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<th>First Synthesis of an Aziridinyl Fused Pyrrolo[1,2-a]benzimidazole and Cytotoxicity Evaluation of Various Imidazobenzimidazolequinones Towards Human Normal and Cancer Cell Lines</th>
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<tr>
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Dedicated to my family and Vinnie
Chapter 1: First Synthesis of an Aziridinyl Fused Pyrrolo[1,2-\(a\)]benzimidazole and Toxicity Evaluation Towards Normal and Breast Cancer Cells

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1.1.2 Aziridinomitosenes
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1.1.3 Aziridinyl substituted benzimidazoles and benzimidazolequinones
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Abstract

Chapter 1 provides a review of aziridine containing anti-tumour agents and some key syntheses of the aziridinomitosene skeleton. A novel protocol for aziridine ring fusion is described leading to the preparation of the first aziridinyl fused pyrrolo[1,2-\(a\)]benzimidazole. This diazole analogue of aziridinomitosene was formed via selective lithiation of the aziridine leading to an anionic aromatic \textit{ipso}-substitution onto the benzimidazole-2-position. A non-fused aziridine containing benzimidazole is also prepared for cytotoxicity comparisons. Both novel aziridine compounds are evaluated towards a human normal skin fibroblast cell line (GM00637) and two breast cancer cell lines (MCF-7) and (HCC1937). The latter is breast cancer tumour suppressor gene deficient (BRCA1 deficient), and showed hypersensitivity to mitomycin C (MMC). BRCA1 is reported to play a key role in the repair of DNA damage. Both aziridine benzimidazole compounds are more cytotoxic towards the breast cancer cell lines than the normal cell line. The evidence provided indicates that different pathways mediate cellular response to benzimidazole containing-aziridine compounds compared to MMC.

Chapter 2 begins with an introduction to the enzyme-directed approach to chemotherapy, and a review of polycyclic quinones. A series of dialicyclic ring fused imidazo[5,4-\(f\)]benzimidazolequinones were evaluated using the MTT assay towards two human cancer cell lines; cervical (HeLa) and prostate (DU145) and a normal cell line (GM00637). Dipyrido[5,4-\(f\)]imidazobenzimidazolequinone is found to possess similar toxicity to its [4,5-\(f\)] isomer, while an oxygen atom in the alicyclic fused ring ([1,4]oxazino) is found to dramatically increase toxicity towards all three cell lines. The toxicity of pyrido-fused compounds is found to be less than the \textit{N}-butyl analogues, and increasing the alicyclic ring size from five to seven membered reduces activity. Iminoquinone is found to be \textasciitilde12 times more toxic towards prostate cancer than towards the normal cell line. The iminoquinone shows a moderate to strong correlation towards cell lines expressing high levels of NQO1 activity, confirmed by further testing at the National Cancer Institute (NCI-60 program).

Chapter 3 describes in detail the experimental procedures for Chapters 1 and 2.
Acknowledgements

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I would also like to acknowledge Dr. Michael Carty (Department of Biochemistry) and his postgraduate students for allowing use of the tissue culture laboratory and for their expertise and advice.

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I am indebted to my parents, Helen and Pat, my brother Paul and sister Emer, for their endless love, support and encouragement. My sincere thanks to my friend Claire, for her constant support and walks along the Salthill prom. Last but not least, I wish to thank Vinnie, for being with me every step of the way (literally), for his endless support and encouragement and for always having faith in me.
Abbreviations

Ac  acetyl
ACCN  1,1′-azobis(cyclohexanecarbonitrile)
AcOH  acetic acid
AIBN  2,2′-azobis(isobutyronitrile)
APBI  acetamidopyrrolo[1,2-a]benzimidazolequinone
AT  adenine-thymine
ATR  universal attenuated total reflectance
AZQ  diaziquone
B:  base
Bn  benzyl
Boc  tert-butoxycarbonyl
Bu  butyl
n-BuLi  n-butyl lithium
t-BuOK  potassium tert-butoxide
C  Celsius
Calcd  calculated
CNS  central nervous system
CSA  camphorsulfonic acid
DBU  1,8-diazabicycloundec-7-ene
DCVC  dry column vacuum chromatography
DEAD  diethyl azodicarboxylate
DEPT  distortionless enhancement by polarization transfer
DIAD  diisopropyl azodicarboxylate
DIBAL  diisobutylaluminium hydride
DMAP  4-(N,N-dimethylamino)pyridine
DMEM  Dulbecco’s modified Eagle’s Medium
DMF  N,N-dimethyl formamide
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
ED_{50}  effective dose: concentration at which 50% of maximal effect is observed
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>EO9</td>
<td>apaziquone</td>
</tr>
<tr>
<td>Equiv.</td>
<td>equivalent</td>
</tr>
<tr>
<td>EWG</td>
<td>electron withdrawing group</td>
</tr>
<tr>
<td>FA</td>
<td>Fanconi anemia</td>
</tr>
<tr>
<td>FAD</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>GI&lt;sub&gt;50&lt;/sub&gt;</td>
<td>growth inhibition: concentration required to inhibit cell growth by 50%</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HCR</td>
<td>hypoxic cytotoxicity ratio</td>
</tr>
<tr>
<td>HMQC</td>
<td>heteronuclear multiple quantum coherence</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectra</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>concentration required to reduce cell viability by 50%</td>
</tr>
<tr>
<td>i-Pr</td>
<td>iso-propyl</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LiAlH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>lithium aluminium hydride</td>
</tr>
<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>lethal concentration: resulting in a 50% reduction of cells measured at the end compared to that at the start</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeLi</td>
<td>methyl lithium</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MEM</td>
<td>minimum essential media</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>MOM</td>
<td>methoxymethyl</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MMC</td>
<td>mitomycin C</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>Ms</td>
<td>mesyl (methanesulfonyl)</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>Nos</td>
<td>nosyl (4-nitrobenzenesulfonate)</td>
</tr>
<tr>
<td>NQO1</td>
<td>NAD(P)H:quinone oxidoreductase</td>
</tr>
<tr>
<td>obsd</td>
<td>observed</td>
</tr>
<tr>
<td>OTf</td>
<td>triflate (trifluoromethanesulfonate)</td>
</tr>
<tr>
<td>PBI</td>
<td>pyrrolo[1,2-α]benzimidazolequinone</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PhMe</td>
<td>toluene</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>psi</td>
<td>pound per square inch</td>
</tr>
<tr>
<td>py</td>
<td>pyridine</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetra-n-butylammonium fluoride</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TEMPO</td>
<td>(2,2,6,6-tetramethylpiperidin-1-yl)oxy</td>
</tr>
<tr>
<td>TGI</td>
<td>total growth inhibition</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>Tr</td>
<td>trityl (triphenylmethyl)</td>
</tr>
<tr>
<td>Ts</td>
<td>tosyl (toluenesulfonyl)</td>
</tr>
</tbody>
</table>
Chapter 1

First Synthesis of an Aziridinyl Fused Pyrrolo[1,2-$a$]benzimidazole and Toxicity Evaluation Towards Normal and Breast Cancer Cells
1.1 Introduction

Bioreductive prodrugs require reductive metabolism in order to exert cytotoxic activity. Quinones are widely employed as bioreductive prodrugs whereby reduction of the quinone moiety can achieve selective toxicity. Two approaches can be employed either singularly or in combination with other regimes to achieve selective toxicity towards cancer cells. A hypoxia selective approach, in which reductive activation of the quinone is catalyzed by single electron reductases (e.g. NADPH: cytochrome P450), resulting in an oxygen sensitive cytotoxic intermediate more favoured under hypoxic conditions (regions of solid tumours containing low concentrations of oxygen caused by poor vasculature). The second route is an enzyme-directed approach, which targets two electron reductase enzymes (e.g. NAD(P)H: quinone oxidoreductase 1 (NQO1) that are over-expressed in certain tumour tissues relative to healthy tissue. This is considered an oxygen independent route and can occur under both aerobic and hypoxic conditions. The mechanisms of toxicity following reductive activation of the quinone are numerous. Some can behave as DNA alkylating agents (e.g. mitomycin C, MMC), some generate reactive oxygen species through redox cycling, and others act as DNA intercalating agents or as topoisomerase inhibitors (Chapter 2).
1.1.1 Mitomycins and mitomycin C

![Chemical structures of mitomycin A, B, K, C (MMC), and porfiromycin.](image)

**Figure 1.1:** Examples representing the mitomycin family

The mitomycin natural products are a family of pyrrolo[1,2-a]indolequinones containing a fused aziridine ring. **Mitomycins A** and **B** were first isolated in 1956 from the fermentation broths of *Streptomyces caespitosus* and found to possess potent antibiotic and anti-tumour activity.\(^3\) Subsequent investigations by a Japanese group in 1958 identified **mitomycin C (MMC)** from the same bacterium.\(^4\) In 1960 **porfiromycin**, the \(N\)-methylated analogue of MMC was isolated from the bacterium *Streptoverticillium ardus*\(^5\), in 1962 these four mitomycin compounds were isolated from one bacterium *Streptomyces verticillatus* (Figure 1.1).\(^6\) The early mitomycins were divided into three structural types based on the nature and stereochemistry of their side chains, of which **mitomycins A, B and K** are representative.\(^7\) MMC is the best known and clinically the most relevant mitomycin to date.

**MMC** can be reduced by both single and two electron reductase enzymes which act upon the quinone moiety triggering a cascade of transformations, resulting in cytotoxic damage through DNA alkylation and crosslinking.\(^8^{\text{--}10}\) The bio-activation mechanism of MMC has been extensively studied and reviewed.\(^8^{\text{--}15}\)
Single electron reduction of MMC (Scheme 1.1) generates a reactive semiquinone radical which under aerobic conditions can be rapidly oxidized with molecular oxygen to regenerate the MMC prodrug with the simultaneous production of superoxide radicals known as redox cycling. Rapid re-oxidation of the semiquinone radical prevents significant DNA alkylation under aerobic conditions. Under hypoxic conditions reoxidation is less efficient, giving a longer lived semi-quinone radical which can alkylate DNA, or alternatively undergo disproportionation or further reduction to give the hydroquinone intermediate also capable of alkylating DNA.

Scheme 1.1: Mechanism for single electron reductive activation of MMC

Two-electron reduction of MMC yields the hydroquinone species directly (Scheme 1.2). The hydroquinone can form under both normal and hypoxic conditions and undergoes much slower oxidation than the semi-quinone radical. Following formation of the hydroquinone the N-4 indole lone pair is released from conjugation allowing for loss of the methoxy group at C-9a. The resulting iminium ion undergoes aromatization via loss of a proton from C-9, yielding the aromatic leuco-aziridinomitosene species. Formation of a quinone methide species and ring opening of the aziridine generates an electrophilic site at C-1 which allows for DNA alkylation. Loss of the carbamate group generates a second electrophilic site at C-10 allowing for the formation of a DNA crosslinked adduct or when water is trapped a decarbamoyl DNA mono-adduct. DNA alkylation occurs with specificity at the N-2 position of guanine.
Scheme 1.2: Mechanism for the two-electron reductive activation-alkylation cascade of MMC
Since its discovery MMC has been widely used in chemotherapy treatments. MMC can be employed as a single agent or in combination regimes for the treatment of several solid tumour types and is most effective in the treatment of non-small-cell lung cancer, head and neck cancer and for the intravesical (directly into the bladder) treatment of superficial bladder cancer.\textsuperscript{16,17} MMC also shows marginal preferential cytotoxicity under hypoxic conditions, however clinical usefulness has been limited by significant toxicity and myelosuppression\textsuperscript{16-20}

\textbf{Porfiromycin} has been shown to be preferentially more cytotoxic towards tumour cells under hypoxic conditions than aerobic conditions and exhibits a greater hypoxic cytotoxicity ratio (HCR) than MMC.\textsuperscript{18,21} HCR is defined as the IC\textsubscript{50} value under aerobic conditions divided by the IC\textsubscript{50} value obtained under hypoxic conditions (IC\textsubscript{50} is the drug concentration required to reduce cell viability by 50\%). Preclinical studies initially demonstrated the usefulness of \textit{porfiromycin} as a potential adjunct to radiotherapy for treatment against solid tumours.\textsuperscript{22} Phase I clinical trials were conducted with \textit{porfiromycin} for the adjuvant treatment of squamous cell cancer of the head and neck with radiotherapy. However despite previous indications as a hypoxia-selective prodrug, randomized stage III trials have so far concluded that \textit{porfiromycin} is inferior to MMC for this treatment.\textsuperscript{23}
1.1.2 Aziridinomitosenes

Aziridinomitosenes belong to a class of compounds called the mitosenes and can be formed as a result of back oxidation of the leuco-aziridinomitosenes intermediate (Scheme 1.2). Patrick and co-workers were first to elucidate the aziridinomitosenes structure from degradation products derived from initial studies on the mitomycins (Figure 1.2).²⁴ Aminoaziridinomitosenes 1 has been shown to react with nucleophiles and DNA in the absence of reductant (Scheme 1.3).²⁵,²⁶ The proposed S₉¹ mechanism proceeds by protonation of the aziridine and formation of a carbocation intermediate which can react with nucleophiles including DNA.

Scheme 1.3: Proposed mechanism of action for 7-aminoaziridinomitosene in the absence of reductant

Studies by Remers and co-workers revealed some good anti-tumour activity of various 7-substituted aziridinomitosenes on leukaemia xenograph models.²⁷ Several synthetic approaches towards the aziridinomitosene tetracyclic skeleton have been reported and reviewed.²⁸-⁴⁸ Some key syntheses are summarised in the following section.
1.1.2.1 Synthetic routes to the aziridinomitosene skeleton

In 1969 Hirata and co-workers reported the first synthesis of the tetracyclic skeleton of aziridinomitosene, represented by compound 5 (Scheme 1.4).28

Aziridine fused pyrrolo[1,2-\textit{a}]indole 5 was accessed by an iodine azide addition on to the alkene bond of 3\textit{H}-pyrrolo[1,2-\textit{a}]indole 2. The resulting iodo azide adduct 3 was catalytically hydrogenated using palladium-charcoal in the presence of hydrogen chloride to give the ammonium chloride salt 4. Deprotonation of 4 with sodium methoxide in methanol gave a crystalline mixture, which was directly converted to aziridinomitosene 5 using triethylamine (TEA) and methylchloroformate.

Franck et al accessed the tetracyclic skeleton of aziridinomitosene 8 via the 1,3-dipole cycloaddition of both phenyl and benzyl azides to the conjugated double bond of lactam 6 (Scheme 1.5). Photolysis of the intermediate triazolines 7 resulted in the facile elimination of nitrogen and the formation of various substituted benzyl- and phenyl aziridinomitosenes 8.29,30

Scheme 1.4: Preparation of aziridinomitosene using iodine azide

Scheme 1.5: Aryl azide cycloaddition approach to the aziridinomitosene skeleton
In 1983 Cory and Ritchie reported an intramolecular bicycloannulation methodology to access aziridinomitosene 13 (Scheme 1.6). Treatment of 2-(N-phenylformimidoyl)indole 9 with sodium hydride followed by addition of \( \alpha \)-bromoacrylic ester (methyl 2-bromopropenoate) 10, gave intermediates 11 and 12. Nucleophilic substitution of bromine gave the substituted mitosene tetracycle 13.\(^{31}\)

\[
\begin{align*}
\text{9} & \quad \text{i. NaH} \\
& \quad \text{ii. } \text{CO}_2\text{Me} \\
& \quad \text{10} \quad \text{Br} \\
\rightarrow \quad & \quad \text{11} \\
& \quad \text{Br} \\
& \quad \text{12} \\
& \quad \text{CO}_2\text{Me} \\
\rightarrow \quad & \quad \text{13}
\end{align*}
\]

**Scheme 1.6:** A single-step route to the tetracyclic skeleton of aziridinomitosene

Rapoport *et al* carried out a photochemical rearrangement of bromoquinone 14 in sunlight, resulting in the formation of a benzoazole intermediate 15 which decomposed to form an iminium salt intermediate 16 and following proton transfer the vinylogous carbamate hydroquinone 17 was formed. (Scheme 1.7) Oxidation of the hydroquinone to the quinone in air, and an intramolecular palladium catalyzed Heck reaction facilitated ring closure to give aziridinomitosene 18.\(^{32}\)

\[
\begin{align*}
\text{14} & \quad \text{hv} \\
& \quad \text{15} \\
& \quad \text{16} \\
\rightarrow \quad & \quad \text{17} \\
& \quad \text{18}
\end{align*}
\]

**Scheme 1.7:** Photochemical route to 7-methoxyaziridinomitosene
Natsume and co-workers prepared 2-substituted indole diastereomers 21a and 21b from the nucleophilic addition of 2-lithio-1-(phenylsulfonyl)indole 19 onto Boc-protected oxazolidine aldehyde 20; removal of the phenylsulfonyl group was carried out under reductive conditions (Scheme 1.8).33

Scheme 1.8: Two step preparation of deprotected 2-Boc-protected oxazolidine substituted indoles

One-pot acid hydrolysis of the N,O-acetonide group of oxazolidine 21a, followed by acetylation of the resulting alcohol moieties gave diacetate 22. Vilsmeier-Haack formylation at the 3-position of indole gave 3-formylindole 23, followed by base induced ring closure on to the terminal mesylate substituent of 24 by the indolyl anion gave amino alcohol 25. Aziridine 28 was obtained by amine displacement of the chloride group of 27, formed via substitution of mesylate 26 (Scheme 1.9).

Scheme 1.9: Nucleophilic displacement route to the aziridinomitosene skeleton
Edstrom and Yu describe the synthesis of functionalized 7-aminoaziridinomitosene 33 (Scheme 1.10).34 Dehydroaromatization of ethyl ester mesylate 29 using DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) gave 2,3-dihydro-1H-pyrrolo[1,2-a]indole 30. Bromination of 30 gave bromoquinone 31 which underwent nucleophilic substitution with sodium azide followed by reduction, and thermal disproportionation to give the desired 7-aminoquinone 32. Ring closure of the aziridine was achieved using the Staudinger reaction (triphenylphosphine and TEA) to give 7-aminoaziridinomitosene 33.

![Scheme 1.10: Synthesis of 7-aminoaziridinomitosene using the Staudinger reaction to give the aziridine ring](image)

Jimenez and Dong were first to report a fully synthetic functionalized aziridinomitosene, containing the three key structural groups, a quinone, a carbamate and an aziridine ring (Scheme 1.11).35 The key steps involved an intramolecular Corey-Chaykovsky reaction by reacting the indole anion 34 with diisopropylvinylsulfonium triflate 35 to give transient epoxide 36, which was ring opened in situ using sodium azide to give azido alcohol 37. Several conventional reactions gave the fully functionalized aziridinomitosene 38.
Scheme 1.11: Corey-Chaykovsky reaction route to functionalized aziridinomitosene

Michael et al reported the key 1,2-azido alcohol for aziridine formation via ketal hydrolysis of 39 to a 1,2-diol, which upon treatment with thionyl chloride formed the cyclic sulfite 40. The azido alcohol 41 was formed by nucleophilic ring opening of the sulfite using sodium azide (Scheme 1.12).36

Scheme 1.12: Ketal hydrolysis route to the aziridinomitosene

Vedejs and co-workers accessed aziridinomitosene 47 by an intramolecular N-alkylation of oxazole 42, to give oxazolium salt intermediate 43 (Scheme 1.13).37 Addition of a cyanide anion resulted in electrocyclic ring opening of transient oxazoline 44 to give the azomethine ylide 45. The [3+2] cycloaddition of 45 on to the alkyne dipolarophile and subsequent oxidative removal of the TBDMS groups gave quinone 46. Conventional reactions installed the carbamate moiety at C-10 but attempts to remove the trityl group from aziridinomitosene 47 were
unsuccessful. Similar aziridinomitosenes have also been prepared using this methodology.\textsuperscript{38-40}

\begin{center}
\begin{tikzpicture}
\node[anchor=center] (a) at (0,0) {\textbf{Scheme 1.13: Cycloaddition [3+2] route to 7-methoxyaziridinomitosenes}};
\end{tikzpicture}
\end{center}

Sulikowski \textit{et al} reported the preparation of metal carbenoid 49, from treatment of diazo-ester 48 with a bis-oxazole copper (I) complex generated \textit{in situ} (Scheme 1.14).\textsuperscript{41} Intramolecular carbon-hydrogen insertion of the carbenoid resulted in mitosane insertion products, which were directly oxidized using chloranil (tetrachloro-\textit{p}-benzoquinone) to the mitosene derivatives 50a and 50b in a ratio of 9:1. Hydrolysis of 50a gave an amino-alcohol 51a, which was converted to chloride 51b. The latter underwent base-induced three-membered intramolecular substitution to give aziridine 52.
Scheme 1.14: Intramolecular carbon-hydrogen insertion reaction to give aziridinomitosene

The preparation of the aziridinomitosene tetracyclic skeleton usually involves the installation of the fragile aziridine function late in the synthesis, however synthesis of aziridinomitosenes via the early introduction of a fully formed aziridine ring have been reported.

Ziegler and Belema carried out aziridinyl radical cyclizations of Barton esters 54 (prepared from acid 53) onto the indole-2-position, but yields of substitution products (e.g. 56) were low due to the formation of a dimer 55 (31%) and recovery of acid 53 (Scheme 1.15). The authors suggest that dimerization is facilitated by resonance stabilization of the intermediate cyclized radical by the adjacent cyano group as later studies on indoles in the absence of a cyano group resulted in the formation of no dimerized products. Yields for the aromatic aziridinomitosene were always low using this radical cyclization approach.
Scheme 1.15: Radical approach to an aziridinomitosene

Photolysis of dimer 55 resulted in the formation of homolytic aromatic substitution product, indole 56 (22%) and the cyclized reduced hydroindole 57 (37%) (Scheme 1.16).42

Scheme 1.16: Photolysis of indole dimer

Vedejs and co-workers have reported the formation of an aziridin-3-yl anion from tin-lithium exchange which undergoes an intramolecular Michael addition on to the activated C-2 position of indole (Scheme 1.17).45,46 Initial tin-lithium exchange studies found that competitive indole lithiation was occurring and it was necessary to introduce a deuterium blocking group at the C-2 position to circumvent this undesirable side-reaction. The indol-3-yl anion generated upon cyclization was quenched with phenylselenyl chloride in order to facilitate re-aromatization via expulsion of phenyl selenium deuteride to afford tetracycle 58.
Scheme 1.17: Intramolecular Michael addition onto the 2-position of indole to give aziridinomitosene

1.1.3 Aziridinyl substituted benzimidazoles and benzimidazolequinones

Figure 1.3: Aziridinyl substituted quinones reported by Skibo and co-workers

In the 1990s Skibo and co-workers prepared and evaluated a series of pyrrolo[1,2-\(a\)]benzimidazolequinones (PBIs) (Figure 1.3). The PBIs are reduced via two-electron reduction mediated by NQO1 to convert the PBI quinone into a reactive hydroquinone species, which hydrogen bonds to the DNA major groove at the AT base pair. Subsequent nucleophilic attack by the phosphate anion at the protonated
aziridine leads to ring opening, alkylation and hydrolytic cleavage of the phosphate backbone of DNA (Scheme 1.18).  

