivity also needs to possess appropriate pharmacological and pharmacokinetic properties (i.e. absorption, metabolism, excretion and bioavailability). Unfavourable pharmacological properties are one of the main causes of attrition in drug discovery and development.[153] The physicochemical properties of compounds (i.e. solubility, Log P values and $pK_a$) can profoundly affect the above biological properties and hence before the isocryptolepine derivatives were biologically evaluated a variety of important physicochemical properties were determined for the compounds under investigation.

### 4.2.1. Solubility

Isocryptolepine derivatives, in the free base form, displayed poor aqueous solubility as expected due to their polyaromatic nature. However, solubility markedly increased if the compounds were converted to their hydrochloride salts. All cryptolepine and neocryptolepine derivatives that have previously been biologically evaluated were presented in their salt forms, presumably also due to solubility issues associated with the free base forms.

Even though the salt did improve aqueous solubility, it did not allow adequately high concentrations of compounds for satisfactory biological evaluation. Low concentrations of ethanol, acetic acid or DMSO in water were compatible with the biological cells but initial investigations found that compounds were insoluble in water-ethanol or water-acetic acid mixtures, but aqueous solubility improved with the addition of DMSO.

Whilst solubility was not quantitatively determined, some observations were made. The parent alkaloid **16**, 9-methylisocryptolepine **97**, 8-bromoisocryptolepine **104** and 8-bromo-9-methylisocryptolepine **108** were soluble in a 50% solution of DMSO in water and 15 mM solutions were achievable. MIQ **31** and 8-chloroisocryptolepine **109** were moderately soluble (10 mM solutions were achievable). 3-Chloroisocryptolepine **64**, 8-bromo-3-chloroisocryptolepine **105**, 2,8-dibromoisocryptolepine **106** and 8-bromo-2-chloroisocryptolepine **107** were poorly soluble and an 80% solution of DMSO in water was needed for complete dissolution

to give 5 mM concentrations. 8-Bromo-3-chloroisocryptolepine **105** was the least soluble as precipitation occurred upon refrigeration at these concentrations.

### 4.2.2. Stability

The stability of the resultant water-DMSO solutions was also investigated to assess if compound degradation was apparent during the biological testing. Initially the percentage purity of the compound (as hydrochloride salts) was assessed by HPLC analysis *via* a similar method previously applied to assess the purity of neocryptolepine derivatives.[58,80] The HPLC conditions used were the same as those previously applied to monitor the Buchwald-Hartwig and C-H arylation reactions, (Section 2.4) in addition to the electrophilic substitution of isocryptolepine (Section 3.4). If compound purity was below 95% the samples were further purified by flash column chromatography. These relatively pure compound samples were subsequently used to prepare stock solutions (in water-DMSO) for biological testing purposes. A selection of these stock solutions were later re-examined post testing to assess their percentage purity over a period of three months. The purity analysis results pre- and post-biological testing is summarised in Table 4.1.

**Table 4.1:** Preliminary Stability Analysis

| Compound | Initial % purity | % Purity in solution (days) | |
|---|---|---|---|
| | | 1st test | 2nd test |
| Isocryptolepine **16** | 99.9 | 96.5 (40) | 94.0 (107) |
| MIQ **31** | 99.1 | - | 99.5 (107) |
| 3-Chloroisocryptolepine **64** | 98.9 | 98.9 (25) | 71.5 (93) |
| 9-Methylisocryptolepine **97** | 98.7 | 99.5 (32) | - |
| 8-Bromoisocryptolepine **104** | 99.5 | 100 (40) | 100 (107) |
| 8-Bromo-3-chloroisocryptolepine **105** | 97.6 | 98.5 (25) | 89.1 (93) |
| 8-Bromo-9-methylisocryptolepine **108** | 97.5 | - | 95.2 (99) |

The majority of the compound solutions did not display reduced percentage purity over the three month period and can be considered relatively stable. However, after 90 days in solution 3-chloroisocryptolepine **64** and 8-bromo-3-chloroisocryptolepine **105** displayed reduced percentage purity (< 90%). Consequently all compounds were

considered stable for a period of up to 25 days, after which time fresh solutions were made for testing purposes.

### 4.2.3. Log P values

The log P value of a compound describes its tendency to partition into non-polar over aqueous environments. It is often quantitatively described by the log of the equilibrium distribution of a compound between octanol and water. The log P of a compound is in essence an indicator of a compound's lipophilicity but also provides information relating to aqueous solubility. A high log P can be associated with issues such as low aqueous solubility, which may in some cases lead to poor oral absorption.[154] The log P values of the isocryptolepine derivatives were estimated using the ACD/I-Lab web service,[137] as previously described in Section 3.2.3 for the estimation of $pK_a$ values for certain 11*H*-indolo[3,2-*c*]quinolines. Based on these calculated log P values all isocryptolepine derivatives were moderately lipophilicity with a log P in the range 2.06 to 4.13. According to Lipinski's rule of five,[155] moderate compound lipophilicity (i.e. Log P < 5) is associated with compounds that display a good balance between aqueous solubility and cell membrane permeability. Thus these compounds may possess acceptable oral absorption *in vivo*.

## 4.3. Antimalarial Evaluation

The *in vitro* antimalarial activity of the selected isocryptolepine derivatives was determined by assessing their ability to inhibit the growth of *Plasmodium falciparum*. Percentage growth inhibition was determined using the standard [$^3$H]-hypoxanthine assay[156,157] and involves supplying the parasite with [$^3$H]-hypoxanthine, which is essential for parasite growth.[158] The difference in [$^3$H]-hypoxanthine uptake between the drug sample and a drug-free control provides a measure of percentage parasite growth which can then be used to calculate $IC_{50}$ values for the compounds under investigation.

Stock solutions of the isocryptolepines derivatives were prepared in either 50% sterile DMSO in water or 80% DMSO in water, as described in Section 4.2.1. Stock solutions of 8-bromo-3-chloroisocryptolepine **105** were prepared fresh on the day of testing due to the precipitation issues. Stock solutions were serially diluted with cell media into 96-well plates and the optimum concentration range for testing pre-determined by a pilot study. Initially, a working standard of 1600 μM for isocryptolepine **16** was tested resulting in a tested concentration range of 12.5 - 800 μM, but was later found to be too concentrated. A working standard of 12 μM, giving a concentration range of 94 - 6000 nM, was found to be adequate on a second attempt. Consequently other derivatives were initially tested in this range and the concentrations adjusted accordingly.

Each compound was tested in triplicate with a drug-free control, chloroquine **2** was used as the positive control and statistically significant data was assured by conducting the assay a minimum of three separate times on each strain of *P. falciparum*. Due to the high dilution factors undertaken the concentration of DMSO was always below 0.05% and did not affect parasite growth.

The results of the antimalarial evaluation of the isocryptolepines are summarised in Table 4.2. The parent alkaloid **16** displayed *in vitro* antimalarial activity ($IC_{50}$ = 1177 nM) against the chloroquine resistant strain (W2mef) at levels consistent with previous literature reports ($IC_{50}$ = 780 nM, K1).[59] Similarly chloroquine **2** was active against the resistant strain ($IC_{50}$ = 144 nM) at comparable levels to published data ($IC_{50}$ = 171 - 246 nM).[57,59,75,157] The structural isomer MIQ **31** also showed *in vitro* antimalarial activity ($IC_{50}$ = 273 nM, W2mef) and although it was nearly 4-fold more

potent than isocryptolepine **16** its antimalarial activity was not as high as previously reported by Van Baelen *et al.* ($IC_{50}$ = 40 nM, K1).[82]

**Table 4.2:** Antimalarial Activity of Selected Isocryptolepine Derivatives

| Compound | Antiplasmodial activity; $IC_{50}$ (nM) [a] | |
|---|---|---|
| | 3D7 | W2mef |
| Isocryptolepine **16** | 665 ± 221 | 1177 ± 390 |
| MIQ **31** | 58.5 ± 16.0 | 273 ± 95.6 |
| 3-Chloroisocryptolepine **64** | 130 ± 11.8 | 316 ± 205 |
| 9-Methylisocryptolepine **97** | 448 ± 83.0 | 760 ± 268 |
| 8-Bromoisocryptolepine **104** | 84.9 ± 33.1 | 184 ± 46.9 |
| 8-Bromo-3-chloroisocryptolepine **105** | 50.4 ± 4.38 | 100 ± 16.3 |
| 2,8-Dibromoisocryptolepine **106** | 127 ± 98.0 | 112 ± 11.7 |
| 8-Bromo-2-chloroisocryptolepine **107** | 57.4 ± 14.3 | 85.0 ± 5.64 |
| 8-Bromo-9-methylisocryptolepine **108** | 62.2 ± 38.9 | 131 ± 55.1 |
| 8-Chloroisocryptolepine **109** | 117 ± 15.9 | 218 ± 34.6 |
| Chloroquine **2** [b] | 20.4 ± 26.6 | 144 ± 10.5 |

[a] $IC_{50}$ ± standard deviation. [b] Evaluated as a diphosphate.

Isocryptolepine derivatives were found to be more bioactive than the parent compound, against both strains of *P. falciparum*. Of the monosubstituted derivatives, 8-bromoisocryptolepine **104** ($IC_{50}$ = 184 nM, W2mef) was the most potent, being approximately 6-fold more active than the parent. 9-Methylisocryptolepine **97** ($IC_{50}$ = 760 nM, W2mef) was the least active of all derivatives, albeit 1.5-fold more active than the parent alkaloid.

The disubstituted analogues 8-bromo-3-chloroisocryptolepine **105** ($IC_{50}$ = 100 nM, W2mef) and 8-bromo-9-methylisocryptolepine **108** ($IC_{50}$ = 131 nM, W2mef) were more active than either of their corresponding mono-substituted counterparts. 8-Bromo-2-chloroisocryptolepine **107** ($IC_{50}$ = 85.0 nM, W2mef) was the most potent of the derivatives, being nearly 14-fold more active than the parent. 8-Bromo-3-chloroisocryptolepine **105** and 2,8-dibromoisocryptolepine **106** ($IC_{50}$ = 112 nM, W2mef) were the next most bioactive derivatives, being 12-fold and 11-fold more potent respectively compared to the parent alkaloid. The analogous cryptolepine derivative of **107**, 7-bromo-2-chlorocryptolepine **23** ($IC_{50}$ = 30 nM, K1; Figure 1.13), was found to be the most potent derivative in the study by Onyeibor *et al.*[75] In

addition 7-bromo-3-chlorocryptolepine **24** ($IC_{50}$ = 37 nM, K1) and 2,7-dibromo-cryptolepine **25** ($IC_{50}$ = 49 nM, K1), analogues of **105** and **106** respectively, displayed significantly enhanced antimalarial activity in comparison to cryptolepine **14**. This similarity in the effects of ring substituents on the enhancement of antimalarial activity suggests that these compounds may act upon the *Plasmodium* parasite in a similar manner.

Most derivatives were more bioactive against the chloroquine sensitive strain, rather than the chloroquine resistant, which may indicate that they have a similar mode of action to chloroquine **2**. 2,8-Dibromoisocryptolepine **106** was the only exception, being slightly more potent against W2mef ($IC_{50}$ = 112 nM) than 3D7 ($IC_{50}$ = 127 nM). However, there is unlikely to be a practical difference between these $IC_{50}$ values and formal mechanistic studies would be required to ascertain if this indicates an alternative mode of action to chloroquine.

Recently Wong *et al.*[157] reported the antimalarial activity of some current antimalarial drugs against the W2mef and 3D7 strains of *P. falciparum* using the same methods to determine parasite growth inhibition as described above. Consequently these results are directly comparable to that particular report. The study found dihydroartemisinin **10** (Figure 1.6) and lumefantrine **11** (Figure 1.7) possessed $IC_{50}$ values of 3.1 nM and 55.5 nM respectively, against the W2mef strain. Whilst the isocryptolepine derivatives are not as potent as dihydroartemisinin **10** certain compounds, notably 8-bromo-2-chloroisocryptolepine **107** ($IC_{50}$ = 85.0 nM), are in the same range as lumefantrine **11** and may possess adequate potency for therapeutic applications.

### 4.3.1. Cross Resistance Estimation

Cross-resistance between two drugs is often estimated using the Spearman correlation coefficient (r), which measures the statistical dependence between two variables.[159] Chloroquine cross-resistance with isocryptolepine derivatives was estimated, where the significance level (P) was set at 0.05. A significant positive correlation was found between chloroquine **2** and the derivatives 3-chloroisocryptolepine **64** ($r$ = 0.73; P = 0.031), 8-bromoisocryptolepine **104** ($r$ = 0.75; P = 0.026), 8-bromo-2-chloroisocryptolepine **107** ($r$ = 0.89, P = 0.033) and 8-chloroisocryptolepine **109** ($r$ = 0.70; P = 0.043). A positive correlation may suggest

common modes of action, drug uptake or resistance mechanism between the derivatives and chloroquine **2**.[160] It should be noted that the Spearman correlation test is normally conducted when field isolates are analysed, but in this case the same strains of *P. falciparum* were used for testing. This may indicate that the results are not statistically viable and can only be used as an indication of a possible correlation.

### 4.3.2. Vacuole Accumulation Estimation

As mentioned in Section 1.1.2, chloroquine **2** accumulates in the food vacuole of the *plasmodium* parasite wherein it inhibits haemozoin. The extent of vacuole accumulation of various cryptolepine derivatives has previously been estimated by Onyeibor *et al.*[75] using Equation 4.1, which affords the vacuole accumulation ratio. This ratio is a percentage of vacuole drug concentration ($[Q]_v$) against external drug concentration ($[Q]_e$). The two values $[H^+]_v$ and $[H^+]_e$ denote the vacuole and external ion concentrations respectively, where the vacuole and external pH are assumed to be 5.5 and 7.4 respectively.[161]

$$\frac{[Q]_v}{[Q]_e} = \frac{1 + \frac{[H^+]_v{}^2}{K_a}}{1 + \frac{[H^+]_e{}^2}{K_a}}$$

**Equation 4.1:** Equation used to estimate vacuole accumulation[75]

Whilst Onyeibor *et al.*[75] concluded that there is no correlation between vacuole accumulation and antimalarial activity (or haemozoin inhibition), this ratio does provide an indication of compound accumulation in the food vacuole. It is proposed that if a compound does not accumulate but still shows good antimalarial activity then the compound may possess a different mode of action or may be extremely potent against haemozoin at low levels.

In order to apply the above equation, $K_a$ values of the isocryptolepine derivatives were required. These values were estimated from calculated $pK_a$ values obtained using the ACD/I-Lab web service[137] and are presented in Table 4.3. The $pK_a$ of isocryptolepine **16** was predicted to be 8.9 (± 0.20), which deviated slightly from the value of 9.8 reported by Grycová *et al.*[162] (obtained *via* NMR spectroscopy). However the predicted $pK_a$ values of cryptolepine **14** (11.19 ± 0.20) and neocryptolepine **19** (7.58 ± 0.20) were found to be close to the reported values (11.0

and 7.1 respectively) and were therefore deemed suitable for the $pK_a$ estimation. The $pK_a$ values of the isocryptolepine derivatives were subsequently calculated and found to lie in the range 8.92 to 4.58.

The calculated $pK_a$ value for MIQ **31** (14.05 ± 0.20) was found to be unusually high which suggested very high compound basicity. As a result a conventional laboratory based investigation of its $pK_a$ was undertaken. The $pK_a$ values of neocryptolepine **19** (included as a reference) and MIQ **31** were determined spectrophotometrically as previously reported,[75,133,163] and found to be 7.75 (± 0.46) and 8.71 (± 1.96) respectively. The values obtained for neocryptolepine **19** was consistent with the ACD/I-Lab value in addition to previously reported experimental values but it is unclear why the ACD/I-Lab software predicted such a high $pK_a$ value for MIQ **31**. It should be noted that the spectrophotometric determinations were only conducted in duplicate and were envisioned to only act as a guide that would quickly indicate if the ACD/I-Lab $pK_a$ value for MIQ **31** was reasonable.

The above $pK_a$ values were adjusted to afford $K_a$ values, which were applied in the calculation of the vacuole accumulation ratios for the derivatives (Table 4.3).

**Table 4.3:** $pK_a$ and Vacuole Accumulation of Selected Isocryptolepine Derivatives

| Compound | $pK_a$ [a] | Vacuole accumulation (%) |
|---|---|---|
| Cryptolepine **14** | 11.16 | 79 |
| Neocryptolepine **19** | 7.58 | 48 |
| Isocryptolepine **16** | 8.90 | 77 |
| MIQ **31** | 14.05 | 76 [b] |
| 3-Chloroisocryptolepine **64** | 6.02 | 4 |
| 9-Methylisocryptolepine **97** | 8.92 | 77 |
| 8-Bromoisocryptolepine **104** | 5.83 | 3 |
| 8-Bromo-3-chloroisocryptolepine **105** | 5.33 | 2 |
| 2,8-Dibromoisocryptolepine **106** | 4.58 | 1 |
| 8-Bromo-2-chloroisocryptolepine **107** | 4.65 | 1 |
| 8-Bromo-9-methylisocryptolepine **108** | 6.26 | 6 |
| 8-Chloroisocryptolepine **109** | 5.78 | 3 |

[a] Values obtained *via* the ACD/I-Lab web service[137]; $pK_a$ ± 0.20. [b] Value obtained using experimental $pK_a$ of 8.71.

With the exception of 9-methylisocryptolepine **97** and MIQ **31**, all isocryptolepine derivatives displayed low vacuole accumulation ratios ranging from 1 - 6%. Given that the derivatives also displayed higher antimalarial activity compared to isocryptolepine **16**, which has a high vacuole accumulation ratio (77%), there may be an alternative mechanism in operation with respect to their antimalarial activity. It should be noted that an alternative mode of action to chloroquine **2** would be highly advantageous for novel antimalarial drugs, which may display delayed emergence of drug resistance. This result suggests further, more formal mechanistic studies are warranted with these compounds in order to confirm this prediction.

## 4.4. Cytotoxicity Evaluation

The cytotoxicity of the selected isocryptolepine derivatives was determined by assessing their ability to inhibit the growth of mouse embryonic fibroblasts (3T3 cells). Percentage growth inhibition was determined using the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) colorimetric assay.[164,165] This assay involves measuring cell population after a period of incubation with compound solutions by adding MTT, which is reduced to blue formazan in the presence of living cells. Blue formazan is dissolved by the addition of DMSO and its absorbance measured spectrophotometrically. The difference in absorption of drug treated cells compared to drug-free controls provides a measure of the percentage growth inhibition, which can be used to calculate compound $IC_{50}$ values.

Stock solutions of the isocryptolepine derivatives were prepared as for the antimalarial evaluation in either 50% or 80% sterile DMSO in water (5 - 10 mM). The optimum concentration range of each compound for testing was pre-determined *via* a pilot study, with the concentrations applied for the antimalarial testing utilised as a starting point. A concentration range of 100 - 0.001 μM was found to be adequate for all derivatives. The pilot study also revealed the fragile nature of the 3T3 cells (which can readily deabsorb from the plate surface and perish). However this particular issue was negated by culturing the cells on plates which were pre-coated with gelatine.

Each compound was tested in quadruplicate with a drug-free control, isocryptolepine **16** was used as the positive control and statistically significant data was assured by conducting the assay a minimum of three separate times. The highest DMSO concentration (1.6 %) was found to affect cell growth and a vehicle control arm was including in the experiments. Due to time constraints not all the derivatives previously assessed for antimalarial activity could be assessed for cytotoxicity. Cytotoxicity data on the dihalogenated isocryptolepines were deemed most important, as dihalogenated isocryptolepines displayed the best antimalarial activity, so these were examined first. Secondly, the monosubstituted derivatives were assessed but the assessment of MIQ **31**, deemed low priority because it was a known compound, was not undertaken. Chloroquine **2**, although also a known compound, was assessed because the inclusion of an established antimalarial drug for direct

comparison was regarded as essential. The results of the cytotoxicity evaluation are summarised in Table 4.4.

**Table 4.4:** Cytotoxicity and Selectivity Indices of Selected Isocryptolepine Derivatives

| Compound | Cytotoxicity; $IC_{50}$ (μM) [a] | Selectivity Index (SI) |
|---|---|---|
| Isocryptolepine **16** | 2.19 ± 0.35 | 1.9 |
| 3-Chloroisocryptolepine **64** | 2.26 ± 0.46 | 7.2 |
| 9-Methylisocryptolepine **97** | 2.07 ± 0.33 | 2.7 |
| 8-Bromoisocryptolepine **104** | 1.97 ± 0.36 | 11 |
| 8-Bromo-3-chloroisocryptolepine **105** | 2.64 ± 0.66 | 26 |
| 2,8-Dibromoisocryptolepine **106** | 2.59 ± 0.53 | 23 |
| 8-Bromo-2-chloroisocryptolepine **107** | 9.01 ± 3.75 | 106 |
| 8-Bromo-9-Methylisocryptolepine **108** | 2.50 ±0.35 | 19 |
| 8-Chloroisocryptolepine **109** | 2.10 ±0.30 | 9 |
| Chloroquine **2** [b] | 72.0 ±19.5 | 499 |

[a] $IC_{50}$ ± standard deviation. [b] Evaluated as a diphosphate.

The selectivity indices of compounds were calculated using the chloroquine resistant (W2mef) antiplasmodial activity data. As previously mentioned (Section 1.3) the selectivity index provides a guide for assessment of a compound potential as an antimalarial drug whereby a high SI value (artemisinin **7**; SI > 10,000)[60] indicates a compound is more therapeutically viable.

