Towards the Synthesis of Makaluvamine-analogues

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 17 December 2014
Abstract

Cancer is one of the leading causes of death in developed countries and rising fast as a cause of death in developing countries. The increase of cancer prevalence in developing countries can be attributed to westernisation trends, with lifestyle cancers such as colorectal and lung cancer being amongst the most commonly reported malignant neoplasms. This means that the development of novel methods of treatment is essential in combatting this disease in the developing world. Combinational chemotherapy is one of the best candidates for treatment, but it is reliant on effective compounds targeting different modes of action. It also means that these compounds should be easily and cheaply available.

Makaluvamines have been identified as a class of compounds that may have a novel mode of action on top of being known as topoisomerase II inhibitors. This study attempted to devise a short and concise synthetic strategy, based on reported procedures, to construct makaluvamine C analogues. This involved the introduction of a methyl group to an indole intermediate (7,8-dimethoxy-1,3,4,5-tetrahydropyrrolo[4,3,2-de]quinoline), before oxidation to a quarternized pyrroloiminoquinone (7-methoxy-5-methyl-8-oxo-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-5-ium chloride). The introduction of this methyl group proved problematic, as the indole substrate proved to be difficult to handle and tended to degrade under reaction conditions. The lack of initial success prompted the deviation from the initial route by quarternizing a quinoline intermediate to form a quinolinium iodide salt (4-(dimethoxymethyl)-6,7-dimethoxy-1-methyl-5-nitroquinolin-1-ium iodide). Upon reduction to give 4-(dimethoxymethyl)-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroquinolin-5-amine, it was discovered that the subsequent ring-closing reaction to produce 7,8-dimethoxy-5-methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-de]quinoline was still problematic.

The synthesis of the target compounds has not yet been successfully completed, but will still be pursued so these compounds can be evaluated for their anticancer activity and have their mode of action tested.
Uittreksel

Kanker lewer van die grootste bydrae tot mortaliteit in ontwikkelde lande en is vining aan die toeneem in ontwikkelende lande. Die toename van kanker voorvalle in ontwikkelende lande kan toegedra word aan die verwesteringsstendens, met kankers soos kolo-rektale- en long kanker onder die mees algemene kwaadaardige neoplasmsas wat aangemeld word – kankers wat gekoppel word aan leefstyl keuses. Dit beteken dat daar ’n dringende nood is aan nuwe metodes van behandeling van die siekte in ontwikkelende lande. Kombinasie chemoterapie is een van die beste kandidate vir behandel, sienende dat dit gebruik maak van middels was verskillende aspekte van die siekte uitbuit. Om effektief te wees, moet die antikanker middels goedkoop en maklik beskikbaar te wees.

Makaluvamines is geïdentificeer as ’n klas van antikanker middele wat moontlik ’n nuwe metode van inhibisie het, tesame met hul topoïsomerase II inhibisie. Hierdie study het daarom gepoog om ’n korter en meer bondige sintetiese roete saam te stel, wat gebaseer is op literatuur prosedures, om analoë van makaluvamine C te produseer. Dit het die aanhegging van ’n metiel group aan ’n indool tussenproduk (7,8-dimetoksie-1,3,4,5-tetrahidropirolo[4,3,2-de]kinolien) behels, gevolg deur die oksidasie tot die kwaternêre piroloiminokwinoon (7-metoksie-5-metiel-8-oxo-1,3,4,8-tetrahidropirolo[4,3,2-de]kinolin-5-ium chloried). Om hierdie metiel groep aan te voeg was, nietemin, problematies, aangesien die indool produk moeilik was om te hanteer sienende dat dit onder reaksie toestande gedegradeer het. Die aanvanklike onsuksesvolle pogings het daartoe gelei dat die sintetiese roete herdink was en is aangepas om eerder gebruik te maak van ’n kinolinium jodied sout (4-(dimetoksiemetiel)-6,7-dimetoksie-1-metiel-5-nitrokinolin-1-ium jodied). Die reduksie van hierdie sout en agtereenvolgende annulasie reaksie om 7,8-dimetoksie-5-metiel-1,3,4,5-tetrahidropirolo[4,3,2-de]kinolien te vorm was egter steeds problematies.

Die sintese van die beoogde produktes was tot dusver nog nie suksesvol nie, maar sal egter steeds aangedurf word om hulle ten einde suksesvol te sintetiseer en dan te stuur om hulle biologiese eienskappe te toets. Dit sluit hulle antikanker aktiwiteit in asook hul metode van inhibisie.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TSG</td>
<td>Tumour Suppressor Gene</td>
</tr>
<tr>
<td>HDI</td>
<td>Human Development Index</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>The concentration where cellular response is reduced by 50%</td>
</tr>
<tr>
<td>m-AMSA</td>
<td>Amsacrine</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol 3′-kinase</td>
</tr>
<tr>
<td>FAS</td>
<td>Fatty Acid Synthase</td>
</tr>
<tr>
<td>CAN</td>
<td>Cerium Ammonium Nitrate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>MVK</td>
<td>Methyl vinyl ketone</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>IBX</td>
<td>2-Iodoxybenzoic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
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Chapter 1

1.1 Introduction

Cancer is caused when there are mutations in genes with high cancer susceptibility, such as oncogenes and tumour-suppressor genes (TSGs), and genes that generally cause genetic instability.\(^1\) It is currently one of the largest diseases worldwide, with 12.7 million new cases of various cancers reported in 2008, according to a world cancer factsheet published by Cancer Research UK in conjunction with the World Health Organization.\(^2\) According to this factsheet, 40% of these newly reported cases were lung, female breast, colorectal and stomach cancers. The deaths reported due to the various cancers in 2008 amounted to 13% of the total global deaths at a number of 7.6 million deaths.\(^2\) On top of these statistics, cancer is also set to become an even bigger challenge to overcome in the next 16 years, with the global population expected to rise to approximately 8.3 billion by 2030. The predicted number of new cases is then expected to be around 20.3 million cases and 13.2 million deaths.\(^2\)

Cancer used to be a more predominant cause of death in high-income countries, but the trends suggest that it will become just as large a cause of mortality in medium to lower income areas.\(^3\) Cancer Research UK reported that 47% of new cancer cases and 55% of cancer deaths are from the less developed countries or regions – countries with a low or medium Human Development Index (HDI). HDI is an index of three components: life expectancy at birth, educational attainment and income. Countries are classified into four levels of HDI by the United Nations Development Programme. Only 5 African countries fall into the high HDI category as of 2014 and the majority of the other countries are in the low human development category.\(^4\) The International Agency for Research on Cancer (IARC) reported that 715,000 new cases were reported in Africa along with 542,000 deaths.\(^5\) This is close to 6% of the global total in new cases and just over 7% in global deaths when considering the statistics given by Cancer Research UK.\(^2\) These numbers are, conservatively, considered to double by 2030 due to the increases in so-called “westernisation” – the lifestyles and diets associated with economic development.\(^2,6\)

As it stands, cancer is still considered a lower priority in comparison with diseases such as tuberculosis, malaria and the biggest pandemic in terms of number of people infected, acquired immune deficiency syndrome (AIDS) brought on by the human immunodeficiency virus (HIV).\(^6\) HIV/AIDS was the leading cause of death in Africa in 2004, contributing 13.3% to the total death toll, whereas it ranked only 8\(^{th}\) worldwide (3.5% of the total number of
deaths recorded). Almost the inverse of these statistics are seen when comparing the contribution of malignant neoplasms to the death toll. Malignant neoplasms accounted for 12.6% of recorded deaths in worldwide in the same year, but only 4.5% of deaths in Africa. The occurrences of cancer and the prevalence of the different types thereof, differ greatly when comparing Africa to a continent with an HDI ranging from high to very high such, as North America. The types of cancers present in these high to very high HDI countries are more lifestyle related, cancers such as lung cancer brought on by tobacco and colorectal cancers exacerbated by obesity and a high consumption of fast food. A decrease in physical activity that generally accompanies economic development also strongly contributes to the high number of colorectal cancers reported. The ‘westernization’ trend of developing countries such as those in Africa are showing increases in the number of reported cases of these lifestyle-associated cancers. This means that as the developing countries strive to increase their HDI level, an increase in reported cancers will also be seen.

It is imperative that, since the statistics mentioned above suggest a definite increase in sufferers from non-communicable diseases like cancer, novel methods of combatting these diseases are developed along with refining and improving upon the current treatments. Chemotherapy has long been used to combat cancer and other diseases. The broad definition of chemotherapy is the use of artificial remedies to treat specific infections, as published by Dale in Science in 1924. According to Dale, the aim of chemotherapy is to find new remedies and improve upon existing ones to suppress a specific infection rather than simply treating the symptoms.

Chemotherapy as a method of treatment for cancer started in the early 1940’s with Louis Goodman and Alfred Gilman, who used nitrogen mustard to treat non-Hodgkin’s lymphoma. This stemmed from their analysis of the autopsy reports on soldiers that died during the First World War due to exposure to some of the chemical warfare agents used in that time. These soldiers exhibited lymphoid hypoplasia as well as myelosuppression, two conditions that Goodman and Gillman postulated could be induced – by controlling the administered dosage of the chemical agents – in certain tumour types as well. They indeed found it to be the case in the lymphoid tumours transplanted into mice. The tumour growth was not only stopped, but showed regression. Continued studies by these researchers also suggested that tumour cells were more sensitive to toxins than healthy cells, which lead to probes into compounds that induce apoptosis. All of this lead to what is considered the dawn of modern chemotherapy - the work of Hitchings and Ellion on antileukemic drugs. Their research demonstrated that these antileukemic agents inhibited cell proliferation via the interference with early stages in RNA and DNA synthesis of the tumour cells.
In the treatment of other diseases, such as tuberculosis and sub-acute bacterial endocarditis, combinations of drugs in a single treatment were already commonplace, as the different drugs targeted different sites of action in the disease. This was termed combination chemotherapy and in the case of cancer - even in the early stages of chemotherapy, the same principle seemed to apply as the treatment of a tumour with a single antiproliferative agent was rarely very effective.\textsuperscript{10} Frei and co-workers stated that the combination of a minimum of three active anticancer agents are necessary to effectively cure a certain cancer.\textsuperscript{11} An active anticancer agent is a compound which can produce a minimum response rate of 30\% when used in isolation for the treatment of a certain type of cancer. The reason cited for this was that it overcomes the drug resistance of the tumours due to the multi-pronged treatment.\textsuperscript{10} Peters \textit{et al.} noted that this combination of various antiproliferative agents and development of methods to test the efficacy of these combinations have led to the treatment of tumour types that were previously considered untreatable.\textsuperscript{12} Another advantage of combination chemotherapy is that the percentage of complete remission of certain cancer types were much higher under this method of treatment was much higher than when a single antiproliferative agent was used. This was reported by Frei and co-workers in a study of the combination of chemotherapeutic agents along with radiation therapy. Complete remission was observed in 12 of their 14 patients, 9 of which remained in remission.\textsuperscript{11}

Though the combination of anticancer agents is beneficial, there is also the toxicity of these compounds to consider. Chemotherapeutic treatments should increase the lifespan and, more importantly, the quality of life of the patient. This is where many of even the most promising antiproliferative compounds come short, as the benefits of the treatments are sometimes counteracted by the toxicities and side-effects of these compounds. The toxicity innate to all anticancer agents is due to the fact that normal cells with rapid cell proliferation are just as susceptible to these compounds as the cancerous cells that are the intended targets. This makes the selection and combination critical.\textsuperscript{13}

Extending on the work initially done by Frei and co-workers,\textsuperscript{10} Bertino and co-workers reported the successful use of combination chemotherapy to treat large cell lymphomas.\textsuperscript{14} The researchers reported a high degree of success in the treatment of patients using combination sequential chemotherapy, with 5 out of 8 patients diagnosed with diffuse histiocytic lymphoma remaining in remission for periods between 55 and 65 months.\textsuperscript{14} The successes thus far relied on the use of a handful of efficient antiproliferative agents and combinations of these compounds to sensitize and treat these malignant neoplasms. This called for the development of new and more efficient anticancer agents, but the field was
considered high risk due to the small returns on research products, as new compounds proved either ineffective or too costly to produce.\textsuperscript{8}

In the two decades following 1975, fewer than 10\% of the newly developed anticancer drugs entering clinical trials were approved by the Food and Drug Administration (FDA) of the United States. Von Hoff cited three main reasons for the low success rate of newly developed compounds: high toxicity of the new anticancer agents; compounds showing no efficacy; and poor understanding of the mechanism of action of the new compounds when the clinical trials were designed.\textsuperscript{15} The progress in chemotherapy development slowed down markedly by the mid 1980’s, due to no marked improvement on older compounds by novel chemotherapeutic agents and only limited success on large, long term trials. Another issue was the models used for testing, as the success in the animal models utilized, did not translate into clinical success. This prompted the development of the National Cancer Institute’s (NCI) 60 human tumour cell line screen.\textsuperscript{8}

This new screen has not been markedly more successful than the previous models in the identification of new lead compounds, but it has given a benchmark for the comparison of new compounds. It does, however, not aid in the identification of new mechanisms of tumour growth inhibition, as all new compounds discovered in these screens are generally antimitotic agents or topoisomerase I and II poisons.\textsuperscript{8} However, to exploit these mechanisms, the basis of all malignant neoplasms need to be understood. Hanahan and Weinberg identified six biological characteristics that offer more insight into the progression of tumour development. These six characteristics are acquired as a normal cell progressed to a tumour cell. The six characteristics, as they are named by Hanahan and Weinberg, are: sustaining proliferative signalling; resisting cell death; inducing angiogenesis; enabling replicative immortality; activating invasion and metastasis; and lastly, evading growth suppressors.\textsuperscript{16} By understanding the mechanism of action of new anticancer compounds, the biological pathways and mechanisms that give rise to these six characteristics could be targeted to inhibit cell proliferation.

As mentioned, the constant improvement of combination chemotherapy needed more lead compounds and the biosphere has proven to be a great source of new pharmaceutical leads.\textsuperscript{17,18} This will be discussed in more depth in the following section (Section 1.2), where more background on the natural products – the makaluvamines - that served as inspiration for this study, will be given. Following the discussion on the makaluvamines, the mechanism of action of these compounds will be discussed. As Von Hoff reported, finding novel mechanisms of action could be the highly advantageous in developing more potent clinical trials. The mechanism by which these compounds, the makaluvamines, inhibit cell
proliferation will then be discussed in Section 1.3, with attention being paid to the mechanisms suggested by Zhang and co-workers.\textsuperscript{19}
1.2 Background

The importance of natural products in the discovery of biologically active compounds has been highlighted by the large percentage of cancer drugs that are based on natural compounds.\textsuperscript{17} A review by Newman and Cragg on cancer compounds used from 1981 to 2010 showed that close to half (48.6\%) of the compounds used in treatment are natural product related.\textsuperscript{18} Marine sources have been a major source of these compounds, with the biologically relevant products existing as secondary metabolites in a variety of marine invertebrates. Considering that the oceans make up about 95\% of the planet’s biosphere, they do present a possible endless source of biologically viable compounds. This is due to the inherent defence mechanisms of these creatures against predation or adaptation to limited resources.\textsuperscript{20} These invertebrates include sponges, bryazoa, tunicates and ascidians, with sponges being the greatest source of novel structures.\textsuperscript{21}

Makaluvamines are alkaloid metabolites that share a general skeleton structure with a variety of other marine alkaloids from the *Latrunculiidae* and *Acarnidae* families of marine sponges.\textsuperscript{22} The sponges of the *Latrunculiidae* family are common in the cold coastal waters of Antarctica, New Zealand, Australia, Tasmania and South Africa.\textsuperscript{23} The general structures found in this family are pyrrolo[4,3,2-de]quinoline 1, pyrrolo[4,3,2-de]pyrrolo[2,3-h]quinoline 2, pyrido[2,3-h]pyrrolo[4,3,2-de]quinoline 3 and pyrroloiminoquinones 4 (Shown in Figure 1.1).