Scheme 1.18: Events leading to cleavage of DNA phosphate backbone by the PBI hydroquinone at the AT base pair

The PBIs exhibit \textit{in vitro} and \textit{in vivo} activity towards a range of cancer cell lines including melanoma, breast, ovarian, CNS and non-small cell lung cancers. Structure-activity relationship studies carried out using the National Cancer Institute (NCI)-60 human tumour cell line screen revealed that the PBIs have a unique spectrum of activity and were most active \textit{in vitro} towards melanoma cell lines and completely inactive towards leukaemia cell lines. The \textit{iso} PBI-A, (when the aziridine ring substituent is moved from the C-6 position to the C-7 position) resulted in an overall decrease in potency by one order of magnitude. Furthermore it was found that increasing the ring size in the PBI system from a pyrrolidine ring to a piperidine ring to give tetrahydropyrido[1,2-\textit{a}]benzimidazole (TPBI) resulted in diminished cytotoxicity most apparent in the melanoma cell lines. This was attributed to the instability of the TPBI solutions which decomposed within a few hours. The non-ring fused benzimidazole (BI) system showed negligible difference in activity overall compared to the PBIs, implying that the pyrrolo ring was not required to exert cytotoxic action. Structure–activity studies of the PBI system identified the requirement of an aziridine ring at the C-6 position, a methyl substituent at the C-7 position, thought to be required for steric and electronic reasons and the replacement of an \textit{O-}
substituent (e.g. acetate or carbamate) with a basic N-substituent at the C-3 position, in the form of an amine or carbamido results in further potency probably due to more efficient hydrogen bonding in the major groove leading to enhanced DNA alkylation.\textsuperscript{54}

Ahn \textit{et al} prepared various C-7 amine substituted PBIs and C-6 substituted BIs containing methyl, ethyl, propyl and butyl groups at the R\textsuperscript{1} position and acetate or hydroxyl groups at the R\textsuperscript{2} position; four analogues containing ester groups were also prepared (Figure 1.4).\textsuperscript{55,56} Cytotoxic studies on three cancer cell lines found the \textit{iso} PBI-A compound to be most active compound of PBI series towards the human gastric cancer cell line, with the acetate and hydroxyl substituents on the BI series showing greater cytotoxicity overall than the ester analogues.

\textbf{Figure 1.4:} Aziridinyl substituted quinones reported by Ahn and co-workers

O’Donovan and Aldabbagh reported 4,7-dimethoxy-N-[(aziridin-2S-yl)methyl]benzimidazole 60 representing the first time that N-diazole nucleophiles have been used to substitute at the methyl carbon of substituted methylaziridines 59 (Scheme 1.19).\textsuperscript{57,58}

\textbf{Scheme 1.19:} Preparation of N-methylaziridinyl benzimidazolequinone
4,7-Dimethoxy-N-aziridinyl benzimidazole 60 was converted to the corresponding benzimidazolequinone 61 using a mild demethylation/oxidation protocol involving N-bromosuccinimide (NBS) and a catalytic amount of H2SO4. The hydrobromic acid/ferric chloride mediated route previously used by the Aldabbagh group to access benzimidazolequinones from dimethoxy precursors was not feasible in the presence of the sensitive aziridine moiety.20,59,60 Aziridine containing benzimidazoles 60 and 61 were evaluated against Fanconi anemia (FA) cell lines. FA is a rare human genetic disease, leading to a high incidence of cancer in early adulthood. FA cells are mutant in one of a number of genes encoding proteins in the FANC multi-protein complex, which plays a key role in processing induced DNA-damage. Mutant FA cells are hypersensitive to DNA-crosslinking agents, including MMC.

The N-[(aziridinyl)methyl]benzimidazolequinone 61 was found to induce hypersensitive killing of FA cells lacking the FANCD2 protein in the nanomolar range (10⁻⁹M), similar to MMC. This hypersensitivity was partially corrected upon introduction of the missing FANCD2 protein illustrating that DNA damage induction and the FANC pathway are important for the cytotoxicity of 61. Further studies found that 60 also induced hypersensitive killing of FA cells lacking the FANCD2 protein despite not having a quinone functionality.58 This implies that it is the aziridine and not the quinone functionality that is the primary cause of induced hypersensitive toxicity towards FA cells. Both 60 and 61 have only one site for DNA alkylation at the aziridine C-1 position suggesting that compounds 60 and 61 do not require the formation of crosslinks to induce DNA damage.

Fahey and Aldabbagh prepared methoxy and aziridinyl substituted pyrrolo-, pyrido-, and azepino benzimidazolequinones 62a-63c for cytotoxic evaluation (Scheme 1.20).58,61 Aziridinyl substituted benzimidazolequinones 63a-c were more cytotoxic than the methoxy precursors 62a-c towards the human normal skin fibroblast cell line (GM00637). Increasing the alicyclic ring size from the pyrrolo-to pyrido- and azepino membered compounds reduced potency with pyrrolobenzimidazolequinones 62a and 63a being more cytotoxic than the methoxy and aziridinyl substituted pyrido- and azepino- analogues.
Further studies with 62a and 63a on the FA cell line lacking FANCD2 found that 6-aziridinyl substituted pyrrolo[1,2-α]benzimidazolequinone 63a was cytotoxic in the nanomolar range (10⁻⁹ M). The cytotoxicity induced by 63a was corrected upon introduction of the missing FANC protein. When the aziridinyl substituent was absent in 6-methoxypyrrolo[1,2-α]benzimidazolequinone 62a, negligible cytotoxicity towards the FA cell line was observed. This implies that the aziridine functionality is mainly responsible for the sensitive toxicity of FA cells (lacking FANCD2 protein) towards these compounds.
1.1.4 Other alkylating anti-tumour agents

Figure 1.5: Examples of alkylating anti-tumour agents

Chlorambucil is a clinically used aromatic mustard. It is used alone or in combination with other chemotherapeutic drugs for the treatment of chronic lymphocytic leukaemia and Hodgkin’s disease, with reduced associated toxicity reported than for other mustines.\textsuperscript{62-64}

Bendamustine is a benzimidazole based $N$-mustard developed in the 1960s as a water soluble anti-cancer agent. It has been widely used in Germany for the treatment of chronic lymphocytic leukaemia and non-Hodgkin’s lymphoma and has been recently approved in the USA for similar treatments.\textsuperscript{65,66} Side effects of the drug are relatively mild with myelosuppression reported as the dose limiting toxicity.\textsuperscript{66} Along with the successful treatment of lymphoid malignancies, Bendamustine has been investigated through phase II trials as a potential salvage chemotherapeutic for the treatment of breast cancer.\textsuperscript{67}

The bi-functional $N$-mustards represent the earliest alkylating agents employed in cancer chemotherapy. The alkylating mechanism of the $N$-mustards proceeds via displacement of the chloride ion intramolecularly to form an electrophilic aziridinium intermediate capable of alkylating DNA (Scheme 1.21).\textsuperscript{68,69} $N$-Mustards can generate two possible alkylation sites which allow for the formation...
of DNA crosslinks. Differences in chemical reactivity and clinical use can be achieved by altering the R substituent on the amine functionality.

Scheme 1.21: Alkylation of DNA by nitrogen mustards

**Diaziridinylquinones**

**Diaziquone (AZQ)** was one of several compounds proposed as a CNS anti-tumour agent reported in 1976 by Driscoll et al. and identified as the most active compound of the series. AZQ is a substrate for NQO1 and exerts damage through alkylation, crosslinking and strand breaks. AZQ has shown activity against leukaemia, lymphomas and solid tumours, however high levels of associated toxicity have precluded clinical entry.

**RH1** is a recently developed water soluble potential chemotherapeutic agent, shown to be selectively cytotoxic towards cell lines with elevated levels of NQO1. RH1 exerts cytotoxic damage through the formation of DNA crosslinks following bioreduction. Preclinical studies of RH1 has shown good activity towards non-small cell lung cancer, colon and ovarian cancers, and clinical evaluation in the UK is in progress.

**EO9** (Apaziquone) is a synthetically derived analogue of MMC reported by Oostveen and Speckamp in the 1980s. EO9 undergoes reductive activation to unmask three reactive sites for DNA alkylation, with loss of the hydroxyl groups at positions C-1, C-10 and ring opening of the aziridine. EO9 is a substrate for
both one and two electron reductases and studies under aerobic conditions have
correlated NQO1 levels on a panel of tumour cell lines with cell sensitivity.\textsuperscript{79} \textbf{EO9} was entered into clinical trials based on its selective toxicity towards solid
tumours and hypoxic cells and its associated low toxicity. However rapid
metabolism \textit{in vivo} prevented significant anti-tumour activity.\textsuperscript{80} \textbf{EO9} has recently
entered phase II and III trials for the intravesical treatment of non-muscle invasive
bladder cancer.\textsuperscript{81}
1.2 Aims and Objectives

To prepare the first aziridinyl fused pyrrolo[1,2-\(\alpha\)]benzimidazole 64

There are no known examples of aziridinyl fused pyrrolo[1,2-\(\alpha\)]benzimidazoles despite the extensive research into aziridinyl substituted benzimidazoles and benzimidazolequinones.\(^{49-54,56-58,61}\) The retrosynthesis of this new heterocyclic system is shown in Scheme 1.22.

Scheme 1.22: Retrosynthetic analysis of aziridinyl fused pyrrolo[1,2-\(\alpha\)]benzimidazole target

To evaluate and compare the cytotoxicity of 64 and non-fused \(N\)-[1-tritylaziridin-(2S)-yl]methyl]-1H-benzimidazole 65 towards a human normal skin fibroblast cell line (GM00637) and breast cancer cell lines (MCF-7) and (HCC1937). Previously reported aziridine containing diazoles were found to induce a hypersensitive response to a FANCD2 deficient cell line similar to MMC (Section 1.1.3).\(^{57,58}\) The BRCA1 protein (breast cancer tumour suppressor protein) is reported to interact with FANCD2 in response to DNA damage.\(^{82,83}\) Novel diazoles 64 and 65 were evaluated against the MCF-7 cell line (BRCA1 positive), and the HCC1937 cell line (BRCA1 mutated), to determine the role of BRCA1 in DNA damage response pathway of these compounds.

Figure 1.6: Synthetic aziridine heterocycles
1.3 Results and Discussion

1.3.1 Preparation of the cis-substituted aziridine fragment

The retrosynthesis of cis-substituted aziridine is shown in Scheme 1.23.

Scheme 1.23: Retrosynthetic analysis of the aziridine fragment

Modifications to the literature reported synthesis of aziridine 74 allowed for the preparation of new cis 2,3-substituted aziridine 75 through a shorter synthetic route. The Bu3Sn functionality allows formation of a reactive lithiated aziridinyl anion intermediate which is used in the subsequent intramolecular ipso-substitution to give aziridinyl fused pyrrolo[1,2-α]benzimidazole 64.
Trityl protection of the amine functionality of (S)-serine methyl ester hydrochloride 79 was carried out using trityl chloride (TrCl) and TEA to give N-trityl-S-serine methyl ester 80 in 91% yield (Scheme 1.24).85

![Reaction Scheme](image)

Scheme 1.24: Trityl protection of (S)-serine methyl ester hydrochloride

Alcohol 80 was treated with (tert-butyldimethylsiloxymethyl)chloride (TBDMSOCH2Cl) 85, a silicon protecting group initially employed by Vedejs et al.47 The TBDMSOCH2Cl group is not commercially available and was prepared in three synthetic steps (Scheme 1.25).86,87

![Synthesis Scheme](image)

Scheme 1.25: Synthesis of (tert-butyldimethylsiloxymethyl)chloride

Hydroxymethylation of ethanethiol with paraformaldehyde and a catalytic amount of sodium methoxide in methanol yielded (ethylsulfanyl)methanol 81 and by-product (ethylsulfanylmethoxy)methanol 82 in a 11:1 ratio determined by 1H NMR. Attempts to separate 81 from by-product 82 by vacuum distillation were unsuccessful and the mixture was used directly in the next step. Alcohols 81 and 82, were reacted with tert-butyldimethylsilyl chloride (TBDMS-Cl) in the presence of 4-(N,N-dimethylamino)pyridine (DMAP) and TEA at room temperature, to give (tert-butyldimethylsiloxymethyl)ethylethyl sulfide 83, contaminated with (tert-butyldimethylsiloxymethyl)methoxymethyl ethyl sulfide 84 in a
ratio of 18:1 by $^1$H NMR. Following sulfuryl chloride mediated cleavage of the ethylsulfide group, impure (tert-butyldimethylsiloxy)methyl chloride 85 was afforded in 96% yield contaminated with traces of unidentifiable impurities. The chloromethyl reagent 85 was highly unstable and degradation occurred over a few hours.$^{87}$

Scheme 1.26: Protecting the alcohol moiety with TBDMSOCH$_2$Cl

Protection of $N$-trityl-$S$-serine methyl ester 80 was achieved using impure (tert-butyldimethylsiloxy)methyl chloride 85 to give $N$-trityl-$O$-(tert-butyldimethylsiloxymethyl)-$S$-serine methyl ester 66 in 48% yield (Scheme 1.26, route a). $N$-trityl-$O$-(tert-butyldimethylsiloxy)-$S$-serine methyl ester 67 by-product was also isolated in 19% yield. Replacing $N,N$-diisopropylethylamine base with TEA significantly reduced the amount of protected esters 66 and 67 formed (Scheme 1.26, route b). The formation of 67 occurred regardless of the type of amine base employed. The proposed mechanism for the formation of ester 67 (Scheme 1.27) is based on the ability of the (tert-butyldimethylsiloxy)methyl chloride 85 to eliminate a molecule of formaldehyde.

Scheme 1.27: Proposed mechanism for the formation of $N$-trityl-$O$-(tert-butyldimethylsiloxy)-$S$-serine methyl ester
Ester 67 could be prepared in high yields according to a procedure by Lee et al.\textsuperscript{88}, by reversing the order of protection of the NH/OH functional groups of (S)-serine methyl ester hydrochloride 80 it was possible to protect both groups in one reaction, to give 67 in 81% yield (Scheme 1.28).

\textbf{Scheme 1.28:} One-pot protection of (S)-serine methyl ester hydrochloride

Methyl ester 66 was efficiently reduced with LiAlH\textsubscript{4} (2.1 equiv.) in THF (Scheme 1.29, route a) to give the corresponding alcohol N-trityl-O-(tert-butyldimethylsiloxymethyl)-R-serinol 68 (92%).

\textbf{Scheme 1.29:} Preparation of serinols through reduction of methylesters

When methylester 67 was employed under the same reductive conditions only known diol, 2-(tritylamino)propane-1,3-diol 86\textsuperscript{89} (73%) was obtained, a product resulting from cleavage of the TBDMS protecting group (Scheme 1.29, route a). De Vries \textit{et al} propose a mechanism for the intramolecular reductive cleavage of TBDMS ethers using LiAlH\textsubscript{4} (Scheme 1.30).\textsuperscript{90} Following reduction of the ester moiety to the primary alcohol, the amine group can be deprotonated and the resulting anion stabilized by AlH\textsubscript{3}. The resulting complex brings the aluminium
moiety close to the silicon group allowing for intramolecular hydride transfer to take place. Loss of tert-butylidimethylsilane and acid aqueous work up affords diol 86.

Scheme 1.30: Mechanism for the reductive loss of the TBDMS protecting group

Diisobutylaluminium hydride (DIBAL, 2.5 equiv.) in toluene was employed as an alternative reductant (Scheme 1.29, route b) to convert esters 66 and 67 to the corresponding serinols, 68 and N-trityl-O-(tert-butyldimethylsiloxyl)-R-serinol91 69 in excellent yields of over 95%.

Two methods of oxidation (TEMPO/NaOCl92 and Swern84) were employed to convert serinols 68 and 69 into aldehydes N-trityl-O-(tert-butyldimethylsiloxymethyl)-S-serinal 70 and N-trityl-O-(tert-butyldimethylsiloxyl)-S-serinal 71 (Scheme 1.31).

Scheme 1.31: Preparation of serinals

Tributyltin lithium (Bu3SnLi) was prepared at -20 °C by deprotonation of tributyltin hydride (Bu3SnH) with lithium diisopropylamide (LDA). Nucleophilic addition to the carbonyl group of aldehydes 70 and 71 by the tributyltin anion at -78 °C yielded intermediate stannyl amino alcohols 72 and 73 (Scheme 1.32). Amino alcohols 72 and 73 were unstable to purification and were directly converted to the cis-substituted aziridines (2S,3R)-2-[(1-tert-
butyl(dimethyl)silyl]oxy)methoxy)methyl]-3-(tributylstanny)1-tritylaziridine 74 and (2S,3R)-2-([tert-butyl(dimethyl)silyl]oxy)methyl)-3-(tributylstanny)1-tritylaziridine 75 via the Mitsunobu ring closure reaction using triphenylphosphine and diisopropyl azodicarboxylate (DIAD)\(^93,94\), as an alternative to the diethyl azodicarboxylate (DEAD) reagent originally reported\(^47,84\) (Scheme 1.33). Aziridine 75 represents a shorter route to achieving the desired cis-substituted aziridine fragment.

Scheme 1.32: Addition of the Bu\(_3\)Sn nucleophile onto serinals

Scheme 1.33: Mitsunobu ring closure of stannyl amino alcohol intermediates

Aziridine 87 (2R)-2-([tert-butyl(dimethyl)silyl]oxy)methyl)-1-tritylaziridine (Scheme 1.34) was occasionally isolated and possibly occurs from the LDA reduction of aldehyde 71 without formation of Bu\(_3\)SnLi. Kowalski et al proposed a mechanism for the LDA reduction of \(\alpha\)-substituted ketones, and this is applied to aldehyde 71.\(^95\)
Scheme 1.34: Proposed mechanism for LDA reduction followed by Mitsunobu ring closure

The aziridinyl hydrogens of cis-substituted aziridines 74 and 75 were obscured in the $^1$H NMR spectra and no coupling constant could be calculated. The cis-stereochemistry of the aziridines was later confirmed by mesyl aziridine 77 (Scheme 1.36). The larger coupling constant ($J_{2,3} \approx 6.8$ Hz) for mesylate 77 was consistent with reported literature values for other cis-substituted aziridines (Figure 1.7) and spectral data reported by Vedejs et al.47

Figure 1.7: Literature examples of cis and trans aziridines with $^1$H NMR coupling constants ($J$) 88-89 96, 90 97

Deprotection of aziridine 75 was carried out using n-tetrabutylammonium fluoride (TBAF) to afford [(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methanol 76 in 96% yield (Scheme 1.35).
Scheme 1.35: TBDMS-deprotection to afford aziridinol

The hydroxyl group of aziridinol 76 was treated with TEA and methanesulfonyl chloride at -78 °C in dichloromethane to give desired mesylate, [(2S,3R)-3-(tributylstanny1)-1-tritylaziridin-2-yl]methyl methanesulfonate 77 in 89% yield. Alternatively treatment of aziridinol 76 with TEA, DMAP and 4-nitrobenzenesulfonyl chloride gave nosylate, [(2S,3R)-3-(tributylstanny1)-1-tritylaziridin-2-yl]methyl 4-nitrobenzenesulfonate 78 in 75% yield (Scheme 1.36).47

Scheme 1.36: Conversion of the alcohol moiety into a good leaving group
1.3.2 Literature reported intramolecular aromatic radical ipso-substitutions

Organosulfur groups have been previously displaced in radical ipso-substitution reactions.\textsuperscript{98-104}

Caddick \textit{et al} reported the preparation of [1,2-\textit{a}] alicyclic ring fused indoles 92a-c using intramolecular radical ipso-substitutions of phenyl sulfide, sulfoxide, and sulfone groups at the 2-position of indoles 91a-c (Scheme 1.37).\textsuperscript{100}

Scheme 1.37: Alkyl radical ipso-substitution at the 2-position of indoles

Aldabbagh and Bowman reported intramolecular homolytic aromatic ipso-substitutions using \(N\)-alkylphenyl selenide radical precursors of imidazole and benzimidazole (Scheme 1.38).\textsuperscript{101} The alkyl radicals displaced tosyl or phenylsulfanyl radical leaving groups from the 2-position of imidazole and benzimidazole respectively. The more activating tosyl leaving group was required for the substitution of imidazoles, where as the phenylsulfanyl group was sufficiently activating for benzimidazole substitution, due to the reduced aromaticity of the imidazole ring in benzimidazole.

Scheme 1.38: Alkyl radical ipso-substitution at the 2-position of imidazole and benzimidazole
1.3.3 **Annulation of aziridine onto pyrrolo[1,2-\(\text{a}\)]benzimidazole**

\(N\)-trityl protection of benzimidazole was carried out in the presence of TEA and TrCl to give 1-trityl-\(1H\)-benzimidazole 93 in 66% yield (Scheme 1.39).\(^{101}\)

![Scheme 1.39: Preparation of 1-trityl-\(1H\)-benzimidazole](image)

Lithiation of benzimidazole 93 with n-butyl lithium (n-BuLi) and quenching the resulting carbanion with diphenyl disulfide (PhS-SPh) gave 2-(phenylsulfanyl)-1-trityl-\(1H\)-benzimidazole 94 in 61% yield (Scheme 1.40). Removal of the trityl group was achieved by heating 94 at reflux in wet methanol in the presence of concentrated hydrochloric acid to give 2-(phenylsulfanyl)-\(1H\)-benzimidazole 95 in 78% yield.

![Scheme 1.40: Preparation of 2-(phenylsulfanyl)-\(1H\)-benzimidazole](image)

2-(Phenylsulfonyl)-\(1H\)-benzimidazole 96 was obtained in 71% yield according to literature procedure.\(^{105}\) 2-(Phenylsulfanyl)-\(1H\)-benzimidazole 95 was added to a solution of hydrogen peroxide and ammonium heptamolybdate catalyst and allowed to react for 24 hours at room temperature (Scheme 1.41).

![Scheme 1.41: Preparation of 2-(phenylsulfonyl)-\(1H\)-benzimidazole](image)
Initial attempts to alkylate 2-organosulfur substituted benzimidazoles 95 and 96 using mesylate 77 to give the respective 2-(phenylsulfanyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole 97 and 2-(phenylsulfonyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole 98 were unsuccessful (Scheme 1.42).

Scheme 1.42: Attempted synthesis of 2-phenylsulfanyl and 2-phenylsulfonyl benzimidazoles

Our focus then turned to the preparation of the non-substituted benzimidazole analogue 99 (Scheme 1.43). Benzimidazole was successfully alkylated using mesylate 77 to give 1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole 99 in 43% yield.

Scheme 1.43: Alkylation of benzimidazole with mesyl aziridine

[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl 4-nitrobenzenesulfonate 78 containing the more reactive nosylate leaving group was employed in place of mesylate 77 in order to decrease the alkylation reaction time. Benzimidazole was alkylated with nosyl aziridine 78 to give 99 in 41% yield overnight (Scheme 1.44).
Scheme 1.44: Alkylation of benzimidazole with nosyl aziridine

Attempts to alkylate 2-substituted organosulfur benzimidazoles 95 and 96 with nosyl aziridine 78 remained unsuccessful. It was thought at this point that steric factors between the phenylsulfanyl and phenylsulfonyl groups and the tributylstannyl substituent of aziridines 77 and 78 may have prevented direct alkylation of the 2-substituted organosulfur benzimidazoles.

An alternative route to access phenylsulfanyl annulation precursor 97 was established (Scheme 1.45).

Scheme 1.45: Lithiation route to cyclization precursors

Selective lithiation at the benzimidazol-2-yl position of 99 was investigated as a method of introducing the necessary C-2 substituent. Benzimidazole 99 was treated with n-BuLi (1.1 equiv.) in THF at -78 °C. The solution turned deep red indicating the formation of the benzimidazol-2-yl anion which was quenched with deuterated methanol (MeOD) after 15 minutes. The solution was slowly warmed to room temperature over 30 minutes, to give 2-deutero-1-\{((2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl)methyl\}-1H-benzimidazole 100 in 88% yield (Scheme 1.46). The $^1$H NMR spectrum indicated the incorporation of deuterium by the disappearance of the imidazole-2-H at 7.79 ppm.
Scheme 1.46: Deuterium incorporation at the benzimidazol-2-yl position

Following successful deuterium incorporation diphenyl disulfide (PhS-SPh) was employed as an alternative electrophile. The benzimidazol-2-yl anion of 99 was quenched with diphenyl disulfide to give the 2-(phenylsulfanyl) substituted benzimidazole 97 in 60% yield (Scheme 1.47).