Although chloroquine **2** cytotoxicity has previously been evaluated against 3T3 cells, these studies did not apply the MTT assay and thus direct comparison is not possible. Riddell *et al.*[166] compared the cytotoxicity of chloroquine **2** using three different cytotoxicity assays (neutral red uptake, kenacid blue and highest tolerated dose method) and obtained three very different results ($IC_{50}$ = 43 - 139 μM). Nevertheless the cytotoxicity data obtained using the MTT assay for chloroquine **2** ($IC_{50}$ = 72.0 μM) was within the range reported. Similarly the cytotoxicity of isocryptolepine **16** had previously only been reported against L-6 cells and is not directly comparable. However, the selectivity index for isocryptolepine **16** derived from the present study (1.9) was consistent with previously reported (1.5)[59] data.

The majority of the isocryptolepine derivatives ($IC_{50}$ = 1.97 - 2.59 μM) had cytotoxicity similar to that of the parent alkaloid **16** ($IC_{50}$ = 2.19 μM) but due to their improved antimalarial activity displayed improved selectivity indices ranging from 2.7 to 26. 8-Bromo-2-chloroisocryptolepine **107** ($IC_{50}$ = 9.01 μM) was the only derivative which displayed a significant reduction in cytotoxicity, being approximately 4-fold less cytotoxic compared to isocryptolepine **16**. This derivative was also the most potent compound against *P. falciparum* (W2mef) and therefore had the highest SI of 106. 8-Bromo-2-chloroisocryptolepine **107** is therefore approximately 56-fold more biologically acceptable compared to the parent. Although this derivative is not as biologically acceptable as chloroquine (SI = 499), it still represents a distinct improvement. It has a higher SI compared to any of the cryptolepis compounds; cryptolepine **14** (SI = 9.3), isoneocryptolepine **30** (SI = 19), neocryptolepine **19** (SI = 1.2) and MIQ **31** (SI = 33).

Compared to the previously reported neocryptolepine derivatives, isocryptolepine derivatives are superior with respect to antimalarial activity. Whilst similar neocryptolepine derivatives also showed improved bioactivity (i.e. 3-chloroneocryptolepine displayed higher antimalarial activity and less cytotoxicity) compared to the parent neocryptolepine **19**, these derivatives are not sufficiently active for biological application as they only possess $IC_{50}$ values in the micro-molar range (1.7 - 1.4 μM).[58] In addition, compared to previously reported similar cryptolepine derivatives, isocryptolepine analogues appear to display a level of superiority. The most potent antimalarial cryptolepine derivative, 7-bromo-2-chlorocryptolepine **23** (Figure 1.13), possessed an $IC_{50}$ value of 30 nM (against K1 *P. falciparum*) but was more cytotoxic compared to its parent cryptolepine **14**.[75] 2,7-Dibromocryptolepine **25** was the most selective of the cryptolepine derivatives (SI = 123) in the same study but is more cytotoxic ($IC_{50}$ = 6.04 μM, MAC15a) compared to 8-bromo-2-chloroisocryptolepine **107**, the most selective compound identified in the present study. Therefore isocryptolepine derivatives represent superior therapeutic lead compounds in comparison to either cryptolepine or neocryptolepine derivatives and there is no justification for the previous lack of interest in this particular series of compounds.

## 4.5. Summary

Preliminary investigations have indicated that isocryptolepine derivatives are reasonably stable in solution and also possess log P values that are conducive to therapeutic application. Antimalarial evaluation of the derivatives has concluded that, in general, isocryptolepine derivatives are more potent than the parent alkaloid. This study has also identified that dihalogenated derivatives represent the most potent compounds. Preliminary cross-resistance and vacuole accumulation estimations suggest that many of these derivatives may act upon the *plasmodium* parasite in a different manner to chloroquine **2** but formal mode of action studies are required to confirm these initial findings. Cytotoxicity evaluation of the derivatives concluded that ring substituents do not appear to result in an increased cytotoxicity, as was the case for many cryptolepine analogues. Furthermore the derivative 8-bromo-2-chloroisocryptolepine **107** was identified as the only compound to display a significant reduction in cytotoxicity and thus represents a potential novel lead compound for antimalarial drug development.

# Chapter 5

## Conclusions and Future Directions

## 5.1. Conclusions

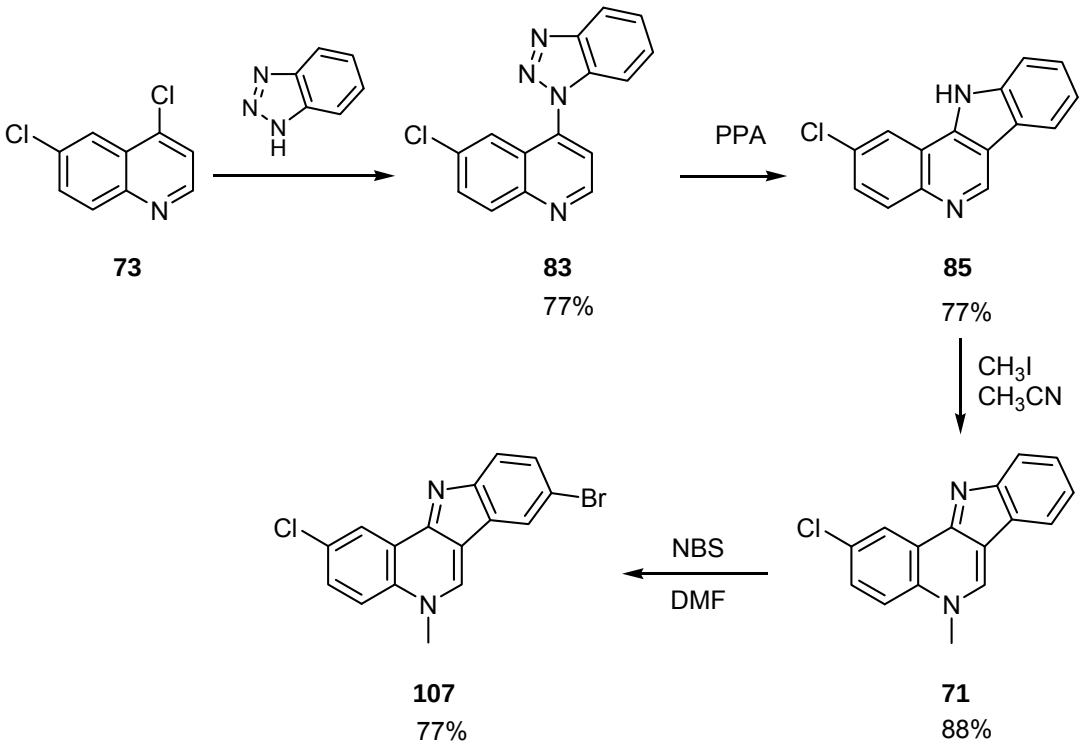
The potential of the indoloquinoline alkaloid isocryptolepine **16** as a lead compound in antimalarial drug design was investigated during the course of this project. A variety of synthetic routes for a range of mono and disubstituted isocryptolepine derivatives were developed and a series of novel isocryptolepine compounds were prepared and fully characterised. A selection of these derivatives was evaluated for both antimalarial activity and cytotoxicity. This investigation represents the first of its kind in relation to the improvement of bioactivity for ring substituted isocryptolepine derivatives and at its conclusion has identified a potential novel lead compound for future antimalarial therapy.

Literature methods for the synthesis of isocryptolepine **16** were discussed in Chapter 2, and the Jonckers and Molina Methods were found to be the most promising with respect to the synthesis of derivatives. The Jonckers Method was optimised and isocryptolepine **16** could be prepared by this methodology in two steps from 4-bromoquinoline **59** (Scheme 2.13) in high yield (77%). This optimised 'domino' Jonckers Method represents the highest yielding method for the synthesis of isocryptolepine **16** to date. The Molina Method was also reproduced and allowed the preparation of isocryptolepine **16** in three steps from 4-chloroquinoline **33** (Scheme 2.14) in high yield (61%).

The preparation of fourteen derivatives of isocryptolepine was outlined in Chapter 3. Substituted 4-chloroquinolines were applicable to the Molina Method and derivatives with halogen ring substituents at positions C2 and C3 were easily prepared *via* this methodology. The optimised 'domino' Jonckers Method was found to be more suitable to substituted anilines and was used to prepare 9-methylisocryptolepine **97** in addition to the isomeric derivative MIQ **31**. A further range of halogenated derivatives (**104** - **109**, **113** and **114**) were prepared *via* electrophilic aromatic substitution of the indoloquinoline ring system. It was found that isocryptolepine **16** and the monosubstituted derivatives prepared *via* the previous two methods could be selectively brominated, iodinated and chlorinated with exclusive substitution occurring at position C8.

Biological evaluation of a selection of isocryptolepine derivatives for antimalarial activity and cytotoxicity was described in Chapter 4. All derivatives

were more potent against *P. falciparum* compared to the parent alkaloid and dihalogenated compounds were clearly the most promising therapeutic candidates. In particular 8-bromo-2-chloroisocryptolepine **107** (Scheme 5.1) was the most potent analogue against the chloroquine resistant strain of *P. falciparum* (W2mef), being approximately 14-fold more potent compared to the parent alkaloid. Cytotoxicity testing ascertained that most derivatives possessed a similar cytotoxicity compared to the parent alkaloid. 8-Bromo-2-chloroisocryptolepine **107** was the exception, being the only derivative to display a reduced cytotoxicity; an approximate 4-fold reduction. This particular compound has an SI of greater than 100, representing a significant improvement in bioactivity compared to the parent alkaloid, and has been identified as a promising novel lead compound. Scheme 5.1 summarises the synthetic route to 8-bromo-2-chloroisocryptolepine **107**, previously outlined in Chapter 3, which allows the target derivative to be obtained in an overall yield of 40% from 4,6-dichloroquinoline **73**.




**73** **83** 77% **85** 77%

**107** 77% **71** 88%

**Scheme 5.1:** The synthesis of the novel lead compound 8-bromo-2-chloroiso-cryptolepine **107** from 4,6-dichloroquinoline **73**

In conclusion, isocryptolepine **16** represents a lead compound in the design and development of antimalarial drugs. As a demonstration of this fact a superior potential novel lead compound has been identified from an investigation of its

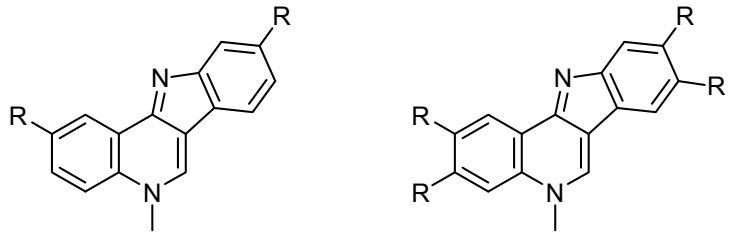
derivatives. This research represents the first study of the synthesis and biological evaluation of isocryptolepine derivatives which has demonstrated that there is the potential to significantly increase the antimalarial activity and decrease the cytotoxicity of these compounds with the addition of ring substituents. Unlike similar cryptolepine derivatives, these isocryptolepine derivatives do not display increased cytotoxicity. Similarly unlike neocryptolepine derivatives, isocryptolepine derivatives possess antimalarial activity in the therapeutically applicable nano-molar range.

## 5.2. Future Directions

Future investigations of isocryptolepine derivatives could follow two different pathways. The first would involve additional *in vitro* and *in vivo* studies conducted on the promising novel lead compound **107**. A second investigative pathway may involve preparing a further range of derivatives based on this compound.

In relation to the first suggestion, formal mechanistic studies are certainly warranted. It has been suggested that cryptolepis based compounds act *via* at least two different mechanisms, one being similar to chloroquine **2**. Initial investigations in the present study have also indicated that there is most likely an additional mode of action to the chloroquine-analogous mechanism, which may either involve DNA intercalation or a mechanism unknown as yet. Furthermore *in vivo* testing on *P. berghei* infected mice is a possible route of investigation and formal solubility, $pK_a$ and bioavailability assessments could also be conducted.

In relation to the proposed second investigative pathway a further range of derivatives could be synthesised and assessed. A possible series of compounds could focus on substituents at positions C2 and C8 and may involve the preparation of iodo, bromo, chloro, fluoro or trifluoromethyl derivatives (Figure 5.1; Series I). A second series could focus on dihalogenated compounds with bromo or chloro substituents at positions C2, C3, C8 and C9 (Figure 5.1; Series II).




**Series I** **Series II**

R = Cl, Br, I, F, $CF_3$ etc. R = Cl, Br

**Figure 5.1:** Suggestions for a second generation of isocryptolepine derivatives

The synthetic methodologies developed in this thesis should provide suitable routes to many of the proposed second generation derivatives. Further studies could also improve the methodologies investigated. In relation to the optimised 'domino' Jonckers Method, an investigation of the effect of electron-withdrawing and donating

groups on the reaction rate and yield, in addition to the positioning of the substituent, is warranted. Furthermore a method for the preparation of disubstituted derivatives, *via* electrophilic aromatic substitution, may be developed based on the initial investigations presented here to represent a superior method compared to those previously applied to prepare these particular compounds.

Despite cytotoxicity issues, the cryptolepis alkaloids are still an attractive group of compounds as demonstrated by the high number of publications in the last year relating to both the synthesis and biological activities of compounds based on these indoloquinoline alkaloids.[79,132,167-170] Notable is the recent cryptolepine derivative study, reported early in 2011 by Lavrado *et al.*[66] This thorough investigation of cryptolepine derivatives substituted with basic side chains discovered that some such derivatives (i.e. piperidine) displayed selectively indices of >1000. Similar derivatives of isocryptolepine represent an additional avenue of investigation and the derivatives presented in the thesis may also provide useful starting points for the synthesis of such compounds.

# Chapter 6

## Experimental

## 6.1. General

### Solvents and Reagents

Solvents were purchased as analytical grade from Biolab (Australia) and dried as required using literature procedures.[171] Anhydrous THF and dioxane were prepared by pre-drying over sodium before distillation under nitrogen from sodium/benzophenone. DMF and dichloromethane were pre-dried over calcium sulfate and subsequently distilled, the former under reduced pressure. Ether refers to diethyl ether and hexane to 95% *n*-hexane. Deuterated chloroform ($CDCl_3$) was purchased from Cambridge isotope laboratories and deuterated DMSO ($d_6$-DMSO) was purchased from Sigma-Aldrich. All reagents were purchased from Sigma-Aldrich and used as received.

### Reactions and Chromatography

All reactions were carried out in standard oven-dried glassware. Lithiation reactions were conducted using standard Schlenk glassware. Hydrogenation reactions were carried out using a simple hydrogenation apparatus based on literature descriptions.[172] Reaction temperatures refer to bath temperatures; oil bath (> 50 °C), acetone and dry ice (< -50 ºC), ice and salt (-10 °C) or iced water (0 °C). Thin layer chromatography was performed with Merck Silica Gel 60 or Merck $F_{254}$ Neutral Alumina aluminium supported sheets. Flash column chromatography was performed with Fluka silica gel 60 (0.035 – 0.07 mm) or Macherey-Nagel neutral alumina 90 (0.05 - 0.2 mm). Final products were oven dried (< 40°) under high vacuum overnight.

Analytical HPLC was performed using an Apollo C18 5 μm (4.6 mm × 150 mm) reverse phase column fitted with a Waters 486 tuneable spectrophotometric detector. A gradient solvent system was used whereby 20% acetonitrile in water with 0.5% formic acid was increased to 80% acetonitrile in water with 0.5% formic acid over a ten minute period. A flow rate of 1.5 mL $minute^{-1}$ was applied and compound spectrophotometric detection was performed at a wavelength of 300 nm.´

### Compound Analysis and Characterisation

Melting points (Mp) were recorded with a Barnstead Electrothermal digital melting-point apparatus and (d) refers to decomposition of the compound at its melting point.

Ultraviolet-visible spectra (UV) were recorded using a Hewlett-Packard 8452A diode array spectrophotometer and absorption peaks are expressed as wavelength ($\lambda_{max}$) values in nm. Infrared spectra (IR) were recorded with a Perkin Elmer FT-IR spectrometer and absorption peaks are expressed as frequency ($\nu_{max}$) values in $cm^{-1}$.

Mass spectra (MS) were obtained by Dr Tony Reeder (School of Biomedical, Biomolecular and Chemical Sciences; UWA) *via* either electrospray ionization (EI) or fast atom bombardment (FAB) using a VG Autospec Mass Spectrometer. Mass spectral data is expressed as m/z (relative intensity) and only spectral peaks with intensity greater than 15% are reported. High resolution mass spectrometry (HRMS) was used to determine the accurate mass of the molecular ion *in-lieu* of elemental analysis.

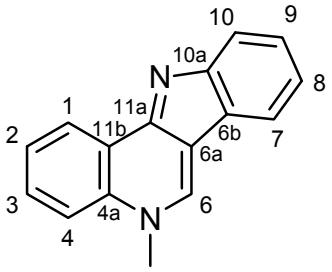
Nuclear magnetic resonance (NMR) spectra were recorded using Varian Gemini (200 MHz, $^{1}H$; 50 MHz, $^{13}C$), Bruker AV400 (400 MHz, $^{1}H$; 100 MHz, $^{13}C$) or Bruker AV600 (600 MHz, $^{1}H$) spectrometers. Spectra recorded on the Bruker AV600 were obtained by Dr Lindsay Byrne (School of Biomedical, Biomolecular and Chemical Sciences; UWA). Chemical shifts ($\delta$) are expressed in ppm relative to either $d_6$-DMSO ($^{1}H$, 2.49 ppm; $^{13}C$, 39.5 ppm) or $CDCl_3$ ($^{1}H$, 7.26 ppm; $^{13}C$, 77.0 ppm). Coupling constants (*J*) are expressed in Hertz (Hz). Assignment of $^{1}H$ and $^{13}C$ spectra were routinely made with the aid of COSY, HSCQ and HMBC 2D experiments performed using the Bruker AV400 spectrometer. Confirmation of ring substitution positions in the preparation of compounds **105 - 109**, **113** and **114** were undertaken with the aid of 1D NOE difference spectrometry conducted on the Bruker AV400 or AV600 spectrometers.

Known compounds were confirmed by NMR spectroscopy. Mass spectra and infrared spectra are only reported for these compounds if not previously reported in the literature. The carbon NMR spectra of known compounds was not normally assigned, expect in the cases of **16** and **30** as full assignment of these spectra was advantageous to the assignment of their novel derivatives. Novel compounds were fully characterised *via* NMR, mass and infrared spectroscopy. High resolution mass spectra were only recorded for the novel isocryptolepine derivatives, and their novel intermediates, which underwent biological evaluation.

Experimental procedures and characterisation data for prepared compounds are summarised in the following section and are arranged numerically in compound order.

## 6.2. Preparation of Compounds

**Isocryptolepine 16**




The above known compound was prepared *via* two different methods, which were both adaptations of previously reported synthetic procedures, and are summarised as follows.[84,88,94]

*Method 1:* A solution of 2-[2-(*N*-methyl)formylaminophenyl]-*N*-[2-(trimethylsilyl)ethoxymethyl]indole **46** (172 mg, 0.45 mmol) in ethanolic sulfuric acid (10%, 5 mL) was refluxed for 4 hours. The reaction mixture was cooled and the solvent subsequently removed *in vacuo*. The residue was redissolved in dichloromethane (10 mL) and extracted with aqueous hydrochloric solution (1M, 3 × 10 mL). The aqueous layer was basified with aqueous sodium hydroxide solution (10%), re-extracted with ethyl acetate (3 × 10 mL) and the solvent removed *in vacuo*. The residue obtained was recrystallised from ethanol and water to give isocryptolepine **16** as a yellow crystalline solid (24 mg, 23%).

*Method 2:* To a solution of 11*H*-indolo[3,2-*c*]quinoline **36** (128 mg, 0.58 mmol) in acetonitrile (11 mL), iodomethane (3.5 mL, 56.22 mmol) was added and the resulting mixture refluxed for 20 hours. The reaction mixture was cooled, the solvent removed *in vacuo* and the residue obtained dissolved in a 1:1 solution of aqueous ammonia (30%) and dichloromethane (140 mL). The organic layer was extracted with dichloromethane (4 × 35 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue obtained was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:4:1) to give isocryptolepine **16** as a yellow crystalline solid (128 mg, 94%).

The spectroscopic data acquired was consistent with that published in the literature.[48,84,88,91]

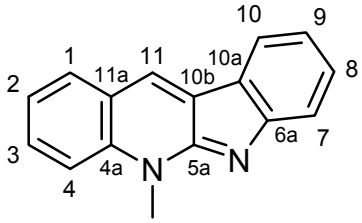
Mp: 138-139 ºC (lit.,[91] 132-133 °C).

$^{1}$H NMR (400 MHz, $d_6$-DMSO) δ: 4.25 (3H, s, $NCH_3$), 7.26 (1H, ddd, *J* = 7.8, 7.0, 0.8 Hz, H-8), 7.44 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, H-9), 7.71 (1H, ddd, *J* = 7.8, 7.0, 0.8 Hz, H-2), 7.79 (1H, d, *J* = 8.0 Hz, H-10), 7.84 (1H, ddd, *J* = 8.7, 7.1, 1.5 Hz, H-3), 8.04 (1H, d, *J* = 8.4 Hz, H-4), 8.12 (1H, d, *J* = 7.6 Hz, H-7), 8.78 (1H, dd, *J* = 8.0, 1.2 Hz, H-1), 9.36 (1H, s, H-6).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.0 ($NCH_3$), 115.9 (C-6a), 117.4 (C-4), 117.9 (C-10), 119.4 (C-7), 119.8 (C-8), 120.6 (C-11b), 123.7 (C-1), 125.1 (C-2), 125.2 (C-9), 125.4 (C-6b), 129.2 (C-3), 135.3 (C-4a), 138.2 (C-6), 151.9 (C-11a), 153.5 (C-10a).