![General Skeletal structures](image)

Figure 1.1: General Skeletal structures

The most abundant motif amongst these is the pyrroloiminoquinone motif 4, of which the makaluvamines [such as makaluvamine A (5a)] and isobatzellines [such as isobatzellines C (5b)] are structurally the closest. Slight variations and combinations of structure 4 with structures 2 and 3 form the basis of compounds such as the damirones 6, batzellines 7, makaluvone 8, discorhabdins 9, epinardins 10 and tsitsikammamines 11 (see representative examples in Figure 1.2).\textsuperscript{22,24} Most of these structures are also abundant in the genus *Zyzzya*, family *Acarnidae*, with makaluvamines A-P being isolated from 2 species in this genus – *Z. marsailis* (from Fiji), *Z. fuliginosa* – and therefore not exclusive to just one family.\textsuperscript{22,25}
Figure 1.2: Representative marine alkaloids

The makaluvamines were first reported by Ireland and co-workers as novel cytotoxic topoisomerase II inhibitors, isolated from the Fijian sponge *Zyzzya cf. marsailis*.\(^{26}\) These compounds have been of great interest due to their remarkable biological activity, with the makaluvamines showing antitumour activity against various cancer cell lines.\(^{27}\) The biological activity of makaluvamines is not however limited to cancer cell lines, some makaluvamines have even been shown to have antiplasmodial activity against *Plasmodium falciparum*. Makaluvamines J (12), G (13) and L (14) showed activities comparable to that of chloroquine (15) (Figure 1.3), further highlighting the importance of these molecules.\(^{28}\)
The iminoquinone motif in these structures appear to be of importance, as a study by Carney et al. noted that the isobatzellines 5b and discorhabdins 9 exhibited potent cytotoxicity against the P388 murine leukaemia cell line, unlike the damirones 6 and batzellines 7, which proved inactive. The lack of the iminoquinone motif was cited as a possible reason for the lack of activity. Barrows and co-workers tested the components extracted from *Zyzzya fuliginosa* (collected from three different locations off the Australian coast) and *Zyzzya* sp. in the NCI 60 cell line human tumour screen. The different compounds showed varying levels of potency during the screen and correlations were drawn between the potency and cytotoxicity as well as unsaturation and substitution on the compounds. The researchers noted the difference in activity between compounds such as makaluvamine A (5a) and B (16). This was also evident in the work of Ireland and co-workers. This difference in activity will be elaborated on below, when the activity of the different makaluvamines and the synthetic analogues are discussed.

In the initial paper by Ireland and co-workers, the novel makaluvamines were tested against the HCT-116 cancer cell line and it was interesting to note that the extra unsaturation in makaluvamine B (16) drastically reduced its activity compared to makaluvamine A (5a). Makaluvamine A had an IC\textsubscript{50} value of 1.3 \(\mu\)M against the HCT-116 cell line, whereas makaluvamine B had an IC\textsubscript{50} value of more than 50 \(\mu\)M. These researchers also tested makaluvamines C – F (17-20, Figure 1.4) against the same cell line. Makaluvamines C and D (17, 18) were not as effective as makaluvamine A, with IC\textsubscript{50} values of 36.2 \(\mu\)M and 17.1

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Figure 1.3: Makaluvamines (12-14) with antiplasmodial activity and chloroquine (15)
µM respectively. Makaluvamines E (19) and F (20) were very potent though, with values of 1.2 µM and 0.17 µM, respectively. The IC\textsubscript{50} values of the compounds tested are summarized in Table 1.1.

With makaluvamines D (18) and E (19) there was also a large difference in activity, despite only minor structural differences. In the screen of the NCI's 60 cell lines by Barrows \textit{et al.}, makaluvamine H (21) (Figure 1.4) showed great potency against lung, central nervous system, melanoma, ovarian and breast cancer cell lines with IC\textsubscript{50} values ranging from 0.15 – 1.05 µg/mL.\textsuperscript{27} Makaluvamine C (17) (Figure 1.4) showed comparable cytotoxicity to makaluvamine H (21), varying against in two areas: it had relatively poor activity against the lung cancer cell lines, but showed excellent cytotoxicity against the OVCAR-3 ovarian cancer cell line (IC\textsubscript{50}: 0.06 µg/mL).\textsuperscript{27}

![Figure 1.4: Structures of additional makaluvamines](https://scholar.sun.ac.za)
Table 1.1: Summary of the antiproliferative activities of makaluvamines A-F against the HCT-116 cell line

<table>
<thead>
<tr>
<th>Makaluvamine</th>
<th>IC\textsubscript{50} against HCT-116 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (5a)</td>
<td>1.3</td>
</tr>
<tr>
<td>B (16)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>C (17)</td>
<td>36.2</td>
</tr>
<tr>
<td>D (18)</td>
<td>17.1</td>
</tr>
<tr>
<td>E (19)</td>
<td>1.2</td>
</tr>
<tr>
<td>F (20)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Considering the potent anticancer activity shown by these molecules and the variation in structure at position 7 [numbering shown on Makaluvamine I (22) in Figure 1.5], various synthetic analogues have been prepared and tested for their anticancer activity. A study by Shinkre et al. investigated the activities of synthetic benzyl- and phenethyl makaluvamine analogues (23a-g and 24a-g in Figure 1.5) against the MCF-7 breast cancer cell line by comparing them to the known topoisomerase II targeting, anticancer agents, etoposide (25) and amsacrine (m-AMSA) (26).\textsuperscript{25}
Figure 1.5: Structure of makaluvamine I (22), synthetic makaluvamine analogues and the known topoisomerase II poisons, etoposide (25) and amsacrine (26).
When the results of this study and those of an earlier study by the same research group [in which a different library of synthetic makaluvamine analogues were produced (27a-e and 28a-c in Figure 1.5)] were compared to the activity of two naturally occurring makaluvamines (17 and 21) and compounds 25 and 26, it was evident that these synthetic analogues had similar potency to not only the natural compounds, but also to the two compounds which were known to be effective topoisomerase II poisons. The IC\textsubscript{50} values of these compounds against the MCF-7 cell line are given in Table 1.2.

Table 1.2: Cytotoxicity results of the synthetic makaluvamine analogues and known standards against MCF-7 Cell line

<table>
<thead>
<tr>
<th>Tested Compounds</th>
<th>IC\textsubscript{50} (µM)</th>
<th>Tested Compounds</th>
<th>IC\textsubscript{50} (µM)</th>
<th>Tested Compounds</th>
<th>IC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>2.6</td>
<td>25</td>
<td>35.6 ± 3.4</td>
<td>27a</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>21</td>
<td>2.3</td>
<td>26</td>
<td>21.7 ± 2.5</td>
<td>27b</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>23a</td>
<td>2.3 ± 0.8</td>
<td>24a</td>
<td>2.3 ± 0.6</td>
<td>27c</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>23b</td>
<td>3.6 ± 1.2</td>
<td>24b</td>
<td>3.4 ± 1.1</td>
<td>27d</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>23c</td>
<td>6.6 ± 0.8</td>
<td>24c</td>
<td>13.7 ± 1.8</td>
<td>27e</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>23d</td>
<td>34.7 ± 5.9</td>
<td>24d</td>
<td>4.6 ± 0.2</td>
<td>28a</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>23e</td>
<td>5.2 ± 0.9</td>
<td>24e</td>
<td>2.4 ± 0.4</td>
<td>28b</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>23f</td>
<td>1.8 ± 0.7</td>
<td>24f</td>
<td>2.8 ± 0.2</td>
<td>28c</td>
<td>1.2 ± 0.03</td>
</tr>
<tr>
<td>23g</td>
<td>2.8 ± 0.3</td>
<td>24g</td>
<td>5.1 ± 0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Even when considering the activities of compounds 23d and 24c, the two least active of these analogues, compound 23d has similar activity to etoposide (25) and compound 24c has a cytotoxic activity comparable to amsacrine (26). It should be noted that etoposide (25) and amsacrine (26) have been successfully utilised in the treatment of acute lymphoblastic leukaemia in children, therefore proving to be valid chemotherapeutic compounds.

The interest in makaluvamines is thus not only due to the excellent \textit{in vitro} anticancer activities against a wide variety of cancer cell lines, but also their mode of action. Etoposide (25) and amsacrine (26) are known topoisomerase II poisons and the makaluvamines seem to inhibit cell proliferation through a similar mechanism. Interestingly, changes to the main scaffold structure seem to affect the mode of action. Some analogues can inhibit both topoisomerase I and II, whilst others are more strictly topoisomerase II inhibitors. Compounds such as the 7-lexitropsin-derived makaluvamine (29) and the water-soluble synthetic analogues 30a and 30b appear to target both DNA topoisomerases (shown in Figure 1.6). The addition of the lexitropsin group in the 7-position seemed to increase the
The study by Zhao et al. produced different analogues of compound 29 by varying the repeating unit from 1 to 3. By using doxorubicin (31), another commercial chemotherapeutic agent (shown in Figure 1.6) as standard, their research found compound 29 to have the greatest in vitro cytotoxicity against KB, HCT-116, L1210, L1210/Adr, MCF-7 and CHO cancer cell lines. The water-soluble analogues synthesized by Bénéteau et al. (compounds 30a and 30b) were also reported to have excellent antiproliferative activities with IC$_{50}$ values of 0.3 and 0.38 μM, respectively, and the compounds were also found to have a nonspecific effect on the L1210 cell cycle.

Figure 1.6: Lexitropsin-derived and water-soluble makaluvamine analogues and doxorubicin

In another study, by the Velu group, makaluvamines with substitutions that have different characteristics – increased steric bulk, hydrophobicity and hydrophilicity - were synthesized over various studies and tested for their anticancer activity. The activities against three cell lines (MCF-7, MDA-MB-468 and HCT-116) all ranged from 0.56 to 11 μM. Interestingly, differences in substitution patterns – primarily in the 1-, 5- and 7- positions of the makaluvamine scaffold (shown in Figure 1.7, denoted by $R^1$, $R^2$ and $R^3$, respectively) - caused the products to show more selectivity for different cell lines. For instance, compound 27a (Figure 1.5) showed potent activity against the MCF-7 breast cancer cell line with an IC$_{50}$ value of 1.0 μM whilst compound 28b (Figure 1.5), which differed in the 7-position by having an extra methylene linker, was more effective against the HCT-116 human colon tumour cell line with an IC$_{50}$ value of 0.5 μM. The significant difference between the two was
that compound 28b has a tosyl-group on the pyrrolo nitrogen and this substitution caused the compound to be more effective against a different cell line.19

The N-tosyl substituted makaluvamines (5-position on the general makaluvamine scaffold) do seem to have fairly potent activity profiles, as one of the synthetic analogues (compound 32, Figure 1.7), synthesized by Nag et al., proved to be highly effective at inducing apoptosis and S-phase cell cycle arrest in lung cancer cell lines.19 The compounds caused down-regulation of MDM2 both dependently and independently of p53. This group also reported five novel makaluvamine analogues (33a-e, Figure 1.7) that were very effective against a broad spectrum of cancer cell lines. They also noted that breast cancer cells were especially sensitive to these novel analogues, most especially towards compound 33b.19

![General Makaluvamine Scaffold](image1)

![32](image2)

![33a-e](image3)

**Figure 1.7: Novel makaluvamine analogues produced by Nag et al.**

Since these compounds seem to act via different mechanisms of inhibition depending on the substitution of the makaluvamine, Nag et al. suggested that they might inhibit cell proliferation via a novel mode of action.19
1.3 Mechanism of action

Von Hoff cites the neglect of the importance of the mechanism of action of potential anticancer leads as one of the three main reasons a large percentage of promising compounds never pass clinical trials.\textsuperscript{15} This is because greater emphasis is placed on whether the compound causes tumour shrinkage (either partial or complete) than on how the compound of interest effects the inhibition of cell proliferation. This leads to premature clinical trial failure, with many compounds failing even before they reach this point.\textsuperscript{15} The following section will discuss topoisomerase II inhibition, as it is the accepted mechanism of action of the makaluvamines, and then consider the possible new mechanisms of action that have been suggested.

1.3.1 Topoisomerase II inhibition

Ireland and co-workers reported the makaluvamines A-F (Shown in Figure 1.4 in Section 1.2) to be topoisomerase II inhibitors as the mechanism of action involved cleavage of the DNA double-stranded complex. This was confirmed through the use of a decatenation assay and a neutral filter assay, in which the makaluvamines were compared to amsacrine (26, shown in Figure 1.5). The makaluvamines compared well with amsacrine, with makaluvamines A (5a) and F (20) were active at concentration similar to amsacrine.\textsuperscript{26}

Topoisomerase II inhibitors are enzymes that effect strand breaks in DNA and are critical to the survival of all cells. The topoisomerase II inhibitors are classified as either type I or type II, with the type indicating whether they effect single-strand DNA breakage (type I) or double-stranded DNA breakage (type II).\textsuperscript{34} As mentioned, the makaluvamines are topoisomerase II inhibitors, therefore the discussion will focus mostly on the type II enzyme, as only a few makaluvamines (natural and synthetic) had exhibited some form of type I inhibition.\textsuperscript{19}

The function of the topoisomerase enzymes is to maintain the topology of the DNA double helix, which is essential for healthy cell functionality. The topology is disrupted during the replication process when the double helix is unwound to open up for RNA transcription. As DNA strands are either anchored to membranes or chromosomal scaffolds, the tension due to unwinding can cause coils to form in the DNA strand. These coils are referred to as supercoils and are classified as either positive or negative coils. Positive supercoils are formed on the side the replication is approaching, whereas negative coils are formed behind the replication process where the double helix is recombining. This sort of tension can translate into knots within the same strand that could have serious implications during further replication or other processes. The biggest problems arise during meiosis and mitosis, as
these knots can lead to DNA molecules getting entangled to the extent that they cannot be resolved. This can lead to cell death. To avoid these problems, the topoisomerases regulate the topological state of the DNA strands by initiating breaks in the chain and releasing the tension. This action eliminates the coils and knots that are formed. 

Topoisomerase II is present in two isoforms, topoisomerase IIα and topoisomerase IIβ, which differ in mass (the α-isoform is 10 kDa smaller than the β-isoform) and their activity in the removal of supercoils. The α-isoform is more efficient at the relaxation of the positive supercoils than the negative supercoils, whereas the β-isoform does not distinguish between the two types of supercoils.

The relaxation of these coils happens by the formation of topoisomerase II-DNA cleavage complexes which exist in cleavage/religation equilibrium. The cleavage complexes are short-lived species and generally only account for 0.5-1% of the concentration of the topoisomerase II enzyme present in the reaction mixture. This equilibrium is fairly sensitive though, as increases or decreases in the concentration of the topoisomerase II-DNA cleavage complex could both cause cell death (Figure 1.8). A decrease in the concentration leads to the impediment of cellular mitosis, whereas an increase results in apoptosis. It is this increase in concentration of the topoisomerase II-DNA cleavage complex that is exploited therapeutically by the use of antiproliferative agents. Topoisomerase II poisons act by stabilizing the cleavage complex and thus increases the concentration of these complexes within the DNA scission mixture. This prevents DNA replication, while promoting apoptosis.

It is interesting to note that makaluvamine A produced strand scission factor comparable to that of amsacrine.
Along with being known as topoisomerase II inhibitors, the makaluvamines have also been found to be DNA intercalators, attributed to its charged, planar structure. The makaluvamines continue to be seen and analysed as topoisomerase II inhibitors, but there have been research groups that have postulated that these compounds might inhibit cellular proliferation by different or novel modes of action. Danieli and co-workers also reported observing DNA fragmentation in HeLa cells treated with three makaluvamines.\(^{36}\)

### 1.3.2 Novel mechanisms

Along with topoisomerase II inhibition and the other pathways by which apoptosis is induced, there have been probes into possible new mechanisms by which cell death is promoted. Zhang and co-workers reported on various new possible mechanisms, based on trials on a fluorobenzyl analogue of the makaluvamines (33b).\(^1^9\) The new mechanisms (dashed lines) along with the established mechanisms (solid lines) are shown in Figure 1.9.

One mechanism proposed by Zhang and co-workers is the activation of p53 through the down-regulation of MDM2. This p53 activation stimulates apoptosis and cell cycle arrest. This was confirmed in \textit{in vitro} and \textit{in vivo} models, both showing an increase in p53/p-53 expression levels and decreased expression levels of MDM2. Another proposed mechanism relates to the levels of reactive oxygen species (ROS) in the cell. ROS levels were noted to be heightened as treatment with the makaluvamines resulted in the decrease of mitochondrial membrane potential and thereby leading to mitochondrial collapse, followed by
cell death. This is an especially useful pathway to target, as the heightened metabolisms of cancer cells lead to increased production of ROS. This is attributed to the tumorigenic properties of ROS as it leads to an increase cell proliferation. It also heightens the survival rate of these cells and aids in cellular migration. The cell proliferation of ROS is due to its ability to initiate tumour growth. Due to this, cancer cells have heightened levels of antioxidant pathways to successfully deal with the increased concentrations of ROS. These pathways include the up-regulated mechanisms of glutathione, superoxide dismutase and catalase, leading to hyposensitivity towards ROS. The inhibition or down-regulation of these pathways have been shown to be effective in the selective killing of tumour cells, as the cancer cells are terminated by the ROS.

These researchers also investigated the activation of the phosphatidylinositol 3'-kinase (PI3K) and the PI3K-Akt pathway. The activation of PI3K in cancer cells is generally triggered by an over-expression of the fatty acid synthase (FAS), an enzyme that forms part of the metabolism of fatty acids to cater to the cancer cells increased energy demands. The fluorobenzyl makaluvamine analogue (33b) was found to down-regulate the activation of PI3K and thereby affected a decrease in the production of phosphorylated Akt (p-Akt). This down-regulation caused a suppression of the PI3K-Akt pathway and along with the down-regulation of MDM2, proved to be a different mode of inhibition to what has previously reported for this class of compounds. It was also postulated, that the makaluvamines might affect the expression of FAS, but this was not verified experimentally.
Based on the report by Zhang and co-workers, it is therefore clear that more probes into the mechanism of action of the makaluvamines are necessary. Therefore the aim of this study is also to produce analogues of the makaluvamines that can be tested not only for their antiproliferative activity, but also for their mechanism of action. This could serve to add to the literature on these compounds and improve their standing as promising clinical agents. Some of the compounds synthesized by Zhang and co-workers [the benzylamine- (33a) and fluorobenzyl analogues (33b)] have already been identified as potential clinical leads.