Scheme 1.47: Phenyl sulfide incorporation at the benzimidazol-2-yl position

The cyclization precursor 97 was treated with methyl lithium, a less bulky organolithium reagent, at -78 °C to generate the aziridin-3-yl anion in situ (Scheme 1.48). The anion adds onto the electrophilic benzimidazol-2-yl position followed by re-aromatization and elimination of the phenyl sulfide group. The latter anionic aromatic ipso-substitution gave novel tetracycle 64 in 75% yield with 2-(phenylsulfanyl)-1-{{(2R)-1-tritylaziridin-2-yl}methyl}-1H-benzimidazole 101 formed in 18% yield.
Scheme 1.48: Anionic aromatic ipso-substitution to access novel tetracyclic target

In this ionic ipso-substitution the phenylsulfide group protects the otherwise acidic 2-position of benzimidazole from potential lithiation, as well as, acting as an effective leaving group. The formation of sulfide by-product 101 indicates the in situ generation of the aziridin-3-yl anion, which was quenched by advantageous hydration.

Figure 1.8: Novel cis-fused tetracycle 64 and literature reported aziridinyl fused tetracycles 102, 103 and 104.

Tetracycle 64 (1aS,8aS)-1-trityl-1,1α,8,8a-tetrahydroazireno[2’,3’:3,4]pyrrolo[1,2-a]benzimidazole represents the first diazole analogue skeleton of aziridinomitosene and is the first time an aziridine has been fused on the pyrrolo[1,2-a]benzimidazole framework. Tetracycle 64 was compared with other reported cis-fused aziridine systems. The $J$ values and shifts for H$_a$ the point of
ring fusion are in close agreement with those reported in literature confirming 64 as the cis fused aziridinyl pyrrolo[1,2-a]benzimidazole (Figure 1.8).

1.3.4 Nomenclature for (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2’,3’:3,4]pyrrolo[1,2-a]benzimidazole

Tetracycle 64 was named according to International Union of Pure and Applied Chemistry (IUPAC) rules.\textsuperscript{106} The base component of diazole 64 is identified as benzimidazole with the pyrrolidine and aziridine rings being labelled as first order and second order components respectively. The outside bonds of the base component benzimidazole are labelled with letters beginning at the 1-NH (Figure 1.9). The atoms of the first and second order components are numbered beginning at the nitrogen heteroatom.

This numbering system assigns the fusion name as azireno[2’, 3’: 3, 4]pyrrolo[1, 2-a]benzimidazole. The molecule is drawn with the maximum number of rings in a horizontal line using the available shapes allowed in order to assign peripheral numbers (Figure 1.10). Numbering begins with the most counter-clockwise non-fusion atom of the ring furthest to the right in the upper right-hand quadrant (nitrogen of the aziridine) and proceeds in a clockwise direction. If it is possible to place a double bond between two saturated positions, then these are hydro positions and are assigned in the name (hydro positions occur between 1-1a and 8-8a). Incorporation of the trityl substituent into the numbering system gives the name (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2’,3’:3,4]pyrrolo[1,2-a]benzimidazole.

\textbf{Figure 1.9:} Numbering of base, 1\textsuperscript{st} order and 2\textsuperscript{nd} order components
**Figure 1.10:** Peripheral numbering with maximum number of rings in a horizontal line
1.3.5 Preliminary studies on 4,7-dimethoxy-1H-benzimidazole

Dimethoxy substituents on the benzimidazole benzene moiety have been used as precursors to access benzimidazolequinones using a demethylation-oxidation protocol.\textsuperscript{20,57,59,60}

Preparation of 4,7-dimethoxy-1H-benzimidazole

Dinitration of commercially available 1,4-dimethoxybenzene \textsuperscript{105} using concentrated glacial acetic acid and nitric acid gave two 1,4-dimethoxydinitrobenzenes \textsuperscript{106} and \textsuperscript{107} which were separated using column chromatography (Scheme 1.49).\textsuperscript{107}

\begin{center}
\textbf{Scheme 1.49:} Preparation of 1,4-dimethoxydinitrobenzenes
\end{center}

1,4-dimethoxy-2,3-dinitrobenzene \textsuperscript{107} was reduced under 40 psi hydrogen for 18 hours in the presence of a palladium catalyst and ethanol to give 1,4-dimethoxy-2,3-diaminobenzene \textsuperscript{108} in 94% yield (Scheme 1.50).

\begin{center}
\textbf{Scheme 1.50:} Preparation of 1,4-dimethoxy-2,3-diaminobenzene
\end{center}

Condensation of 1,4-dimethoxy-2,3-diaminobenzene \textsuperscript{108} with formic acid for 4 hours at reflux, gave 4,7-dimethoxy-1H-benzimidazole \textsuperscript{109} in 72% yield (Scheme 1.51).
Scheme 1.51: Preparation of 4,7-dimethoxy-1H-benzimidazole

*N*-alkylation of 4,7-dimethoxy-1H-benzimidazole 109 with mesyl aziridine 77 was carried out using sodium hydride in DMF to give 4,7-dimethoxy-*N*-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridine-2-yl]methyl\}-1H-benzimidazole 110 in 43% yield (Scheme 1.52).

Scheme 1.52: Alkylation of 4,7-dimethoxy-1H-benzimidazole with mesyl aziridine

The alkylation of 109 with nosyl aziridine 78 was also carried out using sodium hydride in DMF to give the alkylated product 110 (32%) in 24 hours (Scheme 1.53). The reaction indicates that the 4,7-dimethoxy substituents do not hinder the coupling of the bulky Bu₃Sn-substituted aziridine.

Scheme 1.53: Alkylation of 4,7-dimethoxy-1H-benzimidazole with nosyl aziridine
Selective lithiation at the 2-position of \textbf{110} was carried out using the addition of n-BuLi (3 equiv.) to a solution of \textbf{110} at -78 °C to give 2-deutero-4,7-dimethoxy-\textit{N}-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridine-2-yl]methyl\}-1H-benzimidazole \textbf{111} in 82% yield (Scheme 1.54). The \textsuperscript{1}H NMR spectrum indicated the incorporation of deuterium by the disappearance of the imidazole-2-H at 7.63 ppm. Thus, the benzimidazol-2-yl anion should be easily quenched with diphenyl disulfide, as an alternative to MeOD to give the suitably functionalized cyclizations precursor.

**Scheme 1.54**: Selective deuterium incorporation at the 2-position of 4,7-dimethoxybenzimidazole

Future work will involve further studies to investigate selective lithiation and addition of phenyl sulfide group at the benzimidazol-2-yl position, followed by generation of an aziridin-3-yl anion to allow anionic aromatic \textit{ipsso}-substitution to give novel dimethoxy-substituted tetracycle \textbf{112}. Elaboration to the quinone \textbf{113} would be carried out using NBS-mediated demethylation/oxidation to give aziridinyl ring fused benzimidazolequinone (Scheme 1.55).\textsuperscript{57}

**Scheme 1.55**: Future elaboration to the aziridine containing quinone tetracycle
1.3.6 Preparation of \(N\)\-\{(1-tritylaziridin-(2\(S\))-yl)methyl\}\-1\(H\)-benzimidazole
the non-fused aziridinyl benzimidazole

The retrosynthesis of non-fused aziridinyl benzimidazole is outlined (Scheme 1.56).

![Scheme 1.56: Retrosynthesis of \(N\)\-\{(1-tritylaziridin-(2\(S\))-yl)methyl\}\-1\(H\)-benzimidazole](image)

Compound 65 was prepared by the \(N\)-alkylation of benzimidazole with \([(2\(S\))-1-tritylaziridin-2-yl]methyl methanesulfonate 116. Mesyl aziridine 116 was prepared according to a protocol previously reported by the Aldabbagh group\(^{57}\).

\(N\)-trityl-S-serine methyl ester 80 was converted to known aziridine (2\(S\))-1-tritylaziridine-2-carboxylic acid methyl ester 114 using TEA and sulfuryl chloride (Scheme 1.57). DIBAL reduction of ester 114 gave (2\(S\))-1-tritylaziridine-2-methanol 115 in 78% yield, followed by mesylation of the hydroxy group moiety of aziridinol 115 to give mesyl aziridine 116 in 75% yield.

![Scheme 1.57: Preparation of mesyl aziridine](image)
Initial attempts to react benzimidazole with mesylate 116 were unsuccessful due to base-induced ring-opening of the benzimidazole aziridine adduct, leading to the formation of a single product (3E)-3-(1H-benzimidazole-1-yl)-N-tritylprop-2-en-1-amine 117 in 46% yield (Scheme 1.58). $^1$H NMR data confirmed 117 to be the E-isomer with a coupling constant of 14.2 Hz between the vinyl hydrogen atoms.

Scheme 1.58: Unexpected preparation of an allylic amine

The reason for the ring-opening is the presence of an excess of sodium hydride (1.4 equiv.). We propose that the formation of amine 117 occurs via base-induced deprotonation of the benzylic position with subsequent ring-opening of the strained aziridine (Scheme 1.59).

Scheme 1.59: Proposed mechanism for base mediated deprotonation of aziridine

Mordini et al.\textsuperscript{108} reported the base-promoted deprotonation of aziridine at the benzylic position leading to ring opening and isomerization. This involved a superbase mixture of lithium diisopropylamide/potassium tert-butoxide (LIDAKOR) at 25 °C in pentane (Scheme 1.60).

Scheme 1.60: Selective deprotonation of aziridines to yield corresponding allylic amines
Alkylation of benzimidazole with mesyl aziridine 116 was achieved by reducing sodium hydride equivalents to 1.02 and heating the reaction to 30 °C in THF, to afford desired compound \( \text{N-}[(1\text{-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole} \) 65 in 83% yield (Scheme 1.61).

**Scheme 1.61: Preparation of non-fused aziridinyl benzimidazole**

To investigate the earlier proposal that steric factors prevented alkylation of 2-phenylsulfanyl and phenylsulfonylbenzimidazoles 95 and 96 with tributyltin containing aziridines 77 and 78. 2-(Phenylsulfanyl)-1H-benzimidazole 95 was successfully alkylated with mesyl aziridine 116 to give 2-(phenylsulfanyl)-1-\{[(2S)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole 118 in 72% yield (Scheme 1.62).

**Scheme 1.62: Preparation of 2-phenylsulfanyl aziridine substituted benzimidazole**
1.3.7 Cytotoxicity evaluation

MTT Assay

The MTT assay is a standard colorimetric assay used for measuring cell viability.\textsuperscript{109} Metabolically active (living) cells can reduce the water-soluble yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the water-insoluble purple formazan (Scheme 1.63). This process is mediated by the mitochondrial succinate-tetrazolium reductase system, active only in viable cells.

\textbf{Scheme 1.63:} MTT is reduced to purple formazan in viable (living) cells

The MTT assay is widely employed for measuring cell viability in assays of cell proliferation and cytotoxicity. This rapid and reproducible assay can be employed for studies on a broad range of cell types.

\textbf{MMC} was used as a positive control on both the human normal skin fibroblast cell line (GM00637) and human breast cancer cell lines (MCF-7) and (HCC1937). An IC\textsubscript{50} of 0.77 ± 0.18 µM was obtained for \textbf{MMC} under aerobic conditions on the human normal skin fibroblast (GM00637) cell line, which is in line with previous reports by our group for \textbf{MMC} under the same conditions (IC\textsubscript{50} = 0.80 µM\textsuperscript{57} and 0.90 µM\textsuperscript{58}). \textbf{MMC} is reported to be more active than other chemotherapeutic compounds like cisplatin and docetaxel towards the human breast cancer cell line (MCF-7).\textsuperscript{110} The breast cancer cell line HCC1937 carries a mutation for BRCA1, breast cancer susceptibility gene and has reported hypersensitivity to \textbf{MMC} and cisplatin.\textsuperscript{111-114} The main reason for this hypersensitive response is that both chemotherapeutic agents induce DNA
crosslinks, and BRCA1 is thought to play an essential role in the repair of this specific type of DNA damage (Scheme 1.64).

Scheme 1.64: A model of the interactions between FANCD2 and BRCA1 proteins in response to DNA damage

The normal DNA damage response pathway triggered by DNA crosslinking agents, involves activation of the FANCD2 protein, which occurs downstream from the FANC multi-protein complex. Activation usually occurs by the attachment of an ubiquitin label to the target protein, (ubiquitin is a small protein tag which directs the newly labelled protein to its active site or for degradation). Once activated the FANCD2 protein recruits other DNA damage response proteins such as BRCA1, BRCA2/FANCD1 and RAD51, which relocate to the DNA damage site in order to repair the damage and restore cellular stability. Cell lines containing mutated DNA damage response proteins (e.g. FANCD2 or BRCA1) lack the ability to repair DNA damage; this can lead to enhance hypersensitivity towards DNA damaging agents.
Figure 1.11: Aziridines evaluated for cytotoxicity against the human normal fibroblast cell line (GM00637) and human breast cancer cell lines (MCF-7 and HCC1937)

Aziridinyl fused pyrrolo[1,2-a]benzimidazole 64 was found to be largely non-toxic at concentrations of \( \leq 1 \text{ µM} \), with non-fused aziridinyl benzimidazole 65 and MMC showing greater toxicity towards the human normal fibroblast cells (GM00637) (Figure 1.12). Aziridinyl fused compounds 64 and MMC show similar toxicity towards the breast cancer cell line (MCF-7), while cell response towards benzimidazole 65 was greater (Figure 1.13).

In contrast, the BRCA1 deficient breast cancer cell line (HCC1937) showed a significantly greater response towards MMC only (Figure 1.14). Non-fused aziridinyl benzimidazole 65 was more toxic than aziridinyl fused 64 towards the three cell lines evaluated.
Figure 1.12: Viability of the human normal skin fibroblast cell line (GM00637) determined using the MTT assay following treatment with (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2′,3′:3,4]pyrrolo[1,2-a]benzimidazole 64 (●), N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 65 (■) and MMC (♦) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
Figure 1.13: Viability of the human breast cancer cell line (MCF-7) determined using the MTT assay following treatment with (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2',3':3,4]pyrrolo[1,2-a]benzimidazole 64 (●), N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 65 (■) and MMC (♦) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
Figure 1.14: Viability of the human breast cancer cell line (HCC1937) determined using the MTT assay following treatment with (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2',3':3,4]pyrrolo[1,2-a]benzimidazole 64 (●), N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 65 (■) and MMC (♦) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
Table 1.1: Effect of aziridines on human normal skin fibroblast cell line (GM00637) and human breast cancer cell lines (MCF-7) and (HCC1937)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50}^{a}GM00637 [µM]</th>
<th>IC_{50}^{a}MCF-7 [µM]</th>
<th>IC_{50}^{a}HCC1937 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.77 ± 0.18</td>
<td>0.93 ± 0.11</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>64</td>
<td>3.11 ± 0.44</td>
<td>0.84 ± 0.14</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td>65</td>
<td>1.26 ± 0.13</td>
<td>0.22 ± 0.04</td>
<td>0.16 ± 0.03</td>
</tr>
</tbody>
</table>

*IC_{50} represents the compound concentration required for the reduction of the mean cell viability to 50% of the control after incubation for 24 h at 37 °C, as determined using the MTT assay.

Table 1.1 shows the aziridine-containing benzimidazole 64 and 65 to be approximately 4-8 times more cytotoxic towards the breast cancer cell lines than towards the normal cells. In contrast to the clinically used MMC which shows similar cytotoxicity towards the normal cells and the breast cancer cell line MCF-7. For MMC it is reported that the formation of crosslinks are primarily responsible for cell death and hypersensitivity of BRCA1 deficient cells (HCC1937) towards MMC implicates BRCA1 in cellular response to crosslinks. The comparatively smaller cytotoxicity of benzimidazole containing aziridines 64 and 65 towards HCC1937 supports an alternative mechanism for cell response. The structures of 64 and 65 do not allow the formation of crosslinks, because there is only one site for DNA alkylation (at the aziridine) and the absence of a quinone moiety indicates lack of bioreductive activation. This is in line with previous reports of DNA alkylation and reactions of aziridinomitosenes with nucleophiles in the absence of reductant.

The overall greater toxicity of non-ring fused aziridinyl benzimidazole 65 compared to aziridinyl ring-fused benzimidazole 64 is in agreement with previously reported 2-aromatic ring substituted benzimidazolequinones 119 and 120 (Figure 1.15) displaying greater toxicity compared to analogous fused systems 121 and 122 towards human normal and cancer cell lines.
Figure 1.15: Range of IC\textsubscript{50} values for 2-aromatic ring substituted benzimidazolequinones compared to ring-fused analogues on human normal and cancer cell lines\textsuperscript{115}

The enhanced toxicity towards the human normal skin fibroblast cell line (GM00637) by the quinone moiety precluded its requirement in the present work. Previously reported benzimidazolequinone 61, was more cytotoxic than the dimethoxy analogue 60 towards the human normal skin fibroblast cells (GM00637) (60, IC\textsubscript{50} = 1.26 µM and 61, IC\textsubscript{50} = 0.15 µM\textsuperscript{58}). This enhanced cytotoxicity towards the human normal skin fibroblast cell line (GM00637) by the presence of the quinone moiety was further investigated by preparing and evaluating N-ethyl-4,7-dimethoxybenzimidaole 123 and the corresponding quinone 124 (both lacking an aziridine functionality). Dimethoxy benzimidazole 123 was non-toxic towards the human normal skin fibroblast cell line (GM00637) at concentrations of <10 µM, however quinone 124 exhibited enhanced cytotoxicity IC\textsubscript{50} of 0.58 µM (Figure 1.16).\textsuperscript{58}
Figure 1.16: Comparison of IC$_{50}$ values for N-substituted diazoles reported by the Aldabbagh group on the human normal fibroblast cell line (GM00637)
1.4 Conclusions

A new protocol for fusion of aziridine using anionic aromatic ipso-substitution is described. This gave the first diazole analogue of aziridinomitosene. The latter along with non-fused, N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 65 are shown to possess 4-8 times greater toxicity towards human breast cancer cell lines (MCF-7 and HCC1937) than towards a human normal fibroblast (GM00637) cell line, which has potential therapeutic advantages. Aziridine-ring fusion onto diazole was found to reduce overall toxicity. The mitomycin C mechanism for cytotoxicity involving bioreductive activation with subsequent formation of DNA-crosslinks is ruled out for the new benzimidazole compounds because of differences in their chemical structure. Bioreduction cannot take place since there is no aminoquinone motif as with MMC, and the formation of crosslinks is not possible since there is only one position for DNA-alkylation. The inability to form DNA crosslinks is supported by the comparatively smaller cell response of the BRCA1-mutated cell line, HCC1937 towards the new aziridine compounds.
Chapter 2

Cytotoxicity Evaluation of Various Imidazo[5,4-f]benzimidazolequinones Towards Human Normal and Cancer Cell Lines
2.1 Introduction

2.1.1 NAD(P)H: quinone oxidoreductase 1 (NQO1) - an enzyme-directed approach to chemotherapy

Enzyme-directed drug development involves the identification of prodrugs which are bioactivated by a particular reductase to the active form. The elevated levels of NAD(P)H: quinone oxidoreductase 1 (NQO1) in many solid tumours compared to normal tissue and its ability to activate a wide range of quinones, make it a good candidate for enzyme-directed therapy.²,¹¹⁶,¹¹⁷

NQO1, also known as DT-diaphorase (EC 1.6.5.2) is a ubiquitous flavoenzyme which catalyzes the two-electron reduction of several quinones to their corresponding hydroquinones. NQO1 was isolated in 1958 by Ernster and Navazio and initially named DT-diaphorase for its ability to efficiently use both di-or triphosphopyridine nucleotides (DPNH i.e. NADH) or (TPNH i.e. NADPH) electron donor cofactors (Figure 2.1).¹¹⁸ NQO1 has traditionally been regarded as a detoxification enzyme catalyzing the reduction of exogenous quinones to non-toxic hydroquinones.

![Nicotinamide Adenine Dinucleotide (NADH), R = OH](image1)

![Nicotinamide Adenine Dinucleotide Phosphate (NADPH), R = PO₃²⁻](image2)

**Figure 2.1:** Electron donor cofactors NAD(P)H
NQO1 also requires a flavin adenine dinucleotide (FAD) redox cofactor that remains bound to the NQO1 protein during the catalytic cycle and undergoes reduction by NAD(P)H (Scheme 2.1).

**Scheme 2.1**: Structure of Flavin Adenine Dinucleotide (FAD) and mechanism for FAD reduction by NAD(P)H electron donor cofactor\(^{119,120}\)

The NQO1 mechanism of action is described as a ping-pong mechanism as both NAD(P)H and quinone must independently occupy the same binding site. The catalytic cycle begins with the formation of a NQO1-FAD-NAD(P)H complex. The FAD electron acceptor cofactor is reduced to FADH\(_2\) by NAD(P)H and the resulting NAD(P)\(^+\) is lost. The quinone substrate can now enter the binding site and be reduced to a hydroquinone by FADH\(_2\). The substrate is then expelled as the hydroquinone from the binding site, which regenerates the NQO1 enzyme (Scheme 2.2).\(^{119,120}\)
Scheme 2.2: Ping-pong mechanism of NQO1

\[\text{NQO1-FAD} \rightarrow \text{NQO1-FAD-NAD(P)H} \rightarrow \text{NQO1-FAD}_{2}-\text{Quinone} \rightarrow \text{Quinone} \rightarrow \text{NQO1-FAD}_{2} \rightarrow \text{Hydroquinone} \rightarrow \text{NQO1-FAD} \rightarrow \text{NQO1-FAD-NAD(P)H} \]
2.1.2 Polycyclic quinones as anti-tumour agents

Many polycyclic quinones have been reported as anti-tumour agents. One of the most important mechanisms of cytotoxic action for coplanar polycyclic heteroaromatic quinones is DNA intercalation and topoisomerase inhibition. Intercalating agents insert between the base pairs of DNA, resulting in distortion and interference with the DNA replication process. Topoisomerases are DNA modifying enzymes, regulating DNA topology through single (topoisomerase I) or double (topoisomerase II) strand breaks, and are essential for DNA replication. Examples of some known polycyclic quinone intercalating agents and topoisomerase inhibitors are shown in Figure 2.2.

**Figure 2.2:** DNA-intercalating agents and topoisomerase inhibitors

**Streptonigrin** is a functionalized 7-aminoquinoline-5,8-dione, isolated from *Streptomyces flocculus* and highly active against a variety of tumour cell lines. Streptonigrin is a known topoisomerase II inhibitor and a substrate for the reductase enzyme NQO1. Severe toxicity has limited the clinical use of Streptonigrin as an anti-tumour agent.

**Daunorubicin** and **doxorubicin** (Adriamycin) are clinically used anthracycline antibiotic anti-tumour agents. Both are intercalating agents and topoisomerase II inhibitors which differ by one hydroxyl group. However clinically daunorubicin...
is primarily used for the treatment acute leukaemia, while *doxorubicin* has widespread activity against a range of solid tumours and leukaemia.\textsuperscript{128} Clinical usefulness has been limited by cardiomyopathy (heart muscle toxicity).