UV (MeOH) $\lambda_{max}$: 202, 232, 284, 347.

**Neocryptolepine 19**




The above known compound was prepared as for isocryptolepine **16** (Method 2) but starting from 6*H*-indolo[2,3-*b*]quinoline **124** (260 mg, 1.19 mmol) and iodomethane (1.5 mL, 24.10 mmol) in acetonitrile (24 mL). The methiodide salt was purified by silica flash column chromatography eluting with a mixture of dichloromethane and methanol (1:0 increasing to 48:2) and then converted to the free base with a 1:1 solution of aqueous ammonia (30%) and dichloromethane (150 mL). The organic layer was extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give neocryptolepine **19** as an orange crystalline solid (103 mg, 37%).

The NMR data acquired was consistent with that published in the literature.[48,58]

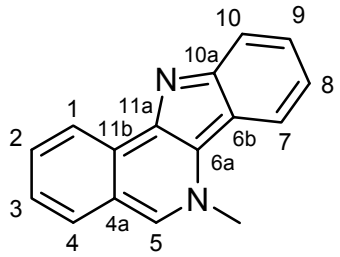
Mp: 106-108 ºC (lit.,[58] 102 ºC).

$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 4.34 (3H, s, $NCH_3$), 7.22 (1H, td, *J* = 7.3, 1.0 Hz, H-9), 7.48-7.66 (3H, m, H-2, H-7 and H-8), 7.89 (1H, ddd, *J* = 8.4, 7.0, 1.4 Hz, H-3), 8.01 (1H, d, *J* = 8.4 Hz, H-4), 8.16-8.20 (2H, m, H-1 and H-10), 8.97 (1H, s, H-11).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 32.5, 114.7, 117.0, 119.0, 120.0, 121.2, 121.6, 123.7, 126.7, 128.5, 128.8, 129.7, 130.5, 136.4, 155.1, 155.2.

UV (MeOH) $\lambda_{max}$: 207, 273, 282, 3325, 351.

**6-Methyl-6*H*-indolo[3,2-*c*]isoquinoline 31**




The above compound was prepared as for isocryptolepine **16** (Method 2) but starting from 11*H*-indolo[3,2-*c*]isoquinoline **117** (241 mg, 1.11 mmol) and iodomethane (6.0 mL, 96.38 mmol) in toluene (15 mL). The methiodide salt was purified by silica flash column chromatography eluting with a mixture of methanol and dichloromethane (10:90 increasing to 15:85) and then converted to the free base with a 1:1 solution of aqueous ammonia (30%) and dichloromethane (400 mL). The organic layer was extracted with dichloromethane (6 × 100 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give 6-methyl-6*H*-indolo[3,2-*c*]quinoline **31** as a red crystalline solid (230 mg, 89%).

This above compound was reported during the course of this project and the NMR and MS data acquired was consistent with that published in the literature.[82]

Mp: 214-215 ºC (lit.,[82] 208-210 °C (d)).

$^1$H NMR (400 MHz, $d_6$-DMSO) δ: 4.85 (3H, s, $CH_3$), 7.19 (1H, t, *J* = 7.4 Hz, H-8), 7.44 (1H, t, *J* = 7.6 Hz, H-9), 7.76 (1H, t, *J* = 7.6 Hz, H-3), 7.83 (1H, d, *J* = 8.0 Hz, H-10), 8.00 (1H, t, *J* = 7.6 Hz, H-2), 8.29 (1H, d, *J* = 7.6 Hz, H-7), 8.30 (1H, d, *J* = 7.6 Hz, H-4), 8.81 (1H, d, *J* = 8.4 Hz, H-1), 9.10 (1H, s, H-5).

$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 45.9 ($NCH_3$), 117.2 (C-6b), 117.6 (C-8), 118.1 (C-10), 120.7 (C-7), 122.3 (C-1), 123.7 (C-4a), 124.4 (C-6a), 124.7 (C-9), 126.7 (C-3), 128.6 (C-11b), 128.9 (C-4), 132.0 (C-2), 132.8 (C-5), 141.5 (C-11a), 150.4 (C-10a).

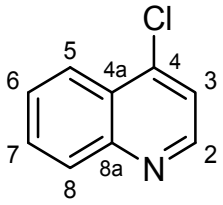
MS (FAB): 154 (18), 233.1 (20), 233.1 (100, $[M+1]^+$), 234.1 (20).

HRMS (FAB): 233.1069 ($C_{16}H_{13}N_2[M+1]^+$ requires 233.1079).

IR (KBr) $\nu_{max}$: 741, 1236, 1371, 1417, 1627, 3061, 3500.

UV (MeOH) $\lambda_{max}$: 227, 291, 391.

**4-Chloroquinoline 33**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[120] Phosphorus oxychloride (2.50 mL, 27.31 mmol) was added to 4-quinolinol **61** (622 mg, 4.29 mmol) and the solution obtained refluxed for 19 hours. The reaction mixture was cooled, quenched with iced water, made alkaline with aqueous ammonia and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with water (50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give 4-chloroquinoline **33** as a pale yellow solid that was used without further purification (555 mg, 79%).

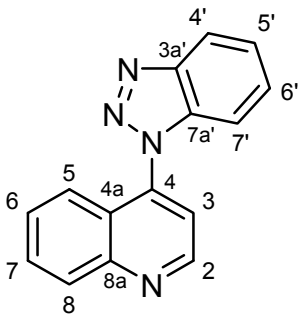
The spectroscopic data acquired was consistent with that published in the literature.[120,151]

Mp: 30-32 °C (lit.,[120] 28-29 °C).

$^1$H NMR (200 MHz, $CDCl_3$) δ: 7.51 (1H, d, *J* = 4.8 Hz, H-3), 7.70 (1H, ddd, *J* = 8.3, 6.8, 1.2 Hz, H-6), 7.81 (1H, ddd, *J* = 8.2, 6.8, 1.6 Hz, H-7), 8.17 (1H, d, *J* = 8.0 Hz, H-5), 8.26 (1H, dd, *J* = 8.0, 1.4 Hz, H-8), 8.82 (1H, br d, *J* = 3.6 Hz, H-2).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 120.5, 123.4, 127.1, 128.5, 130.0, 142.6, 147.5, 148.4.

**4-(1-Benzotriazolyl)quinoline 35**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[84] Benzotriazole (1.30 g, 10.88 mmol) and 4-chloroquinoline **33** (1.62 g, 9.89 mmol) were heated at 110 - 120 ºC for 30 minutes. The resulting solid was cooled to room temperature, quenched with

water and collected by filtration (washing with water). The solid was recrystallised from ethanol to give 4-(1-benzotriazolyl)quinoline **35** as a white crystalline solid (1.87 g, 77%).

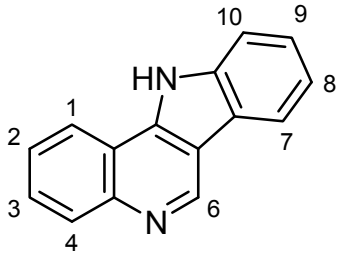
The proton NMR data acquired was consistent with that published in the literature.[84]

Mp: 132-133 ºC (lit.,[83] 132-133 ºC).

$^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.53-7.65 (3H, m, H-4′, H-5′ and H-6′), 7.76 (1H, t, *J* = 7.4 Hz, H-6), 7.88 (1H, br s, H-3), 7.99 (1H, ddd, *J* = 8.0, 7.2, 0.8 Hz, H-7), 8.13 (1H, d, *J* = 8.4 Hz, H-5), 8.26 (1H, d, *J* = 8.4 Hz, H-7′), 8.61 (1H, *J* = 8.8 Hz, H-8), 9.28 (1H, br s, H-2).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 110.6 (C-4′), 117.7 (C-3), 119.7 (C-7′), 122.4 (C-4a), 122.8 (C-5), 124.9 (C-6′), 128.2 (C-5′), 129.0 (C-6), 129.5 (C-7), 130.6 (C-8), 133.4 (C-3a′), 139.4 (C-4), 145.3 (C-7a′), 149.3 (C-8a), 149.5 (C-2).

**11*H*-Indolo[3,2-*c*]quinoline 36**




The above known compound was prepared *via* three different methods, which were adaptations of previously reported synthetic procedures, and are summarised as follows.[84,94,128]

*Method 1:* To a degassed solution of solution of $Pd(OAc)_2$ (2.4 mg, 2 mol%) and BINAP (6.6 mg, 2 mol%) in dry DMF (5 mL), 4-(2-bromophenylamino)quinoline **60** (155 mg, 0.52 mmol) and potassium carbonate (1.46 g, 10.57 mmol) were added. The suspension was flushed with nitrogen and heated at 150 ºC for 24 hours under nitrogen. Upon cooling, the reaction mixture was filtered through celite, washed with dichloromethane (50 mL) and the solvent removed *in vacuo.* The residue obtained was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and methanol (100:0 increasing to 85:15) to give 11*H*-indolo[3,2-*c*]quinoline **36** as a cream solid (80.6 mg, 71%)**.**

*Method 2:* To a degassed solution of $Pd(OAc)_2$ (30 mg, 2.8 mol%) and BINAP (70 mg, 2.3 mol%) in dry DMF (50 mL), 4-bromoquinoline **59** (994 mg, 4.78 mmol),

2-bromoaniline (932 mg, 5.42 mmol) and potassium carbonate (13.34 g, 96.52 mmol) were added. The suspension was flushed with nitrogen and refluxed for 24 hours under nitrogen. Upon cooling, the mixture was filtered through celite, washed with dichloromethane (200 mL) and the solvent removed *in vacuo*. The residue obtained was washed with dichloromethane to give 11*H*-indolo[3,2-*c*]quinoline **36** as a cream solid (853 mg, 82%).

*Method 3:* To 4-(1-benzotriazolyl)quinoline **35** (204 mg, 0.83 mmol), polyphosphoric acid (7.17 g) was added and the mixture heated at 150 ºC for 1 hour. The pink syrupy mixture obtained was cooled, quenched with water and the resulting precipitate collected by vacuum filtration. The solid was re-suspended in water, made alkaline with aqueous sodium hydroxide solution (10%) and collected as previously. The residue obtained was washed with dichloromethane to give 11*H*-indolo[3,2-*c*]quinoline **36** as a cream solid (152 mg, 84%).

The NMR data acquired was consistent with that published in the literature.[86,92]

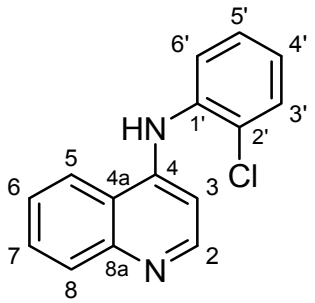
Mp: >300 ºC (lit.,[92] >250 ºC).

$^{1}$H NMR (400 MHz, $d_6$-DMSO) δ: 7.34 (1H, ddd, *J* = 8.0, 7.2, 0.8 Hz, H-8), 7.50 (1H, ddd, *J* = 7.9, 6.7, 1.2 Hz, H-9), 7.69 (1H, ddd, *J* = 8.0, 6.8, 1.4 Hz, H-2), 7.73 (1H, d, *J* = 8.4 Hz, H-10), 7.74 (1H, ddd, *J* = 8.2, 7.0, 1.4 Hz, H-3), 8.15 (1H, dd, *J* = 8.8, 1.2 Hz, H-4), 8.32 (1H, d, *J* = 7.6 Hz, H-7), 8.54 (1H, dd, *J* = 8.0, 1.2 Hz, H-1), 9.60 (1H, s, H-6).

$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 111.9 (C-10), 114.3 (C-6a), 117.1 (C-11b), 120.1 (C-7), 120.6 (C-8), 121.9 (C-6b), 122.1 (C-1), 125.5 (C-9), 125.7 (C-2), 128.0 (C-3), 129.6 (C-4), 138.8 (C-10a), 139.8 (C-11a), 144.8 (C-6), 145.5 (C-4a).

UV (MeOH) $\lambda_{max}$: 237, 274, 291.

**4-(2-Chlorophenylamino)quinoline 37**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[94] To a degassed solution of

$Pd(OAc)_2$ (30 mg, 2.2 mol%) and BINAP (80 mg, 2.1 mol%) in dry dioxane (20 mL), 4-chloroquinoline **33** (1.0 g, 6.11 mmol), 2-chloroaniline (860 mg, 6.74 mmol) and potassium carbonate (16.95 g, 122.6 mmol) were added. The suspension was flushed with nitrogen and the reaction mixture refluxed for 24 hours under nitrogen. After cooling, the mixture was filtered through celite, washed with dichloromethane (200 mL) and the solvent removed *in vacuo.* The residue obtained was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and hexane (1:1 increasing to 1:0) to give 4-(2-chlorophenylamino)quinoline **37** as a white solid (971 mg, 62%).

The NMR data acquired was consistent with that published in the literature.[85,94]

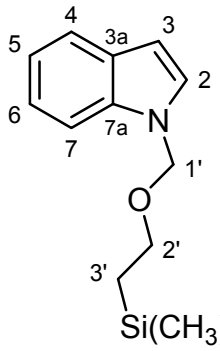
Mp: 144-145 ºC (lit.,[85] 142 °C).

$^1$H NMR (200 MHz, $CDCl_3$) δ: 7.04 (1H, br s, H-3), 7.10-7.17 (2H, m, H-4′ and H-6′), 7.34 (1H, t, *J* = 7.3 Hz, H-5′), 7.54 (1H, d, *J* = 8.4 Hz, H-3′) 7.60 (1H, t, *J* = 7.0 Hz, H-6), 7.77 (1H, t, *J* = 7.0 Hz, H-7), 8.07 (1H, d, *J* = 8.4 Hz, H-5), 8.14 (1H, d, *J* = 8.4 Hz, H-8), 8.70 (1H, d, *J* = 5.2 Hz, H-2).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 103.0, 119.0, 119.7, 120.8, 123.5, 125.0, 126.8, 128.7, 129.4, 136.4, 145.2, 148.5, 150.0.

UV (MeOH) $\lambda_{max}$: 202, 323.

**_N_-[2-(Trimethylsilyl)ethoxymethyl]indole 41**




The above known compound was prepared *via* an adaptation of previously reported synthetic procedures which is summarised as follows.[105,106] Indole **48** (501 mg, 4.27 mmol) was added to a stirred solution of sodium hydride (268 mg; 60% dispersion in mineral oil, 6.75 mmol) in dry DMF (25 mL) and the suspension stirred at room temperature for 30 minutes. A further portion of sodium hydride (2.14 mmol) was added and the reaction mixture stirred for a further hour. SEM chloride (830 μL, 4.69 mmol) was subsequently added and the reaction mixture stirred for a further 3 hours.

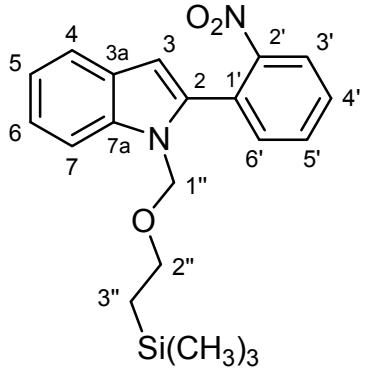
The reaction was quenched with water and the suspension obtained extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate solution (10 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue obtained was purified by flash column chromatography, on neutral alumina, eluting with a mixture of hexane and ether (100:0 increasing to 99:1) to give *N*-[2-(trimethylsilyl)ethoxy-methyl]indole **41** as a yellow oil (885 mg, 84%).

The spectroscopic data acquired was consistent with that published in the literature.[105]

$^1$H NMR (200 MHz, $CDCl_3$) δ: 0.02 (9H, s, 3 × $CH_3$), 0.96 (2H, t, *J* = 8.2 Hz, H-3′), 3.55 (2H, t, *J* = 8.2 Hz, H-2′), 5.56 (2H, s, H-1′), 6.61 (1H, d, *J* = 3.4 Hz, H-3), 7.22-7.27 (2H, m, H-2 and H-6), 7.33 (1H, d, *J* = 7.6 Hz, H-5), 7.58 (1H, d, *J* = 8.2 Hz, H-7), 7.72 (1H, dd, *J* = 6.8, 1.1 Hz, H-4).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: -1.30, 17.8, 60.3, 75.7, 102.5, 109.8, 120.2, 121.0, 122.2, 128.1, 129.2, 136.5.

**2-(2-Nitrophenyl)-*N*-[2-(trimethylsilyl)ethoxymethyl]indole 43**




The above known compound was prepared *via* an adaptation of previously reported synthetic procedures which is summarised as follows.[88,173]

i) To a solution of *N*-[2-(trimethylsilyl)ethoxymethyl]indole **41** (2.63 g, 10.6 mmol) in dry THF (20 mL) under nitrogen at 0 ºC, *n*-butyllithium hexane solution (8.0 mL; 1.6 M, 12.8 mmol) was added and the solution stirred for 10 minutes. A further portion of *n*-butyllithium hexane solution (3.3 mL; 1.6 M, 5.28 mmol) was added and the reaction mixture stirred at room temperature for 1 hour. The solution was cooled to -78°C, tributyltin chloride (3.7 mL, 13.64 mmol) added and allowed to warm to room temperature over 30 minutes. The reaction was quenched with water and extracted with ether (3 × 100 mL). The combined extracts were washed with

water (50 mL), brine (50mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The oil obtained, 2-(tributylstannyl)-*N*-[(2-trimethylsilyl)ethoxymethyl]indole **42**, was used without further purification.

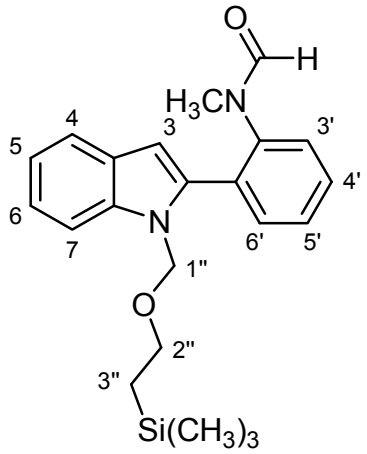
ii) To a degassed solution of iodonitrobenzene (2.60 g, 10.44 mmol) and $Pd(PPh_3)_4$ (0.25 g, 2 mol%) in dry THF (20 mL) under nitrogen, the above stannane **42** was added and the solution refluxed for 72 hours. The reaction mixture was quenched with water and extracted with ether (3 × 100 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue obtained was purified by flash column chromatography, on neutral alumina, eluting with a mixture of heptane and ether (100:0 increasing to 80:20) to give 2-(2-nitro-phenyl)-*N*-[2-(trimethylsilyl)ethoxymethyl]indole **43** as a orange oil (2.45 g, 63%).

The proton NMR data acquired was consistent with that published in the literature.[173]

$^1$H NMR (200 MHz, $CDCl_3$) δ: -0.01 (9H, s, 3 × $CH_3$), 0.90 (2H, t, *J* = 8.4 Hz, H-3″), 3.46 (2H, t, *J* = 8.3 Hz, H-2″), 5.37 (2H, s, H-1″), 6.59 (1H, s, H-3), 7.20-7.39 (2H, m, H-5 and H-6), 7.59 (1H, d, *J* = 8.0 Hz, H-7), 7.62-7.75 (4H, m, H-4, H-4′, H-5′ and H-6′), 8.10 (1H, d, *J* = 8.0 Hz, H-3′).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: -2.4, 17.0, 65.0, 72.5, 103.5, 109.4, 119.8, 120.0, 122.0, 123.3, 126.4, 127.3, 128.8, 131.3, 132.9, 134.4, 137.0, 149.2.

**2-[2-(*N*-methyl)formylaminophenyl]-*N*-[2-(trimethylsilyl)ethoxymethyl]indole 46**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[88]

i) To a stirred solution of 2-(2-nitrophenyl)-1-[2-(trimethylsilyl)ethoxymethyl]-indole **43** (107 mg, 0.29 mmol) in absolute ethanol (20 mL), palladium on carbon (17 mg, 5.5 mol%) was added and the suspension stirred vigorously under an atmosphere of hydrogen for 20 hours. The reaction mixture was filtered through celite (washing with methanol) and the solvent removed *in vacuo* to give 2-(2-aminophenyl)-*N*-[2-(trimethylsilyl)ethoxymethyl]indole **44**, which was used without further purification.

ii) To a solution of compound **44** in dry THF (10 mL) at -10 °C, acetic formic anhydride solution (220 μL, 1.48; prepared as described in the literature[88]) was added. The solution was stirred for 15 minutes then allowed to warm to room temperature. The solvent was removed *in vacuo* to give 2-(2-formylaminophenyl)-*N*-[2-(trimethylsilyl)ethoxymethyl]indole **45** as an orange oil, which was used without further purification.