In the following section, various synthetic strategies will be discussed. The strategies start with the first reports after the discovery of the makaluvamines and subsequent increases in the efficacy of the synthesis or shortening thereof. Shortcomings of the various strategies will be highlighted, ultimately providing a rationale for the choice of the strategy by Joule and co-workers as the basis for this study.
1.4 Synthetic Approaches

Since their discovery in 1993, the makaluvamines have received attention for their potent anticancer activity.\textsuperscript{26} The initial report by Ireland and co-workers showed that these compounds were especially potent against the HCT-116 colon cancer cell line. Makaluvamines A, E and F (5a, 19, 20 in Figure 1.4) were very potent with IC\textsubscript{50} values of 1.3 μM, 1.2 μM and 0.17 μM, respectively.\textsuperscript{26} Makaluvamine G (13, Figure 1.4) was reported in the same year by Carney and Scheuer and also exhibited moderate cytotoxicity against the P388, MCF-7, A549, HT-29 and KB cancer cell lines.\textsuperscript{29} They also reported moderate immunomodulatory activity and also noted that compound 13 was not toxic towards mice in \textit{in vivo} testing, even at the highest tested dose of 210 mg/kg.\textsuperscript{29} This is especially promising in terms of treatment possibilities, as it could possibly inhibit tumour growth without adversely affecting healthy cell function.

In the light of these promising results, the interest turned to the synthesis of these compounds. The first reported synthesis came from Yamamura and co-workers.\textsuperscript{41} This was a fairly short synthetic strategy, but did rely on the availability of a fairly complex starting reagent. Their synthesis started with the lactamization of a tetra- or pentasubstituted indole 34 (Scheme 1.1), followed by a reduction and an oxidation to deliver the 7-methoxy pyrroloiminoquinone 35. This compound could then be converted into the desired makaluvamine analogue by reacting it with an appropriate amine [the use of ammonia solution is shown in Scheme 1.1 to produce makaluvamine A (5a)]. Therefore, the following discussion of synthetic strategies will investigate the construction of the 7-methoxy pyrroloiminoquinone scaffold, as it allowed for the generation of a diverse library from this central precursor. It should be noted that the various strategies do have different substitution patterns in the 1- and 5-positions (shown on the general makaluvamine scaffold in Figure 1.7), such as methyl groups and tosyl-groups.
The main drawback to the strategy by Yamamura and co-workers was the reliance on a fairly complicated starting material, a compound which had to be synthesized as well. Yamamura and co-workers initially reported the synthesis of makaluvamines A to D, but later updated their strategy to include makaluvamine E as well.

White et al. reported some synthetic studies where they explored synthetic routes starting with veratraldehyde (36, Scheme 1.2) and 2,3-methoxybenzoic acid (37, Scheme 1.3) - much simpler starting materials than used by Yamamura and co-workers. The first strategy (from Scheme 1.2) started from veratraldehyde (36), unfortunately they encountered significant problems with this strategy in the final steps to construct the desired 7-methoxy pyrroloiminoquinone (35). The synthesis started with a Knoevenagel condensation to form the ester (38), followed by a hydrocyanation/decarboxylation to give product 39. Successive hydrogenation and acetylation reactions then gave products 40 and 41. This was followed by an oxidation and cyclization with hypervalent iodine to furnish the iminoacetal (42). Another hydrogenation step produced the tetrahydroquinoline (43) and treatment of this product with cerium ammonium nitrate produced the iminoquinone (44a). From here on, significant issues arose with construction of the pyrrole ring (44b) and the researchers thus decided to abandon this route. This unsuccessful route is reported here, as the strategy differed from the strategy reported by Yamamura and co-workers in that it started from a much simpler starting material and attempted the construction of a tetrahydroquinoline basis (43), before
arriving at an iminoquinone structure (44a). This compound (44a) was also reported to be fairly unstable.\textsuperscript{43} As the coupling of the exo-cyclic nitrogen to the “ortho-position” of the iminoquinone to produce compound 44b proved inefficient, it also serves to highlight that even early on, researchers were posed with difficult synthetic challenges.

Scheme 1.2: First synthetic strategy by White \textit{et al.}\textsuperscript{43} i) NCCH\textsubscript{2}CO\textsubscript{2}Et, piperidine, toluene, 110 °C; ii) NaCN, CHCl\textsubscript{3}, EtOH, H\textsubscript{2}O, Δ; iii) H\textsubscript{2}, Pd/C, Ac\textsubscript{2}O; iv) H\textsubscript{2}, Pd/C, MeOH, HCl; v) NH\textsubscript{3}, CHCl\textsubscript{3} vi) Phl(OAc)\textsubscript{2}, CF\textsubscript{3}CH\textsubscript{2}OH; vii) H\textsubscript{2}, Pd/C, MeOH; viii) CAN, MeCN (Yields for steps i and ii are not given).

Whilst the first route focused on the initial construction of a tetrahydroquinoline structure, in the second route White \textit{et al.} focused on the initial construction of an indole backbone. The synthetic route started with 2,3-dimethoxybenzoic acid (37, Scheme 1.3) and was converted into the carbamate 45 via a Curtius rearrangement. The carbamate was next converted into the aniline (46) and subsequently into the phenyl hydrazine (47). This compound was next
treated with 2,3-dihydrofuran in hydrochloric acid to give a mixture of compounds 48 and 49 in a 1:1 ratio. The mixture was subsequently converted into indole 50 by means of a Fischer indolization, along with the formation of indole 51 as a side product via an ipso-substitution of the ortho-methoxy group. This side product proved to be difficult to remove and the mixture was used without purification for the subsequent protection step, which included the tosylation of the hydroxyl group as well as the indole nitrogen. The compounds were then readily separated and nitrated to produce compounds 52a and 52b in a 1:1 mixture.

This is problematic for this synthetic route, as half of the material is lost in this separation by forming the undesired isomer. The isomers were easily separated by means of column chromatography. Reduction of the 4-nitro derivative (52a) using Adams’ catalyst then gave compound 53. The air-sensitivity of compound 53 prompted the immediate treatment of this compound with N,N-diisopropylethylamine to produce compound 54 in a very good yield of 85% over the two steps. This product was oxidized to produce the iminoquinone 55, which was then converted into its tosylate salt, compound 56. White et al. thus managed to synthesize the 7-methoxy pyrroloiminoquinone (56) in 13 steps. The synthetic strategy was fairly efficient in the early stages (compound 47 was synthesized in 64% yield over 5 steps), but the yields were very poor after this step. This was due to the formation of inseparable mixtures and unstable products, which meant yields could not be accurately reported. These researchers also noted that for a successful substitution at C-7 by a desired amine, the imino-nitrogen should be protonated (Shown in Section 3.2, Scheme 3.6).
Scheme 1.3: Successful makaluvamine core synthesis by White et al.\textsuperscript{43} i) (PhO)\textsubscript{2}PON\textsubscript{3}, EtOH, Et\textsubscript{3}N; ii) KOH, EtOH; iii) NaNO\textsubscript{2}, HCl; iv) SnCl\textsubscript{2}, HCl; v) NaOH; vi) 2,3-dihydrofuran, HCl; vii) ZnCl\textsubscript{2}, Ethylene glycol; viii) \( p \)-TsCl, NaH, THF; ix) AcONO\textsubscript{2}, Ac\textsubscript{2}O; x) H\textsubscript{2}, PtO\textsubscript{2}, EtOH; xi) (\( i \)-Pr\textsubscript{2}NEt; xii) CAN, MeCN, H\textsubscript{2}O; xiii) \( p \)-TsOH
A significant drawback to this synthesis arose after the construction of the hydrazine (47). The treatment of compound 47 with 2,3-dihydrofuran in acidic medium gave a mixture of compounds (48 and 49), which the authors mention do not detract from the synthesis as this mixture still gave compound 50. This was not exclusive though, as the researchers reported the ipso-substitution to also give compound 51 as a side product. The mixture of compounds 50 and 51 was isolated in a poor yield of 32%. What makes this even less desirable, is that the ratio of the desired product to the undesired product was 1:7. The subsequent step also gave rise to an almost 1:1 ratio of the 4-nitro-substituted indole (52a) and 2-nitro-substituted indole (52b). Therefore, this route was also not considered.

It should be noted that the successful use of an indole to construct the makaluvamine backbone was also achieved by Sadanandan et al., when they reported the use of a trimethoxyindole to synthesize the tricyclic pyrroloiminoquinone backbone. Their strategy involved starting off by the conversion of 2,4,5-trimethoxybenzaldehyde (57, Scheme 1.4) into the corresponding azidocinnamate (58) via a Knoevenagel condensation. The researchers utilized the Hemetsberger indolization to convert compound 58 into the indole-2-carboxylic ester (59). After a saponification and decarboxylation reactions, 4,6,7-trimethoxyindole (60) was isolated in 73% yield. This indole was then converted into the N-BOC-3-ethylaminoindole (61) over 5 steps. This compound was then oxidised to furnish the pyrroloquinone (62) and the protecting group was removed by treatment with trifluoroacetic acid. This produced the ammonium salt (63) which when dissolved and stirred in chloroform, underwent the ring closure to give the desired 7-methoxy pyrroloiminoquinone (64).
The synthesis by Sadanandan et al. was fairly successful, as compound 62 was synthesized in an overall yield of 28% and a quantitative conversion to compound 64. The construction of the 3-ethylamino-indole (61a) following the synthesis of compound 60 involves two reduction steps separated by the tosyl-protection of the indole nitrogen, the second of which is a fairly strong fire hazard, if a nitrogen atmosphere is not maintained. The long reaction time for this reaction means constant monitoring would be necessary. The precursor to compound 61a, the N,N-dibenzylindole (61b) was reduced using palladium black and ammonium formate in absolute ethanol and immediately protected with BOC anhydride, due to the sensitivity of the free amine (Scheme 1.5). The reduction using palladium black was the step reported as a fire hazard.

Scheme 1.4: Makaluvamine synthesis by Sadanandan et al.\textsuperscript{33} i) $\text{N}_2\text{CH}_2\text{COOMe}$, NaOMe/MeOH ii) Xylene, $\Delta$ iii) NaOH iv) HCl v) Ba(OH)$_2$, $\Delta$ vi) CAN, $n$-Bu$_4$NHSO$_4$, DCM vii) TFA, DCM viii) CHCl$_3$
Scheme 1.5: Reduction and successive protection to afford compound 61a.33 i) Pd black, Ammonium formate, EtOH, HCOOH; ii) (BOC)$_2$O, Et$_3$N, DMAP, CH$_2$Cl

Peat and Buchwald reported the synthesis of an indole intermediate (65, Scheme 1.6), and applied this compound to the synthesis of various makaluvamines and damirones, using zirconocene and palladium catalysts for the formation of the tetrahydroquinoline and pyrrole rings respectively (Shown in Scheme 1.6).44 This synthesis started with the bromination of 3,4-dimethoxyaniline (66) to produce compound 67. This compound was treated with 4-bromobut-1-ene to give product 68 and subsequent methylation introduced the methyl group that is characteristic of makaluvamines C and G (17 and 13, Figure 1.4) to give compound 69. The tetrahydroquinoline structure was then formed using a zirconocene catalyst, followed by iodination and substitution with benzylamine to produce compound 70. The pyrrole ring was then formed by palladium-catalysed ring closing reaction to produce compound 71, which subsequently underwent aromatization to transform from the dihydropyrrole to the pyrrolo-moiety with the removal of the benzyl group under reductive conditions to finally generate the indole 65. According to the authors, oxidation of these structures to afford the 7-methoxy pyrroloiminoquinone (72) precursors have been reported (no references were given in the paper) as part of the total synthesis of makaluvamine C (17). Investigation into this did not deliver results specific to makaluvamine C, but considering that these pyrroloiminoquinone moieties are generally furnished by treatment with cerium ammonium nitrate. Peat and Buchwald thus provided a short and efficient synthesis of these makaluvamine precursors, but still with a reliance on transition metals for the ring closing steps.
Scheme 1.6: Synthetic route by Peat and Buchwald.\textsuperscript{44} i) Bu\textsubscript{4}NBr\textsubscript{3}, MeOH, DCM; ii) 4-bromobut-1-ene, K\textsubscript{2}CO\textsubscript{3}, NaI; iii) MeI, K\textsubscript{2}CO\textsubscript{3}; iv) Cp\textsubscript{2}Zr(Me)Cl, t-BuLi, THF; v) I\textsubscript{2}, DCM; vi) BnNH\textsubscript{2}, THF; vii) 2.5 mol\% Pd\textsubscript{2}(dba)\textsubscript{3}, P(o-tolyl)\textsubscript{3}, NaO\textsubscript{t}Bu, toluene; viii) 10 mol\% Pd/C, HCO\textsubscript{2}NH\textsubscript{4}, MeOH

In comparison, Joule and co-workers reported an even shorter synthesis with a slightly lower dependence on some of the heavier and more expensive metals reported in syntheses up to this point, with the exception of NiCl\textsubscript{2}.\textsuperscript{45} The toxicity of nickel and the nickel borides that form during the reduction are highly toxic, but can be safely worked with and disposed of. Thus, the method of Joule and co-workers is more cost-effective in terms metal catalysts used. The reported synthesis started off with the chlorination of the 8-position of 6,7-dimethoxy-4-methyl-5-nitroquinoline, previously synthesized in two steps (73, Scheme 1.7), with N-chlorosuccinimide to afford the \textit{penta}-substituted quinoline 74. Oxidation of this compound by means of Vismara’s procedure selectively gave the aldehyde 75, which was then converted into the acetal (76). This product was then reduced using NaBH\textsubscript{4} and NiCl\textsubscript{2}.6H\textsubscript{2}O, which reduced both the nitro group and the quinoline to a tetrahydroquinoline, to furnish the diamine (77). It is at this point in the synthetic strategy that something fairly interesting happened. The diamine was treated with aqueous 1N HCl in tetrahydrofuran in hopes of deprotecting the acetal and forming the pyrrole ring. However, instead of the expected
product, the 7-methoxy pyrroloiminoquinone (78) was isolated. The researchers speculated that the acidic medium had catalysed the elimination of the chlorine and thus, formed the iminoquinone. This shortened the synthesis and avoided the need for an oxidation step using cerium ammonium nitrate to produce compound 78. Although this synthetic route is very short, it makes the derivatization of the 5-position (shown on compound 78 in Scheme 1.7) on the pyrroloiminoquinone a bit more problematic. A distinct advantage of the route reported by Peat and Buchwald (as described earlier) was that this position could be easily derivatized.