**Mitoxantrone** was one of several anthracenediones developed based on the anti-tumour activity of the anthracycline antibiotics.\textsuperscript{129} **Mitoxantrone** is a DNA intercalating agent and interacts with topoisomerase II, it is approved for the treatment of leukaemia, lymphoma and breast cancer.\textsuperscript{130}
2.1.2.1 Development of polycyclic quinones as anti-tumour agents

Rao and Kingston first isolated furanonaphthaquinone 125 an anti-cancer compound from the bark of *Tabebuia cassinoides* which showed good activity (ED$_{50}$ = 4.2 µM, where ED$_{50}$ is effective dose: concentration required to produce the desired effect in 50% of the test population) in the KB cell culture assay (a cell line derived from a human carcinoma of the nasopharynx used for screening plant extracts by the NCI$^{131}$) (Figure 2.3).$^{132}$ In 1998 a series of thiophene synthetic analogues 126-129 were developed as potential anti-tumour agents. Thiophene 126 showed an increase in activity (ED$_{50}$ of 1.6 µM) towards the KB cell culture assay compared to the furan natural product 125.$^{133}$

![Chemical structures of 125, 126, 127, 128, and 129](image)

**Figure 2.3:** The natural product Furanonaphthaquinone and some synthetic analogues

The related benzodithiophene-4,8-diones 127a-128f were prepared and evaluated against 8 cancer cell types at the National Cancer Institute (NCI). Both mono- and di-substituted compounds (127a-128f) showed good overall activity with mean GI$_{50}$ values ranging from 40 nM (127e) up to 1.2 µM (127f) (GI$_{50}$ is the concentration required to inhibit cell growth by 50%). Compound 127e displayed the highest potency overall, however its corresponding isomer 128e was among the least active of its series. In both series mono-hydroxyethyl substituted compounds 127c and 128c showed excellent overall cytotoxicity. The mono-substituted compounds were generally shown to have better selectivity than the di-substituted compounds. Mono-substituted compounds 127c and 128a were most selective towards the melanoma cell lines (GI$_{50}$ 33 to < 10 nM) and were
chosen for further in vivo studies. Similar compounds represented by \(129a\) and \(129b\) were also recently prepared to improve water solubility and cytotoxicity, with compound \(129a\) identified as a promising anti-leukemic drug candidate.

Johnson and co-workers have shown that the number and position of nitrogen heteroatoms had a marked influence on the ability of a series of dichloro substituted nitrogen containing quinones to cleave DNA (Figure 2.4). Increasing cytotoxic activity followed the order of naphthalenes < quinolines < isoquinolines < diazanaphthalenes for this series.

![Figure 2.4: Influence of position and number of N-heteroatoms on activity.](image)

Garuti et al also showed the influence of the location of N-heteroatom in benzimidazolequinones substituted with pyridine at the 2-position (Figure 2.5). Shifting the nitrogen from the 4-position to the 3- and 2-position of pyridine resulted in the progressive increase of anti-proliferative activity towards human leukaemia and colon cancer cell lines independent of the quinone substituent.

![Figure 2.5: Influence of pyridine on activity for 2-pyridinylbenzimidazolequinones](image)
Numerous fused and non-fused substituted nitrogen containing polycyclic quinones have been explored as potential anti-tumour agents (Figure 2.6).

**Figure 2.6:** Fused and non-fused heterocyclic quinones

Imidazoquinoxalinediones 130a-d were evaluated towards three human cancer cell lines (gastric, lung and colon), with compound 130d being more cytotoxic than doxorubicin (3.13 µM) and the most cytotoxic quinoxalinedione towards human gastric cancer (IC$_{50}$ = 1.30 µM). However all compounds 130a-d were significantly less active than doxorubicin (0.29 µM) towards human lung cancer and showed no activity towards the colon cancer cell line. Imidazoquinolininediones represented by 131b-132c were subsequently studied, following previously reported imidazonaphthoquinone 131a which showed high selective toxicity towards ovarian cancer cell lines in the NCI screen. Compounds 131b-132c exhibited *in vitro* cytotoxicity towards a range of human cancer cell lines including lung, ovarian, melanoma, brain and colon, but lower activity compared to doxorubicin. Compound 132b (X = O) was the most potent analogue of this series (mean IC$_{50}$ 0.16 µM). Later imidazophthalazinediones 133a-c with various substituents at the N-1 position were explored. The methyl, iso-propyl and phenyl analogues (mean IC$_{50}$ from 0.02-0.11 µM) were found to be more active than doxorubicin (mean IC$_{50}$ 0.32 µM) on ovarian, melanoma, CNS (central nervous system) and colon cancer cell lines suggesting these compounds to be superior to the quinoline series 131b-c.  

---

**Diagram:**

- **130a, R = CH$_3$**
- **130b, R = Et**
- **130c, R = Ph**
- **130d, R = 4-bromophenyl**
- **131a, X = CH, R = Et**
- **131b, X = N, R = Me**
- **131c, X = N, R = i-Pr**
- **131d, X = N, R = Ph**
- **132a, X = CH$_2$**
- **132b, X = O**
- **132c, X = S**
- **133a, R = Me**
- **133b, R = i-Pr**
- **133c, R = Ph**
2.1.3 Imidazo[4,5-f]benzimidazolequinones as anti-tumour agents

![Chemical structures](image)

Figure 2.7: APBI and dialicyclic imidazo[4,5-f]benzimidazolequinones reported by Skibo et al

In 2000 Skibo and Schulz developed the dipyrroloimidazo[4,5-f]benzimidazoles 134a-d as potential topoisomerase II inhibitors inactivated by NQO1 (Figure 2.7). Unlike the PBI s (Chapter 1, Section 1.1.3) which were reductively activated by NQO1, the 6-acetamidopyrrolo[1,2-a]benzimidazolequinones (APBIs) showed an inverse correlation to NQO1 levels, suggesting that reduction by NQO1 leads to an inactive hydroquinone species. Quinones 134a-d were investigated in an effort to further limit NQO1 reduction, by increasing steric bulk around the quinone moiety. NQO1 kinetic studies found compounds 134a and 134b to be excellent NQO1 substrates, however introduction of two acetate groups onto the dipyrroloimidazobenzimidazole system (134c and 134d) resulted in loss of NQO1 substrate activity presumably due to steric factors. When evaluated towards the NCI-(60) human tumour cell line panel, the NQO1 inactivated APBI-A showed selective cytotoxicity towards both melanoma and renal cell lines. Contrary to the APBI system, quinone 134a which is a substrate for NQO1 also displayed high selectivity towards the melanoma cell lines. NQO1 substrate quinone 134b was inactive against all cell lines, perhaps due to efficient NQO1 reductive inactivation. Both quinones 134c and 134d were not NQO1 substrates, however 134c was indiscriminately cytotoxic towards all cell lines and 134d was largely non-toxic overall. The active form of 134c may be the quinone form, while compound 134d may require reductive activation in order to exert cytotoxicity. Compounds 134a, 134c and 134d were also found to be topoisomerase II inhibitors, suggesting a possible mechanism for cytotoxicity.
2.1.4 Iminoquinones as anti-tumour agents

In the 1990s several aromatic pyrroloiminoquinone natural products were isolated from marine sponges and an ascidian (Figure 2.8). Wakayin was isolated from ascidian Clavelina sp. and exhibited in vitro cytotoxicity against a human colon tumour cell line, topoisomerase II inhibition and antimicrobial activity against Bacillus subtilis. Marine metabolites, makaluvamines A-F, H-M, N and P were isolated from the marine sponge Zyzzya. These were reported to exhibit in vitro and in vivo cytotoxicity toward several tumour cell lines and some showed strong inhibition of topoisomerase II. Bis-pyrroloiminoquinones tsitsikammamine A and B were also isolated from marine sponges and showed anti-fungal and cytotoxic activity however these compounds were not topoisomerase inhibitors.

![Chemical structures](image)

**Figure 2.8:** Natural pyrroloiminoquinone products

Synthetic pyrazole derivatives of wakayin and tsitsikammamines A and B were subsequently evaluated. Pyrazole compounds 135b and 136b were the most active topoisomerase inhibitors, but showed no cytotoxic activity on the five cancer cell lines tested. Conversely pyrazole compounds 135a, 136a and 136c showed no topoisomerase inhibition but significant growth inhibition with IC\textsubscript{50} values in the micromolar range, suggesting an alternative mechanism of cytotoxic action for these compounds (Figure 2.9).
Figure 2.9: Synthetic pyrazole iminoquinones

Imidazole analogues 137a-d (Figure 2.10) were evaluated against the NCI-60 human tumour cell line panel. Compounds 137b and 137c were selective towards melanoma and colon cancers, giving similar cytotoxicity profiles to the Makaluvamine natural products, suggesting a similar mechanism of action. Compound 137b was also found to be a topoisomerase II poison. Compounds 137a and 137d were also active across the 60-cell panel, but showed different toxicity profiles to compounds 137b and 137c and did not correlate to the Makaluvamine products.152

Figure 2.10: Imidazoiminoquinone synthetic analogues

The di-imidazole analogues 138a-d showed varying cytotoxicity towards the NCI-60 human tumour cell line panel. Compound 138d showed high cytotoxic
activity towards leukaemia and renal cancer cell lines. However its NCI cytotoxic profile showed that it had a different mode of action to that of naturally occurring iminoquinone **Wakayin**. A good correlation was obtained between its cytotoxicity and the concentration of the molecular target Flt-1, a receptor for Vascular Endothelial Growth Factor (VEGF induces vasculogenesis and angiogenesis, important for tumour growth).\(^{153}\)

### 2.1.5 Preparation of alicyclic ring fused imidazo[5,4-\(f\)]benzimidazolequinone anti-tumour agents

The alicyclic ring fused imidazo[4,5-\(f\)]benzimidazolequinone heterocyclic system is known, and evaluated for anti-cancer activity by Skibo *et al* (see Figure 2.7)\(^{143,154}\). The imidazo[5,4-\(f\)]benzimidazolequinone system was unknown prior to this Ph.D., and compounds were prepared by my colleague in the Aldabbagh group Vincent Fagan.\(^{155}\)

Bu\(_3\)SnH/1,1'-azobis(cyclohexanecarbonitrile) (ACCN)-mediated five, six and seven-membered alkyl radical cyclizations allowed one-pot double intramolecular homolytic substitutions to give the imidazo[5,4-\(f\)]benzimidazoles and dialycyclic ring fused isomers imidazo[4,5-\(f\)]benzimidazoles using 1,5- and 1,7-dialkylphenylselene radical precursors **139a-c** and **140a-c** respectively (Scheme 2.3).\(^{156}\)

\(\text{PhSe} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{PhSe} \quad \text{n} \quad \text{i. Bu}_3\text{SnH, ACCN} \quad \text{ii. Sunlight or 350 nm} \quad \text{PhSe} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{PhSe} \quad \text{n} \quad \text{i. Bu}_3\text{SnH, ACCN} \quad \text{ii. Sunlight or 350 nm} \quad \n\)

\(139\text{a-c, n}=1, 2, 3 \quad 141\text{a-c, n}=1 \quad (47\%)^* \quad 2 \quad (90\%) \quad 3 \quad (54\%)^* \quad 142\text{a-c, n}=1 \quad (48\%)^* \quad 2 \quad (81\%) \quad 3 \quad (48\%)^* \quad \)

*S* CSA or *AcC\(_2\)O* added

**Scheme 2.3:** One-pot double homolytic aromatic substitutions to give imidazobenzimidazoles
Dipyridoimidazobenzimidazoles 141b and 142b were obtained in yields of 81-90% with no radical reduction products formed. The more difficult 5- and 7-membered cyclizations gave significant amounts of reduced products. Quaternization of the 3,7-N basic sites using camphorsulfonic acid (CSA) or acetic anhydride gave optimized yields of 5- and 7-membered dialicyclic ring fused imidazobenzimidazoles of about 50%. Access to the mono-aicyclic ring fused compound 1-butyl pyridoimidazo[5,4-f]benzimidazole 146 was achieved through formation of the mono-alkylated 1-butyl-5(7)H-imidazo[5,4-f(4,5-f)]benzimidazole 143, which was reacted with a second alkylating chain 4-chlorobutyl phenyl selenide to yield the dialkylated radical precursor isomers 144 (43%) and 145 (40%) bearing only one PhSe-radical leaving group. Bu3SnH-mediated radical cyclization via phenylselenide 144 gave the six-membered fused product 146 in 87% yield (Scheme 2.4).

\[
\text{Scheme 2.4: Preparation of 1-butyl pyridoimidazo[5,4-f]benzimidazole}
\]

1,5-dibutyl 147 and 1,7-dibutyl 148 imidazobenzimidazoles (Scheme 2.5) were prepared by alkylation of imidazo[5,4-f(4,5-f)]benzimidazole with 1-chloro butane, resulting in equal amounts of 1,5- and 1,7- isomers, in 45% and 43% yield respectively.
Scheme 2.5: Preparation of 1,5-dibutyl and 1,7-dibutyl imidazobenzimidazoles

An alternative protocol to access imidazo[5,4-f]benzimidazoles involved oxidative cyclization of cyclic amines 149 and 150 onto neighbouring acetamides using Oxone® and formic acid giving the dialicyclic ring fused benzimidazoles 151 and 152 (Scheme 2.6).155

Scheme 2.6: Preparation of dialicyclic ring fused imidazo[5,4-f]benzimidazoles via oxidative cyclizations

Elaboration of benzimidazoles to their respective quinones was achieved by nitration, reduction and oxidation sequence in high yields (Scheme 2.7). Iminoquinone 154 was formed in 91% yield during the Frémy’s salt (K₂NO(SO₃)₂) oxidation of 6-amino dipyridoimidazo[5,4-f]benzimidazole 153 at pH 4 in the presence of phosphate buffer; acid hydrolysis at lower pH gave the corresponding quinone 155.156
Scheme 2.7: Protocol for the preparation of iminoquinone and quinone moiety of imidazo[5,4-f]benzimidazoles
2.2 Aims and Objectives

This chapter deals with the cytotoxicity evaluation of the imidazobenzimidazolequinones 154-161 using the MTT colorimetric assay\textsuperscript{109} (Figure 2.11) Two human cancer cell lines known to express high levels of NQO1: cervical cancer (HeLa) and prostate cancer (DU145) were used.\textsuperscript{116,157} This was compared with toxicity towards a normal human skin fibroblast cell line (GM00637).

\textbf{Figure 2.11}: Imidazobenzimidazolequinones biologically evaluated against three human cell lines; GM00637, HeLa and DU145
The following Structure-Activity Relationships were determined with comparisons made to dipyridoimidazo[5,4-f]benzimidazolequinone 155, which was viewed as the parent molecule.


2. The effect of iminoquinone versus quinone moiety of imidazo[5,4-f]benzimidazoles (154 versus 155) on toxicity.

3. The effect of the alicyclic fused ring size onto imidazo[5,4-f]benzimidazolequinones (155 versus 157 versus 158) on toxicity (pyrrolo versus pyrido versus azepino). Note – that compound 158 is evaluated, and not the dipyrrolo analogue: pyrrolo[1,2-a]pyrrolo[1′,2′:1,2]imidazo[5,4-f]benzimidazolequinone due to the poor solubility of the latter.

4. The effect of alicyclic ring fusion on toxicity (155 versus 159 versus 160).

5. The effect of an oxygen atom within the alicyclic ring (155 versus 161) on toxicity.
2.3 Results and Discussion

Cytotoxicity evaluations
Cytotoxicity evaluations were carried out using the MTT assay. MMC was used as a positive control for the biological assessments (Table 2.1).

2.3.1 The effect of the imidazo[5,4-f]benzimidazolequinone versus imidazo[4,5-f]benzimidazolequinone ring system on toxicity

![Chemical structures](image)

**Figure 2.12:** Dipyridoimidazo[5,4-f(4,5-f)] benzimidazolequinones evaluated to compare the effect of alicyclic ring orientation on cytotoxicity

**Table 2.1:** The effect of MMC and dipyridoimidazo[5,4-f(4,5-f)] benzimidazolequinones on three human cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;GM00637 [µM]</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; HeLa [µM]</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; DU145 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.46 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>155</td>
<td>&gt; 5</td>
<td>1.67 ± 0.05</td>
<td>1.99 ± 0.39</td>
</tr>
<tr>
<td>156</td>
<td>&gt; 5</td>
<td>3.33 ± 0.47</td>
<td>1.73 ± 0.23</td>
</tr>
</tbody>
</table>

<sup>a</sup> IC<sub>50</sub> represents the compound concentration required for the reduction of the mean cell viability to 50% of the control after incubation for 72 h at 37 °C, as determined using the MTT assay.

Skibo previously reported the preparation of dipyridoimidazo[4,5-f]benzimidazolequinone 156<sup>154</sup> but no biological evaluations were carried out. Table 2.1 shows the IC<sub>50</sub> values obtained for MMC, novel dipyridoimidazo[5,4-f]benzimidazolequinone 155 and dipyridoimidazo[4,5-f]benzimidazolequinone 156 evaluated on three cell lines. Both imidazobenzimidazolequinone
compounds 155 and 156 showed negligible toxicity towards the human normal skin fibroblast cell line (GM00637) at $\leq 5 \, \mu M$ concentration. Novel dipyridoimidazo[5,4-f]benzimidazolequinone 155 (Figure 2.13) showed enhanced cytotoxicity towards both HeLa and DU145 cancer cell lines compared to the normal human skin fibroblast cell line (GM00637). Dipyridoimidazo[4,5-f]benzimidazolequinone 156 (Figure 2.14) was more active towards the cervical (HeLa) cancer cell line than the normal skin cell line and up to ~ 3 times more active towards the prostate (DU145) cancer cell line (Table 2.1).
Figure 2.13: Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 1,2,3,4,8,9,10,11-octahydropyrido[1,2-a]pyrido[1’,2’:1,2]imidazo[5,4-f]benzimidazol-6,13-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
Figure 2.14: Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 1,2,3,4,8,9,10,11-octahydropyrido[1,2-\(a\)]pyrido[1′,2′:1,2]imidazo[4,5-f]benzimidazol-6,13-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
2.3.2 The effect of iminoquinone versus quinone moiety of imidazo[5,4-
\textit{f}]benzimidazoles on toxicity

![Iminoquinone and dipyridoquinone](image)

**Figure 2.15:** Iminoquinone and dipyridoquinone were compared to assess the
effect of the iminoquinone moiety on toxicity

**Table 2.2:** The effect of 6-iminodipyridoimidazo[5,4-
\textit{f}]benzimidazol-13-one compared to dipyridoimidazo[5,4-
\textit{f}]benzimidazolequinone on three cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ GM00637 [$\mu$M]</th>
<th>$IC_{50}$ HeLa [$\mu$M]</th>
<th>$IC_{50}$ DU145 [$\mu$M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.46 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>155</td>
<td>&gt; 5</td>
<td>1.67 ± 0.05</td>
<td>1.99 ± 0.39</td>
</tr>
<tr>
<td>154</td>
<td>3.63 ± 0.52</td>
<td>1.55 ± 0.35</td>
<td>0.30 ± 0.01</td>
</tr>
</tbody>
</table>

The iminoquinone 154 (Figure 2.16) showed enhanced cytotoxicity towards all
three cell lines compared to the parent dipyridoquinone 155 (Table 2.2).
Iminoquinone 154 was particularly cytotoxic towards the prostate cancer cell line
(DU145), being the most active compound of the series towards this cell line.
Iminoquinone 154 was further evaluated through the NCI-60 human tumour cell
line panel Section 2.3.6.
Figure 2.16: Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 6-imino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-a]pyrido[1′,2′:1,2]imidazo[5,4-f]benzimidazol-13-one under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
2.3.3 The effect of fused alicyclic ring size on toxicity

Figure 2.17: Dipyridoquinone, pyridopyrroloquinone and diazepinoquinone were compared to evaluate the cytotoxic effect of alicyclic ring size

Table 2.3: Comparison of pyridopyrroloimidazo[5,4-f]benzimidazol-5,12-dione and diazepinoimidazo[5,4-f]benzimidazolequinone to dipyridoimidazo[5,4-f]benzimidazolequinone on three human cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ GM00637 [µM]</th>
<th>IC₅₀ HeLa [µM]</th>
<th>IC₅₀ DU145 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.46 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>155</td>
<td>&gt; 5</td>
<td>1.67 ± 0.05</td>
<td>1.99 ± 0.39</td>
</tr>
<tr>
<td>157</td>
<td>1.27 ± 0.05</td>
<td>0.95 ± 0.10</td>
<td>0.79 ± 0.05</td>
</tr>
<tr>
<td>158</td>
<td>≥ 5</td>
<td>1.78 ± 0.19</td>
<td>4.46 ± 0.16</td>
</tr>
</tbody>
</table>

The effect of the alicyclic ring size on the cytotoxicity was evaluated by comparing the cytotoxicity of pyrido[1,2-a]pyrrolo[1′,2′:1,2]imidazo[5,4-f]benzimidazol-5,12-dione 157 (Figure 2.19) and diazepinoimidazo[5,4-f]benzimidazolequinone 158 (Figure 2.20). Pyrido-pyrroloimidazo[5,4-f]benzimidazol-5,12-dione 157 was more cytotoxic towards all three cell lines tested when compared to dipyridoimidazo[5,4-f]benzimidazolequinone 155 (Table 2.3), but retained preferential toxicity towards the cancer cell lines similar to dipyrido[5,4-f]benzimidazolequinone.

Diazepinoimidazo[5,4-f]benzimidazolequinone 158 was marginally more active than compound 155 towards the human normal skin fibroblast cell line (GM00637) at concentrations of ≤ 5 µM (Figure 2.20). Both quinones 157 and 158 were about equally active towards the cervical cancer cell line (Table 2.3). However the most noteworthy observation was the loss of selective cytotoxicity.
towards the prostate cancer cell line (DU145), IC$_{50}$ = 4.46 µM compared to the compound 155 (IC$_{50}$ = 1.99 µM). Increasing the alicyclic ring size in the methoxy substituted alicyclic ring fused[1,2-a]benzimidazolequinones 62a-62c reported by the Aldabbagh group has also been shown to affect the biological activity of these compounds (Figure 2.18). 

Figure 2.18: Increasing alicyclic ring size in methoxy substituted [1,2-a]benzimidazolequinones showed decreasing toxicity towards the human normal skin fibroblast cell line (GM00637)
Figure 2.19: Viability of normal human skin fibroblast (GM00637) (♦) HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 2,3,7,8,9,10-hexahydro-1H-pyrido[1,2-a]pyrrolo[1’,2’:1,2]imidazo[5,4-f]benzimidazol-5,12-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
Figure 2.20: Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 2,3,4,5,10,11,12,13-octahydro-1H,9H-azepino[1,2-a]azepino[1’,2’:1,2]imidazo[5,4-f]benzimidazol-7,15-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
2.3.4 The effect of alicyclic ring fusion on toxicity

![Imidazo[5,4-f]benzimidazolequinones with decreasing alicyclic ring fusion](image)

**Figure 2.21**: Imidazo[5,4-f]benzimidazolequinones with decreasing alicyclic ring fusion

Mono-alicyclic ring fused quinone 159 (Figure 2.22) and 1,5-dibutylquinone 160 (Figure 2.23) were evaluated and compared to compound dipyridoimidazo[5,4-f]benzimidazolequinone 155 (Table 2.4).