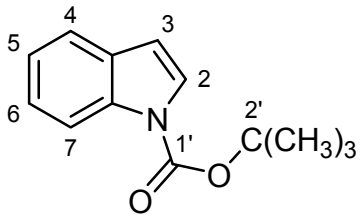
iii) To a solution of compound **45** in dry THF (10 mL), sodium hydride (19 mg; 60% dispersion in mineral oil, 0.47 mmol) was added and the mixture stirred at room temperature for 30 minutes. Iodomethane (100 μL, 1.6 mmol) and *t*-butanol (1 drop) were then added and the solution stirred for 2 hours. The reaction was quenched with aqueous ammonia and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with brine (10 mL), dried ($Na_2SO4$) and the solvent removed *in vacuo.* The residue obtained was purified by silica flash column chromatography eluting with a mixture of hexane and ethyl acetate (100:0 increasing to 80:30) to give 2-[2-(*N*-methyl)formylaminophenyl]-*N*-[2-(trimethylsilyl)ethoxymethyl]indole **46** as a pale yellow oil (49 mg, 48%).

The proton NMR data acquired was consistent with that published in the literature.[88]

$^1$H NMR (200 MHz, $CDCl_3$) δ: 0.13 (9H, s, 3 × $CH_3$), 0.88 (2H, t, $J$ = 8.3 Hz, H-3″), 2.97 (3H, s, $NCH_3$), 3.38 (2H, t, $J$ = 8.3 Hz, H-2″), 5.35 (2H, s, H-1″), 6.56 (1H, s, H-3), 7.23-7.35 (2H, m, H-5 and H-6), 7.53-7.65 (6H, m, H-4, H-7, H-3′, H-4′, H-5′ and H-6′), 8.34 (1H, s, HC=O).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: -2.4, 16.9, 31.9, 65.1, 72.0, 104.1, 109.6, 119.7, 120.0, 121.8, 126.2, 126.7, 127.5, 128.5, 129.0, 132.3, 135.6, 136.8, 140.6, 162.0.

***N*-(*tert*-Butoxycarbonyl)indole 47**



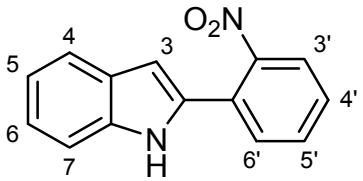

The above known compound was prepared *via* an adaptation of previously reported synthetic procedures which is summarised as follows.[96,174] To a stirred solution of indole **48** (2.00 g, 17.07 mmol) and DMAP (422 mg, 3.45 mmol) in dry dichloromethane (60 mL), di-*tert*-butyl dicarbonate (3.69 g, 16.88 mmol) was added and the reaction mixture stirred at room temperature for 24 hours. The resulting solution was concentrated *in vacuo* and the residue obtained purified by silica flash column chromatography eluting with a mixture of dichloromethane and hexane (0:100 increasing to 30:70) to afford *N*-*tert*-butoxycarbonyl indole **47** as a colourless oil (2.54 g, 70%).

The spectroscopic data acquired deviated slightly from that published in the literature, which was acquired in $CDCl_3$.[96,174]

$^1$H NMR (200 MHz, $d_6$-DMSO) δ: 1.67 (9H, s, 3 × $CH_3$), 6.72 (1H, d, *J* = 3.8 Hz, H-3), 7.29 (1H, td, *J* = 7.4, 1.4 Hz, H-5), 7.35 (1H, ddd, *J* = 8.0, 7.3, 1.4 Hz, H-6), 7.65 (1H, m, H-4), 7.69 (1H, d, *J* = 3.6 Hz, H-2), 8.12 (1H, dd, *J* = 7.4, 0.6 Hz, H-7).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 27.4, 83.5, 107.2, 114.5, 120.9, 122.4, 124.0, 125.8, 130.0, 134.4, 148.9.

**2-(2-Nitrophenyl)indole 51**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[95]

i) To a stirred solution of *N*-*tert*-butoxycarbonyl indole **47** (107 mg, 0.49 mmol) in dry THF (5 mL) at -78 ºC, under nitrogen, *n*-butyllithium hexane solution (600 µL; 1.6 M, 0.96 mmol) was added and the solution stirred for 2 hours. The reaction was allowed to warm to -20 ºC over 1 hour, subsequently re-cooled to -78 ºC and then

tributyltin chloride (150 μL, 0.55 mmol) added. The reaction mixture was stirred for a further hour before warming to room temperature and quenching with water. The solution was extracted with ethyl acetate (3 × 15 mL) and the combined extracts washed with brine (10 mL) and dried ($Na_2SO_4$). The solvent was removed *in vacuo* to give the stannyl product **49** which was used without further purification.

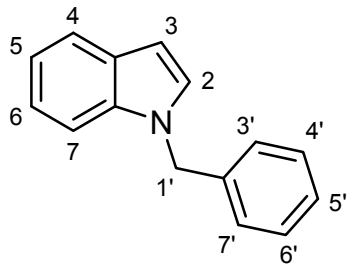
ii) To a degassed solution of iodonitrobenzene (110 mg, 0.44 mmol) and $Pd(PPh_3)_4$ (11 mg, 1.9 mol%) in dry DMF (5 mL) under nitrogen, the above stannane **49** was added and the solution heated at 100 ºC for 20 hours. The reaction was quenched with water and extracted with ethyl acetate (3 × 15 mL). The combined extracts were washed with brine (10 mL), dried ($Na_2SO_4$) and the solvent removed *in vacuo*. The residue obtained was purified by flash column chromatography, on neutral alumina, eluting with a mixture of hexane and ethyl acetate (10:0 increasing to 3:8) to give 2-(2-nitrophenyl)indole **51** as an orange solid (42 mg, 36%).

The spectroscopic data acquired deviated slightly from that published in the literature, which was acquired in $d_6$-acetone.[99]

$^1$H NMR (200 MHz, $CDCl_3$) δ: 6.78 (1H, s, H-3), 7.17-7.35 (2H, m, H-5 and H-6), 7.48 (1H, d, *J* = 7.8 Hz, H-7), 7.57 (1H, d, *J* = 7.8 Hz, H-4), 7.66-7.78 (3H, m, H-4′, H-5′ and H-6′), 7.88 (1H, d, *J* = 7.8 Hz, H-3′), 8.55 (1H, br s, N-H).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 103.6, 110.4, 119.6, 120.1, 122.4, 123.4, 126.1, 127.5, 127.8, 130.8, 131.5, 136.1, 148.1.

**_N_-Benzylindole 52**




The above known compound was prepared *via* an adaptation of the previously reported synthetic procedure applied to prepare 3-acetyl-*N*-benzylindole and the synthesis is summarised as follows.[102] To a solution of sodium hydride (660 mg; 60% dispersion in mineral oil pre-washed with hexane, 16.50 mmol) in dry DMF (50 mL) at 0 °C, indole **48** (1.01 g, 8.60 mmol) was added and the mixture stirred at room temperature for 30 minutes. To the resulting suspension benzyl chloride (1.5 mL, 13.04 mmol) was added in a dropwise fashion and the reaction mixture stirred

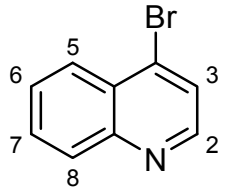
for 3 hours at room temperature. The reaction was quenched with saturated sodium hydrogen carbonate solution and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with water (50 mL), brine (50 mL), and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue purified by silica flash chromatography eluting with a mixture of hexane and dichloromethane (8:2 increasing to 0:1) to give *N*-benzylindole **52** as a yellow oil (1.69 g, 95%).

The spectroscopic data acquired was consistent with that published in the literature.[175,176]

$^1$H NMR (200 MHz, $CDCl_3$) δ: 5.42 (2H, s, H-1′), 6.70 (1H, d, *J* = 3.0 Hz, H-3), 7.20-7.32 (5H, m), 7.38-7.44 (4H, m), 7.81 (1H, dd, *J* = 7.4 Hz, H-7).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 49.3, 100.9, 108.9, 118.8, 120.2, 120.9, 126.0, 126.8, 127.5, 127.9, 135.6, 136.8.

**4-Bromoquinoline 59**




The above known compound was prepared *via* an adaptation of a previously reported synthetic procedure which is summarised as follows.[121] To a solution of 4-quinolinol **61** (1.96 g, 13.48 mmol) in DMF (50 mL), phosphorus tribromide (1.4 mL, 14.90 mmol) was added and the mixture stirred under nitrogen for 30 minutes. To the resulting suspension, iced water was added and the solution stirred for a further 30 minutes. The solution was then made alkaline with aqueous sodium hydroxide solution (20%) and extracted with ethyl acetate (3 × 100 mL). The combined extracts were dried ($Na_2SO_4$) and the solvent removed *in vacuo* to give 4-bromoquinoline **59** as an pale yellow oil which was used without further purification (2.24 g, 80%).

The proton NMR data acquired was consistent with that published in the literature.[121,177]

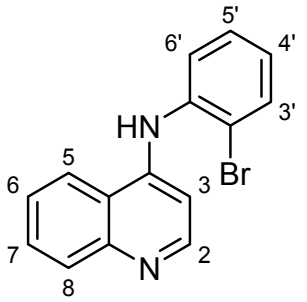
$^1$H NMR (400 MHz, $CDCl_3$): δ 7.62 (1H, t, *J* = 7.6 Hz, H-6), 7.70 (1H, d, *J* = 4.4 Hz, H-3), 7.76 (1H, t, *J* = 7.2 Hz, H-7), 8.13 (1H, d, *J* = 8.4 Hz, H-5) 8.18 (1H, d, *J* = 8.4 Hz, H-8), 8.67 (1H, br s, H-2).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 125.2, 127.0, 128.1, 129.7, 130.7, 134.8, 148.6, 149.6.

IR (nujol) $\nu_{max}$: 1377, 1461, 2854, 2924.

UV (MeOH) $\lambda_{max}$: 227, 290.

**4-(2-Bromophenylamino)quinoline 60**




The above compound was prepared as for compound **37** but starting from 4-bromoquinoline **59** (207 mg, 0.99 mmol), 2-bromoaniline (232 mg, 1.35 mmol), $Pd_2(dba)_3$ (9.8 mg, 1 mol%), XANTPHOS (13 mg, 2.3 mol%), and caesium carbonate (435 mg, 1.34 mmol) in dry dioxane (10 mL). The product was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and hexane (8:2 increasing to 1:0) to afford 4-(2-bromophenylamino)quinoline **60** as a off-white solid (215 mg, 72%).

Mp: 138-140 ºC.

$^1$H NMR (400 MHz, $CDCl_3$) δ: 6.93 (1H, d, *J* = 5.6 Hz, H-3), 7.03 (1H, ddd, *J* = 8.3, 7.1, 1.2 Hz, H-4′), 7.33 (1H, ddd, *J* = 8.0, 7.4, 1.2 Hz, H-5′), 7.53 (1H, dd, *J* = 8.0, 1.6 Hz, H-6′), 7.57 (1H, t, *J* = 8.0 Hz, H-6), 7.65 (1H, dd, *J* = 8.0, 1.2 Hz, H-3′), 7.72 (1H, td, *J* = 7.7, 1.2 Hz, H-7), 8.11 (1H, d, *J* = 8.4 Hz, H-8), 8.16 (1H, *J* = 8.4 Hz, H-5), 8.56 (1H, d, *J* = 5.2 Hz, H-2).

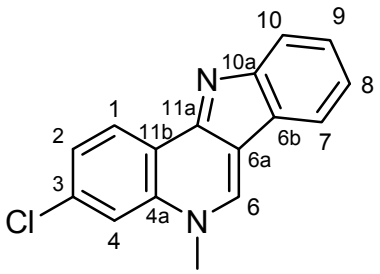
$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 103.2 (C-3), 117.5 (C-2′), 120.0 (C-4a), 120.6 (C-5), 123.0 (C-6′), 125.7 (C-4′), 126.2 (C-6), 128.6 (C-5′), 129.0 (C-8), 130.3 (C-7), 133.7 (C-3′), 138.0 (C-1′), 147.6 (C-4), 147.8 (C-8a), 149.5 (C-2).

MS (EI): 218 (40), 219 (100), 220 (19), 298 (33, $[M]^+$, $^{79}$Br), 300 (32, $[M]^+$, $^{81}$Br).

IR (KBr) $\nu_{max}$: 744, 762, 812, 1338, 1458, 1473, 1568, 2911, 3061.

UV (MeOH) $\lambda_{max}$: 217, 322.

**3-Chloroisocryptolepine 64**




The above compound was prepared as for isocryptolepine **16** (Method 2) but starting from 3-chloro-11*H*-indolo[3,2-*c*]quinoline **68** (438 mg, 1.73 mmol) and iodomethane (11 mL, 176.7 mmol) in acetonitrile (30 mL). The product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:2:1) to give 3-chloroisocryptolepine **64** as a yellow crystalline solid (281 mg, 61%).

Mp: 268-270 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.21 (3H, s, $NCH_3$), 7.25 (1H, t, *J* = 7.5 Hz, H-8), 7.44 (1H, ddd, *J* = 7.8, 7.2, 0.6 Hz, H-9), 7.71 (1H, dd, *J* = 9.0, 1.8 Hz, H-2), 7.79 (1H, br d, *J* = 8.4 Hz, H-10), 8.09-8.10 (2H, m, H-4 and H-7), 8.73 (1H, d, *J* = 9.0 Hz, H-1), 9.27 (1H, s, H-6).

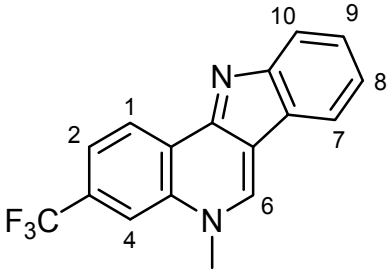
$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 42.2 ($NCH_3$), 116.8 (C-6a), 117.2 (C-4), 118.6 (C-10), 119.6 (C-11b), 119.7 (C-7), 120.1 (C-8), 125.4 (C-2), 125.6 (C-6b), 125.7 (C-1), 125.8 (C-9), 133.9 (C-3), 136.2 (C-4a), 138.4 (C-6), 152.1 (C-11a), 154.7 (C-10a).

MS (FAB): 147 (18), 267 (100, $[M+1]^+$, $^{35}$Cl), 268 (23), 269 (35, $[M+1]^+$, $^{37}$Cl).

HRMS (FAB): 267.0685 ($C_{16}H_{12}N_2Cl$ $[M+H]^+$ requires 267.0689).

IR (KBr) $\nu_{max}$: 739, 1125, 1224, 1329, 1456, 1599, 1638, 3426.

**3-Trifluoromethylisocryptolepine 65**




The above compound was prepared as for isocryptolepine **16** (Method 2) but starting from 3-triflurormethyl-11*H*-indolo[3,2-*c*]quinoline **69** (223 mg, 0.78 mmol) and

iodomethane (4.8 mL, 77.10 mmol) in acetonitrile (13 mL). The methiodide salt was purified by silica flash column chromatography eluting with a mixture of methanol and dichloromethane (10:90 increasing to 15:85) and then converted to the free base with a 1:1 solution of aqueous ammonia (30%) and dichloromethane (200 mL). The organic layer was extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give 3-trifluoromethylisocryptolepine **65** as a yellow crystalline solid (147 mg, 63%).

Mp: 238-240 ºC.

$^1$H NMR (600 MHz, $d_6$-DMSO) δ: 4.31 (3H, s, $NCH_3$), 7.31 (1H, t, *J* = 7.5 Hz, H-8), 7.49 (1H, t, *J* = 7.5 Hz, H-9), 7.83 (1H, d, *J* = 8.4 Hz, H-10), 7.99 (1H, d, *J* = 8.4 Hz, H-2), 8.13 (1H, d, *J* = 7.8 Hz, H-7), 8.32 (1H, s, H-4), 8.93 (1H, d, *J* = 8.4 Hz, H-1), 9.39 (1H, s, H-6).

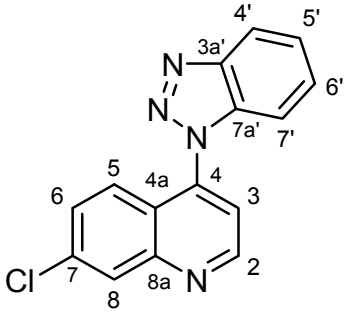
$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 42.3 ($NCH_3$), 115.2 (C-4, *J* = 4.4 Hz,), 117.1 (C-6a), 119.0 (C-10), 119.8 (C-7), 120.5 (C-8), 120.8 (C-2, *J* = 3.3 Hz), 123.4 (C-11b), 124.2 ($CF_3$, *J* = 271 Hz), 125.3 (C-1), 125.6 (C-6b), 126.0 (C-9), 128.7 (C-3, *J* = 32 Hz), 134.8 (C-4a), 139.1 (C-6), 151.6 (C-11a), 154.8 (C-10a).

MS (EI): 300 (100, $[M]^+$), 301 (19).

HRMS (EI): 300.0880 ($C_{17}H_{11}N_2F_3$ $[M]^+$ requires 300.0874).

IR (KBr) $\nu_{max}$: 745, 1086, 1113, 1131, 1225, 1320, 1356, 1642, 2931, 3367.

**4-(1-Benzotriazolyl)-7-chloroquinoline 66**




The above compound was prepared as for compound **35** but starting from 4,7-dichloroquinoline **62** (392 mg, 1.98 mmol) and benzotriazole (265 mg, 2.22 mmol). The product 4-(1-benzotriazolyl)-7-chloroquinoline **66** was obtained as a white crystalline solid (432 mg, 78%).

Mp: 190-192 ºC.

$^1$H NMR (600 MHz, $CDCl_3$) δ: 7.48 (1H, d, *J* = 8.4 Hz, H-4′), 7.52 (1H, t, *J* = 7.8 Hz, H-6′), 7.56 (1H, dd, *J* = 9.0, 2.4 Hz, H-6), 7.59 (1H, t, *J* = 7.8 Hz, H-5′), 7.62

(1H, d, *J* = 4.8 Hz, H-3), 7.83 (1H, d, *J* = 9.0 Hz, H-5), 8.24 (1H, d, *J* = 8.4 Hz, H-7′), 8.29 (1H, d, *J* = 1.8 Hz, H-8), 9.14 (1H, d, *J* = 4.2 Hz, H-2).

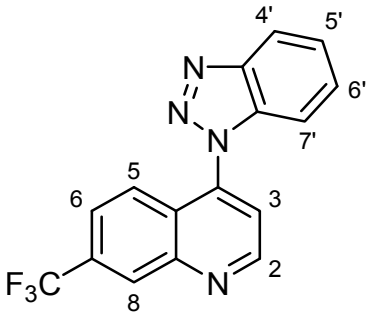
$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 110.3 (C-4′), 117.0 (C-3), 120.9 (C-7′ and C-4a), 125.2 (C-6′ and 5), 129.1 (C-8), 129.3 (C-5′), 129.4 (C-6), 133.8 (C-3a′), 137.2 (C-7), 141.0 (C-4), 146.4 (C-7a′), 150.4 (C-8a), 151.5 (C-2).

MS (EI): 99 (20), 135 (17), 162 (38), 190 (21), 217 (62), 252 (100), 253 (21), 254 (34), 280 (25, $[M]^+$).

HRMS (EI): 280.0520 ($C_{15}H_9N_4Cl$ $[M]^+$ requires 280.0516).

IR (KBr) $\nu_{max}$: 769, 1032, 1074, 1288, 1455, 1502, 1562, 1615, 3052.

**4-(1-Benzotriazolyl)-7-trifluoromethylquinoline 67**




The above compound was prepared as for compound **35** but starting from 4-chloro-7-trifluoromethylquinoline **63** (1.00 g, 4.34 mmol) and benzotriazole (545 mg, 4.57 mmol). The product 4-(1-benzotriazolyl)-7-trifluoromethylquinoline **67** was obtained as an off-white crystalline solid (1.05 g, 77%).

Mp: 160-162 ºC.

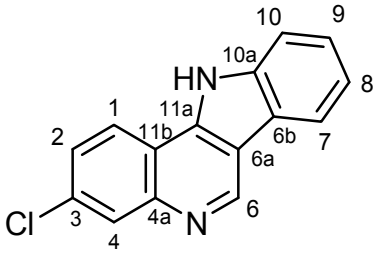
$^1$H NMR (400 MHz, $CDCl_3$) δ: 7.50 (1H, dd, *J* =8.8, 1.6 Hz, H-4′), 7.54 (1H, ddd, *J* = 8.2, 7.0, 1.0 Hz, H-6′), 7.62 (1H, ddd, *J* = 8.1, 7.1, 1.1 Hz, H-5′), 7.78 (1H, d, *J* = 4.4 Hz, H-3), 7.80 (1H, dd, *J* = 9.2, 1.6 Hz, H-6), 8.08 (1H, d, *J* = 9.2 Hz, H-5), 8.26 (1H, dd, *J* = 8.4 Hz, 1.6 Hz, H-7′), 8.63 (1H, s, H-8), 9.31 (1H, d, *J* = 4.4 Hz, H-2).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 110.2 (C-4′), 118.5 (C-3), 121.0 (C-7′), 123.7 ($CF_3$, *J* = 271 Hz), 124.1 (C-6, *J* = 3 Hz), 124.7 (C-4a), 125.5 (C-5 and C-6′), 127.7 (C-8, *J* = 4.3 Hz), 129.5 (C-5′), 132.9 (C-7, *J* = 33 Hz), 133.8 (C-3a′), 141.2 (C-4), 146.4 (C-7a′), 148.9 (C-8a), 151.8 (C-2).

MS (EI): 169 (30), 196 (32), 286 (100), 287 (21), 314 (22, $[M]^+$).

IR (KBr) $\nu_{max}$: 747, 769, 829, 1032, 1067, 1163, 1198, 1287, 1336, 1314, 1454, 1519, 1612, 3040.