Scheme 1.7: Synthetic route reported by Joule and co-workers\(^45\) i) NCS, DMF, 60 °C; ii) I\(_2\), t-Bul, FeCl\(_2\), TFA, DMSO, 80 °C; iii) MeOH, HCl, reflux; iv) NaBH\(_4\), NiCl\(_2\), MeOH, 0 °C; v) aq. 1N HCl, THF, 40 °C

It should be noted that, Kita et al. also managed to synthesize compound 78 in 6 steps by starting with 6,7-dimethoxyindole (79, Scheme 1.8).\(^46\) This compound was converted into the 3-ethylazido indole (80) over 5 steps following the established procedure reported by Sadanandan et al., for the derivatization of the 3-position.\(^33\) Instead of the amino-substituted indole though, Kita et al. synthesized the azido derivative. Compound 80 was successfully converted into the pyrroloiminoquinone (78) in one step through the use of a hypervalent iodine compound – phenyliodine (III) bis(trifluoroacetate) (PIFA).
The shortcoming of this synthesis was that a search on the commercial availability of the starting material, compound 79, delivered no results. It meant that this compound would have to be synthesized as well. Corey and co-workers reported a synthesis of 6,7-dimethoxyindole (79) in 6 steps, thus this synthetic route would be significantly longer.\(^{47}\)

After considering all these design strategies, it was decided to follow a synthetic route based on a variation of the quinoline synthesis reported by Joule and co-workers that formed part of a formal synthesis of batzellines, isobatzellines, discorhabdins and makaluvamine D.\(^{45}\) Joule and co-workers later expanded on this work and published a second paper on the formal syntheses of these compounds, using 6,7-dimethoxy-4-methylquinoline (81, shown in Scheme 2.1 in Section 2.1) as starting point.\(^{40}\) The reason for selecting this route above the other possible routes was that it offered one of the shorter routes to the pyrroloiminoquinone precursor (72) with the option of introducing a methyl group. The strategies reported by White et al. (Scheme 1.3) served to show what was inefficient, as their synthetic strategy ran into considerable issues towards the end.\(^{43}\) The synthesis by Sadanandan et al. was fairly efficient, with good to excellent yields, but only produce the pyrroloiminoquinone precursor in 13 steps. While it does have scope for the introduction of a methyl group, this would have increased the length of the synthesis.\(^{33}\) The procedure by Peat and Buchwald, would perhaps be the second best candidate, but it was not considered due to its reliance on more expensive metal catalysts for ring-closing reactions.\(^{44}\) Similarly, the synthesis by Kita et al. was not considered, due to the need for PIFA in the final step as well as not having scope for the introduction of the methyl group.
Therefore the synthetic strategy reported by Joule and co-workers was selected as the template for constructing the 7-methoxy pyrroloiminoquinone (72), which would serve as precursor to the makaluvamine analogues of interest.\textsuperscript{40} This strategy will be discussed more in depth in the next section, along with the methods investigated for the optimization of certain steps (or possible alternatives in certain instances) and the mechanisms or proposed mechanisms where available.
Chapter 2

2.1 Synthetic aims

As mentioned at the end of Section 1.4, it was decided that the second paper on the formal syntheses of batzellines, isobatzellines, discorhabdins and makaluvamines by Joule and co-workers was to serve as the template for this project. The strategy (Scheme 2.1) involved using the quinoline backbone and constructing the quinoline from 3,4-dimethoxyaniline (66) and methyl vinyl ketone (MVK) (82), as reported by Joule and co-workers in a different study of the structurally related damirones.48 This quinoline would then be nitrated to give compound 73, which would subsequently be oxidised to give compound 83. The aldehyde would then be protected to afford acetal 84 and this product eventually reduced to give the diamine 85. At this point, we would augment the synthetic strategy by selectively introducing a methyl group to the anilino-nitrogen to produce compound 87. This compound would then be oxidised using cerium ammonium nitrate to give the 7-methoxy scaffold (72), a single step away from the desired makaluvamines. The aim was then to synthesize the benzylamine (88), aniline (89) and phenylhydrazine (90) substituted makaluvamine-derivatives. These compounds strongly resemble makaluvamine G (13), shown earlier in Figure 1.4.
Scheme 2.1: Planned synthetic strategy based on work reported by Joule and co-workers\textsuperscript{40}

i) FeCl\textsubscript{3}.6H\textsubscript{2}O, glacial AcOH 140 °C; ii) Fuming HNO\textsubscript{3}, < -40 °C; iii) I\textsubscript{2}, t-BuI, FeCl\textsubscript{2}, TFA, DMSO, 80 °C; iv) MeOH, HCl, reflux; v) NaBH\textsubscript{4}, NiCl\textsubscript{2}, MeOH, 0 °C; vi) aq. 1N HCl, THF, 40 °C; vii) Desired amine, MeOH
2.2 Synthesis

In this section, the synthetic studies and the methods of optimization will be discussed. The experimental data pertaining to each section is described in detail in Section 4.2 of Chapter 4. Unsuccessful and duplicate reaction entries are available in an appendix.

2.2.1 Synthesis of the quinoline backbone (81)

The synthetic strategy was commenced by the synthesis of the quinoline backbone. The starting material was 3,4-dimethoxyaniline (66), which was treated with methyl vinyl ketone (MVK (82)) and iron(III) chloride hexahydrate in glacial acetic acid (Scheme 2.2). The dimethoxyaniline (66) was first dissolved in glacial acetic acid and the iron(III) chloride hexahydrate was added portion-wise as the mixture was heated to 60 °C. The MVK (82) was then added slowly over 30 minutes. After the addition of the MVK, the temperature of the reaction was raised. The first time, the reaction was monitored by TLC at different temperatures. The temperature was first raised to 120 °C and after 30 minutes, no change occurred. The temperature was then raised to 130 °C and after another thirty minutes, some change in terms of TLC spot formation started to occur. The temperature was then finally raised to 140 °C and the reaction was left to stir for 130 minutes, after which all of the starting material had been consumed. Upon cooling of the reaction mixture to room temperature, a solid precipitate crashed out of the solution.

Scheme 2.2: Formation of the quinoline backbone. i) Methyl vinyl ketone (82), FeCl₃·6H₂O, Glacial acetic acid, 140 °C, 2h.

The solid product was collected and the prescribed work-up was followed. Initially, 3.2 g of solid product was collected – which was surmised to be the wet acetate salt of the quinoline – and this was added to a sodium hydroxide solution (50% w/v). A very thick moss-green suspension formed and this was extracted with ethyl acetate, unfortunately giving rise to a very thick emulsion. Further extraction, drying and evaporation only resulted in the isolation of the desired product in 18% yield. This was confirmed by ¹H Nuclear Magnetic Resonance (NMR) spectroscopy, with the spectrum showing the singlet corresponding to the two
methoxy-groups of the starting material (surprisingly at the same ppm), along with a newly formed singlet that corresponded to the methyl group in the 4 position of the quinoline. The protons in the aromatic region also confirmed that it was indeed the product when compared to the chemical shifts reported in the literature.  

Spectrum 2.1: $^1$H NMR spectrum of the methylquinoline 81

This was a very poor yield and did not compare well to the reported literature yield of 66%. Possible reasons for this were considered – with the work-up regarded as one of the main problems, as the formation of the emulsion could hinder efficient extraction of the product. Therefore, rather than using an excess of the sodium hydroxide solution, it was decided to basify the product in a drop-wise manner, as well as to change the solvent used for extraction, as ethyl acetate had a propensity for forming emulsions under the conditions utilized.

The reaction was then repeated and upon completion (confirmed by the disappearance of the starting material spot on TLC), the reaction mixture was cooled on ice for 30 minutes. The solid that formed was collected by means of vacuum filtration and then dissolved in 40 mL of distilled water. This mixture was then basified to a pH of 11 by the addition of a 50% w/v sodium hydroxide solution in a drop-wise manner and rigorously extracted with dichloromethane ($\text{CH}_2\text{Cl}_2$). The product was isolated in a marginally improved yield of 30%.
The strong basic work-up still seemed to be a problem, so it was decided to attempt the following nitration step on the acetate salt (91) of the quinoline (Scheme 2.3) to avoid the unnecessary loss of product. In a repeated experiment, after all of compound 66 had been consumed, the acetate salt (91) was collected and dried under high vacuum. Corresponding to a yield of 73% for the salt, this material was slowly added in portions to fuming nitric acid at -45 °C. The addition was done over an hour, whilst maintaining the temperature between -50 °C and -40 °C and then left for another hour to stir, with the temperature still below -40 °C. The reaction mixture was then poured over ice and basified to pH 12 using a 50% w/v sodium hydroxide solution, followed by a rigorous extraction with CH₂Cl₂. Unfortunately, with this method, the product was isolated in a yield of only 12% over the two steps.

Scheme 2.3: Quinoline formation and successive nitration. i) Methyl vinyl ketone (82), FeCl₃·6H₂O, Glacial acetic acid, 140 °C, 2 h; ii) Fuming HNO₃, < -40 °C, 2 h.

Since the strong basic work-up has proved unsuccessful, it was decided to use a milder base in the work-up procedure. So instead of using the 50% w/v sodium hydroxide solution, the salt was added to CH₂Cl₂ to form a slurry, after which a 10% w/v K₂CO₃ solution was added whilst stirring. This time, after the work-up of the reaction, the product was isolated in a yield corresponding to 36%. The use of a milder base appeared to have a beneficial effect on the work-up, as the yield was doubled from the first attempt where 50% w/v sodium hydroxide solution had been used. Finally, it was decided to use a more concentrated basic work-up.

In the next attempt, after the completion of the reaction, the acetate salt (91, Scheme 2.3) was isolated again and dried. A portion of the product was then used to try a different work-
up. Instead of 10% w/v K$_2$CO$_3$ solution, a saturated solution of sodium bicarbonate was used. Portions of the salt were then slowly added to a stirring solution of saturated sodium bicarbonate. Upon addition, there was an evolution of gas from the reaction mixture and ethyl acetate was added. Subsequently, this was concentrated under reduced pressure and dried under high vacuum to finally give the desired product in a yield of 62%, which was much closer to the 66% reported in literature. The results for all the attempts are summarized in Table 2.1 below:

Table 2.1: Optimization conditions and yields

<table>
<thead>
<tr>
<th>Work-up Base</th>
<th>Amount used (per mass of acetate salts)</th>
<th>Extraction Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 50% w/v NaOH solution</td>
<td>100 mL per 3.2 g</td>
<td>Ethyl Acetate</td>
<td>18</td>
</tr>
<tr>
<td>2 50% w/v NaOH solution</td>
<td>Dropwise on 1.04 g (pH 11)</td>
<td>Dichloromethane</td>
<td>30</td>
</tr>
<tr>
<td>3 10% w/v K$_2$CO$_3$ solution</td>
<td>150 mL per 1.73 g</td>
<td>Dichloromethane</td>
<td>36</td>
</tr>
<tr>
<td>4 Saturated NaHCO$_3$ solution</td>
<td>100 ml per 2.4 g</td>
<td>Ethyl Acetate</td>
<td>62</td>
</tr>
</tbody>
</table>

The mechanism of this reaction was also considered and a possible mechanism is suggested in Scheme 2.4. The mechanism is postulated to start with the protonation of the methyl vinyl ketone (82) by the acidic solvent, thereby increasing the electrophilicity of the compound. The mechanism that follows then resembles the Skraup synthesis of quinolines where the aniline (66) attacks at the Michael-acceptor to give intermediate 92a, which through a loss of a proton and tautomerization, forms compound 92b.$^{49}$ The protonation of compound 92b to form compound 92c, sets it up for an intramolecular nucleophilic attack resulting in the formation of intermediate 93a. A loss of a proton and rearomatization produces the 1,2-dihydroquinoline (93b). Then, whereas the Skraup quinoline synthesis uses nitrobenzene as oxidant, FeCl$_3$.6H$_2$O acts as oxidant in this synthesis to give product 81.
Scheme 2.4: Proposed mechanism of the quinoline formation.

When the synthesis was repeated at a later stage to remake some starting material, the work-up procedure was modified slightly to see if the filtration of the acetate salt could potentially be a point in the synthesis where more product could be isolated. Therefore, instead of filtering off the salt after cooling the reaction mixture on ice, the acetic acid was removed \textit{in vacuo} to give a brown precipitate. The work-up was then continued as usual and the product was isolated in a very good yield of 84%, greatly improving upon the yields of previous attempts, as well as what was reported in the literature.

In summary, the use of a strong basic work-up proved to be detrimental to the success of the reaction, as the continued struggle with emulsions in the early stages along with the use of ethyl acetate meant that the extractions were very inefficient. The exact cause of this was never determined, but the increases in yields observed when a milder basic work-up was
used did seem to support this logic. The removal of acetic acid *in vacuo* also proved to be very successful, as the yields increased remarkably. This change was made due to a suspicion that the glacial acetic acid used for this step had become wet and rather than distil this first, the decision was made to first see what effect *in vacuo* removal would have compared to filtration. The success of this change in the procedure suggested that some product might have been lost during the filtration step. Thus, by increasing the yield to this extent, the synthetic strategy started off more efficiently.

### 2.2.2 Selective nitration of the quinoline (82)

The next step of the synthesis involved the selective nitration of the 5-position of the quinoline scaffold (81, Scheme 2.5). The procedure prescribed by Joule and co-workers used fuming nitric acid (nitric acid with a concentration greater than 99.5%), and it was decided to follow this strategy. The strategy involved nitration at temperatures below -30 °C, otherwise there is a possibility of undergoing nitration in both the 5- and 8-positions to form compound 94.

![Scheme 2.5: Nitration of the quinoline. i) Fuming HNO₃, < -40 °C, 2 h.](image)

The reaction was started by cooling fuming nitric acid to -50 °C in a dry ice and acetone mixture bath under a flow of nitrogen. The first portion of compound 81 was added and it turned out that the nitric acid had frozen. Fuming nitric acid has a melting point of -42 °C, so the reaction mixture was left to warm slightly to -40 °C and the addition was continued. Care was taken to maintain the temperature at about -40 °C during the addition of the quinoline, which was added over an hour. The temperature was monitored using a thermometer that was calibrated for use at temperatures below 0 °C. The reaction was left to stir for another
hour before the reaction was quenched by pouring the reaction mixture over an ice slurry and then basifying to a pH of 12 with 50% w/v sodium hydroxide solution. After the work-up, the product was isolated in a disappointing yield of 28%. The product was confirmed to be the desired product by means of $^1$H NMR spectroscopy, in which the disappearance of one of the aromatic protons was noted. The singlet of the proton in the 5-position of the quinoline was no longer visible and the addition of the nitro-group also led to the resolution of the singlet that corresponded to the two methoxy groups. These two groups now appeared as two singlets that were very close to each other. All of the chemical shifts also correlated well to the literature values.\(^{40}\)

Spectrum 2.2: $^1$H NMR spectrum of the nitration product (73)

Unfortunately, the yield was substantially lower than the reported literature yield of 63% and this was attributed to the freezing of the fuming nitric acid. The reaction was then repeated with the only difference being that the dry ice and acetone ice bath was maintained at -40 °C. This time, after extraction, the product was isolated in 56% yield, thus doubling the previous yield. This reaction was done repeatedly throughout the duration of the project, with only slight changes to the work-up and with this, the yield was increased to 79%, which improved on the literature yield of 63%. The significant change was only in the rate of addition of the sodium hydroxide solution to quench the acid upon completion of the
reaction. In one instance, the sodium hydroxide solution was accidentally added a bit too fast, causing the mixture to heat up whilst still being acidic (pH of the mixture was monitored by universal indicator paper). This caused concern that the compound might have undergone nitration in the 8-position, to form compound 94 as a side product. Fortunately, no side product formed and only compound 73 was isolated in an improved 79% yield, as the $^1$H NMR spectrum showed the singlet corresponding to the 8-position of the quinoline.

2.2.3 Oxidation of the aromatic methyl group of compound 73 to give product 83

After the successful nitration, the quinoline (73) needed to be oxidized to the substituted quinoline-4-carbaldehyde (83). Generally, aromatic methyl groups can easily be oxidised to carboxylic acids by various means, but selectively oxidizing to the corresponding aldehyde has proved to be more difficult. Strategies would, therefore, involve over-oxidizing to the carboxylic acid, followed by a reduction to the alcohol and successive oxidation to the aldehyde by means of the Swern oxidation or by using MnO$_2$. Joule and co-workers made use of a very efficient oxidation initially reported by Vismara et al., which successfully and selectively converted heteroaromatic methyl groups to their corresponding aldehydes in high yields. Their study involved the conversion of lepidine (95) to quinoline-4-carbaldehyde (96) using a new procedure involving the use of iodine, tert-butyl iodide, trifluoroacetic acid (TFA), iron(II) chloride tetrahydrate and dimethyl sulfoxide (DMSO) acting as both solvent and oxidant. In spite of the success of the procedure, there have been no investigations into the mechanism of this oxidation, as far as a literature search was concerned. Vismara and co-workers also speculated as to the mechanism and did investigate the importance of each of the components, although they did not go as far as to suggest a full mechanism. The formal stoichiometry was suggested to be as it is in Scheme 2.6, but this does not take into account the role of the tert-butyl iodide. The formation of dimethyl sulfide as a by-product, does suggest some similarity to the Swern oxidation.

Scheme 2.6: Stoichiometry of the oxidation. i) I$_2$, DMSO.
When the reaction was performed without tert-butyl iodide, a yield of only 13% was achieved compared to the quantitative yield when it is used. This highlighted the importance of the tert-butyl iodide and the stoichiometry was reconsidered (shown in Scheme 2.7). Vismara and co-workers therefore suggested that the major oxidant must be the oxyalkylsulfonium iodide (97), formed by the reaction of DMSO and tert-butyl iodide.

Scheme 2.7: Revised stoichiometry of the oxidation

The stoichiometry also suggests that one equivalent of tert-butyl iodide is regenerated during the oxidation. The reaction also seems strongly dependent on the acid catalyst, as its absence also lowers the yield to 10–12%. It was, therefore, suggested that the acidic medium could cause the formation of the dienamine (98, Scheme 2.8) – even if only in small amounts at equilibrium – which could be very reactive towards the oxyalkylsulfonium iodide species (97).