**Table 2.4**: Comparing the cytotoxic effect of ring fusion towards three human cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ GM00637 [µM]</th>
<th>IC₅₀ HeLa [µM]</th>
<th>IC₅₀ DU145 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.46 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>155</td>
<td>&gt; 5</td>
<td>1.67 ± 0.05</td>
<td>1.99 ± 0.39</td>
</tr>
<tr>
<td>159</td>
<td>2.12 ± 0.04</td>
<td>1.28 ± 0.05</td>
<td>1.35 ± 0.11</td>
</tr>
<tr>
<td>160</td>
<td>1.05 ± 0.15</td>
<td>0.44 ± 0.04</td>
<td>1.34 ± 0.06</td>
</tr>
</tbody>
</table>

The decrease in alicyclic ring fusion from 155 to 160 resulted in increased toxicity (IC₅₀ values decrease with loss of alicyclic ring fusion) (Table 2.4). 1,5-Dibutylquinone 160 was the most cytotoxic imidazobenzimidazolequinone of the series towards the cervical cancer cell line (HeLa, IC₅₀ = 0.44 µM). A similar trend of increasing cytotoxicity upon loss of ring fusion was reported in Chapter 1 for the aziridine containing benzimidazoles.
Figure 2.22: Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 1-butyl-6,7,8,9-tetrahydro-imidazo[5,4-f]pyrido[1,2-a]benzimidazol-4,11-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
**Figure 2.23:** Viability of normal human skin fibroblast (GM00637) (♦), HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 1,5-dibutylimidazo[5,4-f]benzimidazol-4,8-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
2.3.5 The effect of an oxygen atom within the alicyclic ring on toxicity

![Chemical structures](image)

**Figure 2.24:** Dipyridoimidazo[5,4-f]benzimidazolequinone and [1,4]oxazino-pyridoimidazo[5,4-f]benzimidazolequinone

Oxazinopyridoimidazo[5,4-f]benzimidazolequinone 161 containing an oxygen heteroatom was prepared and evaluated towards the cell lines. The IC50 values for oxazinopyridoimidazo[5,4-f]benzimidazolequinone 161 on the three cell lines are represented in Table 2.5 alongside dipyridoimidazobenzimidazolequinone 155 with no heteroatom.

**Table 2.5:** Effect of an oxygen atom within the alicyclic ring on toxicity

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 GM00637 [µM]</th>
<th>IC50 HeLa [µM]</th>
<th>IC50 DU145 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>0.46 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>155</td>
<td>&gt; 5</td>
<td>1.67 ± 0.05</td>
<td>1.99 ± 0.39</td>
</tr>
<tr>
<td>161</td>
<td>0.60 ± 0.03</td>
<td>0.58 ± 0.03</td>
<td>0.66 ± 0.02</td>
</tr>
</tbody>
</table>

Oxazinopyridoimidazo[5,4-f]benzimidazolequinone 162 was equally cytotoxic towards all three cell lines (Figure 2.25). In contrast (Table 2.5) to quinone 155 which showed preferential cytotoxicity towards both the HeLa and DU145 cancer cell lines compared to the human normal skin fibroblast cell line. Oxazinopyridoimidazo[5,4-f]benzimidazolequinone 161 was the most cytotoxic of this benzimidazolequinone series towards the human normal fibroblast cell line (GM00637) (IC50 = 0.60µM), and it is clear that the inclusion of the oxygen atom has significant cytotoxic effect.
Figure 2.25: Viability of normal human skin fibroblast (GM00637) (♦) HeLa (CCL-2) (●) and DU145 (HTB-81) (▲) cell lines determined using the MTT assay following treatment with 3,4,8,9,10,11-hexahydro-1H-[1,4]oxazino[4,3-a]pyrido[1′,2′:1,2]imidazo[5,4-f]benzimidazol-6,13-dione under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments. The lines shown are trend lines.
2.3.6 National Cancer Institute (NCI) -60 DTP human tumour cell line screen

The NCI in vitro cell line screen was implemented in the 1990s under the Development Therapeutics Program (DTP). The screen utilizes 60 different human cell lines representing leukaemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers to identify potential anti-tumour candidates. This screening process results in a unique biological response produced for each compound tested. The toxicity of the compounds are expressed by three parameters the GI$_{50}$ (concentration of compound resulting in 50% reduction in the net cell increase in control cells), TGI (concentration of compound resulting in total growth inhibition) and LC$_{50}$ (concentration of compound resulting in 50% net loss of cells at the end of drug treatment compared to that at the beginning). This unique pattern of activity can be processed using COMPARE software (a pattern recognition program). Compounds which possess similar mechanisms of toxicity will have similar biological response patterns and the COMPARE program can quantify the degree of similarity as a Pearson correlation coefficient. Correlation coefficients fall between -1 and +1, with -1 indicating a perfect inverse correlation, zero indicating no correlation at all and +1 indicating a perfect direct correlation.$^{158}$ A correlation coefficient of 0.3-0.5 is generally accepted as being weak to moderate, 0.5-0.7 as being moderate to strong and above 0.7 strong to very strong. Therefore it is possible to determine the mode of action of new compounds if their biological response patterns are similar to that of compounds whose mode of action is known.

Iminoquinone 154 showed a unique fingerprint of activity against the 60-cell lines tested, showing significant activity towards all melanoma cell lines, activity towards specific cell lines of non-small cell lung cancer, colon, CNS, ovarian, renal, prostate and breast cancer and no activity towards the leukaemia lines at a single dose of $10^{-5}$ M (Figure 2.26). Further five-dose testing ($10^{-4}$ to $10^{-8}$ M) was used to obtain the GI$_{50}$, TGI and LC$_{50}$ for iminoquinone 154 (Figure 2.27).
Figure 2.26: Activity of iminoquinone towards the NCI-60 cell line panel
(Figure courtesy of the NCI-DT program)
Figure 2.27: GI<sub>50</sub>, TGI and LC<sub>50</sub> values for iminoquinone against the 60-cell line panel at the NCI (Figure courtesy of the NCI-DT program)
2.3.6.1 COMPARE analysis

Iminoquinone 154 showed a moderate correlation to NQO1 with a coefficient of 0.51 (Figure 2.28, Appendix). The clinically used quinone drug mitomycin C (MMC) is known to be reductively activated by NQO1 and has the highest correlation of the 171 standard agents at the NCI. However with only a moderate correlation of 0.43 (Figure 2.28, Appendix), iminoquinone 154 compares more favourably. The biological response pattern of 154 was also compared to that of all compounds tested by the NCI. The two highest correlations obtained were to compounds 162 and 163 with coefficients of 0.87 and 0.77 respectively (Figure 2.28, Appendix). Compound 162 had a correlation of 0.64 to NQO1, the second highest of all synthetic compounds in the NCI compound database while compound 163 had a correlation of 0.47. Compound 164 had the highest correlation coefficient of 0.67 to NQO1. Compound 162 and 163 possess structural similarities to 154, all 3 molecules having the iminoquinone motif incorporated in some way. All three are flat aromatic molecules with \( \pi \) stacking capabilities and all have additional groups or positions with hydrogen bonding capabilities.

![Figure 2.28: COMPARE analysis for iminoquinone.](image)

The results obtained here confirm that NQO1 has a role to play in the mode of action of compound 154. However with a moderate to strong correlation
obtained, it seems that other factors also play a significant role. From the excellent correlation obtained between 154 and compounds 162 and 163 it seems that the most significant structural feature of compound 154 is the iminoquinone moiety.
2.4 Conclusions

A series of imidazobenzimidazolequinone compounds and a novel iminoquinone have been evaluated for anti-cancer activity against two human cancer cell lines, cervical cancer cell line (HeLa) and prostate cancer cell line (DU145), and a normal human skin fibroblast cell line (GM00637). Fusion of a [1,4]oxazino ring on the imidazo[5,4-f]benzimidazole system had the most profound effect on toxicity against all three cell lines. Increasing the size of the fused alicyclic ring decreased potency towards the prostate cancer cell line, while replacing the pyrido fused ring with an N-butyl group increased cytotoxicity across all cell lines. A unique iminoquinone was found to have particularly high cytotoxicity towards the prostate cell line (DU145) and further testing at the NCI revealed a moderate to strong correlation to NQO1 reductase enzyme.
Chapter 3
Experimental
3.1 General

3.1.1 Materials
All materials were obtained from Sigma-Aldrich, except (S)-serine methyl ester hydrochloride, which was obtained from TCI Fine Chemicals. Solvents were purified and dried prior to use according to conventional methods. All reactions were carried out under a nitrogen atmosphere apart from those involving aqueous solutions. NaH was obtained as 60% dispersion in oil and used without further purification. n-BuLi was obtained as both 2.5 or 1.6 M solutions in hexanes, MeLi was obtained as a 1.4 M solution in diethyl ether, all alkyl lithium solutions were titrated against diphenylacetic acid before use. Monitoring of reactions by Thin Layer Chromatography (TLC) was carried out on aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F254). Column chromatography and dry column vacuum chromatography (DCVC)\(^{160,161}\) were carried out using Merck Kieselgel silica gel 60 (particle size 0.040-0.063 mm) and Merck Kieselgel silica gel 60 (particle size 0.015-0.040 mm) respectively.

3.1.2 Measurements
Melting points were determined on a Stuart Scientific melting point apparatus SMP3. IR spectra were obtained using a Perkin-Elmer Spectrum 1000 FT-IR spectrophotometer with ATR accessory. NMR spectra were recorded using a JOEL GXFT 400 MHz instrument equipped with a DEC AXP 300 computer workstation. Chemical shifts are reported relative to Me$_4$Si as internal standard and NMR assignments were supported by DEPT for compounds 64-69, 70, 75-78, 86, 87, 97, 99, 100, 101, 110, 114-117 and \(^1\)H-\(^{13}\)C NMR 2D spectra for compounds 64, 65, 67-69, 71, 78, 80, 86, 87, 93-95, 97, 99, 101, 114, 116, 117. Coupling constants (\(J\)) are expressed in Hertz (Hz). High resolution mass spectra (HRMS) for compounds 64, 65, 73, 75, 78, 97, 99, 100, 101, 110, 117 were carried out using electrospray ionization (ESI) on a Waters LCT Premier XE spectrometer by manual peak matching. The precision of all accurate mass measurements is better than 5 ppm. Optical rotations were recorded on a UniPol L1000 polarimeter. HPLC analysis of compounds 64 and 65 was carried out using an Agilent Technologies 1200 series instrument with a UV detector at the specified wavelength. Absorbance was measured in the MTT assay using a Wallac Victor 2 1420 multi-label Counter. All known compounds were compared to their reported experimental literature values.
3.2 Experimental

Experiment 1: Preparation of \textit{N}-trityl-\textit{S}-serine methyl ester (80)

\begin{center}
\begin{align*}
\text{MeO} & \quad \text{NH}_2 \\
\text{HCl} & \quad \rightarrow \\
\text{MeO} & \quad \text{NTr} \\
\text{HO} & \quad \rightarrow \\
\text{HO} & \quad \text{TrCl}, \text{Et}_3\text{N}, \text{CH}_2\text{Cl}_2 \\
& \quad 16 \text{ h, rt}
\end{align*}
\end{center}

Triethylamine (8.96 mL, 64.28 mmol) and trityl chloride (8.96 g, 32.14 mmol) in dichloromethane (20 mL) was added to \textit{S}-serine methyl ester hydrochloride 79 (5.00 g, 32.14 mmol) in dichloromethane (70 mL) at 0 °C. The solution was stirred for 16 hours at room temperature, and the suspended white precipitate (triethylamine hydrochloride) was filtered off. The filtrate was washed with saturated NaHCO$_3$ (2 x 50 mL), citric acid (2 x 50 mL) and water (2 x 50 mL). The organic extracts were dried (MgSO$_4$), evaporated and the residue recrystallized from 1:1 ethyl acetate/hexane to give the \textit{title compound} 80 (10.56 g, 91%) as white crystals; mp 143-146 °C, (mp$^{85}$ 77-78 °C); $\nu_{\text{max}}$/cm$^{-1}$ 3454 (OH), 2948 (OCH$_3$), 1701 (C=O), 1596, 1490, 1443, 1424, 1368, 1331, 1207, 1172, 1126, 1055, 1028; $\delta_H$ 2.28 (t, $J$ 5.6, 1H, OH), 2.93-3.02 (bs, 1H, NH), 3.29 (s, 3H, OCH$_3$), 3.50-3.60 (m, 2H, CH, CH$_2$OH), 3.67-3.74 (m, 1H, CH$_2$OH), 7.17-7.28 (m, 9H, Ph-H), 7.48 (d, $J$ 8.4, 6H, Ph-H); $\delta_C$ 52.11 (OCH$_3$), 58.00 (CH), 65.37 (CH$_2$), 71.26 (CPh$_3$), 126.70, 128.17, 128.94 (Ph-CH), 146.07 (Ph-\textit{ipso}-C), 174.28 (C=O).
Experiment 2: Preparation of (ethylsulfanyl)methanol (81)

A mixture of ethanethiol (14.8 mL, 0.2 mol), paraformaldehyde (6.0 g, 0.2 mol) and 30% sodium methoxide in methanol (60 µL) was heated at 40 °C for 30 minutes. The reaction mixture was allowed to cool to give a viscous pale yellow liquid containing a mixture of the title compound 81 and (ethylsulfanylmethoxy)methanol 82 (16.8 g, ratio of 11:1 by 1H NMR). This mixture was used in the next step without further purification; δH 1.12-1.18 (m, CH2CH3, 81 & 82), 2.50 (q, J 7.2, 2H, CH2CH3, 82), 2.57 (q, J 7.2, 2H, CH2CH3, 81), 3.70-3.89 (bs, 1H, OH, 81), 4.60 (s, 2H, SCH2O, 81), 4.68 (s, 2H, SCH2O, 82), 4.78 (s, 2H, CH2OH, 82).
Experiment 3: Preparation of (tert-butyldimethylsiloxy)methylethyl sulfide (83)

A mixture of 81 & 82 (4.60 g, 50 mmol) in dichloromethane (25 mL) was added to tert-butyldimethylsilyl chloride (8.30 g, 55 mmol), 4-(N,N-dimethylamino)pyridine (DMAP) (0.24 g, 2 mmol) and triethylamine (8.36 mL, 60 mmol) in dichloromethane (25 mL), at room temperature. After stirring for 4 hours, the mixture was diluted with dichloromethane (50 mL) and washed with saturated NH₄Cl (2 x 50 mL) and water (3 x 50 mL). The organic layer was dried (MgSO₄) and evaporated to dryness to give a mixture of the title compound 83, contaminated with tert-butyldimethylsiloxy)methoxymethylethyl sulfide 84 (6.31 g, ratio of 18:1 by ¹H NMR) as a yellow oil; δH 0.07 (s, 6H, CH₃Si, 84), 0.09 (s, 6H, CH₃Si, 83), 0.87 (s, 9H, tBu, 83), 0.88 (s, 9H, tBu, 84), 1.27 (t, 6H, CH₃CH₂, 83 & 84), 2.57-2.67 (q, 4H, CH₂CH₂, 83 & 84), 4.75 (s, 2H, SCH₂O, 84), 4.78 (s, 2H, SCH₂O, 83), 4.96 (s, 2H, OCH₂O, 84); δC -5.12 (CH₃Si, 83), - 3.56 (CH₃Si, 84), 14.88 (CH₃CH₂, 83 & 84), 18.02 (C(CH₃)₃, 83 & 84), 24.35 (CH₃CH₂, 83 & 84), 25.71 (C(CH₃)₃, 83 & 84), 65.85 (CH₂O, 83), 68.18 (SCH₂O, 84), 85.37 (OCH₂O, 84).
Experiment 4: Preparation of (tert-butyldimethylsiloxy)methyl chloride (85)

Sulfuryl chloride (1.67 mL, 20.9 mmol) was added dropwise at 0 °C to impure 83 (4.00 g, 19 mmol) in dichloromethane (30 mL), and the mixture was stirred at 0 °C for 40 minutes. The solvent and reaction by-products were evaporated at room temperature, to give the impure title compound 85 (3.30 g, 96%) as a yellow liquid; δH 0.12 (s, 6H, CH3Si), 0.89 (s, 9H, tBu), 5.49 (s, 2H, CH2).
Experiment 5: Preparation of N-trityl-O-(tert-butylimethylsiloxymethyl)-S-serine methyl ester (66) using N,N-diisopropylethylamine base

\[
\begin{align*}
\text{MeO} & \quad \text{NTr} \\
\text{HO} & \quad \text{TBDMSOCH}_2\text{Cl (85)} \quad \text{12 h, rt} \\
\text{MeO} & \quad \text{NTr} \\
\end{align*}
\]

N,N-diisopropylethylamine (3.25 mL, 18.68 mmol) and (tert-butylimethylsiloxyl)methyl chloride 85 (3.00 g, 16.60 mmol) in dichloromethane (10 mL) were added to N-trityl-S-serine methyl ester 80 (4.50 g, 12.45 mmol) in dichloromethane (50 mL) at 0 °C. The solution was stirred at room temperature for 12 hours then poured into water. The aqueous layer was extracted with dichloromethane and the organic layer washed with saturated NaHCO₃ and dried (MgSO₄). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with 1:9 diethyl ether/hexane as eluent, to give in order of elution; N-trityl-O-(tert-butylimethylsiloxyl)-S-serine methyl ester 67 (1.10 g, 19%) as a white solid; mp 91-92 °C, (mp° 88 88-89 °C); Rf 0.69 (1:9 diethyl ether/hexane); νmax/cm⁻¹ 2931, 1731 (C=O), 1595, 1491, 1462, 1447, 1374, 1326, 1249, 1206, 1176, 1110, 1031, 1009; δH 0.03 (s, 3H, CH₃Si), 0.04 (s, 3H, CH₃Si), 0.86 (s, 9H, tBu), 2.71 (bs, 1H, NH), 3.17 (s, 3H, OCH₃), 3.43-3.48 (m, 1H, CH), 3.64 (dd, 2 9.6, 3 7.6, 1H, CH₂), 7.15-7.19 (m, 1H, CH), 3.91 (dd, 9.6, 3 5.6, 1H, CH₂), 7.49-7.51 (m, 6H, Ph-H); δC -5.41 ((CH₃)₂Si), 18.37 (C(CH₃)₃), 25.85 (C(CH₃)₃), 51.55 (OCH₃), 58.44 (CH), 70.76 (CPh₃), 126.48, 127.90, 128.89 (Ph-CH), 146.07 (Ph-ipso-C), 174.60 (C=O) and the title compound 66 (3.02 g, 48%) as a yellow oil; Rf 0.53 (1:9 diethyl ether/hexane); νmax/cm⁻¹ 2929, 2857, 1735 (C=O), 1596, 1491, 1463, 1447, 1332, 1254, 1207, 1160, 1122, 1032; δH 0.14 (s, 3H, CH₃Si), 0.15 (s, 3H, CH₃Si), 0.96 (s, 9H, tBu), 2.84 (d, 10.0, 1H , NH), 3.24 (s, 3H, OCH₃), 3.56-3.62 (m, 1H, CH), 3.68 (dd, 9.6, 7.0, 1H, CHHH), 3.95 (dd, 9.6, 4.8, 1H, CHH), 4.87 (ABq, 5.6, 1H, OCHH), 4.91 (ABq, 5.6, 1H, OCHHO) 7.19-7.22 (m, 3H, Ph-H), 7.27-7.31 (m, 6H, Ph-H), 7.56 (d, 8.4, 6H, Ph-H); δC -4.91 (CH₃Si), -4.85 (CH₃Si),
18.17 (C(CH$_3$)$_3$), 25.91 (C(CH$_3$)$_3$), 51.98 (OCH$_3$), 56.67 (CH), 70.61 (CH$_2$), 71.07 (CPh$_3$), 90.32 (OCH$_2$O) 126.57, 127.99, 129.04 (Ph-CH), 145.98 (Ph-$ipso$-C), 174.16 (C=O).
Experiment 6: Preparation of N-trityl-O-(tert-butyldimethylsiloxy)-S-serine methyl ester (66) using triethylamine base

![Chemical structure](image)

Triethylamine (4.16 mL, 29.88 mmol) and (tert-butyldimethylsiloxy)methyl chloride 85 (5.40 g, 29.88 mmol) in dichloromethane (10 mL) were added to N-trityl-S-serine methyl ester 80 (7.20 g, 19.92 mmol) in dichloromethane (50 mL) at 0 °C. The solution was stirred at room temperature for 12 hours then poured into water. The aqueous layer was extracted with dichloromethane and the organic layer washed with saturated NaHCO₃ and dried (MgSO₄). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with 1:9 diethyl ether/hexane, as eluent to give N-trityl-O-(tert-butyldimethylsiloxy)-S-serine methyl ester 67 (1.44 g, 15%) as a white solid and the title compound 66 (1.78 g, 18%) as a yellow oil. Physical and spectroscopic data was consistent with that stated in experiment 5 for compounds 66 and 67.
Experiment 7: One-pot preparation of N-trityl-O-(tert-butyldimethylsiloxy)-S-serine methyl ester (67)

\[ \text{MeO} \quad \text{NH}_2 \quad \text{HCl} \xrightarrow{\text{i. TBDMSCl, Et}_3\text{N, CH}_2\text{Cl}_2, 48\text{ h, rt}} \quad \text{MeO} \quad \text{NHTr} \]

\[ \text{TBDMSO} \]

Triethylamine (10.21 mL, 73.28 mmol) and tert-butyldimethylsilyl chloride (5.91 g, 39.21 mmol) were added to a solution of (S)-serine methyl ester hydrochloride 79 (5.00 g, 32.14 mmol) in dichloromethane (70 mL) at -20 °C and the solution stirred at room temperature for 48 hours. Trityl chloride (9.86 g, 35.37 mmol) and triethylamine (5.91 mL, 42.42 mmol) were added and the solution heated to reflux for 5 hours. The solution was cooled to room temperature, added to water (80 mL) and extracted with dichloromethane (3 x 50 mL). The organic layer was washed with brine and dried (MgSO\(_4\)). The solution was evaporated and the residue recrystallized from absolute ethanol to give the title compound 67 (12.38 g, 81%) as a white solid. Physical and spectroscopic data was consistent with that stated in experiment 5 for compound 67.
Experiment 8: Preparation of $N$-trityl-$O$-($\text{tert}$-butyldimethylsiloxymethyl)-$R$-serinol (68) using lithium aluminium hydride reducing agent

$N$-Trityl-$O$-($\text{tert}$-butyldimethylsiloxymethyl)-$S$-serine methyl ester 66 (0.89 g, 1.76 mmol) in THF (10 mL) was added to lithium aluminium hydride (0.14 g, 3.70 mmol) in THF (8 mL) at -20 °C and the solution was stirred for 2 hours. Excess lithium aluminium hydride was quenched with a saturated sodium potassium tartrate solution. The mixture was filtered through Celite and the solids washed with diethyl ether. The filtrate was dried (MgSO$_4$) and the solvent evaporated to give the title compound 68 (0.77 g, 92%) as a clear oil; $\nu_{\text{max}}$/cm$^{-1}$ 3446 (OH) 2929, 1596, 1490, 1448, 1361, 1254, 1210, 1160, 1117, 1032, 903; $\delta_H$ 0.17 (s, 3H, CH$_3$Si), 0.18 (s, 3H, CH$_3$Si), 0.99 (s, 9H, $\text{tBu}$), 2.46-2.56 (bs, 2H, NH, OH), 2.94-3.01 (m, 3H, CH, CH$_2$OH, CH), 3.52-3.55 (m, 2H, CH$_2$OH, CH), 4.68 (d, $J=5.0$, 1H, OCH$_2$O), 4.85 (d, $J=5.0$, 1H, OCH$_2$O), 7.23-7.27 (m, 3H, Ph-H), 7.32-7.36 (m, 6H, Ph-H), 7.69 (d, $J=7.6$, 6H, Ph-H); $\delta_C$ -4.83 (CH$_3$Si), -4.71 (CH$_3$Si), 18.47 ($C(CH_3)_3$), 25.98 ($C(CH_3)_3$), 53.13 (CH), 63.05 (CH$_2$), 69.15 (CH$_2$), 71.30 (CPh$_3$), 90.60 (OCH$_2$O), 126.64, 128.11, 128.89 (Ph-CH), 147.05 (Ph-$ipso$-C).
Experiment 9: Attempted preparation of N-trityl-O-(tert-butyldimethylsiloxy)-R-serinol (69) using lithium aluminium hydride reducing agent

\[
\begin{array}{ccc}
\text{MeO} & \text{NTr} & \text{TBDMOSO} \\
\text{O} & \text{OH} & \text{TBDMOSO} \\
\text{67} & \text{LiAlH}_4 & \text{THF} \\
\rightarrow & \text{2 h, -20 °C} & \\
\text{69} & \\
\end{array}
\]