**3-Chloro-11*H*-indolo[3,2-*c*]quinoline 68**




The above known compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-7-chloroquinoline **66** (993 mg, 3.54 mmol) and polyphosphoric acid (31.32 g). The reaction mixture was heated at 140 °C for 3 hours. The product 3-chloro-11*H*-indolo[3,2-*c*]quinoline **68** was obtained as a cream solid (695 mg, 78%).

The proton NMR data acquired deviated slightly from that published in the literature, which was acquired in salt form in $D_2O$.[134]

Mp: >310 ºC.

$^1$H NMR (600 MHz, $d_6$-DMSO) δ: 7.35 (1H, td, *J* = 7.5, 0.6 Hz, H-8), 7.51 (1H, ddd, *J* = 8.4, 7.2, 0.6 Hz, H-9), 7.73 (1H, dd, *J* = 8.4, 0.6 Hz, H-10), 7.74 (1H, dd, *J* = 8.7, 2.1 Hz, H-2), 8.16, (1H, d, *J* = 2.4 Hz, H-4), 8.33 (1H, dd, *J* = 7.5, 0.6 Hz, H-7), 8.56 (1H, d, *J* = 8.4 Hz, H-1), 9.62 (1H, s, H-6), 12.88 (1H, br s, N-H).

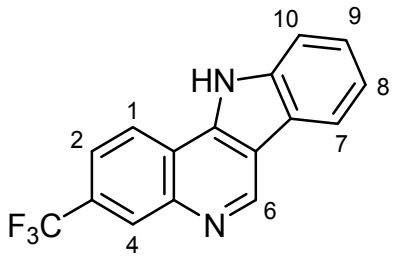
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 111.8 (C-10), 114.5 (C-6a), 115.5 (C-11b), 120.0 (C-7), 120.6 (C-8), 121.5 (C-6b), 124.0 (C-1), 125.7 (C-9), 125.9 (C-2), 128.1 (C-4), 132.2 (C-3), 138.7 (C-10a), 139.3 (C-11a), 145.7 (C-4a), 145.9 (C-6).

MS (EI): 217 (16), 252 (100, $[M]^+$, $^{35}$Cl), 253 (20), 254 (33, $[M]^+$, $^{37}$Cl).

HRMS (EI): 252.0454 ($C_{15}H_9N_2Cl$ $[M]^+$requires 252.0454).

IR (KBr) $\nu_{max}$: 756, 873, 1135, 1278, 1456, 1499, 1562, 1620, 3052.

**3-Trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline 69**




The above compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-7-trifluoromethylquinoline **67** (322 mg, 1.02 mmol) and polyphosphoric acid (10.19 g). The reaction mixture was heated at 140 °C for 3

hours. The product 3-trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline **69** was obtained as a pale yellow solid (115 mg, 39%).

Mp: >350 ºC.

$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 7.42 (1H, t, *J* = 7.1 Hz, H-8), 7.59 (1H, t, *J* = 7.2 Hz, H-9), 7.80 (1H, d, *J* = 7.4 Hz, H-10), 8.02 (1H, d, *J* = 8.4 Hz, H-2), 8.40 (1H, d, *J* = 7.6 Hz, H-7), 8.48 (1H, s, H-4), 8.78 (1H, d, *J* = 8.4 Hz, H-1), 9.77 (1H, s, H-6).

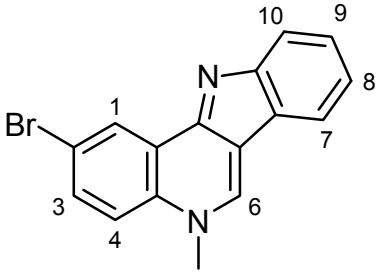
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 112.0 (C-10), 115.5 (C-6a), 119.1 (C-11b), 120.3 (C-7), 120.8 (C-8), 121.4 (C-2, *J* = 5 Hz), 123.8 (C-1), 124.1 ($CF_3$, *J* = 275 Hz), 126.1 (C-9), 126.6 (C-4, *J* = 4 Hz), 127.8 (C-3, *J* = 32 Hz), 138.9 (C-10a and C-11a), 144.0 (C-4a), 146.3 (C-6).

MS (EI): 286 (100), 287 (18).

HRMS (EI): 286.0716 ($C_{16}H_9N_2F_3$ $[M]^+$ requires 286.0718).

IR (KBr) $\nu_{max}$: 739, 1073, 1128, 1170, 1284, 1509, 1572, 2960, 3050.

**2-Bromoisocryptolepine 70**




The above compound was prepared as for isocryptolepine **18** (Method 2) but starting from 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84** (206 mg, 0.69 mmol) and iodomethane (4.3 mL, 69.07 mmol) in acetonitrile (12 mL). The product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:2:1) to give 2-bromoisocryptolepine **70** as a yellow crystalline solid (194 mg, 90%).

Mp: 262-263 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.19 (3H, s, $NCH_3$), 7.26 (1H, t, *J* = 7.5 Hz, H-8), 7.45 (1H, ddd, *J* = 7.8, 7.2, 0.6 Hz, H-9), 7.80 (1H, d, *J* = 7.8 Hz, H-10), 7.92 (1H, dd, *J* = 9.6, 2.1 Hz, H-3), 7.96 (1H, d, *J* = 9.0 Hz, H-4), 8.10 (1H, d, *J* = 7.2 Hz, H-7), 8.82 (1H, d, *J* = 2.4 Hz, H-1), 9.27 (1H, s, H-6).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.0 ($NCH_3$), 116.6 (C-6a), 117.7 (C-2), 118.6 (C-10), 119.5 (C-4), 119.9 (C-7), 120.0 (C-8), 122.3 (C-11b), 125.5 (C-6b),

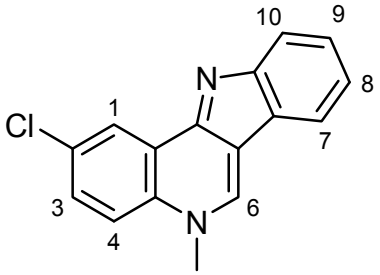
125.6 (C-9 and C-1), 131.4 (C-3), 134.1 (C-4a), 138.1 (C-6), 151.2 (C-11a), 154.5 (C-10a).

MS (EI): 189 (16), 231 (44), 310 (100, $[M]^+$, $^{79}Br$), 311 (19), 312 (88, $[M]^+$, $^{81}Br$), 313 (18).

HRMS (EI): 310.0113 ($C_{16}H_{11}N_2Br$ $[M]^+$ requires 310.0106).

IR (KBr) $\nu_{max}$: 738, 1118, 1217, 1344, 1365, 1447, 1595, 1637, 3047, 3394.

**2-Chloroisocryptolepine 71**




The above compound was prepared as for isocryptolepine **18** (Method 2) but starting from 2-chloro-11*H*-indolo[3,2-*c*]quinoline **85** (51 mg, 0.20 mmol) and iodomethane (1.3 mL, 20.9 mmol), in acetonitrile (3.5 mL). The product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:2:1) to give 2-chloroisocryptolepine **71** as a yellow crystalline solid (47 mg, 88%).

This compound was reported during the course of this project and the spectroscopic data acquired was consistent with that published in the literature.[93]

Mp: 248-249 ºC.

$^1H$ NMR (600 MHz, $d_6$-DMSO) δ: 4.19 (3H, s, $NCH_3$), 7.26 (1H, t, *J* = 7.5 Hz, H-8), 7.45 (1H, t, *J* = 7.5 Hz, H-9), 7.79-7.81 (2H, m, H-3 and H-10), 8.02 (1H, d, *J* = 9.0 Hz, H-4), 8.10 (1H, d, *J* = 7.2 Hz, H-7), 8.66 (1H, d, *J* = 2.4 Hz, H-1), 9.25 (1H, s, H-6).

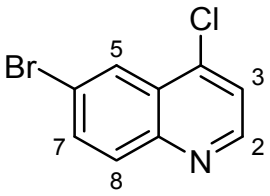
$^{13}C$ NMR (50 MHz, $d_6$-DMSO) δ: 42.0 ($NCH_3$), 116.4 (C-6a), 118.5 (C-4), 119.5 (C-10), 119.7 (C-7), 120.0 (C-8), 121.9 (C-11b), 122.4 (C-1), 125.4 (C-6b), 125.5 (C-9), 128.8 (C-3), 129.5 (C-2), 133.8 (C-4a), 138.1 (C-6), 151.3 (C-11a), 154.4 (C-10a).

MS (EI): 205 (28), 266 (100, $[M]^+$, $^{35}Cl$), 267 (20), 268 (34, $[M]^+$, $^{37}Cl$).

HRMS (EI): 266.0603 ($C_{16}H_{11}N_2Cl$ $[M]^+$ requires 266.0611).

IR (KBr) $\nu_{max}$: 736, 1107, 1219, 1340, 1449, 1597, 1638, 3049, 3338.

**6-Bromo-4-chloroquinoline 72**




The above known compound was prepared *via* an adaptation of the synthetic procedure published by Lin and Loo[135] which is summarised as follows. Phosphorus oxychloride (4.4 mL, 48.07 mmol) was added to 6-bromo-4-quinolone **80** (1.79 g, 8.00 mmol) and the mixture refluxed for 19 hours. The reaction mixture was quenched with iced water and basified with aqueous ammonia. The resulting white precipitate was collected by vacuum filtration (washing with water) to give 6-bromo-4-chloroquinoline **72** as a white solid (1.73 g, 89%) which was used without further purification.

Mp: 110-111 °C (lit.,[135] 111-112°C).

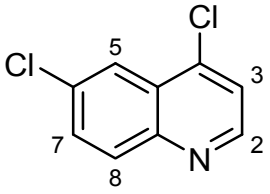
$^{1}$H NMR (200 MHz, $CDCl_3$) δ: 7.49 (1H, d, *J* = 4.8 Hz, H-3), 7.82 (1H, dd, *J* = 9.2, 2.2 Hz, H-7), 7.97 (1H, d, *J* = 9.2 Hz, H-8), 8.37 (1H, d, *J* = 1.8 Hz, H-5), 8.77 (1H, d, *J* = 4.8 Hz, H-2).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 121.1, 121.2, 125.6, 126.8, 130.8, 133.1, 140.7, 147.0, 149.3.

MS (EI): 127 (17), 162 (33), 241 (77, $[M]^+$), 243 (100), 254 (24).

IR (KBr) $\nu_{max}$: 677, 830, 842, 1180, 1342, 1487, 1550, 1578, 3083.

**4,6-Dichloroquinoline 73**




The above known compound was prepared as for 6-bromo-4-chloroquioline **72** but starting from 6-chloro-4-quinolone **81** (381 mg, 2.12 mmol) and phosphorus oxychloride (1.2 mL, 13.10 mmol). 4,6-Dichloroquinoline **73** was obtained as a white solid (301 mg, 72%).

Mp: 103-105°C (lit.,[135] 104-105°C).

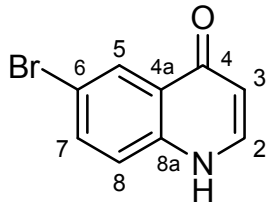
$^{1}$H NMR (200 MHz, $CDCl_3$) δ: 7.50 (1H, d, *J* = 4.6 Hz, H-3), 7.69 (1H, dd, *J* = 9.0, 2.2 Hz, H-7), 8.04 (1H, d, *J* = 9.2 Hz, H-8), 8.20 (1H, d, *J* = 2.2 Hz, H-5), 8.75 (1H, d, *J* = 4.8 Hz, H-2).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 121.1, 122.3, 126.4, 130.5, 130.7, 133.0, 140.7, 146.7, 149.2.

MS (EI): 99 (19), 162 (52), 164 (16), 197 (100), 199 (67, $[M]^+$, $^{35}$Cl).

IR (KBr) $\nu_{max}$: 677, 823, 849, 1086, 1345, 1470, 1557, 1583, 3081.

**6-Bromo-4-quinolone 80**




The above known compound was prepared *via* an adaptation of the synthetic procedure published by Lin and Loo[135] which is summarised as follows.

i) To 4-bromoaniline **74** (988 mg, 5.74 mmol), diethyl ethoxymethylene malonate (1.2 mL, 5.99 mmol) was added and the mixture stirred at room temperature until homogeneous. The reaction mixture was then heated at 100°C for 2 hours after which nitrogen was bubbled through the solution for 30 minutes. Diphenyl ether (10 mL) was added and the solution refluxed for a further 2 hours. The reaction mixture was cooled, hexane added (10 mL) and the precipitate obtained collected by vacuum filtration (washing with hexane) to give 6-bromo-3-carbethoxy-4-hydroxyquinoline **76** as a off-white solid which was used without further purification.

Mp: 322-324 ºC (lit.,[178] 320-322 °C).

ii) A solution of compound **76** in aqueous sodium hydroxide (10%, 10 mL) was refluxed for 2 hours. The mixture was then cooled, acidified with concentrated hydrochloric acid and the precipitate obtained collected by vacuum filtration (washing with water) to give 6-bromo-3-carboxy-4-quinolinol **78** as a white solid which was used without further purification.

Mp: 281-282 ºC (lit.,[179] 297 ºC).

iii) A solution of compound **78** in diphenyl ether (10 mL) was refluxed for 3 hours. The mixture was cooled, hexane added (10 mL) and the precipitate obtained collected by vacuum filtration (washing with hexane). The precipitate was

decolourised with charcoal and recrystallised from methanol to give 6-bromo-4-quinolone **80** as a white solid (786 mg, 61%).

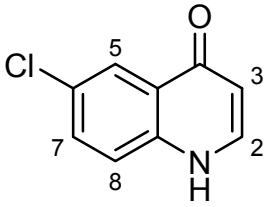
The spectroscopic data acquired was consistent with that published in the literature.[180]

Mp: 290-291 ºC (lit.,[180] 286-291 ºC).

$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 6.06 (1H, dd, *J* = 7.2, 2.2 Hz, H-3), 7.51 (1H, dd, *J* = 8.8, 1.6 Hz, H-8), 7.75 (1H, dd, *J* = 9.2, 2.4 Hz, H-7), 7.92 (1H, dd, *J* = 7.2, 2.2 Hz, H-2), 8.12 (1H, d, *J* = 2.0 Hz, H-5).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 108.8, 115.5, 120.8, 126.9, 134.1, 138.8, 139.7, 175.4.

**6-Chloro-4-quinolone 81**




The above known compound was prepared as for 6-bromo-4-quinolone **80** and the procedure is summarised as follows.

i) Reaction of 4-chloroaniline **75** (1.05 g, 8.20 mmol) and diethyl ethoxymethylene malonate (1.60mL, 7.84 mmol) in diphenyl ether (10 mL) gave 6-chloro-3-carbethoxy-4-hydroxyquinoline **77** as an off-white solid which was used without further purification.

Mp: 310-312 ºC (lit.,[181] >280°C).

ii) Refluxing **77** in aqueous sodium hydroxide (10%, 10 mL) gave 6-chloro-3-carboxy-4-quinolinol **79** as a white solid which was used without further purification.

Mp: 288-290 ºC (lit.,[181] 261 °C).

iii) Decarboxylation of **79** in diphenyl ether (10 mL) gave 6-chloro-4-quinolone **81** as a white solid (701 mg, 49%).

Mp: 266-268 ºC (lit.,[182] 261-263°C).

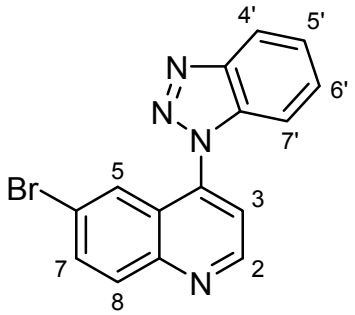
$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 6.06 (1H, d, *J* = 7.2 Hz, H-3), 7.62-7.64 (2H, m, H-7 and H-8), 7.92 (1H, d, *J* = 7.4 Hz, H-2), 7.99 (1H, d, *J* = 1.8 Hz, H-5), 12.00 (br s, NH).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 108.7, 120.7, 123.7, 126.5, 127.6, 131.6, 138.5, 139.7, 175.4.

MS (EI): 89 (19), 151 (46), 153 (16), 179 (100, $[M]^+$, $^{35}Cl$), 181 (32, $[M]^+$, $^{37}Cl$).

IR (KBr) $\nu_{max}$: 826, 1212, 1353, 1514, 1587, 2817, 2892, 3051, 3435.

**4-(1-Benzotriazolyl)-6-bromoquinoline 82**




The above compound was prepared as for compound **35** but starting from 4-chloro-6-bromoquinoline **72** (401 mg, 1.65 mmol) and benzotriazole (212 mg, 1.78 mmol). The product 4-(1-benzotriazolyl)-6-chloroquinoline **82** was obtained as a white crystalline solid (376 mg, 70%).

Mp: 181-182 ºC.

$^1H$ NMR (200 MHz, $CDCl_3$) δ: 7.46-7.57 (3H, m, H-4′, H-5′ and H-6′), 7.61 (1H, d, *J* = 4.4 Hz, H-3), 7.91 (1H, dd, *J* = 9.2, 2.2 Hz, H-7), 8.05 (1H, d, *J* = 1.8 Hz, H-5), 8.14 (1H, d, *J* = 8.8 Hz, H-8), 8.24 (1H, dd, *J* = 7.6, 1.0 Hz, H-7′), 9.13 (1H, d, *J* = 4.6 Hz, H-2).

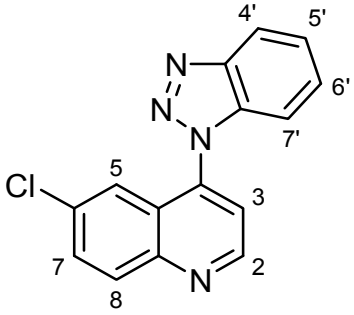
$^{13}C$ NMR (50 MHz, $CDCl_3$) δ: 109.1 (C-4′), 116.6 (C-3), 119.9 (C-7′), 121.8 (C-6), 123.4 (C-4a), 124.2 (C-5), 124.9 (C-6′), 128.3 (C-5′), 130.9 (C-8), 132.9 (C-3a′), 133.5 (C-7), 138.8 (C-4), 145.4 (C-7a′), 147.9 (C-8a), 149.8 (C-2).

MS (EI): 100 (19), 127 (20), 190 (29), 206 (18), 208 (17), 216 (23), 217 (100), 218 (20), 296 (55), 298 (58), 324 (17, $[M]^+$, $^{79}Br$), 326 (17, $[M]^+$, $^{81}Br$).

HRMS (EI): 324.0005 ($C_{15}H_9N_4Br$ $[M]^+$ requires 324.0011).

IR (KBr) $\nu_{max}$: 750, 844, 1034, 1458, 1498, 1585, 3068, 3450.

**4-(1-Benzotriazolyl)-6-chloroquinoline 83**




The above compound was prepared as for compound **35** but starting from 4,6-dichloroquinoline **73** (425 mg, 2.15 mmol) and benzotriazole (274 mg, 2.30 mmol). The product 4-(1-benzotriazolyl)-6-chloroquinoline **83** was obtained as a white crystalline solid (464 mg, 77%).

Mp: 186-187 ºC.

$^{1}$H NMR (200 MHz, $CDCl_3$) δ: 7.45-7.55 (3H, m, H-4′, H-5′ and H-6′), 7.61 (1H,d, *J* = 4.8 Hz, H-3), 7.75 (1H, dd, *J* = 8.8, 2.2 Hz, H-7), 7.86 (1H, d, *J* = 2.2 Hz, H-5), 8.20 (1H, d, *J* = 8.8 Hz, H-8), 8.22 (1H, dd, *J* = 8.8, 1.2 Hz, H-7′), 9.10 (1H, d, *J* = 4.8 Hz, H-2).

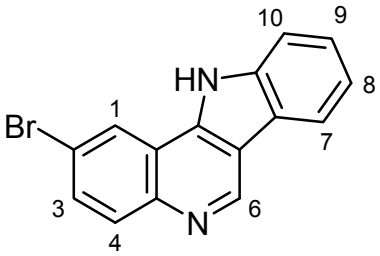
$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 109.1 (C-4′), 116.7 (C-3), 119.9 (C-7′), 121.6 (C-5), 122.9 (C-4a), 124.2 (C-6′), 128.3 (C-5′), 130.9 (C-7 and 8), 132.8 (C-3a′), 133.7 (C-6), 138.9 (C-4), 145.3 (C-7a′), 147.7 (C-8a), 149.7 (C-2).

MS (EI): 99 (44), 126 (15), 127 (18), 134 (31), 162 (49), 164 (19), 190 (29), 216 (17), 217 (81), 252 (100), 253 (19), 254 (35), 280 (37, $[M]^+$, $^{35}$Cl).

HRMS (EI): 280.0515 ($C_{15}H_9N_4Cl$ $[M]^+$ requires 280.0516).

IR (KBr) $\nu_{max}$: 750, 1035, 1461, 1501, 1586, 3062, 3439.

**2-Bromo-11*H*-indolo[3,2-*c*]quinoline 84**




The above compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-6-bromoquinoline **82** (675 mg, 2.08 mmol) and polyphosphoric acid (16.71 g). The reaction mixture was heated at 140 °C for 3 hours. The product

was purified by washing the precipitate with methanol to give 2-bromo-11*H*-indolo[3,2-*c*]quinoline **84** as a cream solid (332 mg, 54%).

Mp: >350 ºC.