Scheme 2.8: Equilibrium under acidic conditions

The importance of the ferrous chloride tetrahydrate and iodine were also investigated, as their absence produced yields of 60–64% and 15%, respectively. All four components were, therefore, essential for the success of this reaction. Due to the efficacy of this reaction, Joule and co-workers, therefore, successfully utilized it in their work. They reported synthesizing compound 83 (Scheme 2.9), in a 73% yield. This reaction was repeated exactly as prescribed in our synthesis. The starting material, 6,7-dimethoxy-4-methyl-5-nitroquinoline (73), was dissolved in DMSO. One equivalent of iodine and 0.06 equivalents of ferrous chloride tetrahydrate were then added to the stirring reaction mixture, followed by 1.25
equivalents of TFA and 0.22 equivalents of tert-butyl iodide. The reaction was stirred for 7 hours at 90 °C, but analysis by means of thin layer chromatography (TLC) showed the reference spot of the starting material and the reaction spot to be at the same $R_f$-value. It appeared that no reaction had occurred in the allotted time and the decision was made to stop the reaction. It was decided to quench the reaction and analyse it anyway, as there was a slight difference in the way the two spots fluoresced under ultraviolet light irradiation. The reaction mixture was diluted with distilled water and added to a stirring solution of saturated sodium thiosulfate. The acid in the reaction mixture was quenched with 10% w/v K$_2$CO$_3$ solution and the product extracted with CH$_2$Cl$_2$. A small amount of the crude product was isolated, but analysis by $^1$H NMR showed that it was not in fact the desired product.

Scheme 2.9: Oxidation of 6,7-Dimethoxy-4-methyl-5-nitroquinoline. i) FeCl$_2$·4H$_2$O, TFA, I$_2$, t-BuI, DMSO, 90 °C, 6 h.

Since the first attempt was unsuccessful, even after following the literature procedure, it was decided to use different equivalents. The reaction was then attempted again, this time altering the order of the addition of the reagents. After first dissolving the quinoline (73) in DMSO, 0.52 equivalents of the ferrous chloride tetrahydrate was added, followed by 5 equivalents of TFA and 1 equivalent of iodine. Finally, 2 equivalents of tert-butyl iodide were added. The reaction was stirred for 7 hours at 90 °C. Subsequent analysis by TLC showed no more starting material (which had a $R_f$-value of 0.45) and a spot on the base line. This was strange, as it would not be expected for there to be such a big difference in polarities between the starting material and product. The reaction was stopped and the work-up modified slightly. Instead of diluting the reaction mixture with distilled water, the saturated sodium thiosulfate solution was added to the reaction and then basified to pH 11 using 50% w/v sodium hydroxide solution. The product was then rigorously extracted with ethyl acetate and a very poor 42 mg of crude product was collected (compared to the 683 mg of starting material used for the reaction). The small amount of product was then analysed by $^1$H NMR spectroscopy, only for the spectrum to be unusable. Excessive line-broadening occurred and the characteristic aldehyde peak one would expect, was absent. This meant that the reaction had failed once more. Considering that the TLC showed a spot on the base line, it was
speculated that the oxidation might have somehow gone too far and that it could have formed the carboxylic acid instead.

Thus, the reaction was attempted again on a 100 mg scale, but the reaction was monitored much more closely. The same equivalents of reagents were used, but the reaction was analysed by TLC after 2 hours. At this stage, the spot on the baseline had already formed – meaning the reaction might have over-oxidized already. The reaction was quenched the same way as in the previous attempt, delivering 44.7 mg of product. The crude product was analysed by $^1$H NMR spectroscopy and it appeared that some of the desired product might have formed, as a singlet appeared at 10.16 ppm in the $^1$H NMR spectrum that could have been indicative of an aldehyde. The sample was still very impure though, so purification would not give enough product to continue the synthesis. It was decided to monitor the reaction after 30 minutes instead and it was also decided to dry the DMSO over 4 Å molecular sieves.

The reaction was again repeated with the same equivalents of reagents and monitored much more closely. This time, there was no spot on the base line and after 3 hours and 15 minutes, analysis by TLC showed two spots at the same $R_f$-value that fluoresced differently. The two spots also reacted differently to the KMnO$_4$-stain, with the spot of the reaction almost not reacting at all as opposed to the starting material developing in the stain. A part of the work-up was also modified to try and maximise the yield. The extractions were done with copious amounts of CH$_2$Cl$_2$ instead of using ethyl acetate. The CH$_2$Cl$_2$ fractions were combined and then washed with a large amount of distilled water, to remove most of the DMSO. The solvent was removed under vacuum to furnish 55 mg of crude product. The product was still impure and purification by chromatography would be impossible as the starting material had the same $R_f$-value as the product. The oxidation was starting to become a hindrance, as attempts to successfully utilise the procedure and optimization thereof, had been unfruitful.

At this stage, other methods for oxidation were being considered, as well as a change in the order of the synthetic strategy. Firstly, it was decided to attempt the oxidation procedure on the methylquinoline (81, Scheme 2.10). The reaction was monitored by TLC and when it appeared to have reached completion, the reaction was worked up. Instead of basifying to a pH of 11 with 50% w/v sodium hydroxide solution, the reaction was basified to a pH of 8 with 10% w/v K$_2$CO$_3$ solution. After this, 93 mg of product was isolated, but analysis by means of $^1$H NMR spectroscopy showed that no reaction had occurred as it was all starting material (81).
Following the failure of this attempt as well, other methods were researched. One method for the oxidation of the methylquinoline (81) that was considered was based on a method reported by Trahanovsky and Young, which employed the use of cerium ammonium nitrate (CAN) in aqueous acetic acid to oxidize toluene (100) into benzaldehyde (101) in 92% yield (Shown in Scheme 2.11).  

The method of Trahanovsky and Young was attempted on the substrate of interest (Scheme 2.12), but this was unsuccessful, as the material appeared to have degraded. It was speculated that a possible product might have been the iminoquinone (102), amongst others. The reasoning behind this comes from considering similarities in the substrate (81) to that of the substrate in the unsuccessful synthesis reported by White et al. (compound 43, Scheme 1.2). These researchers also employed the use of CAN for the oxidation of the tetrahydroquinoline (43) and synthesized a compound (44) similar to what we postulated.
Scheme 2.12: Attempted oxidation procedure reported by Trahanovsky and Young applied to our substrate.\textsuperscript{51} i) CAN, 50% aq. HOAc, 100 °C, 5 h.

The decision was made to have one last attempt at the oxidation procedure reported by Vismara \textit{et al.}\textsuperscript{50} Considering the fact that it appeared that some over-oxidation might be occurring, it was decided to minimize the amount of water in the reaction. The equivalents of ferrous chloride tetrahydrate were thus adjusted to 0.5 equivalents. This corresponded to 2 equivalents of water being added to the reaction. To further ensure that the amount of water in the reaction was minimized, the DMSO was dried over 4 Å molecular sieves. The DMSO was then heated to 60 °C while the methylquinoline (73) was added. 0.125 Equivalents of the ferrous chloride tetrahydrate was then added, followed by the same equivalents of the other reagents as before: 5 equivalents of TFA, 1 equivalent of iodine and 2 equivalents of \textit{tert}-butyl iodide. The reaction was then heated to 90 °C and stirred for 6 and a half hours. The reaction was then analysed by TLC and again, the spots for the starting material and proposed product were at almost the exact same \textit{Rf}-value. Again, under ultraviolet light irradiation, there was a slight difference in the fluorescence of the two spots. The TLC was then stained using an iodine stain and it was noted that the starting material developed in the stain, whereas the product did not develop at all. For extra assurance and since iodine is a reversible stain, the TLC was also stained with \textit{p}-anisaldehyde stain – which is selective for aldehydes. Upon heating the TLC plate, the product spot started turning yellow. The reaction was therefore deemed complete and worked up to give 209 mg of crude product. Analysis by \textsuperscript{1}H NMR spectroscopy confirmed that it was indeed the desired product, showing a singlet at 10.23 ppm that corresponded to the aldehyde proton of the literature value.\textsuperscript{40} This also meant the product was isolated in an excellent yield of 99%.
The reaction was repeated many times and the yields then generally varied between 80 and 95%. It should be noted that the amount of ferrous chloride tetrahydrate clearly had an influence on the success of the reaction, as using 0.125 equivalents delivered very high yields. This corresponded to merely half an equivalent of water, so the results seen up to this point which indicated a possible over-oxidation could have been influenced by an excess of water present. The initial report by Vismara and co-workers utilized 0.06 equivalents of ferrous chloride tetrahydrate (thus, 0.24 equivalents of water in the reaction), less than half of what we found to be efficient. Therefore it did seem that the amount of water added to the reaction in the form of the ferrous salt needed to be kept to a minimum. It should be noted that this was not stipulated or mentioned in the paper published by Vismara and co-workers. Following the success of the reaction and the high yields, the silver(II) oxide and IBX methods were not investigated.

### 2.2.4 Protection of the aldehyde (84a,b)

The next part of the synthesis involved the protection of the aldehyde functionality by forming the acetal (Scheme 2.13). Joule and co-workers formed the acetal (84a) by stirring up the aldehyde (83) in anhydrous methanol with an acid catalyst. Another member of our group had initially attempted the prescribed procedure for the formation of compound 84a, but it was found to be fairly inefficient.

[Scheme 2.13: Formation of the acetal](#)

Therefore, the decision was made to make use of an orthoester to generate the acetal. Methods of interest involved the use of trimethyl orthoformate (103, Scheme 2.14) in anhydrous methanol, in the presence of an organic acid such as camphor sulfonic acid or p-toluene sulfonic acid, to generate the dimethyl acetal. The use of an orthoester in acidic medium served a dual purpose, as it acted as both a dehydrating agent and generated methanol in situ, as shown in Scheme 2.14. This favoured the forward reaction by limiting
the amount of water present for hydrolysis of the acetal. It also avoided the formation of an equilibrium, as it promoted the forward reaction along with the generation of more methanol. As the acetal is formed, the consumption of the alcohol by the aldehyde also favours the forward reaction of the hydrolysis of the orthoester (103). The organic acid first protonates one of the methoxy-groups of the orthoester, after which a lone pair of another oxygen kicks in to eliminate the first equivalent of methanol. The resulting oxonium species (104) then undergoes nucleophilic attack by a water molecule. This consumption of water by the orthoester also serves to inhibit the hydrolysis of the newly formed acetal and makes the forward reaction more favourable. An intramolecular proton transfer converted compound 105 into compound 106, which can now eliminate another equivalent of methanol. By regenerating the acid catalyst, methyl formate (107) is formed as a by-product.

Scheme 2.14: Mechanism for the hydrolysis of the orthoester

Chao et al. reported using p-toluene sulfonic acid as catalyst, thus the consumption of water by the orthoester is especially beneficial as the acid is used in its monohydrate form. The mechanism for the formation of the acetal is shown in Scheme 2.15.
Scheme 2.15: Mechanism for the formation of the acetal.

The reaction was then done by dissolving the aldehyde (83) in anhydrous methanol and then adding the orthoester (103) along with the acid catalyst. A nitrogen atmosphere was established and the reaction was stirred at 30 °C for 24 hours. After 24 hours though, TLC showed that the starting material was still present, so a nitrogen atmosphere was established again and the reaction heated to 60 °C and then stirred for 24 hours. Analysis by TLC then showed that the starting material had been consumed and the reaction had reached completion. The reaction was stopped and worked-up to give 94.3 mg of the crude product in a mere 38% yield. However, even this yield was not obtained as analysis by 1H NMR spectroscopy showed the product to contain a significant number of impurities that could not be identified. Nonetheless, the spectrum did show the peaks corresponding to the two methyl groups of the newly formed acetal at 3.31 ppm and the C-H proton peak of the acetal at 5.50 ppm.

The reaction was then repeated using dry benzene instead of methanol and stirred at 60 °C for 48 hours before the reaction was stopped and worked-up. This time, the product was isolated in 82% yield, but again the 1H NMR spectrum showed there to be small amounts of impurities present. This problem persisted, so the decision was made to synthesize a different acetal. Instead of the dimethyl acetal, the dioxalanyl acetal was formed by stirring...
up the aldehyde with ethylene glycol and an acid catalyst, thus generating compound 84b. The reaction was carried out in dry benzene using a Dean-Stark setup over a period of 72 hours, after which the reaction mixture was concentrated under reduced pressure and the residue redissolved in 10% K$_2$CO$_3$ solution. The resulting solution was extracted with ethyl acetate to give compound 84b (Scheme 2.16) in 79% yield as a pure product, according to the $^1$H NMR spectrum.

![Scheme 2.16: Formation of the dioxalanyl acetal (84b). i) Ethylene glycol, $p$-TsOH, benzene, reflux, 72 h.](image)

The C-H peak of the acetal was visible at 6.18 ppm and the two methylene groups of the dioxalanyl ring overlapped with the peak for the two methoxy groups in the 6- and 7-positions. The greater purity of the product obtained from this method made it preferable to the formation of the dimethyl acetal. The formation of the dioxalanyl acetal was driven by its superior stability to that of the dimethyl acetal. This increased stability was as much a disadvantage as it was an advantage, as it made the deprotection after the reduction step much more problematic. The reaction times had to be increased as the protonation of the dioxalanyl ring (compound 108, Scheme 2.17) led to the formation of the oxonium species 109 that had two possible nucleophiles – the amine on the 5-position of the tetrahydroquinoline and the hydroxyl-group on the ethyl chain. The desired route is for the amino-group to attack the electrophilic site of the oxonium species (109) and thus lead to the formation of the ammonium species 110. But the reverse reaction – with the hydroxyl-group acting as nucleophile – is equally likely and the reaction would reverse to form compound 108 again.
Therefore the decision was made to investigate the use of a dimethyl acetal again and attempt to optimize the reaction conditions. The advantages of using an orthoester were considered again and a literature search was done. Yang et al.\textsuperscript{53} reported a procedure fairly similar to that of Chao et al.,\textsuperscript{52} but instead of using methanol as solvent, they merely used trimethyl orthoformate as solvent and methanol source. The aldehyde (83) was then dissolved in trimethyl orthoformate and 0.5 equivalents of \( p \)-toluene sulfonic acid monohydrate were added. The reaction was next heated to 100 \(^\circ\)C and stirred for 24 hours to reach completion. After the reaction mixture was quenched with a saturated solution of sodium hydrogen carbonate, the product was extracted with ethyl acetate to give the pure dimethyl acetal (84a) in 92\% yield (Scheme 2.18).
Scheme 2.18: Successful acetal formation procedure. i) Trimethyl orthoformate, \(p\)-TsOH, 100 °C, 24 h.

This procedure – with its high yield and purity – was then used. The different sources of alcohol for the formation of the acetals, along with which acid and solvent was used, is summarized in Table 2.2 below:

Table 2.2: Different strategies attempted for the synthesis of Compounds 84a and 84b

<table>
<thead>
<tr>
<th>Entry</th>
<th>Source of alcohol</th>
<th>Acid</th>
<th>Solvent</th>
<th>Acetal</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>Hydrochloric acid</td>
<td>Anhydrous methanol</td>
<td>84a</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>Trimethyl orthoformate</td>
<td>(p)-Toluene sulfonic acid</td>
<td>Anhydrous methanol</td>
<td>84a</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>Trimethyl orthoformate</td>
<td>(p)-Toluene sulfonic acid</td>
<td>Benzene</td>
<td>84a</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>Ethylene glycol</td>
<td>(p)-Toluene sulfonic acid</td>
<td>Benzene</td>
<td>84b</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>Trimethyl orthoformate</td>
<td>(p)-Toluene sulfonic acid</td>
<td>Trimethyl orthoformate</td>
<td>84a</td>
<td>92</td>
</tr>
</tbody>
</table>

2.2.5 Reduction, ring-closure and selective methylation (85-87)

Upon the successful synthesis of compound 84a in high yield and purity, it was used for the synthesis of the diamine 85a (Shown in Scheme 2.19). It should be noted here that compound 84b was also used in these reduction reactions, but as mentioned earlier, the complications that arose during the hydrolysis of the acetal rendered it ineffective as an intermediate in the synthetic strategy. Therefore the use of compound 84b and subsequently that of compound 85b will not be discussed, although it was used in reactions that attempted the construction of the tricyclic scaffold. The planned reduction of compound 84a was expected to bring about a large change in molecular properties, as not only the nitro-group would be reduced to an amine, but the aromaticity of the heterocyclic ring of the quinoline would be changed to produce a substituted aniline. The reduction was done by dissolving compound 84a in methanol along with the nickel(II) salt. Sodium borohydride was then rapidly added in portions to produce a highly exothermic reaction. Both the sodium borohydride and nickel(II) chloride hexahydrate were used in great excess. The reaction was started at room temperature, but after the addition of the sodium borohydride, the reaction
mixture could reach temperatures as high as 80 °C. Upon addition of the first portion of sodium borohydride, the mixture turned black and started giving off gas – which is most probably \( \text{H}_2 \) as transition metals in the presence of sodium borohydride and water tend to evolve hydrogen gas from the reaction mixture.\(^{54}\) The black colour was due to the fine particles of the nickel borides, the \( \text{Ni}_3\text{B} \) and \( \text{Ni}_2\text{B} \) species, that formed as the sodium borohydride interacted with the nickel(II) salt in the alcohol medium.\(^{55}\) Nickel boride is a very active reducing agent with a selectivity for nitro-groups – even aliphatic nitro-groups – and C-C unsaturations, but it is not strong enough to reduce carboxylic acids, esters or amides.\(^{56}\)

The reaction was stirred for 25 minutes after the addition of sodium borohydride, at which point the reaction mixture also stopped bubbling. The reaction mixture was subsequently quenched with distilled water and ethyl acetate was then added to the stirring mixture. The work-up was fairly difficult in terms of effective extraction as the black particles that formed were insoluble in the aqueous phase leading to a heterogeneous mixture. Initial attempts at filtering off the black solid proved troublesome as the fine particles packed very tightly during filtration by means of a sintered glass funnel, such that the filtrate could not pass through. When the product, using compound \( 84a \) as starting material, was finally isolated, it was a faint pink oil with an almost waxy consistency. It was stored below 0 °C overnight, but even at low temperatures, the product seemed prone to degradation. The colour changed from the waxy, light pink to a strong dark pinkish purple colour overnight.