\textit{N}-trityl-O-(\textit{tert}-butyldimethylsiloxy)-S-serine methyl ester 67 (1.00 g, 2.10 mmol) in THF (15 mL) was added to lithium aluminium hydride (0.17 g, 4.41 mmol) in THF (10 mL) at -20 °C and the solution was stirred for 2 hours. Excess lithium aluminium hydride was quenched with a saturated sodium potassium tartrate solution. The mixture was filtered through Celite and the solids washed with diethyl ether. The filtrate was dried (\textit{MgSO}_4) and the solvent evaporated to give 2-(tritylamino)propane-1,3-diol 86 (0.51 g, 73%) as a white solid; mp 121-123 °C (mp\textsuperscript{89} 122-124 °C); \nu max/cm\textsuperscript{-1} 3322 (OH) 3056, 2881, 1595, 1490, 1448, 1201, 1157, 1052, 1030, 1117, 961; \delta\textsubscript{H} 2.23-2.37 (bs, 2H, OH), 2.72-2.76 (m, 1H, CH), 3.01 (dd, J\textsuperscript{2} 10.8, J\textsuperscript{3} 5.3, 2H, CH\textsubscript{2}), 3.40 (dd, J\textsuperscript{2} 10.8, J\textsuperscript{3} 3.1, 2H, CH\textsubscript{2}), 7.18-7.22 (m, 3H, Ph-H), 7.26-7.30 (m, 6H, Ph-H), 7.55-7.57 (m, 6H, Ph-H); \delta\textsubscript{C} 53.41 (CH), 64.76 (CH\textsubscript{2}), 71.22 (CPh\textsubscript{3}), 126.69 , 128.08, 128.72 (Ph-CH), 146.54 (Ph-ipso-C).
Experiment 10: Preparation of $N$-trityl-$O$-(tert-butyldimethylsiloxymethyl)-$R$-serinol (68) using diisobutylaluminium hydride reducing agent

Diisobutylaluminium hydride (11.48 mL of a 1 M solution in toluene, 11.48 mmol) was added dropwise to a solution of ester 66 (2.32 g, 4.59 mmol) in toluene (35 mL) at $-78 \, ^\circ\text{C}$ over a 10 minute period. The resulting solution was stirred for 3 hours and quenched with methanol (8 mL). The reaction was warmed to room temperature, a saturated sodium potassium tartrate solution (35 mL) was added and the mixture stirred vigorously for 20 minutes. The resulting biphasic mixture was extracted with ethyl acetate, washed with brine and dried ($\text{Na}_2\text{SO}_4$). The solution was evaporated to give the title compound 68 (2.08 g, 95%) as a clear oil. Physical and spectroscopic data was consistent with that stated in experiment 8 for compound 68.
Experiment 11: Preparation of \(N\)-trityl-\(O\)-(tert-butyldimethylsiloxy)-\(R\)-serinol (69)

\[
\begin{align*}
\text{MeO} & \quad \text{NTr} \\
\text{TBDSMSO} & \quad \text{DIBAL, PhMe} \\
67 & \quad 3 \text{h}, -78 \degree \text{C} \\
\text{HO} & \quad \text{NTr}
\end{align*}
\]

Diisobutylaluminium hydride (5.25 mL of a 1 M solution in toluene, 5.25 mmol) was added dropwise to a solution of ester 67 (1.00 g, 2.10 mmol) in toluene (20 mL) at -78 \degree C over a 10 minute period. The resulting solution was stirred for 3 hours and quenched with methanol (5 mL). The reaction was warmed to room temperature, a saturated sodium potassium tartrate solution (20 mL) was added and the mixture stirred vigorously for 20 minutes. The resulting biphasic mixture was extracted with ethyl acetate, washed with brine and dried (\(\text{Na}_2\text{SO}_4\)). The solution was evaporated to give the title compound 69 (0.91 g, 97\%) as a colourless oil; \(\nu_{\text{max}}/\text{cm}^{-1} \) 3463 (OH), 3058, 2856, 1596, 1491, 1448, 1389, 1253, 1083, 1031, 907; \(\delta_\text{H} \) 0.04 (s, 3H, \(\text{CH}_3\text{Si}\)), 0.05 (s, 3H, \(\text{CH}_3\text{Si}\)), 0.93 (s, 9H, \(\text{tBu}\)), 2.37-2.57 (bs, 2H, \(\text{NH}, \text{OH}\)), 2.80-2.84 (m, 1H, \(\text{CH}\)), 2.97 (dd, \(J_2 10.7, J_3 5.8, 1H, \text{CH}_2\text{OSi}\)), 3.11 (dd, \(J_2 9.8, J_3 6.0, 1H, \text{CH}_2\text{OH}\)), 3.41 (dd, \(J_2 9.8, J_3 4.0, 1H, \text{CH}_2\text{OH}\)), 3.49 (dd, \(J_2 10.7, J_3 3.0, 1H, \text{CH}_2\text{OSi}\)), 7.22-7.26 (m, 3H, Ph-H), 7.30-7.34 (m, 6H, Ph-H), 7.64 (d, \(J 7.6, 6\text{H}, \text{Ph-H}\)); \(\delta_\text{C} \) -5.24 (\((\text{CH}_3)_2\text{Si}\)), 18.36 (\((\text{CH}_3)_3\)), 26.07 (\((\text{CH}_3)_3\)), 54.05 (CH), 63.84 (CH\(_2\)), 64.81 (CH\(_2\)), 71.04 (CPh\(_3\)), 126.61, 128.09, 128.82 (Ph-CH), 147.04 (Ph-\(\text{ipso}\)-C).
Experiment 12: Preparation of N-trityl-O-(tert-butyltrimethylsiloxyethyl)-S-serinal (70) using TEMPO/Sodium Hypochlorite Oxidation

An aqueous solution of NaOCl, (8.96 mL, 12% w/v, 14.50 mmol), NaHCO₃ (2.96 g, 35.23 mmol) was added dropwise to a solution of alcohol 68 (2.13 g, 4.46 mmol), sodium bromide (0.48 g, 4.66 mmol), TEMPO (83 mg, 0.53 mmol), ethyl acetate (20 mL), toluene (20 mL) and water (4 mL) at 0 °C. The solution was stirred at room temperature for 96 hours. The aqueous layer was extracted with diethyl ether (2 x 50 mL) and the combined organic layers washed with KI in 10% KHSO₄ (2 x 50 mL), 10% sodium thiosulphate (2 x 50 mL), brine (2 x 50 mL) and dried (MgSO₄). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with 1:4 ethyl acetate/hexane as eluent to give the title compound 70 (1.85 g, 87%) as a clear oil; R_f 0.65 (1:4 ethyl acetate/hexane); ν_max/cm⁻¹ 2928, 1725 (C=O), 1661, 1597, 1490, 1447, 1254, 1160, 1118, 1034, 1004; δ_H 0.13 (s, 3H, CH₃Si), 0.14 (s, 3H, CH₃Si), 0.96 (s, 9H, tBu), 3.24 (dd, J 2 9.6, J 3 5.6, 1H, CH₂), 3.51-3.53 (m, 1H, CH), 3.82 (dd, J 2 9.6, J 3 4.1, 1H, CH₂), 4.73 (d, J 5 5.2, 1H, OCH₂O), 4.83 (d, J 5 5.2, 1H, OCH₂O), 7.22-7.24 (m, 3H, Ph-H), 7.30-7.34 (m, 6H, Ph-H), 7.59 (d, J 7 6, 6H, Ph-H), 9.31 (s, 1H, CHO); δ_C -4.98 (CH₃Si), -4.85 (CH₃Si), 18.07 (C(CH₃)₃), 25.80 (C(CH₃)₃), 61.71 (CH), 69.15 (CH₂), 71.07 (CPh₃), 90.49 (OCH₂O), 126.84, 128.20, 128.77 (Ph-CH), 146.08 (Ph-iph⁻C), 204.03 (CHO).
Experiment 13: Preparation of N-trityl-O-(tert-butyldimethylsiloxymethyl)-S-serinal (70) using the Swern Oxidation

DMSO (0.49 mL, 6.96 mmol) in dichloromethane (5 mL) was added to oxalyl chloride (0.41 mL, 4.92 mmol) in dichloromethane (10 mL) at -78 °C. After 5 minutes a solution of the alcohol 68 (2.08 g, 4.35 mmol) in dichloromethane (5 mL) was added and after 10 minutes N,N-diisopropylethylamine (2.41 mL, 13.83 mmol) was added to the mixture. The solution was stirred for 15 minutes at -78 °C and a further 15 minutes at room temperature. The mixture was poured into 10% citric acid and extracted with dichloromethane (2 x 50 mL). The combined layers were washed with saturated NaHCO₃ (2 x 50 mL) and dried (MgSO₄). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with 1:4 ethyl acetate/hexane as eluent to give the title compound 70 (1.89 g, 91%) as a clear oil. Physical and spectroscopic data was consistent with that stated in experiment 12 for compound 70.
Experiment 14: Preparation of N-trityl-O-(tert-butyldimethylsiloxy)-S-serinal (71)

An aqueous solution of NaOCl, (10.8 mL of 12% w/v, 17.48 mmol), NaHCO₃ (2.03 g, 24.17 mmol) was added dropwise to a solution of alcohol 69 (1.37 g, 3.06 mmol), sodium bromide (0.33 g, 3.20 mmol) and TEMPO (57 mg, 0.37 mmol), ethyl acetate (20 mL), toluene (20 mL) and water (4 mL) at 0 °C. The solution was stirred at room temperature for 96 hours. The aqueous layer was extracted with diethyl ether (2 x 50 mL) and the combined organic layers washed with KI in 10% KHSO₄ (2 x 50 mL), 10% sodium thiosulphate (2 x 50 mL) and brine (2 x 50 mL) and dried (MgSO₄). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane to give the title compound 71 (1.25 g, 92%) as a clear oil; $R_f$ 0.72 (1:9 ethyl acetate/hexane); $\nu_{\text{max}}$/cm$^{-1}$ 2950, 2929, 2856, 1732 (C=O), 1573, 1522, 1491, 1447, 1462, 1377, 1330, 1253, 1205, 1176, 1109, 1031, 1005; $\delta_H$ 0.14 (s, 3H, CH₃Si), 0.15 (s, 3H, CH₃Si), 0.97 (s, 9H, tBu), 3.25 (dd, $J^2$ 9.6, $J^3$ 5.6, 1H, CH₂OSi), 3.51-3.54 (m, 1H, CH), 3.82 (dd, $J^2$ 9.6, $J^3$ 4.0, 1H, CH₂OSi), 7.30-7.35 (m, 9H, Ph-H), 7.59-7.61 (m, 6H, Ph-H), 9.31 (s, 1H, CHO); $\delta_C$ -5.50 ((CH₃)₂Si), 18.15 (C(CH₃)₃), 25.64 (C(CH₃)₃), 63.04 (CH), 64.21 (CH₂), 70.76 (CPh₃), 126.90, 128.02, 128.84 (Ph-CH), 146.20 (Ph-ips-o-C), 205.13 (CHO).
Experiment 15: Preparation of \( N\)-trityl-\( O\)-(tert-butyldimethylsiloxy)-S-serinal (71)

DMSO (0.25 mL, 3.55 mmol) in dichloromethane (4 mL) was added to oxalyl chloride (0.20 mL, 2.55 mmol) in dichloromethane (4 mL) at -78 °C. After 5 minutes a solution of the alcohol 69 (1.00 g, 2.23 mmol) in dichloromethane (3 mL) was added and after 10 minutes \( N,N\)-diisopropylethylamine (1.24 mL, 7.10 mmol) was added to the mixture. The solution was stirred for 15 minutes at -78 °C and a further 15 minutes at room temperature. The solution was poured into 10% citric acid and extracted with dichloromethane (2 x 40 mL). The combined layers were washed with saturated \( \text{NaHCO}_3 \) (2 x 30 mL) and dried (\( \text{MgSO}_4 \)). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane to give the title compound 71 (0.87 g, 88%) as a clear oil. Physical and spectroscopic data was consistent with that stated in experiment 14 for compound 71.
Experiment 16: Preparation of (2S,3R)-2-[[[tert-butyl(dimethyl)silyl]oxy]methoxy)methyl]-3-(tributylstannyl)1-tritylaziridine (74)

n-Butyl lithium (2.57 mL of a 1.6 M solution in hexanes, 4.11 mmol) was added to a solution of N,N-diisopropylamine (0.39 mL, 2.74 mmol) in THF (3 mL) at –20 °C. After 20 minutes, tributyltin hydride (0.74 mL, 2.74 mmol) was added and the resultant yellow solution was stirred for 30 minutes. The solution was cooled to -78 °C and a solution of the aldehyde 70 (0.65 g, 1.37 mmol) in THF (2 mL) was added dropwise, the solution turned a blue-black colour. After 1 hour a saturated solution of NH₄Cl (10 mL) was added and the solution extracted with diethyl ether (3 x 40 mL), the organic layers were combined and dried (Na₂SO₄). The solution was evaporated to dryness to give the amino alcohol intermediate as a yellow oil, this was unstable and used directly in the following Mitsunobu ring closure reaction.

Diisopropyl azodicarboxylate (DIAD) (0.54 mL, 2.74 mmol) was added to a solution of amino alcohol and triphenylphosphine (0.72 g, 2.74 mmol) in toluene (35 mL) at 0 °C. The solution was stirred for 48 hours at room temperature. Water (30 mL) was added, and the aqueous layer extracted with dichloromethane (3 x 30 mL) and the combined organic layers dried (Na₂SO₄). The solution was evaporated to dryness to give an orange residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of diethyl ether/hexane to yield the title compound 74 (0.43 g, 42%) as a clear oil; Rᵣ 0.64 (1:9 diethyl ether/hexane); νₓₓₓ/cm⁻¹ 2954, 2926, 2855, 1596, 1490, 1447, 1376, 1254, 1217, 1163, 1116, 1043, 1003; δₓₓ 0.12 (CH₃Si), 0.13 (CH₃Si), 0.86-1.02 (m, 24H, tBu, CH₂CH₃), 1.28-1.33 (m, 7H, CH₂, aziridinyl-CHN), 1.44-1.54 (m, 7H, CH₂, aziridinyl-CHN), 3.56 (dd, J₂ 10.2, J₃ 5.4, 1H, CH₂), 3.88 (dd, J₂ 10.2, J₃ 5.4, 1H, CH₂), 4.84 (ABq J 4.8, 1H, OCH₂O), 4.90 (ABq J 4.8, 1H, OCH₂O), 7.19-7.29 (m, 9H, Ph-H), 7.52 (d J 7.2, 6H, Ph-H); δₓₓ 4.98 (CH₃Si), -4.88
(CH₃Si), 10.15 (CH₂), 13.79 (CH₃), 18.20 (C(CH₃)₃), 24.56 (CH), 25.89 (C(CH₃)₃), 27.56 (CH₂), 29.43 (CH₂), 35.23 (CH), 71.39 (OCH₂), 75.35 (CPh₃), 90.13 (OCH₂O), 126.62, 127.37, 129.84 (Ph-CH), 144.60 (Ph-\textit{ipso}-C).
n-Butyl lithium (2.87 mL of a 1.6 M solution in hexanes, 4.59 mmol) was added to a solution of N,N-diisopropylamine (0.43 mL, 3.06 mmol) in THF (3 mL) at -20 °C. After 20 minutes, tributyltin hydride (0.82 mL, 3.06 mmol) was added and the resultant yellow solution was stirred for a further hour. The solution was cooled to -78 °C and a solution of the aldehyde 71 (0.68 g, 1.53 mmol) in THF (2 mL) was added dropwise, the solution turned a blue-black colour. After 1 hour a saturated solution of NH4Cl was added and the solution extracted with diethyl ether (3 x 40 mL), the organic layers were combined and dried (Na2SO4). The solution was evaporated to dryness to give the amino alcohol intermediate 73 as a yellow oil; HRMS (ESI) m/z (M+H)+ C40H63NO2SiSn calcd 738.3728, obsd 738.3705 (C40H51N2O5SSn) which was unstable to purification and used directly in the following Mitsunobu ring closure.

Diisopropyl azodicarboxylate (DIAD) (0.60 mL, 3.06 mmol) was added to a solution of amino alcohol 73 and triphenylphosphine (0.80 g, 3.06 mmol) in toluene (35 mL) at 0 °C. The solution was stirred for 48 hours at room temperature. Water (30 mL) was added, and the aqueous layer extracted with dichloromethane (3 x 30 mL) and the combined organic layers dried (Na2SO4). The solution was evaporated to dryness to give an orange residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of dichloromethane/hexane to yield the title compound 75 (0.62 g, 56%) as a colourless oil; Rf 0.60 (1:4 dichloromethane/hexane); [α]20D = -6.1° (c=0.30 in CHCl3); νmax/cm⁻¹ 2954, 2926, 2854, 1489, 1462, 1254, 1074, 907; δH 0.04 (s, 3H, CH3Si), 0.06 (s, 3H, CH3Si), 0.88-0.91 (m, 18H, CH2CH3, tBu), 0.93-1.03
(m, 6H, CH₂), 1.26-1.40 (m, 7H, CH₂ and aziridinyl-CHN), 1.43-1.51 (m, 7H, CH₂ and aziridinyl-CHN), 3.65 (dd, J² 10.8, J₃ 5.0, 1H, CH/H), 3.82 (dd, J² 10.8, J₃ 6.4, 1H, CH/H), 7.19-7.23 (m, 3H, Ph-H), 7.26-7.30 (m, 6H, Ph-H), 7.53 (d, J 7.2, 6H, Ph-H); δC - 5.21 (CH₃Si), -5.14 (CH₃Si), 10.24 (CH₂), 13.80 (CH₃), 18.36 (C(CH₃)₃), 24.13 (CH), 25.96 (C(CH₃)₃), 27.52 (CH₂), 29.35 (CH₂), 38.15 (CH), 67.45 (OCH₂), 75.23 (CPh₃), 126.48, 127.35, 129.78 (Ph-CH), 144.58 (Ph- ipso-C). HRMS (ESI) m/z (M+H)⁺ C₄₀H₆₂NOSiSn calcd 720.3623, obsd 720.3625 (C₄₀H₆₂NOSi¹²⁰Sn, 100%), 718 (C₄₀H₆₂NOSi¹¹⁸Sn, 80%), 719 (C₄₀H₆₂NOSi¹¹⁹Sn, 60%).
n-Butyl lithium (4.20 mL of a 1.6 M solution in hexanes, 6.72 mmol) was added to a solution of N, N-diisopropylamine (0.63 mL, 4.48 mmol) in THF (3 mL) at -20°C. After 20 minutes, tributyltin hydride (1.21 mL, 4.48 mmol) was added and the resultant yellow solution was stirred for a further hour. The solution was cooled to -78°C and a solution of the aldehyde (1.00 g, 2.24 mmol) in THF (2 mL) was added dropwise, the solution turned a green-yellow colour. After 1 hour, a saturated solution of NH₄Cl was added and the solution extracted with diethyl ether (3 x 30 mL), the organic layers were combined and dried (Na₂SO₄). The solution was evaporated to dryness and the unstable orange residue was used directly in the Mitsunobu ring closure reaction.

Diisopropylazodicarboxylate, DIAD (0.88 mL, 4.48 mmol) was added to the solution of amino alcohol and triphenylphosphine (1.18 g, 4.48 mmol) in toluene (25 mL) at 0 °C. The solution was stirred for 48 hours at room temperature. Water (30 mL) was added, and the aqueous layer extracted with dichloromethane (3 x 30 mL) and the combined organic layers dried (Na₂SO₄). The solution was evaporated to dryness to give an orange residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of dichloromethane/ hexane as eluent to yield the title compound 87 (0.43 g, 45%) as a colourless oil; Rf 0.48 (1:4 dichloromethane/hexane); ν max/cm⁻¹ 3108, 1657, 1421, 1404, 1392, 1364, 1110, 1077, 1057, 1013, 1030, 981; δ H 0.02 (s, 3H, CH₃Si), 0.03 (s, 3H, CH₃Si), 0.86 (S, 9H, tBu), 1.23 (d, J 6.4, aziridinyl-CH₂), 1.39-1.44 (m, 1H, aziridinyl-CHN), 3.64 (dd, J 10.2, J 5.8, 1H, CH₂O), 3.96 (dd, J 10.2, J 5.2, 1H, CH₂O), 7.17-7.27 (m, 9H, Ph-H), 7.62 (d, J 7.2, 6H, Ph-H); δ C - 5.15 (CH₃Si), -5.12 (CH₃Si), 18.53 (C(CH₃)₃), 25.48 (CH₂), 26.03 (C(CH₃)₃), 34.52 (CH), 65.95 (CH₂O) 73.62 (CPh₃), 126.63, 127.45, 129.62 (Ph-CH), 144.74 (Ph-ipso-C).
Experiment 19: Preparation of [(2S,3R)-3-(tributylstanny)-1-tritylaziridin-2-yl]methanol (76)

Tetra-n-butylammonium fluoride (TBAF) (8.57 mL of a 1 M solution in THF, 8.57 mmol) and aziridine 75 (0.610 g, 0.85 mmol) in THF (10 mL) were stirred for 18 hours at room temperature. Water (30 mL) was added, the mixture extracted with dichloromethane (3 x 30 mL), dried (MgSO₄) and evaporated to dryness to yield a brown residue, which was purified by column chromatography using silica gel as absorbent with 1:1 ethyl acetate/hexane as eluent. Evaporation of the fractions containing the first component gave the title compound 76 (0.49 g, 96%) as a clear oil, $R_f$ 0.44 (1:1 ethyl acetate/hexane); $\nu_{\text{max}}/\text{cm}^{-1}$ 3455 (OH), 2923, 1664, 1584, 1491, 1447, 1377, 1159, 1034; $\delta_{\text{H}}$ 0.86 (t, $J$ 7.2, 9H, CH₂C₃H₃), 0.89-1.00 (m, 7H, CH₂ and aziridinyl-CHN), 1.23-1.32 (m, 6H, CH₂), 1.40-1.48 (m, 6H, CH₂), 1.51-1.54 (m, 1H, aziridinyl-CHN), 2.03 (dd, $J$ 2 7.5, $J$ 3 4.3, 1H, OH), 3.59 (ddd, $J$ 11.2, 7.5, 3.7, 1H, CH/HOH), 3.84 (ddd, $J$ 11.2, 4.3, 3.0, 1H, CH/CH₂), 7.19-7.29 (m, 9H, Ph-H), 7.45 (d, $J$ 7.2, 6H, Ph-H); $\delta_{\text{C}}$ 10.34 (CH₂), 13.77 (CH₃), 23.56 (CH), 27.46 (CH₂), 29.27 (CH₂), 36.78 (CH), 62.87 (CH₂OH), 75.50 (CPh₃), 126.81, 127.62, 129.42 (Ph-CH), 144.51 (Ph-ips-C).
Experiment 20: Preparation of [(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl methanesulfonate (77)