$^{1}$H NMR (400 MHz, $d_6$-DMSO) δ: 7.34 (1H, ddd, *J* = 7.9, 7.2, 0.8 Hz, H-8), 7.50 (1H, ddd, *J* = 8.2, 7.0, 0.8 Hz, H-9), 7.75 (1H, dt, *J* = 7.2, 0.8 Hz, H-10), 7.83 (1H, dd, *J* = 8.8, 2.0 Hz, H-3), 8.06 (1H, d, *J* = 9.2 Hz, H-4), 8.32 (1H, dt, *J* = 7.6, 0.8 Hz, H-7), 8.85 (1H, d, *J* = 2.0 Hz, H-1), 9.62 (1H, s, H-6).

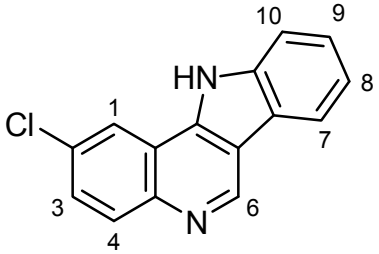
$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 112.3 (C-10), 114.9 (C-6a), 118.3 (C-2), 118.7 (C-11b), 120.3 (C-7), 120.7 (C-8), 121.7 (C-6b), 124.7 (C-1), 125.9 (C-9), 130.8 (C-3), 131.7 (C-4), 139.0 (C-10a), 139.2 (C-11a), 144.0 (C-4a), 145.4 (C-6).

MS (EI): 190 (19), 216 (22), 217 (40), 296 (100, $[M]^+$, $^{79}$Br), 297 (22), 298 (98, $[M]^+$, $^{81}$Br), 299 (17).

HRMS (EI): 295.9944 ($C_{15}H_9N_2Br$ $[M]^+$ requires 295.9949).

IR (KBr) $\nu_{max}$: 740, 822, 1236, 1339, 1363, 1459, 1506, 2983, 3084.

**2-Chloro-11*H*-indolo[3,2-c]quinoline 85**




The above compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-6-chloroquinoline **83** (608 mg, 2.17 mmol) and polyphosphoric acid (19.48 g). The reaction mixture was heated at 140 ºC for 3 hours. The product 2-chloro-11*H*-indolo[3,2-*c*]quinoline **85** was obtained as a cream solid (422 mg, 77%).

Mp: >350 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 7.36 (1H, td, *J* = 7.5, 0.6 Hz, H-8), 7.52 (1H, td, *J* = 7.8, 1.2 Hz, H-9), 7.73-7.75 (2H, m, H-3 and H-10), 8.14 (1H, d, *J* = 9.0 Hz, H-4), 8.33 (1H, dd, *J* = 7.8, 0.6 Hz, H-7), 8.64 (1H, d, *J* = 2.4 Hz, H-1), 9.61 (1H, s, H-6).

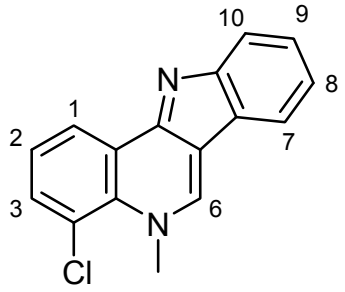
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 111.9 (C-10), 114.7 (C-6a), 117.8 (C-11b), 120.1 (C-7), 120.7 (C-8), 121.1 (C-1), 121.5 (C-6b), 125.8 (C-9), 128.1 (C-3), 129.8 (C-2), 131.4 (C-4), 138.7 (C-10a and C-11a), 143.6 (C-4a), 145.1 (C-6).

MS (EI): 252 (100, $[M]^+$, $^{35}$Cl), 253 (18), 254 (34, $[M]^+$, $^{37}$Cl).

HRMS (EI): 252.0453 ($C_{15}H_9N_2Cl$ $[M]^+$ requires 252.0454).

IR (KBr) $\nu_{max}$: 738, 823, 1088, 1230, 1341, 1364, 1459, 1508, 3085.

**4-Chloroisocryptolepine 91**




The above compound was prepared as for isocryptolepine **18** (Method 2) but starting from 4-chloro-11*H*-indolo[3,2-*c*]quinoline **95** (135 mg, 0.53 mmol) and iodomethane (1 mL, 16.1 mmol) in acetonitrile (10 mL). The product 4-chloroisocryptolepine **91** was obtained as a yellow crystalline solid (36 mg, 25%).

Mp: 225-227 ºC.

$^1$H NMR (600 MHz, $d_6$-DMSO) δ: 4.55 (3H, s, $NCH_3$), 7.27 (1H, t, *J* = 7.2 Hz, H-8), 7.45 (1H, t, *J* = 7.5 Hz, H-9), 7.62 (1H, t, *J* = 7.8 Hz, H-2), 7.79 (1H, d, *J* = 7.8 Hz, H-10), 7.89 (1H, dd, *J* = 7.2, 0.6 Hz, H-3), 8.12 (1H, d, *J* = 7.8 Hz, H-7), 8.80 (1H, d, *J* = 7.8 Hz, H-1), 9.25 (1H, s, H-6).

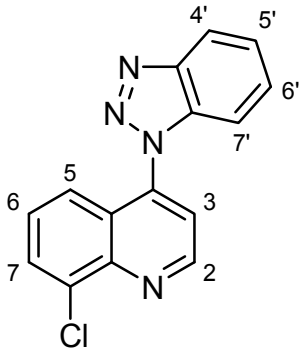
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 47.8 ($NCH_3$), 116.6 (C-6a), 118.5 (C-10), 119.7 (C-7), 120.2 (C-8), 122.1 (C-11b), 123.6 (C-1), 124.1 (C-4), 125.5 (C-9), 125.9 (C-2), 128.9 (C-6b), 132.9 (C-3), 133.4 (C-4a), 141.2 (C-6), 152.4 (C-11a), 154.6 (C-10a).

MS (EI): 251 (21), 252 (16), 266 (100, $[M]^+$, $^{35}$Cl), 267 (23), 268 (34, $[M]^+$, $^{37}$Cl).

HRMS (EI): 266.0616 ($C_{16}H_{11}N_2Cl$ $[M]^+$ requires 266.0611).

IR (KBr) $\nu_{max}$: 756, 1074, 1323, 1480, 1640, 2926, 3326.

**4-(1-Benzotriazolyl)-8-chloroquinoline 93**




The above compound was prepared as for compound **35** but starting from 4,8-dichloroquinoline **89** (165 mg, 0.84 mmol) and benzotriazole (110 mg, 0.92 mmol). The product 4-(1-benzotriazolyl)-8-chloroquinoline **93** was obtained as a white crystalline solid (167 mg, 71%).

Mp: 208-210 ºC.

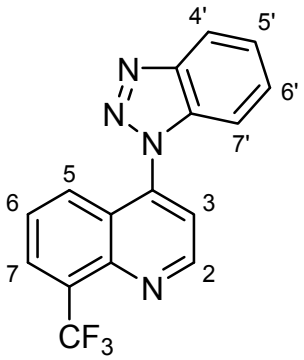
$^{1}$H NMR (200 MHz, $CDCl_3$) δ: 7.48-7.65 (4H, m, H-4′, H-5′, H-6′ and H-6), 7.76 (1H, d, *J* = 4.8 Hz, H-3), 7.83 (1H, dd, *J* = 8.4, 1.2 Hz, H-7), 8.04 (1H, dd, *J* = 7.5, 1.3 Hz, H-5), 8.33 (1H, dd, *J* = 8.8, 1.2 Hz, H-7′), 9.33 (1H, d, *J* = 4.8 Hz, H-2).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 109.3 (C-4′), 117.1 (C-3), 119.8 (C-7′), 121.8 (C-5), 123.8 (C-4a), 124.2 (C-6′), 127.2 (C-6), 128.3 (C-5′), 130.2 (C-7), 133.0 (C-3a′), 133.6 (C-8), 140.4 (C-4), 145.4 (C-7a′), 150.0 (C-2 and C-8a).

MS (EI): 99 (27), 126 (21), 162 (37), 190 (18), 217 (53), 218 (25), 252 (100), 253 (23), 254 (47), 280 (23, $[M]^+$, $^{35}$Cl).

IR (KBr) $\nu_{max}$:782, 769, 814, 1036, 1056, 1216, 1292, 1427, 1506, 1597,1586, 3061.

**4-(1-Benzotriazolyl)-8-trifluoromethylquinoline 94**




The above compound was prepared as for compound **35** but starting from 4-chloro-8-trifluoromethylquinoline **90** (496 mg, 2.14 mmol) and benzotriazole (280 mg, 2.35 mmol). The product 4-(1-benzotriazolyl)-8-trifluoromethylquinoline **94** was obtained as an off-white crystalline solid (446 mg, 66%).

Mp: 215-217 ºC.

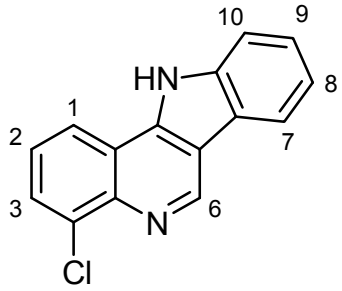
$^{1}$H NMR (600 MHz, $CDCl_3$) δ: 7.47 (1H, dd, *J* =8.4, 1.2 Hz, H-4′), 7.54 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-6′), 7.61 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-5′), 7.68 (1H, br t, *J* = 7.8 Hz, H-6), 7.73 (1H, d, *J* = 4.2 Hz, H-3), 8.09 (1H, dd, *J* = 9.0, 1.2 Hz, H-5), 8.23 (1H, br d, *J* = 7.2 Hz, H-7), 8.27 (1H, dd, *J* = 8.4, 1.2 Hz, H-7′), 9.31 (1H, d, *J* = 4.8 Hz, H-2).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 110.1 (C-4′), 117.8 (C-3), 120.9 (C-7′), 121.3 ($CF_3$, *J* = 260 Hz), 123.8 (C-4a), 125.3 (C-6′), 126.9 (C-5), 128.2 (C-6), 128.7 (C-8, *J* = 30 Hz), 129.3 (C-5′), 129.4 (C-7, *J* = 5 Hz), 133.9 (C-3a′), 141.0 (C-4), 146.3 (C-7a′), 146.6 (C-8a), 151.4 (C-2).

MS (FAB): 315 (100, $[M]^+$), 316 (21).

IR (KBr) $\nu_{max}$: 744, 777, 1059, 1105, 1130, 1214, 2184, 1320, 1513, 1586, 1601, 3089.

**4-Chloro-11*H*-indolo[3,2-c]quinoline 95**




The above compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-8-chloroquinoline **93** (398 mg, 1.42 mmol) and polyphosphoric acid (13.39 g). The reaction mixture was heated at 130 °C for 4 hours. The product 4-chloro-11*H*-indolo[3,2-*c*]quinoline **95** was obtained as a pale yellow solid (201 mg, 56%).

Mp: >350 ºC.

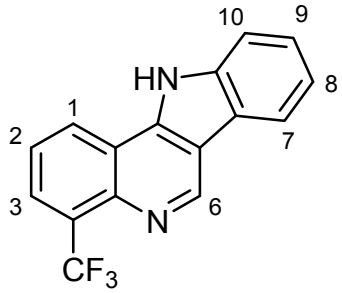
$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 7.40 (1H, t, *J* = 7.5 Hz, H-8), 7.60 (1H, t, *J* = 7.5 Hz, H-9), 7.68 (1H, t, *J* = 7.9 Hz, H-2), 7.78 (1H, d, *J* = 8.0 Hz, H-10), 7.95 (1H, dd, *J* = 7.7, 1.2 Hz, H-3), 8.38 (1H, d, *J* = 7.6 Hz, H-7), 8.57 (1H, dd, *J* = 8.0, 1.0 Hz, H-1), 9.72 (1H, s, H-6).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 112.0 (C-10), 115.0 (C-6a), 118.5 (C-11b), 120.1 (C-7), 120.6 (C-8), 121.3 (C-1), 121.5 (C-6b), 125.5 (C-9), 125.8 (C-2), 128.0 (C-3), 133.1 (C-4), 139.2 (C-10a), 139.9 (C-11a), 141.0 (C-4a), 145.1 (C-6).

MS (EI): 252 (100, $[M]^+$, $^{35}$Cl), 253 (18), 254 (34, $[M]^+$, $^{37}$Cl).

IR (KBr) $\nu_{max}$: 747, 764, 891, 1115, 1235, 1339, 1358, 1503, 3168.

**4-Trifluoromethyl-11*H*-indolo[3,2-*c*]quinoline 96**




The above compound was prepared as for compound **36** (Method 3) but starting from 4-(1-benzotriazolyl)-8-trifluoromethylquinoline **94** (188 mg, 0.60 mmol) and polyphosphoric acid (4.90 g). The reaction mixture was heated at 140 °C for 3 hours.

The product 4-trifluoromethyl-11*H*-[3,2-*c*]quinoline **96** was obtained as a pale yellow solid (73 mg, 43%).

Mp: 312-314 ºC.

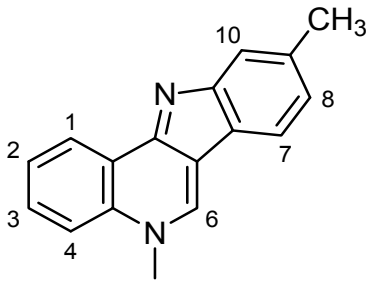
$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 7.38 (1H, ddd, *J* = 7.8, 7.0, 0.8 Hz, H-8), 7.54 (1H, ddd, *J* = 8.1, 7.1, 1.2 Hz, H-9), 7.77 (1H, d, *J* = 8.0 Hz, H-10), 7.81 (1H, t, *J* = 7.8 Hz, H-2), 8.14 (1H, d, *J* = 7.2 Hz, H-3), 8.36 (1H, dd, *J* = 7.6, 0.8 Hz, H-7), 8.87 (1H, d, *J* = 7.6, 0.8 Hz, H-1), 9.72 (1H, s, H-6).

$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 112.2 (C-10), 114.9 (C-6a), 117.9 (C-11b), 120.4 (C-7), 120.9 (C-8), 121.6 (C-6b), 124.7 ($CF_3$, *J* = 240 Hz), 124.5 (C-2), 126.1 (C-9), 126.3 (C-3, *J* = 4.2 Hz), 126.5 (C-4, *J* = 33 Hz), 127.3 (C-1), 139.2 (C-10a), 139.8 (C-11a), 141.4 (C-4a), 145.6 (C-6).

MS (FAB): 286 (31), 287 (100, $[M+H]^+$), 288 (18).

IR (KBr) $\nu_{max}$: 750, 780, 1082, 1272, 1312, 1330, 1456, 1581, 1598, 3251.

**9-Methylisocryptolepine 97**




The above compound was prepared as for isocryptolepine **16** (Method 2) but starting from 9-methyl-11*H*-indolo[3,2-c]quinoline **98** (222 mg, 0.95 mmol) and iodomethane (5.90 mL, 94.77 mmol) in acetonitrile (15 mL). The product 9-methylisocryptolepine **97** was obtained as a yellow crystalline solid (197 mg, 84%).

Mp: 259-260 ºC.

$^{1}$H NMR (600 MHz, $CDCl_3$) δ: 2.58 (3H, s, $CH_3$), 3.97 (3H, s, $NCH_3$), 7.09 (1H, ddd, *J* = 7.8, 1.2, 0.6 Hz, H-8), 7.52-7.55 (2H, m, H-2 and H-4), 7.61 (1H, ddd, *J* = 9.0, 7.8, 1.2 Hz, H-3), 7.73 (1H, d, *J* = 7.8 Hz, H-7), 7.77 (1H, br s, H-10), 8.12 (1H, s, H-6), 8.86 (1H, dd, *J* = 7.2, 2.1 Hz, H-1).

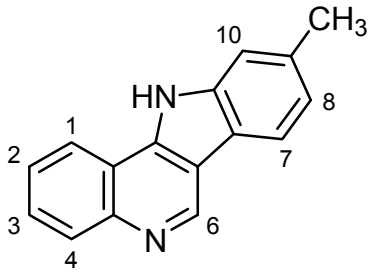
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 21.7 ($CH_3$), 42.1 ($NCH_3$), 116.0 (C-6a), 117.4 (C-4), 117.9 (C-10), 119.1 (C-7), 120.4 (C-11b), 121.5 (C-8), 122.7 (C-6b), 123.8 (C-1), 125.1 (C-2), 129.2 (C-3), 134.9 (C-9), 135.3 (C-4a), 137.8 (C-6), 151.8 (C-11a), 153.7 (C-10a).

MS (EI): 231 (19), 245 (35), 246 (100, $[M]^+$), 247 (19).

HRMS (EI): 246.1151 ($C_{17}H_{14}N_2$ $[M]^+$ requires 246.1157).

IR (KBr) $\nu_{max}$: 752, 806, 1122, 1228, 1241, 1350, 1455, 1600, 1640, 3427.

**9-Methyl-11*H*-indolo[3,2-*c*]quinoline 98**




The above compound was prepared as for compound **36** (Method 2) but starting from 4-bromoquinoline **59** (206 mg, 0.99 mmol), 2-bromo-5-methylaniline (206 mg, 1.11 mmol), $Pd(OAc)_2$ (4.2 mg, 1.9 mol%), BINAP (13.2 mg, 2.1 mol%) and potassium carbonate (2.66 g, 19.24 mmol) in dry DMF (20 mL). The product 9-methyl-11*H*-indolo[3,2-c]quinoline **98** was obtained as a cream solid (171 mg, 74%).

Mp: >340 ºC (d).

$^1$H NMR (600 MHz, $d_6$-DMSO) δ: 2.52 (3H, s, $CH_3$), 7.16 (1H, dd, *J* = 7.8, 1.2 Hz, H-8), 7.51 (1H, d, *J* = 0.6 Hz, H-10), 7.66 (1H, ddd, *J* = 8.1, 6.9, 1.2 Hz, H-2), 7.72 (1H, ddd, *J* = 8.1, 6.9, 1.2 Hz, H-3), 8.11 (1H, dd, *J* = 8.4, 0.6 Hz, H-4), 8.17 (1H, d, *J* = 7.8 Hz, H-7), 8.53 (1H, dd, *J* = 8.4, 1.2 Hz, H-1), 9.53 (1H, s, H-6).

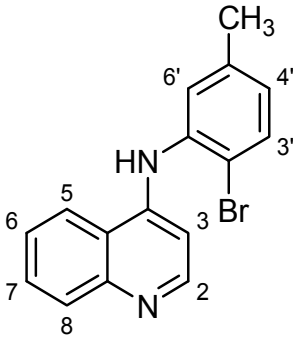
$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 21.7 ($CH_3$), 111.7 (C-10), 114.4 (C-6a), 117.2 (C-11b), 119.6 (C-6b), 119.8 (C-7), 122.0 (C-1), 122.2 (C-8), 125.6 (C-2), 127.8 (C-3), 129.5 (C-4), 135.2 (C-9), 139.3 (C-10a), 139.6 (C-11a), 144.6 (C-6), 145.3 (C-4a).

MS (EI): 231 (63), 232 (100, $[M]^+$), 233 (19).

HRMS (EI): 232.1002 ($C_{16}H_{12}N_2$ $[M]^+$ requires 232.1000).

IR (KBr) $\nu_{max}$: 757, 803, 1155, 1216, 1335, 1363, 1456, 1569, 1593, 2886, 3052.

**4-(2-Bromo-5-methylphenylamino)quinoline 99**




The above compound was prepared as for compound **37** but starting from 4-bromoquinoline **59** (500 mg, 2.40 mmol), 2-bromo-5-methylaniline (490 mg, 2.63 mmol), caesium carbonate (1.08 g, 3.31 mmol), $Pd_2(dba)_3$ (21.7 mg, 1 mol%) and XANTPHOS (29.8 mg, 2.1 mol%) in dry dioxane (10 mL). The product was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and methanol (100:0 increasing to 95:5) give 4-(2-bromo-5-methylphenylamino)quinoline **99** as an off-white solid (572 mg, 76%).

Mp: 155-156 ºC.

$^1$H NMR (600 MHz, $CDCl_3$) δ: 2.33 (3H, s, $CH_3$), 6.84 (1H, dd, *J* = 8.4, 2.4 Hz, H-4′), 7.02 (1H, d, *J* = 5.4 Hz, H-3), 7.34 (1H, d, *J* = 2.4 Hz, H-6′), 7.52 (1H, d, *J* = 7.8 Hz, H-3′), 7.54 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-6), 7.71 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-7), 8.01 (1H, d, *J* = 8.4 Hz, H-8), 8.09 (1H, d, *J* = 8.4 Hz, H-5), 8.63 (1H, d, *J* = 4.8 Hz, H-2).

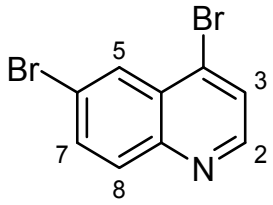
$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 21.3. ($CH_3$), 102.8 (C-3), 114.5 (C-2′), 119.8 (C-4a), 120.8 (C-5), 124.2 (C-6′), 126.3 (C-6), 127.1 (C-4′), 128.3 (C-8), 130.5 (C-7), 133.3 (C-3′), 137.4 (C-5′), 139.0 (C-1′), 147.1 (C-8a), 148.4 (C-4), 148.9 (C-2).

MS (EI): 218 (31), 231 (15), 232 (23), 233 (100), 234 (18), 312 (27, $[M]^+$), 314 (28).

HRMS (EI): 312.0263 ($C_{16}H_{13}N_2Br$ $[M]^+$ requires 312.0262).

IR (KBr) $\nu_{max}$: 594, 757, 1339, 1403, 1499, 1537, 2953, 3213.