This product was analysed by means of \( ^1\text{H} \) NMR spectroscopy, but the product had degraded and the spectrum was unusable. When the reaction was repeated, it was noticed that the colour of the organic layer also started changing during the course of the extraction. The organic layer started off as colourless, but as the work-up continued, the organic layer started to darken and started resembling the colour of the degraded product. The work-up was continued, but the colour had changed to a very dark pinkish red and analysis by means of \( ^1\text{H} \) NMR spectroscopy showed that the product had degraded. It was speculated that the resulting diamine (\( 85a \)) was fairly sensitive to oxidation when exposed to air. This seemed to be supported by the fact that the first attempt saw the degradation of the product when stored under normal atmosphere below 0 °C. Due to the apparent sensitivity of compound \( 85a \), it seemed more logical to only isolate the product after the following step – thus, only isolate the product after the ring-closing step, i.e. isolate the indole \( 86 \). The indole was to be generated by stirring up the intermediate \( 85a \) in acidic medium. Various strategies were attempted, but the first was the strategy proposed by Joule and co-workers.\(^{40}\) In their approach, the diamine (\( 85a \)) was stirred up in aqueous 1 N hydrochloric acid in THF at 40 °C for 1 hour to afford the indole in a respectable 64% yield.
Scheme 2.19: Reduction of the acetal and subsequent ring-closure to form the indole and proposed aniline methylation. i) NiCl₂·H₂O, NaBH₄, MeOH, rt, 15 min; ii) [H⁺]

This approach was therefore followed on the acetal (84a) and after an hour and 30 minutes at 40 °C, no real change was observed. The reaction temperature was then increased to 65 °C, but analysis by means of TLC showed multiple spots and it seemed degradation had occurred. The reaction was attempted again, this time the acetal was dissolved in methanol and the nickel(II) salt added. The addition of sodium borohydride again turned the reaction mixture black as the nickel boride formed. After the addition of all the sodium borohydride, the reaction mixture was left to stir until it stopped bubbling and the reaction was then quenched with distilled water. The reaction mixture was immediately filtered through a sintered glass filter, again proving difficult, as the small, black nickel boride particles packed very tightly and blocked the frit of the sintered glass filter, slowing down the filtration. The filtrate was then extracted with CH₂Cl₂ and the organic layer collected, dried and evaporated under reduced pressure. The residue was then redissolved in aqueous 2 N hydrochloric acid and stirred at 60 °C for 3 hours. When analysed by TLC though, four spots had formed again, but unlike the earlier attempt, these spots looked to be more easily separated by column chromatography.
Firstly, a crude $^1$H NMR spectrum was obtained of the extracted products, rather than investing time in purification. From this crude $^1$H NMR spectrum, some signals suggested that the desired product was in fact present, namely: the two triplets corresponding to the two methylene carbons (numbered 1 and 2 in Spectrum 2.3), the two aromatic singlets corresponding to the tetra-substituted indole (numbered 3 and 4 in Spectrum 2.3) and finally the two methyl groups of the methoxy substituents (numbered 5 and 6 in Spectrum 2.3).

There were also methoxy-peaks present in the crude sample that did not appear in the spectra of the purified products that were collected. It is unclear what happened to these spots.

Spectrum 2.3: Crude $^1$H NMR spectrum of the ring-closing reaction

The crude product was then purified by means of column chromatography, using a gradient elution. The top two spots on the TLC (which required the use of a solvent system of 10% methanol/ethyl acetate as eluent), were collected by using a solvent system of 5% methanol/ethyl acetate as eluent and the third spot was collected using a 15% methanol/ethyl acetate solvent system as eluent. The fourth spot observed on the TLC plate was not collected, as it did not elute when using the polar 15% methanol/ethyl acetate solvent system as eluent. All three of the spots that were collected, were analysed by means of $^1$H NMR spectroscopy and unfortunately the structures of the first two spots could not be
elucidated from their NMR spectra, as these were complex and no structural information could be obtained from them. Significantly, the peaks of the third NMR spectrum matched up with the peaks identified in the crude spectrum. The $^1$H NMR spectrum showed broadening of the peaks, which could not be removed by any processing and the area under these peaks could not be integrated. The chemical shifts did however correspond to the peaks identified in the crude reaction mixture. The reaction was repeated and purified by means of column chromatography to again produce the product, this time in a 19% yield, which delivered a much more interpretable $^1$H NMR spectrum (Spectrum 2.4). It should be noted here, that the deuterated solvents used in our experiment differed from those reported in the literature, as the literature spectrum was recorded in deuterated chloroform and we used a mixture of deuterated chloroform and DMSO due to the sparing solubility of the product in deuterated chloroform. However, when the chemical shifts of the peaks of this spectrum were compared to those reported in the literature, it was found that the peaks in the aliphatic region did not match up to that of the expected product. The triplet signal at a chemical shift of 2.78 ppm, was close to that reported in the literature – 2.99 ppm – for the expected product 86. The other triplet signal, which was expected to appear at a chemical shift of 3.50 ppm, appeared much higher at a chemical shift of 4.15 ppm. This meant that this was in fact not the desired product (this was confirmed by $^{13}$C NMR spectroscopy, which is discussed later).\textsuperscript{40} Analysis of this sample by means of $^1$H NMR spectroscopy introduced more discrepancies between the spectrum of our product and that reported for compound 86 – the peaks did not match up and extra splitting appeared on the signals. Closer inspection also revealed that this splitting pattern had occurred with all proton signals (Shown on Spectrum 2.4). The area under the proton signals were integrated and the signal at 3.75 ppm, which would be expected to be equivalent to 6 protons for the two methoxy groups, only integrated for 3 protons. This indicated a possible loss of a methyl-group. The other peaks did integrate for their expected values, which lead us to believe that this compound might be related to the desired product. Due to the darkening of the colour during column chromatography, an oxidation was considered as a possible side reaction since SiO$_2$ has been reported to act as a mild oxidant.\textsuperscript{57}
Spectrum 2.4: $^1$H NMR spectrum of the compound at first thought to be compound 86.

*It should be noted that an unexplained doubling up of the peaks in the spectrum occurred. Due to the instability of the product, the spectrum could not be rerun. These are not present in the $^{13}$C NMR spectrum.

The $^{13}$C NMR spectrum was also obtained and inspected (Spectrum 2.5), so as to gain insight into the possible structure of the compound which had formed. Upon inspection, a carbon signal in the aliphatic region was also unaccounted for, which confirmed the suspicion that a methyl-group had been “lost” during the reaction.
Spectrum 2.5: $^{13}$C NMR spectrum of the compound.

As an oxidation was suspected to have been possible, the NMR spectroscopic data of the known 7-methoxypyrroloiminoquinone (78) was inspected. Of significance was that the chemical shift values of the spectrum compared very well to that reported in the literature. 40 The chemical shifts of the signals in the $^1$H NMR spectrum were 0.08 – 0.15 ppm from that of the literature value. It must also be noted that the literature spectrum was recorded in CDCl$_3$, but in our analysis, as the product was only sparingly soluble in this solvent, deuterated DMSO was added to aid in dissolving the compound. The difference in the values of the $^{13}$C NMR spectrum and that reported in the literature was also off by 1 – 2 ppm (these results are summarized in Table 2.3).
Table 2.3: Comparison of chemical shifts of compound 78 as reported in the literature and experimentally determined during this study.\textsuperscript{40}

<table>
<thead>
<tr>
<th></th>
<th>( ^1\text{H NMR Chemical shifts (ppm)} )</th>
<th></th>
<th>( ^{13}\text{C NMR Chemical shifts (ppm)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lit.\textsuperscript{40}</td>
<td>Multipl.</td>
<td>Exp.</td>
</tr>
<tr>
<td></td>
<td>6.92</td>
<td>s</td>
<td>6.77</td>
</tr>
<tr>
<td></td>
<td>6.14</td>
<td>s</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>4.19</td>
<td>t, 7.9 Hz</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>3.84</td>
<td>s</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>2.78</td>
<td>t, 7.9 Hz</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>121.8</td>
<td>120.3</td>
<td></td>
</tr>
</tbody>
</table>

\*Literature NMR spectra recorded in CDCl\(_3\) whereas experimental data recorded in CDCl\(_3\)/DMSO-d\(_6\). Experimental \(^1\text{H NMR spectrum also showed unexplained doubling of peaks, as mentioned below Spectrum 2.4.}\)

This means that during the purification (possibly even during the reaction), an unexpected additional reaction had occurred – namely, the oxidation of the desired product (86) into the pyrroloiminoquinone 78 (See Scheme 2.20). This unexpected reaction was of course problematic, as the \(\text{N}\)-methyl group necessary to construct the makaluvamine G analogues, had not yet been introduced.

Scheme 2.20: Reaction scheme of side reaction that occurred. i) NiCl\(_2\).6.H\(_2\)O, NaBH\(_4\), MeOH, rt, 15 min; ii) [H\(^+\)].

Considering the lack of success in generating the indole scaffold (compound 86), different methods for achieving the ring closure were attempted, starting with the reduction of the acetal (84a) and optimization thereof. Different strategies for acidifying the resulting diamine (85a) were then investigated. Instead of acidifying the product (85a) under aqueous conditions, after the reduction was quenched and extracted with ethyl acetate, the residue
that was left when the ethyl acetate was removed under reduced pressure, was redissolved in dry THF. One equivalent of β-toluene sulfonic acid was added, a nitrogen atmosphere established and the reaction was stirred at room temperature for 17 hours. But, even then, no product had formed judging by the thin layer chromatograph analysis. The reaction was then heated to 65 °C and stirred for another 5 hours, but still no product had formed. The same procedure was repeated again, but the reaction mixture with β-toluene sulfonic acid was heated to 65 °C overnight instead of stirring it at room temperature. The reaction was again unsuccessful, as the 1H NMR spectrum of the crude product did not show any of the expected peaks of the indole product (86).

The reduction of the acetal (84a) was then repeated, but in attempt to slow down the reaction, the reaction mixture was cooled down to -20 °C using a crushed ice and coarse salt ice bath. Upon the completion of the reduction step, the reaction mixture was diluted with diethyl ether and the ether layer was isolated. This ether layer was acidified by using hydrochloric acid gas, which was generated by adding concentrated sulphuric acid to sodium chloride and bubbling the gas through the solution of ether and crude product. The hope was that if the ring closure did not occur, the diamine (85a) could at least be isolated as the corresponding ammonium salt.

As soon as the acid started bubbling through the ether, two layers formed. A small yellow layer formed at the bottom, which was suspected to be an aqueous layer containing the newly formed ammonium salts. Another possibility is that it could also be an immiscible salt that appears as an oily residue. This proved that it was imperative to thoroughly dry the ether layer after extraction. The ether was removed under reduced pressure to leave the suspected water layer. A colour change was observed whilst the reaction mixture was on the rotary evaporator: the yellow turning maroon and finally dark brown. This suggested that the ring closure had started taking place in the acidic conditions, therefore two drops of sulfuric acid were added to the mixture and left to stir at room temperature. An analysis by means of 1H NMR spectroscopy indicated the formation of the indole, although the spectrum was very impure. Two singlets that correspond to the two methoxy groups of tetrahydroquinoline scaffold were present. It was very difficult to conclusively identify any other characteristic peaks in the aliphatic region due to a multitude of peaks present. The protons of the two methylene groups on the tetrahydroquinoline structure could therefore not be clearly identified for confirmation of the ring closure. The attempted synthesis of compound 86 was again unsuccessful.

The reduction and subsequent acidification of compound 84a to synthesize compound 86 was attempted again, this time with the only difference that the ether layer was dried over
anhydrous magnesium sulphate to avoid the formation of two immiscible layers again. This did not prove to be sufficient though, as on commencement of the acid addition, small orange droplets formed. These became darker in colour with time. The mixture was then quenched with a solution of saturated sodium bicarbonate. Upon addition, the ether layer became a milky orange, then turned green and finally dark orange. The ether layer was isolated and dried overnight over anhydrous magnesium sulphate so as to dry the organic layer. When the isolated crude product was finally analysed by \(^1\)H NMR spectroscopy, it turned out not to be the desired product. The presence of water in this procedure does seem to cause some problems. Therefore, it is important to attempt to keep this step as water-free as possible and to minimize the amount of oxygen to which the reaction mixture is exposed.

Since repeated attempts to synthesize the indole had been unsuccessful, the isolation of the diamine 85a was attempted (Shown in Scheme 2.23). Therefore, the reduction with nickel(II) chloride and sodium borohydride was repeated. This new attempt was with a new batch of nickel(II) chloride, as well as a new bottle of methanol (both ordered from Sigma Aldrich), with the main difference being that the methanol was used without further purification or drying, whereas the previous attempts involved the use of anhydrous methanol. The sodium borohydride pellets were also crushed to a fine powder, instead of just being broken down into smaller pieces. The acetal was then dissolved in the commercial methanol and the fine sodium borohydride powder added rapidly in small portions.

Scheme 2.21: The reduction of quinoline (84a) giving rise to the tetrahydroquinoline (85a). i) NiCl\(_2\).6H\(_2\)O, NaBH\(_4\), MeOH, rt, 15 min.

Over various attempts, the yields attained using this method were as high as 94%. One of the ways this was achieved was by changing the sintered glass funnel used for the removal of the black nickel boride particles. The frit of the sintered glass funnel that was initially used had a porosity of 4, so it was later repeated but with a sintered glass funnel with a porosity of 0. The filtration was then done using a pad of cotton wool and the filtrate was collected with ease. The colour change was minimal when the reaction was rapidly filtered. It was also noted that the diamine (85a,b) – even though sensitive to oxidation – could be safely stored for three to four days, by storing it under a nitrogen atmosphere below 0 °C.
The successful synthesis of compound 85a, made it possible to focus attempts on the synthesis of the indole (86) again. The dioxalanyl acetal was then deprotected using a procedure reported by Schlosser and co-workers, but again, the procedure proved inefficient for this substrate. According to the procedure followed, the acetal had to be stored in 2 M hydrobromic acid for 24 hours. Instead of simply storing the compound in acid, the reaction was stirred for the same amount of time at room temperature, but the reaction did not proceed. Upon heating, the reaction did proceed but also degraded the organic material, therefore rendering the method ineffective.

Considering the lack of success in synthesizing the indole (86), the authors of the paper that initially reported the synthesis of this product were contacted. Professors Joule and Alvarez responded and confirmed what our research had been suggesting up until this point. According to Professor Alvarez, in their experience as well, the indole was very unstable and tended to degrade under reaction conditions. The researchers attributed this to the benzene ring being very electron rich, due to the methoxy- and nitrogen substituents on the ring. This supported our findings where significant colour changes occurred when handling the indole. These researchers then supplied us with the experimental sections of the thesis of two of their students who had worked on the results published in this paper. Due to the confirmed instability of compound 86, the decision was made to reconsider the synthetic strategy and alter it in order to avoid the synthesis of the indole (86).

2.2.6 Reconsideration of the synthetic route

The initial aim was to introduce a methyl group onto the anilino-nitrogen of the indole and synthesize compound 87, but the poor stability of compound 86 made it impossible to continue the initial synthetic strategy. Therefore, the decision was made to introduce the methyl group at an earlier stage in the synthetic strategy. The method considered was the methylation of the quinoline 84 to produce the quinolinium species 111 (Scheme 2.24). Reduction of this compound would then give rise to the N-substituted diamine 119. Compound 87 would then be synthesized using the same procedure initially attempted for the synthesis of indole 86 – by deprotecting the acetal under acidic conditions. There were concerns, however, that there might not be a marked improvement in the stability of compound 87 over that of compound 86. The extra inductive effects of the methyl group could increase the electron density in the benzene ring even further. It was hoped that the tertiary amine moiety might be more stable under the reaction conditions though. Upon the successful synthesis of compound 87, the synthetic strategy would then continue as initially planned with the oxidation to compound 72 and subsequent substitution.
The revised strategy was then started by the methylation of the quinoline (84).