Triethylamine (1.60 mL, 11.48 mmol) was added to a solution of methanesulfonyl chloride (0.09 mL, 1.14 mmol) and aziridinol 76 (0.46 g, 0.76 mmol) in dichloromethane (10 mL) at -78 °C. After 18 hours water (20 mL) was added, the mixture was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts dried (Na₂SO₄). The solution was evaporated to dryness to yield a yellow oil, which was purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the first component gave the title compound 77 (0.46 g, 89%) as a clear oil. $R_f$ 0.48 (1:1 dichloromethane/hexane); $\nu_{max}/\text{cm}^{-1}$ 2954, 2924, 2851, 1596, 1490, 1447, 1361 (S=O), 1340, 1227, 1175 (S=O), 1075, 1029; $\delta_H$ 0.87 (t, $J_7$ 7.3, 9H, CH₂C₆H₃), 0.93-1.05 (m, 7H, CH₂ and aziridinyl-CHN), 1.24-1.33 (m, 6H, CH₂), 1.42-1.50 (m, 6H, CH₂), 1.60 (ddd, $J_7$ 6.8, 6.8, 5.5, 1H, aziridinyl-CHN), 2.91 (s, 3H, CH₃), 4.19 (dd, $J^2$ 10.1, $J^3$ 5.5, 1H, CHHOMs), 4.34 (dd, $J^2$ 10.1, $J^3$ 6.8, 1H, CHHOMs), 7.19-7.28 (m, 9H, Ph-H), 7.47 (d, $J_7$ 7.2, 6H, Ph-H); $\delta_C$ 10.16 (CH₂), 13.79 (CH₃), 24.51 (CH), 27.47 (CH₂), 29.20 (CH₂), 34.07 (CH), 37.99 (CH₃), 72.73 (OCH₂), 75.47 (CPh₃), 126.36, 127.79, 129.57 (Ph-CH), 143.76 (Ph-ips-o-C).
Experiment 21: Preparation of [(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl 4-nitrobenzenesulfonate (78)

Triethylamine (0.26 mL, 1.90 mmol) was added to a solution of 4-nitrobenzenesulfonyl chloride (0.27 g, 1.24 mmol), DMAP (7 mg, 55 µmol) and aziridinol 76 (0.50 g, 0.83 mmol) in dichloromethane (15 mL) at 0 °C. After 18 hours, water (30 mL) was added, and the mixture extracted with dichloromethane (3 x 30 mL). The combined organic extracts were dried (Na₂SO₄) and evaporated to dryness to give a yellow–orange oil, which was purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the first component gave the *title compound* 78 (0.49 g, 75%) as a yellow oil. *R*ᵣ 0.83 (1:3 ethyl acetate/hexane); *ν*<sub>max/cm⁻¹</sub> 2956, 2926, 1598, 1534 (S=O), 1493, 1448, 1377, 1349, 1187 (S=O), 1073, 1033; δ<sub>Η</sub> 0.84 (t, *J* 7.2, 9H, CH₃), 0.87-0.97 (m, 7H, CH₂ and aziridinyl-CHN), 1.19-1.28 (m, 6H, CH₂), 1.34-1.44 (m, 7H, CH₂ and aziridinyl-CHN), 1.78 (t, *J* 7.2, 9H, CH₃), 1.94-2.05 (m, 7H, CH₂ and aziridinyl-CHN), 1.96-2.05 (m, 7H, CH₂ and aziridinyl-CHN), 2.06-2.17 (m, 7H, CH₂ and aziridinyl-CHN), 4.19 (dd, *J*<sub>2,3</sub> 10.2, *J*<sub>3</sub> 5.4, 1H, CHHONos), 4.28 (dd, *J*<sub>2,3</sub> 10.2, 7.6, 2H, Ar-Nos), 7.18-7.23 (m, 9H, Ph-H), 7.34 (d, *J* 7.6, 6H, Ph-H), 7.96 (d, *J* 8.8, 2H, Ar-Nos), 8.32 (d, *J* 8.8, 2H, Ar-Nos); δ<sub>C</sub> 10.03 (CH₂), 13.60 (CH₃), 24.66 (CH), 27.42 (CH₂), 29.24 (CH₂), 33.86 (CH), 75.00 (CH₂), 75.41 (CPh₃), 124.42 (ArNos-CH), 126.87, 127.49 (Ph-CH), 129.23 (ArNos-CH), 129.44 (Ph-CH), 142.20, 143.83 (C); HRMS (ESI) m/z (M+H)<sup>+</sup> C₄₀H₅₁N₂O₅Sn calcd 791.2541, obsd 791.2547 (C₄₀H₅₁N₂O₅Sn<sup>120</sup>Sn, 100%).
Experiment 22: Preparation of 1-trityl-1H-benzimidazole (93)

\[
\begin{align*}
\text{Et}_3\text{N, TrCl, CH}_2\text{Cl}_2 & \quad 24 \text{ h, rt} \\
\text{N} & \quad \text{H} & \quad \text{N} & \quad \text{Tr}
\end{align*}
\]

Triethylamine (35 mL, 0.25 mol) and trityl chloride (39 g, 0.14 mol) were added over 20 minutes to benzimidazole (15 g, 0.13 mol) in dichloromethane (200 mL) and the solution stirred for 24 hours at room temperature. The solution was evaporated to dryness and the residue recrystallized from absolute ethanol to give the title compound 93 (31.05 g, 66%) as a white solid; mp 174-175 °C (mp\textsuperscript{101} 181-182 °C); \( R_f \) 0.41 (2:3 ethyl acetate/hexane); \( \nu_{\text{max}}/\text{cm}^{-1} \) 1595, 1478, 1443, 1303, 1272, 1221, 1188, 1035, 1013; \( \delta_{\text{H}} \) 6.49 (d, \( J \) 8.0, 1H, BnIm-7-H), 6.89 (dd, \( J \) 7.6, 8.0, 1H, BnIm-6-H), 7.13-7.20 (m, 7H, BnIm-5-H, Ph-H), 7.29-7.31 (m, 9H, Ph-H), 7.78 (d, \( J \) 8.4, 1H, BnIm-4-H), 7.88 (s, 1H, BnIm-2-H); \( \delta_{\text{C}} \) 75.48 (CPh\textsubscript{3}), 115.47 (BnIm-7-CH), 120.35 (BnIm-4-CH), 122.11 (BnIm-6-CH), 122.39 (BnIm-5-CH), 128.09, 128.22, 130.08 (Ph-CH), 134.89, 141.41 (C), 144.21 (BnIm-2-CH), 144.71 (C).
Experiment 23: Preparation of 2-(phenylsulfanyl)-1-trityl-1H-benzimidazole (94)

\[
\begin{align*}
\text{93} & \quad \xrightarrow{i. \text{ n-BuLi, THF, 1 h, -78 °C}} \quad \text{94} \\
& \quad \xrightarrow{\text{ii. PhS-SPh, 4 h, rt}}
\end{align*}
\]

n-Butyl lithium (8.15 mL, of a 1.6 M solution in hexanes, 13 mmol) was added dropwise over 10 minutes to a solution of 1-trityl-1H-benzimidazole 93 (4.0 g 11 mmol) in THF (100 mL) at -78 °C. The solution turned deep red and was allowed to reach room temperature over 1 hour. A solution of diphenyl disulfide (7.3 g, 34 mmol) in THF (15 mL) was added over 10 minutes, and stirred for a further 4 hours at room temperature. The solution was evaporated to dryness to yield a yellow residue, which was purified by column chromatography using silica gel as absorbent and 1:9 ethyl acetate/hexane as eluent. Evaporation of fractions containing the second component gave the title compound 94 (3.14 g, 61%) as a cream powder; mp 164-167 °C (mp101 182-184 °C); \( R_f \) 0.33 (1:9 ethyl acetate/hexane); \( \nu_{\text{max}}/\text{cm}^{-1} \) 1491, 1433, 1267, 1220, 1069, 1024, 905; \( \delta_H \) 6.05 (d, \( J \) 8.4, 1H, BnIm-7-H), 6.85 (dd, \( J \) 8.4, 8.0, 1H, BnIm-6-H), 7.00 (d, \( J \) 8.0, 2H), 7.10 (dd, \( J \) 7.6, 7.2, 1H, BnIm-5-H), 7.16-7.18 (m, 3H), 7.31-7.33 (m, 9H), 7.52-7.54 (m, 6H), 7.62 (d, \( J \) 8.0, 1H, BnIm-4-H); \( \delta_C \) 76.40 (CPh3), 114.68 (BnIm-7-CH), 119.40 (BnIm-4-CH), 121.68, 121.82 (BnIm-5,6-CH), 127.68, 127.90, 128.10, 128.87, 130.15, 132.22 (Ar-CH) 133.79, 136.87, 142.49, 143.99, 153.50 (C).
Concentrated hydrochloric acid (2 mL) was added to a solution of 2-(phenylsulfanyl)-1-trityl-1\(^H\)-benzimidazole \(94\) (1.72 g, 3.67 mmol) in wet methanol (60 mL) and the mixture heated to reflux for 3 hours. The solution was allowed to cool, evaporated to dryness and water (80 mL) was added. The aqueous mixture was extracted with dichloromethane to remove the trityl alcohol and the acidic aqueous layer was concentrated by evaporation to \(\sim 15\) mL of solution. The benzimidazole compound was precipitated as the free base by addition of solid \(\text{Na}_2\text{CO}_3\) until foaming ceased. The precipitate was recovered by filtration and recrystallized from ethanol to give the \textit{title compound} \(95\) (0.65 g, 78%) as a white solid; mp 203-205 °C (mp\(^{162}\) 201.5-202.5 °C); \(\nu_{\text{max}}/\text{cm}^{-1}\) 1619, 1501, 1476, 1440, 1412, 1349, 1266, 1235, 1206, 1179, 1070, 1022, 978; \(\delta_h\) (DMSO) 7.14-7.16 (m, 2H, BnIm-5,6-H), 7.34-7.41 (m, 4H, BnIm-7-H, Ph-\text{para}-H, Ph-\text{meta}-H), 7.46 (dd, \(J\) 8.2, 1.4, 2H, Ph-\text{ortho}-H), 7.53 (d, \(J\) 7.2, 1H, BnIm-4-H); \(\delta_c\) (DMSO) 111.55 (BnIm-7-CH), 118.86 (BnIm-4-CH), 122.15, 123.12 (BnIm-5,6-CH), 128.73, 130.13, 131.79 (Ph-CH), 135.89, 144.27, 147.14 (C).
Experiment 25: Preparation of 2-(phenylsulfonyl)-1H-benzimidazole (96)

Hydrogen peroxide (30%, 1.47 mL, 13.78 mmol) was added to a solution of 2-(phenylsulfanyl)-1H-benzimidazole 95 (0.20 g, 0.88 mmol) and ammonium heptamolybdate catalyst (0.11 g, 88 µmol.) in ethanol. The solution was stirred for 24 hours at room temperature, extracted with ethyl acetate, and dried (Na₂SO₄). The mixture was evaporated to dryness to yield the title compound 96 (0.161 g, 71%) as a white solid; mp 245-247 °C; ν max/cm⁻¹ 3066, 2964, 1709, 1584, 1474, 1434, 1338 (S=O), 1204, 1149 (S=O), 1083, 976; δ H (DMSO) 7.26-7.42 (m, 2H), 7.65-7.68 (m, 4H), 7.73-7.77 (m, 1H), 8.03 (d, J 7.2, 2H, Ph-ortho-H); δ C (DMSO) 113.67, 120.84, 123.92, 126.14, 128.25, 130.46, 135.18 (all Ar-CH), 138.81, 149.23 (C); HRMS (ESI) m/z (M+H)+ C₁₃H₁₁N₂O₂S calcd 259.0541, obsd 259.0538.
Experiment 26: General procedure for attempted synthesis of 2-(phenylsulfanyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1\textit{H}-benzimidazole (97) using mesyl aziridine (77) and nosyl aziridine (78)

\[
\begin{align*}
\text{2-(Phenylsulfanyl)-1\textit{H}-benzimidazole 95} & \quad (48 \text{ mg, 0.21 mmol}) \quad \text{and sodium hydride (6 mg, 0.25 mmol) in THF (10 mL)} \quad \text{were stirred at room temperature for 30 minutes. Aziridine 77 or 78 (0.23 mmol) in THF (3 mL)} \quad \text{was added and the mixture heated to 50 °C for 72 hours. The reaction was cooled and evaporated to dryness to yield a yellow residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane. Only unreacted starting materials were recovered.}
\end{align*}
\]
Experiment 27: General procedure for attempted preparation of 2-(phenylsulfonyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (98)

2-(Phenylsulfonyl)-1H-benzimidazole 96 (55 mg, 0.21 mmol) and sodium hydride (10 mg, 0.25 mmol) in THF (8 mL) were stirred at room temperature 30 minutes. Aziridine 77 or 78 (0.23 mmol) in THF (3 mL) was added and the mixture heated to 50 °C for 72 hours. The reaction was cooled and evaporated to dryness to yield a yellow residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane. Only unreacted starting materials were recovered.
Experiment 28: Preparation of 1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (99) using mesyl aziridine (77)

Benzimidazole (49 mg, 0.41 mmol) and sodium hydride (12 mg, 0.50 mmol) in THF (10 mL) were stirred at room temperature for 30 minutes. Mesyl aziridine 77 (0.310 g, 0.45 mmol) in THF (4 mL) was added and the mixture heated to 50 °C for 72 hours. The cooled mixture was evaporated, and the yellow residue purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of fractions containing the second component gave the title compound 99 (0.124 g, 43%) as a yellow oil; 

$R_f$ 0.28 (1:4 ethyl acetate/hexane); $[\alpha]^D_{20} = -8.6^\circ$ (c.0.50 in CHCl$_3$); $\nu_{\text{max}}$/cm$^{-1}$ 2927, 1597, 1492, 1447, 1216, 1032; $\delta_H$ 0.87 (t, $J$ 7.2, 9H, CH$_3$), 0.94-1.07 (m, 6H, CH$_2$), 1.09 (d, $J$ 6.8, 1H, aziridinyl-CHN), 1.24-1.33 (m, 6H, CH$_2$), 1.43-1.51 (m, 6H, CH$_2$), 1.77 (ddd, $J$ 6.8, 6.8, 4.8, 1H, aziridinyl-CHN), 4.16 (dd, $J^2$ 14.0, $J^3$ 4.8, 1H, NCH$_2$), 4.27 (dd, $J^2$ 14.0, $J^3$ 6.8, 1H, NCHH), 7.14-7.16 (m, 9H, Ph-H), 7.23-7.29 (m, 3H, BnIm-5,6,7-H), 7.35-7.38 (m, 6H, Ph-H), 7.75-7.77 (m, 1H, BnIm-4-H), 7.79 (s, 1H, BnIm-2-H); $\delta_C$ 10.26 (CH$_2$), 13.76 (CH$_3$), 25.79 (CH), 27.48 (CH$_2$), 29.28 (CH$_2$), 35.50 (CH), 49.44 (NCH$_2$), 75.77 (CPh$_3$) 109.60 (BnIm-7-CH), 120.49 (BnIm-4-CH), 122.15, 122.90 (BnIm-5,6-CH), 126.82, 127.46, 129.34 (Ph-CH), 134.07 (C), 142.26 (BnIm-2-CH), 143.89 (C), 148.68 (C); HRMS (ESI) $m/z$ (M+H)$^+$ C$_{41}$H$_{52}$N$_3$Sn calcld 706.3183, obsd 706.3204 (C$_{41}$H$_{52}$N$_3^{120}$Sn, 100%), 705 (C$_{41}$H$_{52}$N$_3^{119}$Sn, 56%), 702 (C$_{41}$H$_{52}$N$_3^{116}$Sn, 42%), 703 (C$_{41}$H$_{52}$N$_3^{117}$Sn, 39%).
Experiment 29: Preparation 1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (99) using nosyl aziridine (78)

Benzimidazole (55 mg, 0.47 mmol) and sodium hydride (13 mg, 0.54 mmol) in THF (5 mL) were stirred at room temperature for 30 minutes. Nosyl aziridine 78 (0.40 g, 0.51 mmol) in THF (3 mL) was added and the mixture heated to 50 °C for 24 hours. The cooled mixture was evaporated, and the yellow residue purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of fractions containing the second component gave the title compound 99 (0.134 g, 41%) as a yellow oil. Physical and spectroscopic data was consistent with that stated for experiment 28 for compound 99.
Experiment 30: General procedure for preparation of 2-substituted benzimidazoles: 2-deutero-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (100) and 2-(phenylsulfanyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (97)

n-Butyl lithium (0.10 mL, of a 1.6 M solution in hexanes, 0.16 mmol) was added dropwise at -78 °C to a stirring solution of benzimidazole 99 (0.10 g, 0.14 mmol) in THF (4 mL). The solution turned deep red and after 15 minutes d$_1$-methanol (MeOD) or diphenyl disulfide (0.28 mmol) was added in THF (1 mL). The resulting solution was warmed to room temperature over 30 minutes. The mixture was evaporated and the yellow residue was purified by column chromatography using silica gel as absorbent with gradient elution of ethyl acetate/hexane. Evaporation of fractions containing the second component gave the 2-substituted benzimidazoles:

2-deutero-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (100) (87 mg, 88%) as a clear oil; $R_f$ 0.21 (1:4 ethyl acetate/hexane); $\nu_{\text{max}}$/cm$^{-1}$ 2929, 1489, 1466, 1439, 1216, 1100; $\delta_H$ 0.86 (t, $J$ 7.2, 9H, CH$_3$), 0.95-1.08 (m, 6H, CH$_2$), 1.09 (d, $J$ 6.8, 1H, aziridinyl-CHN), 1.23-1.33 (m, 6H, CH$_2$), 1.42-1.50 (m, 6H, CH$_2$), 1.76 (ddd, $J$ 6.8, 6.8, 4.8, 1H, aziridinyl-CHN), 4.15 (ddd, $J_1^2$ 14.0, $J_1^3$ 4.8, 1H, NCHH), 4.26 (dd, $J_2^3$ 14.0, $J_3^3$ 6.8, 1H, NCHH), 7.14-7.16 (m, 9H, Ph-H), 7.26-7.27 (m, 3H, BnIm-5,6,7-H), 7.35-7.37 (m, 6H, Ph-H), 7.74-7.77 (m, 1H, BnIm-4-H); $\delta_C$ 10.25 (CH$_2$), 13.76 (CH$_3$), 25.77 (CH), 27.48 (CH$_2$), 29.27 (CH$_2$), 35.48 (CH), 49.40 (NCH$_2$), 75.73 (CPh$_3$) 109.59 (BnIm-7-CH), 120.51 (BnIm-4-CH), 122.12, 122.88 (BnIm-5,6-CH), 126.82, 127.75, 129.33 (Ph-CH), 134.05, 143.89 (C); HRMS (ESI) m/z (M+H)$^+$ C$_{41}$H$_{51}$DN$_3$Sn calcd 707.3205, obsd 707.3204.

2-(phenylsulfanyl)-1-\{[(2S,3R)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl\}-1H-benzimidazole (97) (68 mg, 60%) as a clear oil; $R_f$ 0.56 (1:4 ethyl acetate/hexane); $[\alpha]_{D}^{20}$ = -30.0° (c=0.10 in CHCl$_3$); $\nu_{\text{max}}$/cm$^{-1}$ 2929, 1596,
1490, 1447, 1328, 1251, 1080, 1033, 907; \( \delta_H \) 0.87 (t, \( J = 7.4 \), 9H, CH\(_3\)), 0.97-1.13 (m, 7H, CH\(_2\) and aziridinyl-CHN), 1.25-1.35 (m, 6H, CH\(_2\)), 1.45-1.53 (m, 6H, CH\(_2\)), 2.05 (ddd, \( J = 8.4, 8.4, 2.8 \), 1H, aziridinyl-CHN), 4.15 (dd, \( J_H^1 = 14.4, J_H^2 = 8.4 \), 1H, NCHH), 4.25 (dd, \( J_H^1 = 14.4, J_H^2 = 2.8 \), 1H, NCHH), 7.00-7.04 (m, 9H), 7.06-7.10 (m, 5H), 7.21-7.30 (m, 9H), 7.71-7.73 (m, 1H, BnIm-4-H); \( \delta_C \) 10.29 (CH\(_2\)), 13.78 (CH\(_3\)), 25.53 (CH), 27.51 (CH\(_2\)), 29.31 (CH\(_2\)), 35.51 (CH), 49.77 (NCH\(_2\)), 75.81 (CPh\(_3\)), 110.23 (BnIm-7-CH), 120.05 (BnIm-4-CH), 122.28, 123.13 (BnIm-5,6-CH), 126.59, 127.23, 127.52, 129.41, 129.48, 129.87 (Ph-CH), 132.34, 136.25, 143.57, 143.90, 146.98 (C); HRMS (ESI) \( m/z \) (M+H\(^+\)) C\(_{47}\)H\(_{56}\)N\(_3\)SSn calcld 814.3217, obsd 814.3229 (C\(_{47}\)H\(_{56}\)N\(_3\)S\(_{120}\)Sn, 100%), 812 (C\(_{47}\)H\(_{56}\)N\(_3\)S\(_{118}\)Sn, 66%), 813 (C\(_{47}\)H\(_{56}\)N\(_3\)S\(_{119}\)Sn, 54%).
Experiment 31: Preparation of (1aS,8aS)-1-trityl-1,1a,8,8a-tetrahydroazireno[2',3':3,4]pyrrolo[1,2-a]benzimidazole (64)

Methyl lithium (0.19 mL, of a 1.4 M solution in diethyl ether, 0.26 mmol) was added dropwise at -78 °C to a stirring solution of benzimidazole 97 (60 mg, 74 μmol) in THF (2 mL). The solution turned deep red and was slowly warmed to room temperature over 30 minutes. The resulting mixture was evaporated and the yellow residue purified by column chromatography with gradient elution of ethyl acetate/hexane. Evaporation of fractions containing the second component gave 2-(phenylsulfanyl)-1-[((2R)-1-tritylaziridin-2-yl)methyl]-1H-benzimidazole 101 (7 mg, 18%) as a yellow oil; \( R_f \) 0.65 (1:1 ethyl acetate/hexane); \( [\alpha]^{20}_D = +8.7^\circ \) (c 0.52 in CHCl₃); \( \nu_{\text{max}}/\text{cm}^{-1} \) 1581, 1480, 1441, 1353, 1326, 1281, 1246, 1202, 1154, 1083, 1024; \( \delta_H \) 1.05 (d, \( J = 6.0 \), 1H, aziridinyl-CHH), 1.60-1.65 (m, 1H, aziridinyl-CHN), 1.81 (d, \( J = 2.8 \), 1H, aziridinyl-CHH), 4.23 (dd, \( J^1 = 14.8 \), \( J^3 = 7.6 \), 1H, NCH₂), 4.81 (dd, \( J^2 = 14.8 \), \( J^3 = 4.0 \), 1H, NCH₃/CH₂), 7.20-7.26 (m, 17H), 7.40-7.42 (m, 6H), 7.74-7.76 (m, 1H, BnIm-4-H); \( \delta_C \) 28.05 (CH₂), 31.64 (CHN), 47.65 (NCH₂), 74.19 (CPh₃), 110.02 (BnIm-7-CH), 120.11 (BnIm-4-CH), 122.55, 123.49 (BnIm-5,6-CH), 126.93, 127.66, 129.39, 129.53, 130.28 (Ph-CH), 132.31, 136.11, 143.41, 144.13, 147.34 (C); HRMS (ESI) m/z (M+H)\(^+\) C₃₅H₃₀N₃S calcd 524.2160, obsd 524.2162. Evaporation of fractions containing the third component gave the title compound 64 (23 mg, 75%) as a pale yellow oil; \( R_f \) 0.50 (1:1 ethyl acetate/hexane); \( [\alpha]^{20}_D = -9.3^\circ \) (c 0.15 in CHCl₃); \( \nu_{\text{max}}/\text{cm}^{-1} \) 2929, 1623, 1545, 1490, 1448, 1354, 1265, 1216, 1151, 1051, 909; \( \delta_H \) 2.97 (d, \( J = 4.8 \), 1H, 1a-H), 3.08-3.10 (m, 1H, 8a-H), 4.06 (dd, \( J^1 = 11.2 \), \( J^3 = 4.0 \), 1H, 8-H/H'), 4.42 (d, \( J = 11.2 \), 1H, 8-HH'), 7.23-7.32 (m, 12H), 7.47-7.49 (m, 6H, Ph-H), 7.76-7.79 (m, 1H, 3-H); \( \delta_C \) 33.29 (1a-CH), 40.24 (8a-CH), 45.23 (CH₂), 73.44 (CPh₃), 108.56 (6-CH), 119.48 (3-CH), 120.79, 121.62 (4,5-CH), 126.22, 126.98, 128.25.
(Ph-CH), 132.02, 142.84, 146.93 (C), 157.57 (1b-C); HRMS (ESI) m/z (M+H)^+ 
C_{29}H_{24}N_3 \text{ calcd 414.1971, obsd 414.1970.}
Concentrated nitric acid (70-72%, 18 mL) was added dropwise to a stirring solution of 1,4-dimethoxybenzene 105 (13.80 g, 0.10 mol) in glacial acetic acid (35 mL) at 0 °C. After the vigorous reaction had subsided a further portion (18 mL) of nitric acid was added. The reaction was heated to 80-90 °C for 5-10 minutes to release a red gas. The reaction was cooled and diluted with water (100 mL). The yellow precipitate was filtered and purified by column chromatography using silica gel as absorbent and 1:1 ethyl acetate/hexane as eluent to yield 1,4-dimethoxy-2,5-dinitrobenzene 106 as a yellow solid (3.25 g, 14 %); the second isomer eluted was 1,4-dimethoxy-2,3-dinitrobenzene 107 as a yellow solid (12.65 g, 55%); mp 185-187 °C; \( R_f \) 0.25 (1:1 ethyl acetate/hexane); \( \nu_{\text{max}}/\text{cm}^{-1} \) 2925, 1490, 1352, 1271, 1050; \( \delta_H \) 3.92 (s, 6H, OCH\(_3\)), 7.20 (s, 2H, Ar-H); \( \delta_C \) 57.63 (OCH\(_3\)), 116.86 (Ar-CH), 145.40 (C).
Experiment 33: Preparation of 1,4-dimethoxy-2,3-diaminobenzene (108)