**4,6-Dibromoquinoline 102**




The above known compound was prepared as for 4-bromoquinoline **59** but starting from 6-bromo-4-quinolone **80** (489 mg, 2.18 mmol) and phosphorus tribromide (300 μL, 2.53 mmol) in DMF (12 mL). On basification, the product precipitated and therefore product extraction was unnecessary. The precipitate was collected by vacuum filtration (washing with water) to give 4,6-dibromoquinoline **102** as a white solid which was used without further purification (516 mg, 82%).

The NMR data acquired was consistent with that published in the literature.[121]

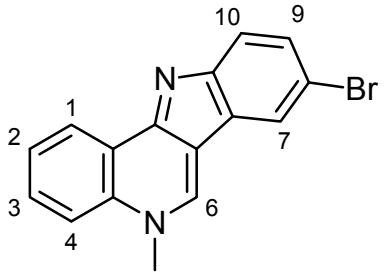
Mp: 140-146 °C.

$^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.78 (1H, d, *J* = 4.8 Hz, H-3), 7.87 (1H, d, *J* = 8.8 Hz, H-8), 8.07 (1H, dd, *J* = 9.2, 2.2 Hz, H-7), 8.38 (1H, s, H-5), 8.70 (1H, d, *J* = 4.8 Hz, H-2).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 122.2, 123.0, 126.0, 126.8, 129.3, 130.9, 134.8, 146.4, 149.3.

IR (KBr) $\nu_{max}$: 659, 840, 1176, 1340, 1490, 1543, 1574, 1604, 3422.

**8-Bromoisocryptolepine 104**




To a solution of isocryptolepine **16** (395 mg, 1.70 mmol) in DMF (20 mL), *N*-bromosuccinimide (344 mg, 1.93 mmol) was added and the solution heated at 150°C for 24 hours. The reaction mixture was cooled, quenched with water and basified with aqueous sodium hydroxide solution (10%). The precipitate obtained was collected by vacuum filtration (washing with water) and recrystallised from ethanol to give 8-bromoisocryptolepine **104** as a yellow crystalline solid (392 mg, 74%).

Mp: 257-258 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.25 (3H, s, $NCH_3$), 7.54 (1H, dd, *J* = 8.4, 1.8 Hz, H-9), 7.71-7.74 (2H, m, H-2 and H-10), 7.85 (1H, ddd, *J* = 8.7, 7.2, 1.5 Hz, H-3), 8.05 (1H, d, *J* = 9.0 Hz, H-4), 8.30 (1H, d, *J* = 2.4 Hz, H-7), 8.76 (1H, dd, *J* = 8.1, 1.5 Hz, H-1), 9.39 (1H, s, H-6).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.3 ($NCH_3$), 111.7 (C-8), 115.2 (C-6a), 117.5 (C-4), 120.0 (C-10), 121.0 (C-11b), 122.0 (C-7), 123.8 (C-1), 125.4 (C-2), 127.5 (C-6b), 127.6 (C-9), 129.4 (C-3), 135.4 (C-4a), 139.2 (C-6), 153.0 (C-10a and C-11a).

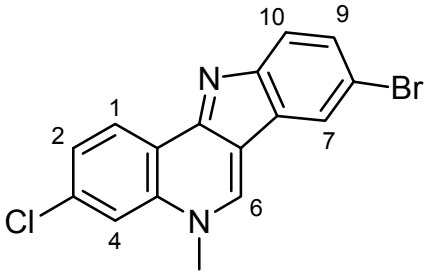
MS (EI): 189 (17), 215 (16), 216 (20), 231 (21), 310 (100, $[M]^+$, $^{79}$Br), 311 (22), 312 (100, $[M]^+$, $^{81}$Br), 313 (19).

HRMS (EI): 310.0110 ($C_{16}H_{11}N_2Br$ $[M]^+$ requires 310.0106).

IR (KBr) $\nu_{max}$: 748, 808, 1126, 1229, 1320, 1438, 1643, 2920, 3400.

UV (MeOH) $\lambda_{max}$: 202, 239, 293.

**8-Bromo-3-chloroisocryptolepine 105**




The above compound was prepared as for compound **104** but starting from 3-chloroisocryptolepine **64** (202 mg, 0.76 mmol) and *N*-bromosuccinimide (149 mg, 0.84 mmol) in DMF (10 mL). The reaction mixture was heated for 24 hours and the product was recrystallised from methanol and water to give 8-bromo-3-chloroisocryptolepine **105** as a yellow crystalline solid (187 mg, 71%).

Mp: 247-250 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.23 (3H, s, $CH_3$), 7.54 (1H, dd, *J* = 8.7, 2.1 Hz, H-9), 7.73 (1H, d, *J* = 8.4 Hz, H-10), 7.76 (1H, dd, *J* = 8.4, 1.8 Hz, H-2), 8.16 (1H, d, *J* = 1.8 Hz, H-4), 8.31 (H, d, *J* = 1.8 Hz, H-7), 8.74 (1H, d, *J* = 8.4 Hz, H-1), 9.40 (1H, s, H-6).

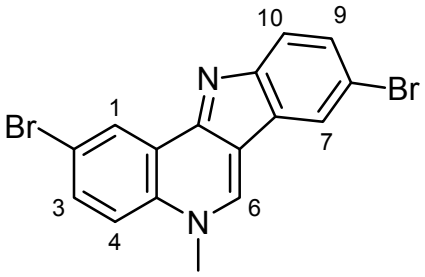
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.4 ($NCH_3$), 112.0 (C-8), 115.7 (C-6a), 117.4 (C-4), 119.6 (C-11b), 120.2 (C-10), 122.2 (C-7), 125.6 (C-2), 125.7 (C-1), 127.4 (C-6b), 127.9 (C-9), 134.0 (C-3), 136.1 (C-4a), 139.6 (C-6), 152.4 (C-11a), 153.2 (C-10a).

MS (FAB): 344 (18), 345 (80, $[M+H]^+$), 346 (39), 347 (100), 348 (25), 349 (26).

HRMS (FAB): 344.9798 ($C_{16}H_{11}N_2ClBr$ $[M+H]^+$ requires 344.9794).

IR (KBr) $\nu_{max}$: 810, 1220, 1338, 1432, 1458, 1594, 1616, 1641, 2854, 3400.

**2,8-Dibromoisocryptolepine 106**




The above compound was prepared as for compound **104** but starting from 2-bromoisocryptolepine **70**(175 mg, 0.56 mmol) and *N*-bromosuccinimide (106 mg, 0.59 mmol) in DMF (7 mL). The reaction mixture was heated for 20 hours and the product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:2:1) to give 2,8-dibromoisocryptolepine **106** as a yellow crystalline solid (155 mg, 71%).

Mp: 324-326 ºC.

$^1$H NMR (400 MHz, $d_6$-DMSO) δ: 4.29 (3H, s, $CH_3$), 7.55 (1H, dd, *J* = 8.8, 2.2 Hz, H-9), 7.74 (1H, d, *J* = 8.8 Hz, H-10), 8.00 (1H, dd, *J* = 8.8, 2.2 Hz, H-3), 8.05 (1H, d, *J* = 9.2 Hz, H-4), 8.33 (H, d, *J* = 2.0 Hz, H-7 ), 8.83 (1H, d, *J* = 2.0 Hz, H-1), 9.44 (1H, s, H-6).

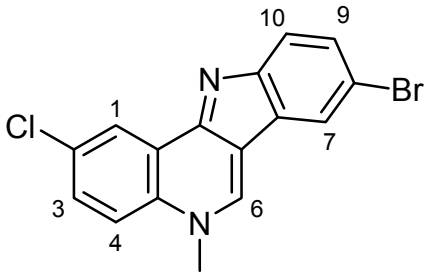
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.4 ($NCH_3$), 112.2 (C-8), 115.6 (C-6a), 118.2 (C-2), 120.3 (C-4 and C-10), 122.2 (C-7), 122.4 (C-11b), 125.6 (C-1), 127.4 (C-6b), 128.0 (C-9), 131.9 (C-3), 134.3 (C-4a), 139.6 (C-6), 151.8 (C-11a), 152.9 (C-10a).

MS (EI): 215 (18), 309 (21), 311 (20), 388 (52, $[M]^+$), 390 (100), 391 (19), 392 (50).

HRMS (EI): 387.9189 ($C_{16}H_{10}N_2Br_2$ $[M]^+$ requires 387.9211).

IR (KBr) $\nu_{max}$: 800, 1223, 1335, 1372, 1436, 1480, 1640, 3360.

**8-Bromo-2-chloroisocryptolepine 107**




The above compound was prepared as for compound **104** but starting from 2-chloroisocryptolepine **71** (116 mg, 0.44 mmol) and *N*-bromosuccinimide (86.6 mg, 0.49 mmol) in DMF (5 mL). The reaction mixture was heated for 2 hours and the product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:4:1) to give 8-bromo-2-chloroisocryptolepine **107** as a yellow crystalline solid (118 mg, 77%).

Mp: 265-266 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.22 (3H, s, $NCH_3$), 7.54 (1H, dd, *J* = 8.4, 2.1 Hz, H-9), 7.73 (1H, d, *J* = 8.4 Hz, H-10), 7.85 (1H, dd, *J* = 9.6, 2.7 Hz, H-3), 8.08 (1H, d, *J* = 9.6 Hz, H-4), 8.30 (H, d, *J* = 2.4 Hz, H-7), 8.66 (1H, d, *J* = 2.4 Hz, H-1), 9.36 (1H, s, H-6).

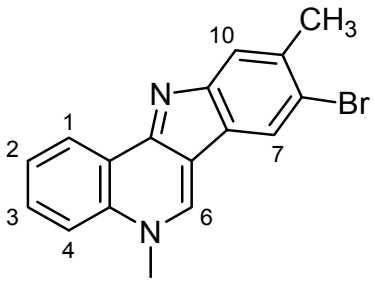
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.4 ($NCH_3$), 112.1 (C-8), 115.5 (C-6a), 120.0 (C-4), 120.2 (C-10), 122.0 (C-11b) 122.1 (C-7), 122.4 (C-1), 127.4 (C-6b), 127.9 (C-9), 129.1 (C-3), 129.9 (C-2), 133.9 (C-4a), 139.3 (C-6), 151.8 (C-11a), 153.0 (C-10a).

MS (EI): 188 (17), 215 (21), 310 (22), 312 (22), 344 (72, $[M]^+$), 346 (100), 347 (19), 348 (25).

HRMS (EI): 343.9727 ($C_{16}H_{10}N_2ClBr$ $[M]^+$ requires 343.9716).

IR (KBr) $\nu_{max}$: 805, 1224, 1337, 1374, 1447, 1482, 1641, 3070, 3356.

**8-Bromo-9-methylisocryptolepine 108**




The above compound was prepared as for compound **104** but starting from 9-methylisocryptolepine **97** (160 mg, 0.65 mmol) and *N*-bromosuccinimide (137 mg, 0.77 mmol) in DMF (7 mL). The reaction mixture was heated for 20 hours and the product was purified by silica flash column chromatography eluting with a mixture of dichloromethane, ethanol and aqueous ammonia (100:0:1 increasing to 100:2:1) to give 8-bromo-9-methylisocryptolepine **108** as a yellow crystalline solid (168 mg, 80%).

Mp: 266-267 ºC.

$^{1}$H NMR (400 MHz, $d_6$-DMSO) δ: 2.53 (3H, s, $CH_3$), 4.23 (3H, s, $NCH_3$), 7.70 (1H, t, *J* = 7.2 Hz, H-2), 7.75 (1H, s, H-10), 7.84 (1H, ddd, *J* = 8.4, 7.2, 1.4 Hz, H-3), 8.03 (1H, d, *J* = 8.8 Hz, H-4), 8.32 (1H, s, H-7), 8.74 (1H, dd, *J* = 8.0, 1.2 Hz, H-1), 9.32 (1H, s, H-6).

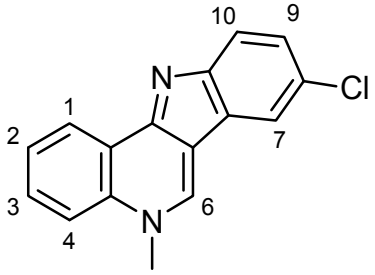
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 23.2 ($CH_3$), 42.1 ($NCH_3$), 114.9 (C-8), 115.3 (C-6a), 117.4 (C-4), 120.1 (C-10), 121.0 (C-11b), 122.5 (C-7), 123.8 (C-1), 125.1 (C-2), 125.4 (C-6b), 129.2 (C-3), 133.1 (C-9), 135.4 (C-4a), 138.3 (C-6), 153.3 (C-11a), 154.1 (C-10a).

MS (EI): 98 (54), 229 (21), 230 (21), 245 (54), 246 (16), 324 (100, $[M]^+$, $^{79}$Br), 326 (94, $[M]^+$, $^{81}$Br), 327 (17).

HRMS (EI): 324.0255 ($C_{17}H_{13}N_2Br$ $[M]^+$ requires 324.0262).

IR (KBr) $\nu_{max}$: 753, 1148, 1226, 1245, 1393, 1453, 1598, 1620, 2921, 3232

**8-Chloroisocryptolepine 109**




The above compound was prepared as for compound **104** but starting from isocryptolepine **16** (222 mg, 0.96 mmol) and *N*-chlorosuccinimide (146 mg, 1.09 mmol) in DMF (5 mL). The reaction mixture was heated for 20 hours and the product 8-chloroisocryptolepine **109** was obtained as a yellow crystalline solid (99 mg, 41%).

This compound was reported during the course of this project and the NMR and MS data acquired was consistent with that published in the literature.[93]

Mp: 257-259 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.25 (3H, s, $CH_3$), 7.40 (1H, dd, *J* = 9.0, 2.1 Hz, H-9), 7.72 (1H, t, *J* = 7.5 Hz, H-2), 7.77 (1H, d, *J* = 9.0 Hz, H-10), 7.85 (1H, ddd, *J* = 8.5, 7.0, 1.2 Hz, H-3), 8.05 (1H, d, *J* = 8.4 Hz, H-4), 8.16 (1H, d, *J* = 2.4 Hz, H-7), 8.75 (1H, dd, *J* = 7.8, 1.2 Hz, H-1), 9.38 (1H, s, H-6).

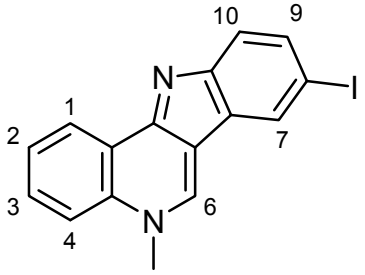
$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 42.6 ($NCH_3$), 114.8 (C-6a), 117.8 (C-4), 118.7 (C-10), 119.2 (C-7), 120.2 (C-11b), 123.8 (C-1), 124.3 (C-8), 125.4 (C-2), 125.9 (C-9), 126.2 (C-6b), 129.9 (C-3), 135.4 (C-4a), 140.0 (C-6), 150.5 (C-11a), 151.6 (C-10a).

MS (EI): 266 (100, $[M]^+$, $^{35}$Cl), 267 (20), 268 (33, $[M]^+$, $^{37}$Cl).

HRMS (EI): 266.0602 ($C_{16}H_{11}N_2Cl$ $[M]^+$ requires 266.0611).

IR (KBr) $\nu_{max}$: 760, 1118, 1223, 1342, 1444, 1610, 1636, 3027, 3200.

**8-Iodoisocryptolepine 113**




To a 1:1 solution of concentrated nitric acid (69%) and glacial acetic acid (10 mL) isocryptolepine methiodide **16.HI** (215 mg, 0.60 mmol) was added and the reaction mixture stirred at room temperature for 24 hours. The reaction was quenched with water and basified with aqueous sodium hydroxide solution (10%). The precipitate obtained was collected by vacuum filtration (washing with water) and recrystallised from methanol to give 8-iodoisocryptolepine **113** as an orange crystalline solid (122 mg, 57%).

Mp: 260-261 ºC.

$^{1}$H NMR (600 MHz, $d_6$-DMSO) δ: 4.22 (3H, s, $NCH_3$), 7.62 (1H, d, *J* = 8.4 Hz, H-10), 7.66 (1H, dd, *J* = 8.4, 1.8 Hz, H-9), 7.70 (1H, br t, *J* = 7.2 Hz, H-2), 7.82 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-3), 8.01 (1H, d, *J* = 8.4 Hz, H-4), 8.46 (1H, d, *J* = 1.8 Hz, H-7), 8.75 (1H, dd, *J* = 8.4, 1.5 Hz, H-1), 9.34 (1H, s, H-6).

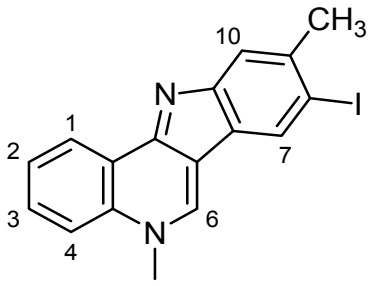
$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 42.5 ($NCH_3$), 83.2 (C-8), 115.0 (C-6a), 117.8 (C-4), 120.7 (C-10), 121.0 (C-11b), 124.0 (C-1), 125.6 (C-2), 128.2 (C-7), 128.4 (C-6b), 129.6 (C-3), 133.4 (C-9), 135.6 (C-4a), 139.3 (C-6), 152.7 (11a), 153.4 (C-10a).

MS (EI): 231 (29), 232 (19), 358 (100, $[M]^+$), 359 (18).

HRMS (EI): 357.9970 ($C_{16}H_{11}N_2I$ $[M]^+$ requires 357.9967).

IR (KBr) $\nu_{max}$: 749, 803, 1116, 1219, 1385, 1637, 2923, 3400.

**8-Iodo-9-methylisocryptolepine 114**




The above compound was prepared as for compound **113** but starting from 9-methylisocryptolepine methiodide **97.HI** (103 mg, 0.27 mmol) and glacial acetic acid (6 mL). The reaction mixture was stirred for 96 hours and the product 8-iodo-9-methylisocryptolepine **114** was obtained as an orange crystalline solid (72 mg, 71%).

Mp: 244-245 ºC.

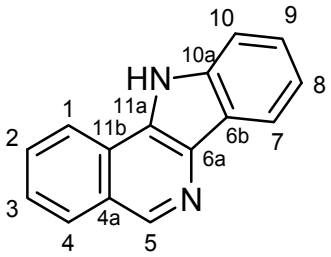
$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ: 2.54 (3H, s, $CH_3$), 4.25 (3H, s, $NCH_3$), 7.77-7.87 (3H, m, H-2, H-3 and H-10), 8.07 (1H, d, *J* = 8.8 Hz, H-4), 8.62 (1H, s, H-7), 8.77 (1H, br d, *J* = 6.8 Hz, H-1), 9.36 (1H, s, H-6).

$^{13}$C NMR (50 MHz, $d_6$-DMSO) δ: 28.3 ($CH_3$), 42.1 ($NCH_3$), 90.0 (C-8), 114.9 (C-6a), 117.4 (C-4), 119.5 (C-10), 120.9 (C-11b), 123.8 (C-1), 125.1 (C-2), 126.1 (C-6b), 129.0 (C-7), 129.2 (C-3), 135.4 (C-4a), 136.2 (C-9), 138.2 (C-6), 153.1 (C-11a), 155.0 (C-10a).

MS (EI): 245 (45), 246 (15), 372 (100, $[M]^+$), 373 (20).

IR (KBr) $\nu_{max}$: 753, 1122, 1227, 1245, 1372, 1389, 1450, 1641, 3228.

**11*H*-Indolo[3,2-*c*]isoquinoline 117**




The above known compound was prepared *via* two different synthetic methods, which were both adaptations of previously reported synthetic procedures, and are summarised as follows. [82,94]

*Method 1:* As for compound **36** (Method 1) but starting from 4-(2-bromophenylamino)isoquinoline **120** (470 mg, 1.57 mmol), $Pd(OAc)_2$ (35 mg, 10 mol%), BINAP (101 mg, 10 mol%) and potassium carbonate (4.28 g, 30.94 mmol) in

dry DMF (14 mL). The product was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and dichloromethane (5:95 increasing to 100:0) to give 11*H*-indolo[3,2-*c*]isoquinoline **117** as a pale yellow solid (199 mg, yield 58%).

*Method 2:* As for compound **36** (Method 2) but starting from 4-bromoisoquinoline **118** (400 mg, 1.93 mmol), 2-bromoaniline (404 mg, 2.35 mmol), $Pd(OAc)_2$ (46 mg, 10 mol%), BINAP (130 mg, 11 mol%) and potassium carbonate (5.35 g, 38.68 mmol) in dry DMF (20 ml). The product was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and hexane (1:9 increasing to 0:10) to give 11*H*-indolo[3,2-*c*]isoquinoline **117** as a pale yellow solid (238 mg, yield 57%).

The spectroscopic data acquired was consistent with that published in the literature.[82,146]

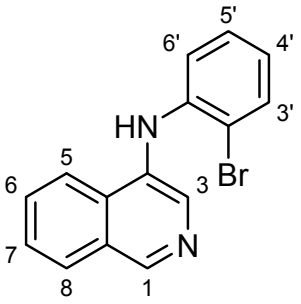
Mp: >300 ºC (lit.,[82] >300 ºC).