### 2.2.7 Methylation of the quinoline (111)

The methylation was done according to a procedure reported by Joule and co-workers.\(^4\) \(\text{Compound 84 was dissolved in dry CH}_2\text{Cl}_2\) and methyl iodide was added. The reaction was stirred at room temperature for two days to produce the product in a low yield of 28%. The quinolinium iodide (111, Scheme 2.25) was isolated by removing the solvent under reduced pressure and the residue product washed with benzene to remove any remaining starting material, unfortunately the \(^1\text{H}\) NMR spectrum indicated that there was still starting material present. The presence of the starting material in the NMR sample was attributed to the fact that the residue was not washed thoroughly enough with benzene. When initially concentrated \textit{in vacuo}, some of the starting material could have been trapped in the residue product along with the product and could not be washed out. The decision was, therefore, made to alter the reaction conditions by changing solvents.

Since the starting material and the product are both soluble in dichloromethane, the decision was made to use toluene instead as the starting material is soluble in this solvent, but the chosen product was expected not to be. Therefore, taking advantage of the charge of the molecule to increase the ease of purification of the reaction, the reaction was reattempted. The starting material was dissolved in toluene and an excess of methyl iodide was added. The reaction was then left to stir for four and a half days. During this time, the colour of the reaction mixture changed from dark orange to a very faint orange-yellow and a residue formed on the sides of the round-bottomed flask. The work-up of the reaction involved
decanting off the mother liquor of the reaction and repeatedly rinsing the reaction with portions of toluene and then drying under high vacuum.

Scheme 2.23: Methylation of the quinoline to form the quinolinium salt (111). i) MeI, Toluene, 35 °C, 4 days.

The desired product was isolated as an orange powder and analysis by $^1$H NMR spectroscopy confirmed that it was the product, as evidenced by the down-field shift of the doublet signal of the proton ortho to the $N$-methyl group to 9.45 ppm. The protons of the new methyl group were also visible, resonating at a chemical shift of 4.64 ppm (Shown in Spectrum 2.7). The product was isolated in 44% yield.

Spectrum 2.6: $^1$H NMR spectrum of the methylated quinoline (111)
2.2.8 Reduction and ring-closure of the quinolinium species (112 & 87)

Upon the successful methylation of the quinoline to produce compound 111, the reduction of the nitro-group and quinoline was performed. This was done with the same degree of success as when using the unmethylated substrate. The conditions and ratios used were exactly the same as described for the successful reaction in Section 2.2.5 and will therefore not be repeated here. Yields for this reaction ranged from 80 to 93%. Instead, synthetic attention was focused on the construction of the elusive indole and the diamine was subjected to acidic conditions. The methods attempted were the initial one reported by Joule and co-workers (the use of aqueous 2N hydrochloric acid in THF), as well as the use hydrochloric acid gas generated in situ using sodium chloride and sulfuric acid.

The reduction of compound 111 also gave rise to a waxy compound, very similar to compound 85a. The difference in appearance between the two compounds was that compound 85a was a light pink waxy residue, whereas compound 112 was greenish-brown in colour. The first attempt at the ring-closure involved the use of the procedure by Joule and co-workers, using aqueous 2 N hydrochloric acid in THF. This procedure yet again proved to be inefficient, as no progress in the reaction was noted after stirring for three hours at room temperature and elevation of the temperature merely caused significant material degradation. The yellowish-brown reaction mixture started to darken and finally turned black. Considering the repeated failures utilizing this procedure, the decision was made to finally abandon this method of ring-closure, as it seemed to be unsuccessful when utilizing both compound 85a and compound 112 as substrate.

The ring-closure was then attempted again, but using hydrochloric gas generated in situ instead. Compound 112 was redissolved in diethyl ether and the acid was bubbled through the mixture whilst stirring (Scheme 2.26). The reaction mixture at first went milky and when left for a 20 minutes, a precipitate crashed out. This was attributed to possible formation of the hydrochloride salt of the diamine (112). When left to stir, the ring-closure is suspected to occur, since the precipitate started redissolving.
Scheme 2.24: Conditions for the revised synthetic route. i) NiCl$_2$.6H$_2$O, NaBH$_4$, MeOH, rt, 15 min; ii) [H$^+$].

Considering the mechanism of the ring-closure reaction (Scheme 2.27): if a proton transfer happened with the hydrochloride salt of the diamine 112 – the ammonium salt could tautomerize to the oxonium species, which could be followed by the ring-closure. Methanol could then be eliminated from the oxonium intermediate to form the methyl oxonium species (113). This electrophilic species could then undergo an intramolecular nucleophilic attack and the neighbouring amine moiety acts as a nucleophile, to form the basis of the tricyclic structure (114). The elimination of the second equivalent of methanol produces the intermediate 115 which through an isomerization would then form the indole (87).

This seems to be what is occurring in the reaction mixture, considering the disappearance of the precipitate. As mentioned earlier, with the synthesis of the unsubstituted indole (86), these indoles seem to be prone to degradation under reaction conditions. The same appeared to apply to the substituted indole (87), as a prolonged exposure to the acidic medium seemed to destroy the substrate. The colour of the reaction mixture darkened and then turned black. When the reaction mixture turned black (or darkened) in the synthesis of the indole 86, the reaction had failed. The same occurred during this reaction.
Since there was a window in the reaction before the colour started to darken and, ultimately, turn black, the decision was made to repeat the reaction. When the reaction was repeated, the changes during the acid step were closely monitored. The precipitate formed first and was then left to stir until it dissolved again. After this, the reaction mixture was concentrated under reduced pressure. Here, again, as the mixture started to get more concentrated due to the removal of the diethyl ether, the colour change started. When all of the diethyl ether was removed, the oil that remained was a dark brown. This was concerning, as all evidence so far had indicated that the darkening of the colour was indicative of degradation.

Instead of spending more time on attempting to isolate compound 87, the decision was made to use the crude product directly for the subsequent step and purify only once the oxidation had been done (Scheme 2.28). The crude mixture was redissolved in dry acetonitrile and cerium ammonium nitrate was added to the reaction mixture. After 30 minutes, the starting material had disappeared (when the reaction mixture was analysed by means of TLC) and the reaction was stopped. The work-up proved a challenge, as the expected product (72) is a charged species and possibly water soluble. Also, an exhaustive
literature search on the purification of the charged analogues proved unfruitful, as most syntheses focused on the synthesis of the unmethylated 7-methoxy analogue (78) instead of the quaternized imminium quinone (72). An analysis of the crude product (with cerium ammonium nitrate anion as counter ion) by $^1$H NMR spectroscopy was unfruitful, as the counter ion appeared to distort the spectrum. The removal of this counter ion seemed to be essential.

Scheme 2.26: Attempted synthesis of the indole (87) and subsequent oxidation. i) HCl (g), Et$_2$O, rt; ii) CAN, MeCN, rt, 30 min.

The decision was made to attempt the removal of the cerium complex counter ion through the use of an anion exchange resin. This resin is expected to retain the positively charged pyrroloiminoquinone species whilst the unwanted counter ion is flushed out (Figure 2.3). As no anion exchange resins were available, a Dowex$^\text{®}$ strong acid resin was repurposed to act as a cation retaining resin. This was done by stirring the resin in a sodium hydroxide solution overnight and then washing the resin with distilled water until all the base had been removed. The crude mixture containing compound 72 was then dissolved in methanol to dry load it onto the resin beads. The resin was loaded onto a column and also washed with copious amounts of distilled water. Acidic methanol (prepared by generating hydrochloric acid gas and bubbling it through methanol) was used as eluent, which would then protonate the resin causing the product to elute as the chloride salt.

Figure 2.2: A diagrammatic representation of the preparation of the resin.

An issue that arose with using the repurposed resin, in that the elution also flushed a large amount of sodium chloride from the column. The sodium chloride was removed by dissolving
the residue in freshly distilled anhydrous methanol, which caused the sodium chloride to precipitate out. When the compound obtained after the removal of the solvent was analysed by $^1$H NMR spectroscopy, none of the characteristic peaks were visible. This procedure was tried a second time as well, unfortunately with the same results.

At this point, the project was concluded due to time constraints, as the synthesis and exploration of different routes to reach the target compounds could unfortunately not be done before the hand in date. Various strategies to overcome the challenges that have so far been identified in this project were however considered and will be discussed in Section 3.2 – Future Work.

In our opinion, the work done in this study will add to the literature on makaluvamines as it highlights shortcomings in a number of the synthetic strategies. With regards to the synthetic strategy ultimately followed, the first half of the synthetic procedure has been optimized to deliver good to excellent yields for the individual steps – in many cases improving on the literature reported yields. Also, in a number of cases, more efficient methods of synthesis and isolation have been identified. This present study also highlighted and explained some of the shortcomings in the literature, particularly where synthetic procedures were not properly described or too vaguely alluded to. Although the latter portion of the envisioned project was not successfully completed, the strategies to overcome the problems identified have been considered and will form part of the discussion under the section “Future work”.

The project will still receive attention and hopefully will be able to deliver the compounds targeted at the onset. These synthetic results along with the biological evaluation results, will however, not form part of this dissertation.
Chapter 3

3.1 Conclusion

This project attempted the synthesis of three makaluvamine-analogues (88-90, Figure 3.1) and the generation of a possible small library upon successful synthesis of these compounds. The project was not successful yet, due to one weak point in the synthetic strategy, but continued attempts at the optimization of the synthetic method, could yet deliver these compounds.

Figure 3.1: Makaluvamines-analogues that were to be synthesized

The initial failure to generate these compounds just further highlighted the need for efficient, concise and short synthetic procedures to generate compounds of interest, as from a medicinal standpoint, the success of an anticancer agent can be undermined if the synthetic route to these compounds are long and inefficient. Thus, even though our probe into a shorter and more efficient route to 1-\(N\)-methylated makaluvamines (1-position shown on the general makaluvamine scaffold in Figure 3.1) had been unsuccessful, it has offered insight into how some of the issues can be overcome. These strategies will be discussed under future work, as they could not be completed within the time-frame of this study.

Makaluvamines are a fascinating class of compounds that still have much to offer in terms of anticancer research. Of the many analogues that have been isolated, synthesized and tested, many have been shown to rival the current clinical agents such as etoposide (25) and amsacrine (26) (Shown in Figure 3.2). Therefore, by experimenting with different substitution
patterns on these makaluvamines and generating new libraries of analogues with novel groups in the 7-position, even more effective antiproliferative agents could be discovered. Combine this with further probes into the mechanism of action of these compounds, they could serve as potent anticancer agents.

Figure 3.2: Current clinical topoisomerase II poisons

### 3.2 Future Work

Our future work involves reconsidering the synthetic strategy once more. The synthetic strategy devised by Joule and co-workers for the synthesis of damirones has been identified as a possible way of avoiding the very unstable indole (86, Scheme 3.1). This strategy provided the methylated indole (87) by going via the quinolinium species (113). The researchers reported generating this species by methylating compound 83 by using trimethyloxonium tetrafluoroborate. This group reported that the use of methyl iodide was ineffective and attributed this to the electron-withdrawing properties of the aldehyde. What was an interesting aside, was that they reported doing the reduction on the quinolinium species (116) without protecting the aldehyde. This had the effect that the reaction did not stop at the diamine (117), but that it immediately underwent the ring-closure to afford compound 87. Our aim is to use this strategy and immediately oxidize compound 87 to afford the 7-methoxy pyrroloiminoquinone (72).
Scheme 3.1: Future strategy to be attempted.

The methylation strategy would be done using trimethylxonium tetrafluoroborate as mentioned above, in CH₂Cl₂ at room temperature, as shown below in Scheme 3.2.

Scheme 3.2: Methylation of the quinoline to be attempted. i) \((\text{Me}_3\text{O}^+)\text{BF}_4^-, \text{DCM}, \text{rt, 24 h.}\)

The methylation would then be followed by the reduction method, using NiCl₂.6H₂O and NaBH₄ that had been used very successfully during this project.

Scheme 3.3: Efficient reduction technique applied to the new quinolinium substrate. i) NiCl₂.6H₂O, NaBH₄, MeOH, rt, 15 min.
Another issue that arose during the project was the removal of the cerium complex as counter ion after the CAN oxidation; therefore oxidation conditions will also be reconsidered. Cerium ammonium nitrate is a one-electron transfer oxidant, thus generating the Cerium(III) complex as counter-ion.

Attempts to remove this compound have so far been unsuccessful in our hands, but more strategies to solve this problem are possible. These include trying to do an exchange with a more lipophilic counter-ion that would cause the product to partition into a more hydrophobic solvent. Counter-ions that are of interest are the PF$_6^-$ anion and the tosylate anion. The other option is to use a different oxidant, one that might be easier to remove after the oxidation. The best candidate seems to be chloranil (118). The reduction of chloranil gives the radical species 119 as side product (shown in Scheme 3.5), which can be quenched to form the phenolic species, compound 120. This should be more easily removed by using a suitable organic solvent.

Once this procedure had been successfully carried out, the final substitution (as shown in Scheme 3.6) with the amines of interest can be done [example shown with benzylamine (121) acting as nucleophile to produce target compound 88].
Scheme 3.6: Substitution of the 7-position

Once these compounds have been successfully synthesized, they will be sent to our collaborators for biological testing – not just for their anticancer activity, but also the mode of action of these compounds will be studied. The results of the planned biological tests could add to the literature on these compounds and aid in the development of these compounds as clinically viable agents, as the understanding of the mode of action of these compounds could lead to the development of more suitable clinical trials.\(^{15}\)

Once the synthetic procedure to afford the methylated pyrroloiminoquinone precursor had been fully optimized, a library of makaluvamine C analogues could be generated to further investigate the effect of substitution of the 7-position. These could possibly later be used to generate structure activity relationship (SAR) data for these compounds.
Chapter 4

4.1 General information regarding experimental procedures

4.1.1 Chemicals and solvents

The chemicals used in this study were either purchased from two suppliers, Sigma Aldrich and Merck, or the chemicals from the De Beers Building chemical stores were used. Ethyl acetate, CH$_2$Cl$_2$ and hexane were distilled open to the atmosphere before use and were used for TLC plate separation and column chromatography. The solvents used for reactions were distilled under inert conditions over a suitable drying agents: CH$_2$Cl$_2$ and acetonitrile over calcium hydride, toluene and tetrahydrofuran (THF) over sodium (benzophenone as indicator for the THF still). These solvents were also stored over 4 Å molecular sieves when distilled.

4.1.2 Laboratory equipment and consumables

Reactions were heated in oil baths (either silicon oil or paraffin oil) using magnetic heater stirrers equipped with temperature probes. Solvents were removed under reduced pressure using Büchi RII Rotovapor rotary evaporators with Büchi V-700 Vacuum Pumps, equipped with V-850 Vacuum controllers, and the final trace amounts of solvents were removed by means of an Edwards high vacuum pump, capable of sustaining a vacuum of 0.08 mmHg. Glassware was dried in an oven at a temperature of 110 °C. Compounds were stored in Bosch refrigerators and freezers, which were kept at 2 °C and -22 °C, respectively.

Machery-Nagel ALUGRAM® Xtra SIL G UV254 aluminium sheets were used for thin layer chromatography (TLC) analyses and a 254 nm UV light used for visualization. Various stains were used for the permanent development of TLC plates, including ninhydrin, potassium permanganate, cerium ammonium molybdate and p-anisaldehyde. Iodine was used as a reversible stain. Column chromatography was done with Merck silica gel (particle size range of 0.063-0.200 mm with 60 Å pores).
4.1.3 Analysis equipment

NMR spectra were recorded on the instruments of the Central Analytical Facility (CAF) of Stellenbosch University. A 300 MHz Varian VNMRS spectrometer and a 400 MHz Varian Unity Inova spectrometer were used for the recording of $^1$H NMR spectra (75 MHz and 101 MHz on these two instruments respectively for $^{13}$C NMR spectra).

4.2 Experimental Procedures

4.2.1 Experiments pertaining to Section 2.2.1 – Synthesis of the quinoline (81)

4.2.1.1 Synthesis of 6,7-Dimethoxy-4-methylquinoline (81)

Iron(III) chloride hexahydrate (9.3 g, 0.034 mol) was dissolved in glacial acetic acid (100 mL) at 60 °C. When all of the Iron(III) chloride had dissolved, 3,4-dimethoxyaniline (5.0 g, 0.033 mol) was added. Methyl vinyl ketone (3.8 mL, 0.045 mol) was then added over 25 min. When all of the methyl vinyl ketone had been added, the temperature was raised to 140 °C and stirred for 2 h 15 min. The reaction was adjudged to have reached completion by means of TLC and cooled on ice. The cooled reaction mixture was then concentrated under reduced pressure to remove the acetic acid, leaving a brown precipitate. The precipitate was added to a mixture of saturated NaHCO$_3$ (250 mL) and extracted with EtOAc (6 x 60 mL). The EtOAc was removed under reduced pressure to give 5.54 g of product (84%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.60 (d, $J$ = 4.5 Hz, 1H), 7. 46 (s, 1H), 7.20 (d, $J$ = 4.5 Hz, 1H), 7.18 (s, 1H), 3.99 (s, 3H), 3.97 (s, 3H), 2.65 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.2, 149.7, 148.1, 145.2, 142.6, 123.7, 120.7, 108.7, 101.7, 56.3, 56.2, 19.1. Spectrum compares well to spectrum reported in the literature.