$^1$H NMR (400 MHz, $d_6$-DMSO) δ: 7.31 (1H, ddd, *J* = 7.8, 7.0, 0.8 Hz, H-8), 7.49 (1H, ddd, *J* = 8.3, 7.2, 1.2 Hz, H-9), 7.69 (1H, d, *J* = 8.0 Hz, H-10), 7.71 (1H, ddd, *J* = 8.0, 7.2, 0.8, H-3), 7.91 (1H, ddd, *J* = 8.2, 7.0, 1.2 Hz, H-2), 8.23 (1H, d, *J* = 8.0 Hz, H-7), 8.28 (1H, d, *J* = 8.0 Hz, H-4), 8.51 (1H, dd, *J* = 8.4, 0.8 Hz, H-1), 9.12 (1H, s, H-5).

$^{13}$C NMR (100 MHz, $d_6$-DMSO) δ: 111.9 (C-10), 119.3 (C-7), 119.8 (C-8), 121.1 (C-1), 122.7 (C-6b), 123.5 (C-11b), 125.5 (C-9), 126.2 (C-3), 126.5 (C-4a), 127.3 (C-6a), 128.6 (C-4), 130.0 (C-2), 133.5 (C-11a), 138.5 (C-10a), 144.6 (C-5).

IR (KBr) $\nu_{max}$: 741, 1220, 1320, 1368, 1459, 1525, 1635, 2980, 3000.

**4-(2-Bromophenylamino)isoquinoline 120**




The above compound was prepared as for compound **37** but starting from 4-bromoisoquinoline **118** (504 mg, 2.42 mmol), 2-bromoaniline (511 mg, 2.97 mmol), $Pd_2(dba)_3$ (110 mg, 5 mol%), XANTPHOS (146 mg, 10 mol%) and caesium

carbonate (2.36 g, 7.26 mmol) in dry dioxane (10 mL). The product was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and dichloromethane (5:95 increasing to 100:0) to give 4-(2-bromophenylamino)-isoquinoline **120** as a reddish brown solid (487 mg, 67%).

The above compound was reported during the course of this project and the NMR data acquired was consistent with that published in the literature.[82]

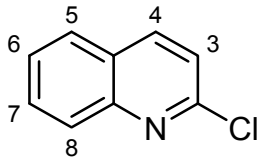
Mp: 94-96 ºC (lit.,[82] 94-96 ºC).

$^{1}$H NMR (400 MHz, $CDCl_3$) δ: 6.51 (1H, br s, NH), 6.81 (1H, ddd, *J* = 8.2, 7.0, 1.2 Hz, H-4′), 6.91 (1H, dd, *J* = 8.2, 1.4 Hz, H-6′), 7.13 (1H, ddd, *J* = 8.4, 7.2, 1.2 Hz, H-5′), 7.58 (1H, dd, *J* = 7.8, 1.4 Hz, H-3′), 7.70 (1H, t, *J* = 8.0 Hz, H-7), 7.78 (1H, ddd, *J* = 8.3, 7.1, 1.2 Hz, H-6), 8.04 (1H, d, *J* = 8.4. Hz, H-5), 8.06 (1H, d, *J* = 7.6 Hz, H-8), 8.41 (1H, br s, H-3), 9.07 (1H, br s, H-1).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 110.6, 114.7, 120.1, 120.7, 126.8, 127.2, 127.5, 129.6, 130.0, 132.0, 136.7, 141.7, 148.3.

IR (KBr) $\nu_{max}$: 736, 753, 782, 1025, 1315, 1410, 1470, 1499, 1560, 2956, 3162.

**2-Chloroquinoline 122**




The above known compound was prepared *via* an adaptation of previously reported synthetic procedures which is summarised as follows.[150,151]

i) To a solution of quinoline **125** (4.62 g, 35.79 mmol) in glacial acetic acid (11 mL), hydrogen peroxide (30%, 4 mL) was added and the mixture heated at 70 - 80 °C for 19 hours. The solution was allowed to cool, quenched with iced water, rendered alkaline with saturated aqueous sodium carbonate solution and extracted with DCM (3 × 200 mL). The combined extracts were washed with aqueous hydrochloric acid (1M, 100 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue obtained was purified by silica flash column chromatography eluting with a mixture of ethyl acetate and hexane (1:1 increasing to 1:0) to give quinoline *N*-oxide **126** as pale brown solid (1.70 g, 33%).

Mp: 63-64ºC (lit.,[150] 60-62 ºC).

ii) Phosphorus oxychloride (6.5 mL, 71.01 mmol) was added to compound **126** (1.70 g, 11.73 mmol) at 0 °C and the mixture refluxed for 20 hours. The reaction

mixture was quenched with iced water, rendered alkaline with aqueous sodium hydroxide solution (10%) and extracted with DCM (3 × 100 mL). The combined extracts were dried ($Na_2SO_4$) and the solvent removed *in vacuo*. The residue obtained was purified by silica flash column chromatography eluting with a mixture of dichloromethane and hexane (1:1 increasing to 4:1) to give 2-chloroquinoline **122** as a yellow solid (333 mg, 17%).

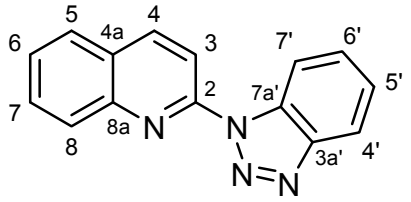
The spectroscopic data acquired was consistent with that published in the literature.[151]

Mp: 33-34°C (lit.,[151] 36-38°C).

$^1$H NMR (200 MHz, $CDCl_3$) δ: 7.43 (1H, d, *J* = 8.8 Hz, H-3), 7.61 (1H, ddd, *J* = 8.1, 7.0, 1.1 Hz, H-6), 7.79 (1H, ddd, *J* = 8.4, 7.0, 1.4 Hz, H-7), 7.86 (1H, d, *J* = 8.0 Hz, H-5), 8.08 (1H, d, *J* = 8.4 Hz, H-8), 8.15 (1H, d, *J* = 8.4 Hz, H-4).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 121.5, 126.0, 126.1, 126.7, 127.7, 129.7, 138.0, 147.0, 149.8.

**2-(1-Benzotriazolyl)quinoline 123**




The above known compound was prepared as for compound **35** but starting from 2-chloroquinoline **122** (596 mg, 3.64 mmol) and benzotriazole (492 mg, 4.13 mmol). The product was recrystallised from methanol to give 2-(1-benzotriazolyl)quinoline **123** as a pale green crystalline solid (673 mg, 75%).

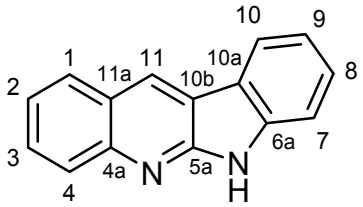
The spectroscopic data acquired was consistent with that published in the literature.[183,184]

Mp: 144-146ºC (lit.,[183] 148-149 ºC).

$^1$H NMR (200 MHz, $CDCl_3$) δ: 7.55-7.68 (2H, m, H-6′ and H-6), 7.74 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, H-5′), 7.85 (1H, ddd, *J* = 8.4, 7.0, 1.4 Hz, H-7), 7.95 (1H, d, *J* = 8.0 Hz, H-5), 8.19-8.24 (2H, m, H-4′ and H-8), 8.42 (1H, d, *J* = 9.2 Hz, H-4), 8.55 (1H, d, *J* = 9.2 Hz, H-3), 9.02 (1H, d, *J* = 8.4 Hz, H-7′).

$^{13}$C NMR (50 MHz, $CDCl_3$) δ: 112.5, 114.6, 119.0, 124.3, 125.8, 126.2, 126.9, 127.9, 128.1, 129.7, 130.8, 138.3, 145.7, 146.1, 149.6.

**6*H*-Indolo[2,3-*b*]quinoline 124**




The above known compound was prepared as for compound **36** (Method 3) but starting from 2-(1-benzotriazolyl)quinoline **123** (441 mg, 1.79 mmol) and polyphosphoric acid (15.27 g). The reaction mixture was heated at 150 °C for 2 hours. The product 6*H*-indolo[3,2-*b*]quinoline **124** was obtained as a pale yellow solid (130 mg, 33%).

The spectroscopic data acquired was consistent with that published in the literature.[85,185]

Mp: >300 ºC (lit.,[85] >300 ºC).

$^{1}$H NMR (200 MHz, $d_6$-DMSO) δ:7.33 (1H, t, *J* = 7.8 Hz, H-9), 7.48-7.57 (3H, m, H-7, H-8 and H-2), 7.76 (1H, t, *J* = 7.1 Hz, H-3), 8.02 (1H, d, *J* = 8.4 Hz, H-4), 8.15 (1H, d, *J* = 8.0 Hz, H-1), 8.30 (1H, d, *J* = 7.6 Hz, H-10), 9.08 (1H, s, H-11), 11.75 (1H, s, NH).

$^{13}$C NMR (100 MHz, $CDCl_3$) δ: 111.0, 117.9, 119.7, 120.3, 121.8, 122.8, 123.7, 127.0, 127.6, 128.2, 128.7, 141.5, 146.4, 152.9.

## 6.3. General Procedures for Optimisation Experiments

### 6.3.1. Buchwald-Hartwig and Domino Reactions

To a degassed solution of a palladium catalyst and ligand in dry dioxane or DMF (2.5 mL), the appropriate quinoline (**33** or **59**; 0.24 mmol), aniline (2-choroaniline or 2-bromoaniline; 0.30 mmol) and base were added. The flask was flushed with nitrogen and the mixture heated at the temperature indicated for 24 hours (unless otherwise stated). At specific time intervals 0.1 mL aliquots were removed and filtered through celite (washed with dichloromethane; 3 mL). The solvent was removed *in vacuo* and the residue obtained made up to 20 mL with methanol. These solutions were filtered and analysed *via* HPLC (using the HPLC conditions outlined in Section 6.1). After a correction factor had been applied to the peak areas the percentage conversion was determined for the appropriate product in each reaction by dividing the peak area by the sum of reactants and products. The correction factor was determined *via* the difference in absorbance of the compounds from analysis of an appropriate standard. One standard contained 4-bromoquinoline **59** (478 μM), 4-(2-bromophenylamino)quinoline **60** (478 μM) and 11*H*-indolo[3,2-*c*]quinoline **36** (475 μM). The other contained 4-chloroquinoline **33** (611 μM), 4-(2-chlorophenylamino)quinoline **37** (613 μM) and 11*H*-indolo[3,2-*c*]quinoline **36** (594 μM).

### 6.3.2. Intramolecular C-H Arylation Reactions

To a degassed solution of a palladium catalyst and ligand in dry dioxane or DMF (2.5 mL), 4-(2-chlorophenylamino)quinoline **37** or 4-(2-bromophenylamino)quinoline **60** (0.24 mmol) and base were added. The flask was flushed with nitrogen and the mixture heated at the temperature indicated for 24 hours (unless otherwise stated). At specific time intervals 0.1 mL aliquots were removed and filtered through celite (washed with dichloromethane; 3 mL). The solvent was removed *in vacuo* and the residue made up to 20 mL with methanol. These solutions were filtered and then analysed *via* HPLC (using the HPLC conditions outlined in Section 6.1). The percentage conversion was determined for the appropriate product as detailed above for the Buchwald-Hartwig and Domino Reactions.

### 6.3.3. Bromination of Isocryptolepine 16 or MIQ 31

To a solution of isocryptolepine **16** or MIQ **31** (0.13 mmol) in glacial acetic acid (3 mL), bromine was added. The solution was stirred at the indicated temperature for 24 hours. At specific time intervals 0.1 mL aliquots were removed and made up to 20 mL with methanol. These solutions were filtered and then analysed *via* HPLC (using the HPLC conditions outlined in Section 6.1).

### 6.3.4. Nitration of Isocryptolepine 16

To a 1:1 solution of concentrated nitric acid (69% or 90%) and glacial acetic acid (3 ml), isocryptolepine **16** (0.13 mmol) was added and the mixture stirred at the indicated temperature for 24 hours. At specific time intervals 0.1 mL aliquots were removed and made up to 20 mL with methanol. These solutions were filtered and then analysed *via* HPLC (using the HPLC conditions outlined in Section 6.1).

## 6.4. General Procedures for Determination of Physicochemical Properties

### 6.4.1. Purity

Isocryptolepine derivatives were prepared as hydrochloride salts by dissolving the free base in a minimal amount of methanol and the dropwise addition of concentrated hydrochloric acid. The volume of solvent was reduced *in vacuo* and the resultant precipitate was collected by filtration and dried under high vacuum. Purity analysis was performed *via* HPLC (using the HPLC conditions outlined in Section 6.1). All compounds had purity of greater than 96.5% - refer to Table 6.1 for further details.

### 6.4.2. Ionisation constant ($pK_a$)

$pK_a$ values were obtained spectrophotometrically according to literature methods.[75,133,163] Stock solutions of neocryptolepine hydrochloride **19.HCl** (0.95 mM) and MIQ hydrochloride **31.HCl** (0.70 mM) were prepared in methanol. Dilute solutions were subsequently prepared in a phosphate buffer at pH 5 and 0.1 M NaOH; **19.HCl** (4.74 μM) and **31.HCl** (7.00 μM). The UV spectrum of the pH 5 buffer (wherein the compound is fully ionised) and NaOH (wherein the compound is fully unionised) were recorded and the wavelength where the pH 5 buffer had a $\lambda_{max}$ was chosen for further investigation; **19.HCl** (280 nm) and **31.HCl** (288 nm). A further range of solutions in phosphate and borax buffers were prepared and photometrically measured at the previously indicated wavelengths; **19.HCl** (pH 7.2 - 9.5), **31.HCl** (pH 8.1 - 10). The pH is related to absorbance by the equation shown in Equation 6.1 and the values A(I) and A(U) signify the absorbance of the fully ionised and fully unionised solutions respectively.

$$\mathrm{p}K_a = \mathrm{pH} + \log\left[\frac{[A - A(\mathrm{I})]}{[A(\mathrm{U}) - A]}\right]$$

**Equation 6.1:** Equation used to estimate $pK_a$[163]

pH was plotted as a function of $\log\left[\frac{[A-A(\mathrm{I})]}{[A(\mathrm{U})-A]}\right]$, for each compound, and the point of intersection with the y-axis gave the compound $pK_a$ values. This experiment was

conducted separately twice for both compounds and the average taken, where the error is represented by the standard deviation of the mean.

## 6.5. General Procedures for Biological Assays

### 6.5.1. Antimalarial Evaluation

**Parasite Cultures**

The laboratory-adapted *Plasmodium falciparum* strains 3D7 and W2mef were cultured in RPMI 1640 HEPES media (Sigma Aldrich) by Ms Rina Wong (Fremantle Unit, School of Medicine and Pharmacology; UWA) according to literature methods.[157,186,187] Parasite cultures were supplemented with 92.6 mg $L^{-1}$ L-glutamine (Sigma Aldrich), 500 μg $L^{-1}$ gentamicin, 50 mg $L^{-1}$ L-hypoxanthine (Sigma Aldrich) and 10% v/v pooled human plasma. Cultures were incubating at 37 °C in a low oxygen atmosphere of (3 - 7%).

**Compound Solutions**

Stock solutions of chloroquine diphosphate (Sigma Aldrich) were freshly prepared in distilled water (100 mM). Stock solutions of hydrochloride salts were prepared in 50% v/v or 80% v/v DMSO in distilled water (5 - 15 mM; Table 6.1). On the day of testing aliquots were freshly diluted with RPMI (without hypoxanthine) to a working standard and added in triplicate to 96-well plates. Further two-fold serial dilutions were conducted (final concentrations of chloroquine; 25 - 1600 nM, final concentrations of isocryptolepines; 8 - 6000 nM).

***In Vitro* Antimalarial Activity**

The [$^3$H]-hypoxanthine growth inhibition assay was used to determine i*n vitro* antimalarial activity and was conducted by Ms Rina Wong, with the assistance of the candidate, according to literature methods.[156,157] To the serial diluted compound-media solutions, infected erythrocytes (final 0.5% parasitemia and 1.5% hematocrit) and [$^3$H]-hypoxanthine (Perkin Elmer; final concentration of 0.5 μCi/well) were added. The plates were incubated for 48 hours and then underwent a freeze-thaw process before harvesting onto 96-well glass-fibre filtermats using a Havaster 96 (Tomtec Incorporated). Filtermats were counted on a 1450 Microbeta Plus liquid scintillation counter (Wallac). The assay was performed a minimum of three separate times on each compound for both strains, chloroquine diphosphate was used as a positive control for antiplasmodial activity and drug-free controls (uninfected and infected) were included in each test.

**Table 6.1:** Purity, cross-resistance and biological assay data for selected isocryptolepine derivatives

| Compound | **Purity** | **Cross-resistance** | | **Antimalarial assay** | **Cytotoxicity assay** |
|---|---|---|---|---|---|
| | Purity; % (Rt; min) [a] | Spearman *r* (XY pairs) | P value | Stock conc.; mM (tested conc. range; µM) | Stock conc.; mM (tested conc. range; µM) |
| Isocryptolepine **16** | 99.9 (5.67) | 0.67 (9) | 0.059 | 10 [b] (6-0.094) | 10 [b] (100-0.001) |
| MIQ **31** | 99.1 (5.20) | 0.83 (9) | 0.026 | 10 [b] (2-0.016) | 10 [b] (100-0.001) |
| 3-Chloroisocryptolepine **64** | 98.9 (6.32) | 0.73 (9) | 0.031 | 5 [c] (2-0.031) | 5 [c] (100-0.001) |
| 9-Methylisocryptolepine **97** | 98.7 (6.59) | 0.71 (6) | 0.136 | 10 [b] (2-0.031) | 10 [b] (100-0.001) |
| 8-Bromoisocryptolepine **104** | 99.5 (6.97) | 0.75 (9) | 0.026 | 10 [b] (2-0.016) | 10 [b] (100-0.001) |
| 8-Bromo-3-chloroisocryptolepine **105** | 97.6 (7.54) | 0.75 (7) | 0.066 | 5 [c] (0.5-0.008) | 5 [c] (100-0.001) |
| 2,8-Dibromoisocryptolepine **106** | 96.5 (8.15) | 0.43 (6) | 0.419 | 5 [c] (0.5-0.008) | 5 [c] (100-0.001) |
| 8-Bromo-2-chloroisocryptolepine **107** | 98.3 (7.99) | 0.89 (6) | 0.033 | 5 [c] (0.5-0.008) | 5 [c] (100-0.001) |
| 8-Bromo-9-methylisocryptolepine **108** | 97.5 (8.01) | 0.48 (8) | 0.243 | 15 [b] (2-0.031) | 10 [b] (100-0.001) |
| 8-Chloroisocryptolepine **109** | 97.6 (6.50) | 0.70 (9) | 0.043 | 10 [b] (2-0.031) | 10 [b] (100-0.001) |
| Chloroquine **2** | - | - | - | 100 [d] (1.6-0.025) | 100 [d] (100-0.1) |

[a] Rt: retention time. [b] Stock in 50% DMSO in water. [c] Stock in 80% DMSO in water. [d] Stock in water.

**Data Analysis**

$IC_{50}$ values were determined by Ms Rina Wong *via* non-linear regression analysis of log-dose response curves (Graphpad Prism 4.0).

**Cross-resistance Analysis**

Chloroquine cross-resistance with isocryptolepines was estimated by Ms Rina Wong *via* the Spearman correlation coefficient[160] where the significance level (P) was set at 0.05 (two-tailed) and results are shown in Table 6.1.

### 6.5.2. Cytotoxicity Evaluation

**Cell Cultures**

3T3 cells (mouse embryonic fibroblasts) were cultured and maintained in RPMI 1640 media (Invitrogen) by Ms Erin Bolitho (Technology Park, School of Pharmacy; Curtin University). Cell cultures were supplemented with 2 mM L-alanyl-L-glutamine (GlutaMAX; Invitrogen), 100 units $mL^{-1}$ penicillin (Invitrogen), 100 μg $mL^{-1}$ streptomycin (Invitrogen) and 10% v/v fetal calf serum. 24 hours prior to testing cells were added to 96-well plates pre-coated with 1% gelatine at 7500 cells per well (final well volume 100 μL) and incubated at 37 °C in a 5% $CO_2$ humidified atmosphere.

**Compound Solutions**

Stock solutions were prepared as for the antimalarial evaluation. On the day of testing aliquots were freshly diluted with RPMI to a working standard and serial dilutions subsequently conducted by Ms Erin Bolitho (final concentrations of chloroquine; 0.1 - 1000 μM, final concentrations of isocryptolepines; 0.001 - 100 μM).

***In Vitro* Cytotoxicity**

The MTT colorimetric assay was used to determine *in vitro* cytotoxicity and was conducted by Ms Erin Bolitho according to literature procedures.[164,165] 100 μL of each compound-media solution was added in quadruplicate to the 96-well plates. The potential DMSO effect was countered by including a vehicle control arm to the experiments. Plates were incubated for 48 hours, media was removed from each well and 100 μL of 1 mg $mL^{-1}$ MTT (Sigma) in RPMI was added. Plates were incubated

for 60 minutes after which time MTT solution was removed and 100 μL DMSO added to each well. The absorbance of each plate solution was measured using an automated plate reader (Biorad) at a wavelength of 595 nm. The assay was performed a minimum of three separate times for each compound, isocryptolepine hydrochloride **16.HCl** was used as a positive control for cytotoxicity and drug-free controls were included in each test.

**Data Analysis**

$IC_{50}$ values were determined by Dr Simon Fox *via* nonlinear regression analysis of log-dose response curves, after correction for the DMSO affects (Graphpad Prism 4.0).

# Chapter 7

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# Appendices

## Appendix 1: Copyright Permission

**Figure 1.1:** The life cycle of the *Plasmodium* parasite