4.2.2 Experiments pertaining to Section 2.2.2 – Selective nitration of the quinoline (82)

4.2.2.1 Synthesis of 6,7-Dimethoxy-4-methyl-5-nitroquinoline (82)

Fuming Nitric Acid (75 mL) was cooled to ca. -40 °C. 6,7-Dimethoxy-4-methylquinoline (4.0 g, 0.020 mol) was added to the reaction mixture in portions over 1 h, keeping the temperature at -40 °C. The reaction was then stirred for an hour, also maintaining...
the temperature at -40 °C. The reaction mixture was then poured over an ice-slurry and basified to pH 12 (50% w/v NaOH solution) in an ice bath of ice/salt (ca. -10 °C). The mixture did heat up as the base was added a bit too rapidly, causing concern for dinitration. The work-up was continued, the product was then rigorously extracted with CH₂Cl₂ (5 x 100 mL) and the solvent removed under reduced pressure to give 3.84 g of product (79% Yield).

1H NMR (400 MHz, CDCl₃) δ 8.69 (d, J = 4.5 Hz, 1H), 7.75 (s, 1H), 7.21 (d, J = 4.5 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 2.59 (s, 3H).

13C NMR (101 MHz, CDCl₃) δ 153.8, 150.3, 145.9, 142.5, 141.3, 141.0, 123.8, 115.1, 111.8, 62.9, 56.6, 18.6. Spectrum compares well to spectrum reported in the literature.

4.2.3 Experiments pertaining to Section 2.2.3 – Oxidation of the aromatic methyl group (83)

4.2.3.1 Synthesis of 6,7-Dimethoxy-5-nitroquinoline-4-carbaldehyde (83)

6,7-Dimethoxy-4-methyl-5-nitroquinoline (1.6 g, 6.5 mmol) and Iron(III) chloride tetrahydrate (0.16 g, 0.81 mmol) was dissolved in DMSO (30 mL) at 60 °C. Trifluoroacetic acid (2.5 mL, 32 mmol), iodine (1.6 g, 6.4 mmol) and 2-iodo-2-methylpropane (1.5 mL, 13 mmol) were then added to the reaction mixture in succession. The reaction was heated to 90 °C and stirred for 330 min. Analysis by TLC showed a spot at the same Rf (0.4 in 60% EtOAc/hexane) as the starting material. The reaction was adjudged to have reached completion. The reaction mixture was then poured into a stirring solution of saturated Na₂S₂O₃ (200 mL) to give a green coloured solution. The mixture was then further diluted with K₂CO₃ (100 mL, 10% w/v) and the reaction mixture turned dark orange. The product was then rigorously extracted CH₂Cl₂ (5 x 50 mL). This extract was then washed with water (150 mL) and dried over anhydrous MgSO₄. Removal of the solvent still left a high boiling point liquid, so the residue was redissolved in CH₂Cl₂ (100 mL) and washed with more H₂O (150 mL). The solvent was then removed under reduced pressure to give 1.52 g of product (90% Yield). 1H NMR (400 MHz, CDCl₃) δ 10.20 (s, 1H), 8.97 (d, J = 4.3 Hz, 1H), 7.78 (d, J = 4.6 Hz, 1H), 7.69 (s, 1H), 4.14 (s, 3H), 4.12 (s, 3H). 13C NMR (101 MHz, CDCl₃) δ 189.5, 155.0, 150.7, 146.5, 145.1, 140.1 137.8, 122.2, 112.6, 111.7, 63.2, 56.8. Spectrum compares well to spectrum reported in the literature.
4.2.4 Experiments pertaining to Section 2.2.4 – Protection of the aldehyde (84a,b)

4.2.4.1 Synthesis of 4-(dimethoxymethyl)-6,7-dimethoxy-5-nitroquinoline (84a)

6,7-Dimethoxy-5-nitroquinoline-4-carbaldehyde (1.75 g, 6.67 mmol) was dissolved in trimethyl orthoformate (50 mL) and p-toluenesulfonic acid monohydrate (635 mg, 3.34 mmol) was then added. The mixture was stirred for 24 h at 100 °C and then quenched with saturated NaHCO₃ solution (40 mL). The mixture was then extracted with EtOAc (2 x 50 mL), dried and the solvent removed to give 1.96 g of pure product (92%). ¹H NMR (300 MHz, CDCl₃) δ 8.88 (d, J = 4.6 Hz, 1H), 7.88 (s, 1H), 7.84 (d, J = 4.6 Hz, 1H), 5.60 (s, 1H), 4.09 (s, 3H), 4.07 (s, 3H), 3.41 (s, 3H), 3.40 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 152.6, 149.1, 145.4, 143.1, 139.7, 127.0, 124.6, 112.8, 111.9, 99.9, 62.8, 56.4, 54.0. Spectrum compares well to spectrum reported in the literature.

4.2.4.2 Synthesis of 4-(1,3-dioxolan-2-yl)-6,7-dimethoxy-5-nitroquinoline (84b)

6,7-Dimethoxy-5-nitroquinoline-4-carbaldehyde (1.00 g, 3.81 mmol) was dissolved in dry benzene (20 mL) and ethylene glycol (0.43 mL, 7.6 mol) was added to the stirring solution. p-Toluenesulfonic acid monohydrate (0.725 g, 3.81 mmol) was then added and the mixture was heated at reflux in a Dean-Stark apparatus. Reaction was stirred for 72 hours and then concentrated under reduced pressure. The residue was dissolved in 10% w/v K₂CO₃ (100 mL) and EtOAc (100 mL) and the organic layer collected. The solvent was removed to give 0.912 g of pure product, which did not require any further purification (79%). ¹H NMR (400 MHz, CDCl₃) δ 8.83 (d, J = 4.6 Hz, 1H), 7.76 (dd, J = 4.6, 0.6 Hz, 1H), 7.66 (s, 1H), 6.18 (s, 1H), 4.04 (s, 3H), 4.03 (s, 3H), 4.00 – 3.94 (m, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 152.8, 149.1, 145.1, 143.0, 139.7, 117.3, 112.2, 111.4, 98.5, 64.1, 61.8, 55.4. IR (ATR, cm⁻¹) 3383, 2943, 2891, 1538, 1489, 1472, 1455, 1418, 1402, 1353, 1325, 1246, 1196, 1091, 1053, 1001, 968, 949, 936, 888, 859, 763. HRMS: calcd for C₁₄H₁₃N₂O₆ [M+H]⁺, 307.0930 – found 307.0932.
4.2.5 Experiments pertaining to Section 2.2.5 – Reduction, ring-closure and selective methylation (85,a,b-86)

4.2.5.1 Synthesis of 4-(dimethoxymethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroquinolin-5-amine (85a)

4-(1,3-Dimethoxymethyl)-6,7-dimethoxy-5-nitroquinoline (743 mg, 2.41 mmol) and NiCl₂.6H₂O (6.87 g, 28.9 mmol) were rapidly stirred in methanol (90 mL). NaBH₄ (6.47 g, 0.171 mol) was crushed using a mortar and pestle and then added to the reaction in portions. Upon the addition of the first portion, the reaction mixture turned black due to a precipitate. The reaction was highly exothermic and the temperature increased to ca. 80 °C. After ca. 20 min, the reaction was quenched by adding distilled water (100 mL). The product was then extracted with EtOAc (3 x 50 mL) and the EtOAc layer dried over anhydrous MgSO₄. The solid was filtered off and the EtOAc removed. The oily residue was redissolved in CH₂Cl₂ (40 mL), transferred to a smaller round bottomed flask and concentrated under reduced pressure to give 578.7 mg of pure product (85% yield).

1H NMR (300 MHz, DMSO-d₆) δ 5.52 (s, 1H), 4.56 (d, J = 8.1 Hz, 1H), 3.87 (s, 3H), 3.75 (s, 3H), 3.41 (s, 3H), 3.36 (s, 3H), 3.20 – 3.16 (m, 2H), 2.21 (t, J = 4.7 Hz, 2H), 1.66 (m, 1H).

13C NMR (101 MHz, DMSO-d₆) δ 152.3, 142.2, 141.0, 127.2, 107.1, 97.5, 88.4, 65.3, 64.4, 60.2, 55.6, 37.3, 35.3, 23.4. Compound was difficult to dissolve in CDCl₃, so spectrum was obtained in DMSO-d₆. Despite different solvents, 1H NMR spectrum correlates well to spectrum reported in the literature. 13C NMR not reported in literature.

4.2.5.2 Synthesis of 4-(1,3-dioxolan-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroquinolin-5-amine (85b)

4-(1,3-Dioxolan-2-yl)-6,7-dimethoxy-5-nitroquinoline (200 mg, 0.663 mmol) and NiCl₂.6H₂O (1.9 g, 7.8 mmol) were rapidly stirred in methanol (15 mL). Sodium borohydride (1.76 g, 46.2 mmol) was crushed using a mortar and pestle and then added to the reaction in portions. Upon the addition of the first portion, the reaction mixture turned black due to a precipitate. Highly exothermic reaction, addition of the powdered sodium borohydride was too rapid and the reaction boiled over. After about 20 min, the reaction was then quenched by adding distilled water (20 mL). The product was then extracted with EtOAc (3 x 10 mL) and the EtOAc layer dried over anhydrous MgSO₄. The solid was filtered off and the EtOAc removed. The oily residue was redissolved in CH₂Cl₂ (10 mL), transferred to a smaller round bottomed flask and
concentrated under reduced pressure to give 133.3 mg of pure product (73% yield).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 5.50 (d, \( J = 1.4 \) Hz, 1H), 4.88 (dd, \( J = 6.0, 1.5 \) Hz, 1H), 3.94 – 3.85 (m, 2H), 3.79 – 3.72 (m, 2H), 3.70 – 3.65 (m, 6H), 3.52 – 3.45 (m, 1H), 3.36 – 3.27 (m, 1H), 3.21 – 3.12 (m, 2H), 2.98 (t, \( J = 4.9 \) Hz, 1H).\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \( \delta \) 152.5, 141.3, 140.9, 128.5, 107.9, 98.6, 88.9, 65.5, 64.5, 60.5, 55.6, 37.9, 35.5, 23.6. IR (ATR, cm\textsuperscript{-1}) 3401, 2930, 2890, 1506, 1464, 1423, 1355, 1293, 1237, 1202, 1114, 1086, 1016, 952, 788, 732. HRMS: calcd for C\textsubscript{14}H\textsubscript{21}N\textsubscript{2}O\textsubscript{4} [M+H]\textsuperscript{+}, 281.1501 – found 281.1498.

4.2.5.3 Synthesis of 7,8-dimethoxy-1,3,4,5-tetrahydropyrrolo[4,3,2-de]quinoline (86)

4-(1,3-Dioxolanyl)-6,7-dimethoxy-5-nitroquinoline (0.500 g, 1.63 mmol) and NiCl\textsubscript{2}.6H\textsubscript{2}O (4.6 g, 20 mmol) were rapidly stirred in methanol (50 mL). NaBH\textsubscript{4} (3.48 g, 92.1 mmol) was then added to the reaction and upon addition of the first portion, the reaction mixture turned black due to a precipitate. The reaction was highly exothermic and a lot of gas was formed. After ca. 20 min, the reaction stopped bubbling and the reaction mixture was then quenched by adding distilled water (100 mL). The reaction mixture was filtered to remove the black precipitate and then transferred into a separation funnel. The mixture was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 50 mL) and the solvent removed. The residue was immediately dissolved in 2N HCl (15 mL) and then heated to 60 °C. After 3 hours, the reaction mixture was neutralized with saturated NaHCO\textsubscript{3} (75 mL) and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 30 mL). Removal of the solvent gave 270 mg of impure product, which was purified by column chromatography. The spot at R\textsubscript{i} 0.19 was collected to afford the desired product (66 mg, 19% yield).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 6.98 (2 x s, 1H), 6.21 (2 x s, 1H), 4.26 (2 x t, \( J = 7.9, 2H \), 3.95 (2 x s, 3H), 2.89 (2 x t, \( J = 7.9, 2H \)).\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \( \delta \) 175.66, 163.49, 161.76, 128.95, 128.07, 126.07, 121.52, 110.90, 61.32, 55.74, 23.33. Spectrum showed unexplained doubling of all signals. These still compare reasonably well to literature values, considering that literature spectrum was reported in CDCl\textsubscript{3} and experimental data collected in CDCl\textsubscript{3}/DMSO-d\textsubscript{6} due to limited solubility of compound.\textsuperscript{40}

4.2.5.4 Synthesis of acetic formic anhydride

Acetyl chloride (10 mL, 0.14 mol) was stirred in dry THF (10 mL) at 0 °C and sodium formate (10 g, 0.22 mol) was added to the mixture. A condenser was attached to the reaction vessel and stirred for 6 hours. The solid was then filtered off and the filtrate was stored in the freezer.\textsuperscript{60}
4.2.6 Experiments pertaining to Section 2.2.7 – Methylation of the quinoline (111)

4.2.6.1 Synthesis of 4-(dimethoxymethyl)-6,7-dimethoxy-1-methyl-5-nitroquinolin-1-ium iodide (111)

4-(1,3-Dimethoxymethyl)-6,7-dimethoxy-5-nitroquinoline (1.83 g, 6.06 mmol) was dissolved in toluene (20 mL). Methyl iodide (30 mL, 4.8 mol) was added and the mixture heated to 35 °C and stirred for 5 days. Product started precipitating out of solution and depositing on the walls of the round bottom flask. The reaction mixture poured off and diluted with hexane. The mixture went murky and when left for 3 days, crystals formed. The solvent was decanted and the deposit was dried under high vacuum and collected as an orange powder, giving 1.20 g of the desired product (44%).

\[ ^1H \text{ NMR (300 MHz, DMSO-}d_6) \delta 9.55 (d, J = 6.3 \text{ Hz, 1H}), 8.28 (d, J = 6.2 \text{ Hz, 1H}), 8.02 (s, 1H), 5.62 (s, 1H), 4.71 (s, 3H), 4.31 (s, 3H), 4.11 (s, 3H), 3.43 (s, 3H), 3.40 (s, 3H). \]

\[ ^{13}C \text{ NMR (151 MHz, DMSO-}d_6) \delta 160.3, 152.6, 148.7, 143.2, 140.6, 140.4, 131.0, 128.4, 123.2, 117.5, 66.3, 61.4, 57.3, 50.1, 23.8. \]

Literature spectrum was run in acetone-\(d_6\), experimental data obtained in DMSO-\(d_6\), but despite this there is a strong correlation between the results. \(^{13}C \text{ NMR spectrum not reported in literature.} \]

4.2.7 Experiments pertaining to Section 2.2.8 – Reduction and ring-closure of the quinolinium species (112 & 87)

4.2.7.1 Synthesis of 4-(dimethoxymethyl)-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroquinolin-5-amine (112)

4-(dimethoxymethyl)-6,7-dimethoxy-1-methyl-5-nitroquinolin-1-ium iodide (690 mg, 1.53 mmol) and NiCl\(_2\).6H\(_2\)O (4.37 g, 18.4 mmol) were rapidly stirred in methanol (50 mL). Sodium borohydride (4.11 g, 0.109 mol) was crushed using a mortar and pestle and then added to the reaction in portions. Upon the addition of the first portion, the reaction mixture turned black due to a precipitate. Highly exothermic reaction, addition of the powdered sodium borohydride was too rapid and the reaction boiled over. After 35 min, the reaction was then quenched by adding distilled water (50 mL). The product was then extracted with EtOAc (3 x 50 mL) and the EtOAc layer dried over anhydrous MgSO\(_4\). The solid was filtered off and the EtOAc removed \textit{in vacuo}. The oily residue was redissolved in CH\(_2\)Cl\(_2\) (50 mL), transferred to
a smaller round bottomed flask and concentrated under reduced pressure to give 419 mg of pure product (92% yield).  $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 5.71 (s, 1H), 4.36 (d, $J$ = 7.8 Hz, 1H), 3.72 (s, 3H), 3.56 (s, 3H), 3.30 (s, 3H), 3.22 (s, 3H), 3.21 – 3.18 (m, 1H), 3.04 (d, $J$ = 11.6, 3.5 Hz, 1H), 2.98 – 2.94 (m, 1H), 2.82 (s, 3H), 2.81 – 2.78 (m, 1H).  $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 154.4, 145.6, 131.1, 128.6, 110.3, 103.6, 90.3, 62.8, 59.3, 58.2, 55.4, 49.5, 37.4, 25.9. IR (ATR, cm$^{-1}$) 3434, 3351, 2929, 2826, 2361, 2341, 1508, 1451, 1426, 1396, 1341, 1308, 1264, 1228, 1197, 1051, 1019, 962, 891, 778, 735, 708. HRMS: calcd for C$_{14}$H$_{21}$N$_2$O$_4^+$ [M+H]$^+$, 297.1814 – found 297.1815.
References


(59) Joule, J. A.; Alvarez, M. Personal communication.