A mixture of 1,4-dimethoxy-2,3-dinitrobenzene 107 (3.10 g, 13.59 mmol) and Pd-C (10%, 0.31 g) in ethanol (200 mL) was agitated under 40 psi H₂ for 18 hours at room temperature. The catalyst was removed by filtration and the filtrate evaporated to dryness to yield the title compound 108 (2.16 g, 94%) as a brown solid; mp 75-77 °C (mp 163-85-87 °C); ν max/cm⁻¹ 3423, 3391, 3327, 2995, 2950, 2830, 1635, 1489, 1440, 1302, 1268, 1206, 1165, 1142, 1087, 1054; δ H 3.50 (bs, 4H, NH), 3.80 (s, 6H, OCH₃), 6.30 (s, 2H, Ar-H); δ C 56.00 (OCH₃), 100.68 (Ar-CH), 124.54, 143.22 (C).
Experiment 34: Preparation of 4,7-dimethoxy-1H-benzimidazole (109)

1,4-Dimethoxy-2,3-diaminobenzene 108 (2.13 g, 12.66 mmol) in formic acid (20 mL, 95-97%) was heated at reflux for 4 hours. The reaction was cooled and basified with ammonium hydroxide to pH 12 and extracted with chloroform (4 x 200 mL). The combined organic extracted were washed with brine (2 x 300 mL) and dried (Na₂SO₄). The solution was evaporated to dryness to yield the title compound 109 (1.62 g, 72%) as a brown solid; mp 222-223 °C (mp¹⁶³ 218-222 °C); νmax/cm⁻¹ 2954, 2791, 1526, 1452, 1405, 1261, 1215, 1169, 1104; δH 3.95 (s, 6H, OCH₃), 6.59 (s, 2H, Ar-CH), 7.97 (s, 1H, BnIm-2-H); δC 56.22 (OCH₃), 102.72 (Ar-CH), 138.87 (C).
Experiment 35: Preparation of 4,7-dimethoxy-N-\[((2S,3R)-3-(tributylstannyl)-1-tritylaziridine-2-yl)methyl]-1H-benzimidazole (110) using mesyl aziridine (77)

4,7-Dimethoxy-1H-benzimidazole 109 (20 mg, 0.11 mmol) and sodium hydride (3 mg, 0.13 mmol) in DMF (10 mL) were stirred at room temperature for 30 minutes. Mesyl aziridine 77 (0.10 g, 0.15 mmol) in DMF (3 mL) was added and the mixture heated to 60 °C for 72 hours. The reaction was cooled and evaporated to dryness to yield a brown residue, which was purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the second component gave the title compound 110 (36 mg, 43%), as a yellow oil; R$_f$ 0.50 (3:7 ethyl acetate/hexane); $\nu_{max}$/cm$^{-1}$ 2852, 1737, 1523, 1462, 1376, 1263, 1096, 706; $\delta_H$ 0.87 (t, $J$ 7.3, 9H, CH$_3$), 0.97-1.10 (m, 6H, CH$_2$), 1.25-1.34 (m, 7H, CH$_2$ and aziridinyl-CHN), 1.43-1.51 (m, 6H, CH$_2$), 1.79-1.82 (m, 1H, aziridinyl-CHN), 3.69 (s, 3H, OCH$_3$), 3.94 (s, 3H, OCH$_3$), 4.34 (dd, $J^2$ 14.0, $J^3$ 4.6, 1H, NCH$_2$), 4.60 (dd, $J^2$ 14.0, $J^3$ 6.6, 1H, NCH$_2$), 6.49 (d, ABq, $J$ 8.4, 1H, BnIm-5,6-H), 6.52 (d, ABq, $J$ 8.4, 1H BnIm-5,6-H), 7.13-7.17 (m, 9H, Ph-H), 7.38 (dd, $J$ 7.8, 1.4, 6H, Ph-H), 7.63 (s, 1H, BnIm-2-H); $\delta_C$ 10.21 (CH$_2$), 13.81 (CH$_3$), 25.44 (CH), 27.50 (CH$_2$), 29.29 (CH$_2$), 37.11 (CH), 51.26 (NCH$_2$), 55.46 (OCH$_3$), 56.14 (OCH$_3$), 75.68 (CPh$_3$), 101.82, 102.89 (BnIm-5,6-CH), 124.96 (C), 126.67, 127.36, 129.40 (Ph-CH), 141.71 (BnIm-2-CH), 144.12, 146.17 (C); HRMS (ESI) $m/z$ (M+H)$^+$ C$_{43}$H$_{56}$N$_3$O$_2$Sn calcd 766.3395, obsd 766.3372
Experiment 36: Preparation of 4,7-dimethoxy-N-[(2S,3R)-3-(tributylstannyl)-1-tritylaziridine-2-yl[methyl]-1H-benzimidazole (110) using nosyl aziridine (78)

4,7-Dimethoxy-1H-benzimidazole 109 (68 mg, 0.38 mmol) and sodium hydride (11 mg, 0.46 mmol) in THF (10 mL) were stirred at room temperature for 30 minutes. Nosyl aziridine 78 (0.42 g, 0.53 mmol) in THF (4 mL) was added and the mixture heated for 24 hours. The reaction was cooled and evaporated to dryness to yield a brown residue, which was purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the second component gave the title compound 110 (94 mg, 32%), as a yellow oil. Physical and spectroscopic data were consistent with that stated in experiment 35 for compound 110.
n-Butyl lithium (35 µL, of a 2.5 M solution in hexanes, 88 µmol) was added dropwise at -78 °C to 4,7-dimethoxybenzimidazole 110 (22 mg, 29 µmol) in THF (1 mL). The solution turned deep red and after 30 minutes d1-methanol (MeOD) (0.3 mL, 29.3 mmol) was added. The resulting solution was stirred for a further 10 minutes at -78 °C, then warmed to room temperature for 30 minutes. The solution was evaporated and the solution was purified using DCVC and gradient elution with ethyl acetate/hexane. Evaporation of fractions containing the first component gave the title compound 111 (18 mg, 82%) as a yellow oil; \( R_f \) 0.82 (1:4 ethyl acetate/hexane); \( \delta_H \) 0.88 (t, \( J \) 7.2, 9H, CH3), 0.99-1.11 (m, 6H, CH2), 1.24-1.34 (m, 7H, CH2 and aziridinyl-CHN), 1.46-1.52 (m, obsc), 3.85 (s, 3H, OCH3), 3.99 (s, 3H, OCH3), 4.85 (d, \( J \) 13.6, 1H, NCH), 5.19 (dd, \( J^1 \) 13.6, \( J^2 \) 8.8, 1H, NCHH), 6.57 (d, \( J \) 8.4, 1H, Bnlm-5,6-H), 6.73 (d, \( J \) 8.4, 1H, Bnlm-5,6-H), 6.98-7.04 (m, 9H, Ph-H), 7.23-7.25 (m, 6H, Ph-H); \( \delta_C \) 10.05 (CH2), 13.88 (CH3), 23.87 (CH), 27.56 (CH2), 29.32 (CH2), 39.38 (CH), 51.23 (CH2), 55.71 (OCH3), 56.01 (OCH3), 101.55, 105.84 (Bnlm-5,6-CH), 126.42, 127.11, 129.29 (Ph-CH), 144.38 (C).
Experiment 38: Preparation of (2S)-1-tritylaziridine-2-carboxylic acid methyl ester (114)

Triethylamine (2.00 mL, 14.35 mmol) was added dropwise to a solution of N-trityl-S-serine methyl ester 80 (2.00 g, 5.53 mmol) in toluene (20 mL) and the solution cooled to -50 °C. Sulfuryl chloride (0.72 mL, 7.21 mmol) was added dropwise and the solution stirred for 4 hours. The organic extracts were washed with brine (50 mL) and dried (Na₂SO₄). The solution was evaporated to dryness and the residue was purified by column chromatography using silica gel as absorbent with 1:9 diethyl ether/hexane as eluent. Evaporation of fractions containing the second component gave the title compound 114 (1.39 g, 73%) as a white solid; \( R_f \) 0.48 (1:9 diethyl ether/hexane); mp 115-117 °C, \( \text{mp}^{85} \) 114-116 °C; \([\alpha]_{20}^{D} = -88.3^{\circ} \) (c. 2.68 in THF), \{\([\alpha]_{20}^{D} = -89.3^{\circ} \) (c. 2.68 in THF)\} \(^{85}\); \( \nu_{\text{max}}/\text{cm}^{-1} \) 1747 (C=O), 1489, 1445, 1329, 1244, 1201, 1180, 1157, 1010; \( \delta_{\text{H}} \) 1.49 (dd, \( J \) 6.3 \( J \) 1.2, 1H, aziridinyl-CH\(\text{HN} \)), 1.97-1.99 (m, 1H, aziridinyl-CH\(\text{HN} \)), 2.33-2.34 (m, 1H, CH), 3.79 (s, 3H, OCH₃), 7.25-7.35 (m, 9H, Ph-H), 7.59 (d, \( J \) 7.2, 6H, Ph-\text{ortho}-H); \( \delta_{\text{C}} \) 28.88 (CH₂), 31.89 (CH), 52.27 (CH₃), 74.59 (CPh₃), 127.15, 127.86, 129.50 (Ph-CH), 143.80 (Ph-\text{ipso}-C), 172.10 (C=O).
Experiment 39: Preparation of (2S)-1-tritylaziridine-2-methanol (115)

Diisobutylaluminium hydride (18.50 mL of a 1 M solution in toluene, 18.50 mmol) was added dropwise to a solution of ester 114 (2.53 g, 7.37 mmol) in toluene (30 mL) at -78 °C over a 5 minute period and the solution was stirred for 3 hours. The reaction was warmed to room temperature, quenched with methanol (5 mL), a saturated sodium potassium tartrate solution (40 mL) was added and the mixture stirred vigorously for 20 minutes. The resulting biphasic mixture was extracted with ethyl acetate (3 x 50 mL), washed with brine (2 x 30 mL) and dried (Na₂SO₄). The solution was evaporated to dryness to give the title compound 115 (1.82 g, 78%) as a white solid; mp 126-128 °C (mp33 127.5-128.5 °C); \([\alpha]^{24}_D = +7.6^\circ \) (c.1.00 in dichloromethane), \([\alpha]^{24}_D = +8.8^\circ \) (c. 1.00 in dichloromethane); \(\nu_{\text{max}}/\text{cm}^{-1} \) 3400 (OH), 1489, 1446, 1215, 1032; \(\delta_H \) 1.14 (d, \(J \) 6.4, 1H, aziridinyl-CHH), 1.59 (dddd, \(J \) 6.4, 3.2, 3.2, 3.2, 1H, aziridinyl-CHN), 1.88 (d, \(J \) 3.2, 1H, aziridinyl-CHHN), 2.27-2.30 (m, 1H, OH), 3.71 (ddd, \(J \) 11.0, 7.8, 3.2, 1H, CHHOH), 3.89 (ddd, \(J \) 11.0, 3.2, 3.2, 1H, CHHOH), 7.21-7.31 (m, 9H, Ph-H), 7.47 (d, \(J \) 7.2, 6H, Ph-ortho-H); \(\delta_C \) 23.89 (CH₂), 33.19 (CH), 61.72 (CH₂), 73.85 (CPh₃), 126.96, 127.80, 129.43 (Ph-CH), 144.39 (Ph-ipso-C).
Experiment 40: Preparation of $N$-[(2S)-1-tritylaziridine-2-yl]methyl methanesulfonate (116)

Triethylamine (2.00 mL, 14.25 mmol) was added dropwise to a solution of methanesulfonyl chloride (0.11 mL, 1.40 mmol), and aziridinol 115 (0.30 g, 0.95 mmol) in dichloromethane (10 mL) at -78 °C. The solution was stirred for a further 18 hours at room temperature. Water was added (30 mL) and the mixture extracted with dichloromethane (2 x 30 mL) and the combined organic extracts were dried (Na$_2$SO$_4$). The solution was evaporated to dryness and the residue was purified by column chromatography using silica gel as absorbent and 1:4 ethyl acetate/hexane as eluent. Evaporation of fractions containing the first component gave the **title compound** 116 (0.28 g, 75%) as a white solid; $R_f$ 0.64 (1:4 ethyl acetate/hexane); mp 90-91 °C; $[\alpha]^{20}_D = -17.2^\circ$ (c.1.00 in dichloromethane); $\nu_{\text{max}}$/cm$^{-1}$ 3022, 1489,1447, 1356 (S=O), 1172 (S=O), 1050, 941; $\delta_H$ 1.31 (d, $J$ 6.4, 1H, aziridinyl-$\text{CH}_2$H), 1.71-1.76 (m, 1H, CH), 1.90 (d, $J$ 3.2, 1H, aziridinyl-$\text{CH}_3$), 2.91 (s, 3H, $\text{CH}_3$), 4.37 (dd, $J^2$ 10.5 $J^3$ 3.3, 1H, $\text{CH}_2$OMs), 4.45 (dd, $J^2$ 10.5 $J^3$ 6.1, 1H, CH2OMs), 7.25-7.35 (m, 9H, Ph-H), 7.57 (d, $J$ 7.6, 6H, Ph-ortho-H); $\delta_C$ 25.62 (CH$_2$), 30.83 (CH), 37.67 (CH$_3$), 71.91 (CH$_2$O), 74.10 (CPh$_3$), 127.10, 127.80, 129.57 (Ph-CH), 144.18 (Ph-ipso-C).
Experiment 41: Attempted preparation of N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole (65)

Benzimidazole (0.23 g, 1.95 mmol) and sodium hydride (65 mg, 2.71 mmol) in DMF (10 mL) were stirred at room temperature for 30 minutes. Mesyl aziridine 116 (0.90 g, 2.29 mmol) in DMF (5 mL) was added and the mixture heated to 60 °C for 24 hours. The reaction was cooled and evaporated to dryness to yield a yellow residue, which was purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the second component gave (3E)-3-(1H-benzimidazole-1-yl)-N-tritylprop-2-en-1-amine 117 (0.374 g, 46%) as a clear oil; Rf 0.72 (1:4 ethyl acetate/hexane); \( \nu_{\text{max}}/\text{cm}^{-1} \) 3057, 2852, 1735, 1672, 1596, 1489, 1453, 1370, 1325, 1289, 1202, 1078, 1031; \( \delta_{\text{H}} \) 3.01 (dd, J 6.0, 1.6, 2H, NCH2), 6.12 (ddd, J 14.2, 6.0, 6.0, 1H, vinyl-CH), 7.07 (d, J 14.2, 1H, vinyl NCH), 7.23-7.25 (m, 3H, BnIm-5,6,7-H), 7.30-7.34 (m, 9H, Ar-H), 7.52-7.54 (m, 6H, Ar-H), 7.80-7.82 (m, 1H, BnIm-4-H), 8.09 (s, 1H, BnIm-2-H); \( \delta_{\text{C}} \) 43.98 (CH2), 71.13 (CPh), 110.41 (BnIm-7-CH), 119.70 (vinyl-CH), 120.72 (BnIm-4-CH), 122.77 (vinyl-NCH) 122.96, 123.76 (BnIm-5,6-CH), 126.65, 128.13, 128.63 (Ph-CH), 141.01 (BnIm-2-CH), 145.87 (C); HRMS (ESI) m/z (M+H)+ C29H26N3 calcd 416.2127, obsd 416.2111.
Experiment 42: Preparation of \(N-[(1\text{-tritylaziridin-(2S)-yl})\text{methyl}] \text{-1H-benzimidazole (65)}\)

Benzimidazole (96 mg, 0.81 mmol) and sodium hydride (20 mg, 0.83 mmol) in THF (5 mL) were stirred at room temperature for 30 minutes. Mesyl aziridine 116 (0.45 g, 1.14 mmol) in THF (3 mL) was added and the mixture heated to 30 °C for 24 hours. The reaction was cooled and evaporated to yield a yellow residue, which was purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the second component gave the title compound 65 (0.281 g, 83%) as a clear oil; \(R_f\ 0.31\) (1:9 ethyl acetate/hexane); [\(\alpha\)]\(^{20}_{D}\) = -58.9° (c.0.18 in CHCl\(_3\)); \(\nu_{\text{max}}/\text{cm}^{-1}\) 2962, 1594, 1490, 1446, 1382, 1261, 1232, 1085, 1029; \(\delta_H\) 1.18 (d, \(J\ 6.0\), 1H, aziridinyl-\(\text{CH}_2\)), 1.67-1.75 (m, 2H, aziridinyl-\(\text{CH}_2\) and aziridinyl-\(\text{CH}_2\)), 4.25 (dd, \(J^2\ 14.8, J^3\ 5.6\), 1H, \(\text{NCH}_2\)), 7.18-7.31 (m, 12H), 7.44-7.46 (m, 6H, Ph-H), 7.76-7.79 (m, 1H, BnIm-4-H), 7.94 (s, 1H, BnIm-2-H); \(\delta_C\) 26.57 (CH\(_2\)), 31.91 (CH), 47.42 (NCH\(_2\)), 74.23 (CPh\(_3\)), 109.78 (BnIm-7-CH), 120.52 (BnIm-4-CH) 122.36, 122.99 (BnIm-5,6-CH), 127.02, 127.73, 129.35 (Ph-CH), 134.18 (C), 143.11 (BnIm-2-CH), 143.75, 144.06 (C); HRMS (ESI) \(m/z\) (M+H)\(^+\) \(C_{29}H_{28}N_3\) calcd 416.2127, obsd 416.2109.
Experiment 43: Preparation of 2-(phenylsulfanyl)-1-[[2S]-1-tritylaziridin-2-yl]methyl]-1H-benzimidazole (118)

2-(phenylsulfanyl)-1H-benzimidazole 95 (0.15 g, 0.66 mmol) and sodium hydride (17.5 mg, 0.73 mmol) in THF (5 mL) were stirred at room temperature for 30 minutes. Mesyl aziridine 116 (0.31 g, 0.79 mmol) in THF (2 mL) was added and the mixture heated to 30 °C for 24 hours. The reaction was cooled and evaporated to yield a yellow residue, which was purified using DCVC with gradient elution of ethyl acetate/hexane. Evaporation of the fractions containing the second component gave the title compound 118 (0.25 g, 72%) as a yellow oil; \([\alpha]^{20}_D = -8.7^\circ\) (c.0.52 in CHCl3). Spectroscopic data was consistent with that reported in experiment 31 for compound 101.
3.2.1 Cell culture and cytotoxicity evaluation

3.2.1.1 Cell lines
An SV40-transformed human normal skin fibroblast cell line (repository number GM00637) was obtained from the National Institute for General Medical Sciences (NIGMS) Human Genetic Cell Repository (Coriell Institute for Medical Research, New Jersey, USA). The MCF-7 breast cancer cell line was obtained from Dr. Adrienne Gorman, Biochemistry, School of Natural Sciences, National University of Ireland, Galway. The HCC1937 breast cancer cell line was obtained from Dr. Paul Mullan, Queens University, Belfast. The HeLa cervical cancer cell line (repository number CCL-2) was obtained from the American Type Culture Collection (ATCC) and the DU145 prostate cancer cell line (ATCC repository number HTB-81) was obtained from Prof. William Watson, School of Medicine & Medical Science, University College Dublin, Ireland.

Cell culture reagents were obtained from Sigma-Aldrich and sterile plastic ware was obtained from Sarstedt AG (Numbrecht, Germany). The SV40-transformed human normal skin fibroblast cell line (GM00637) was grown in Minimum Essential Media (MEM) Eagle-Earle’s BSS supplemented with 15% non heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 2mM L-glutamine, 2X essential and non-essential amino acids and 2X vitamins. MCF-7 and HCC1937 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing high glucose (4.5g/mL) and supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin. HeLa cervical cancer cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% non heat-inactivated fetal bovine serum (FBS), penicillin-streptomycin, 2 mM L-glutamine, and MEM non-essential amino acids. DU145 prostate cancer cells were grown in RPMI-1640 medium supplemented with 10% non heat-inactivated fetal bovine serum, penicillin-streptomycin and 2 mM L-glutamine. All cell lines grew as adherent cultures.
Cell culture procedures were carried out in a Class III Bio-Safety Cabinet (Medical Supply Company, Dublin, Ireland). Disposable sterile plastic ware was used for all cell culture protocols. Surfaces were sprayed with 70% ethanol prior to carrying out procedures. Cells were grown in 75 cm$^3$ flasks in 20 mL of medium, and incubated in an autoflow CO$_2$ water-jacket incubator at 37 °C and 5% CO$_2$. When cells were approximately 80% confluent, they were subcultured by treatment with 2X trypsin-EDTA in Hanks balanced salt solution or for the MCF-7 cell line, 5X trypsin-EDTA for five minutes. Cells were centrifuged at 1,200 rpm in a Rotanta 300 centrifuge and the cell pellet was re-suspended in fresh culture medium. The total cell number was determined using a Kova® Glasstic® Slide 10 combination coverslip-microslip slide. When cells did not need to be counted the GM00637 stock was seeded at 1/4, MCF-7 at 1/10, HCC1937 at 1/2, HeLa at 1/6, DU145 at 1/6 and were added to 20 mL of prewarmed medium in a sterile 75 cm$^3$ flask and incubated at 37 °C and 5% CO$_2$. Cell culture medium was changed every two-three days.

### 3.2.1.2 Cell resuscitation

All cell lines were resuscitated by rapid thawing of the cell suspension at 37 °C. 1 mL of pre-warmed culture medium was added to a 25 cm$^3$ sterile culture flask followed by the thawed cell suspension, and a further 5 mL of pre warmed culture medium was added. The cells were incubated at 37 °C and 5% CO$_2$ and the culture medium was changed the following day.

### 3.2.1.3 Cytotoxicity measurements using the MTT assay

Cell viability was determined using the MTT colorimetric assay. Cells were plated into 96-well plates at a density of 10,000 cells per well (GM00637, 200 μL per well), 1,000 cells per well (MCF-7, 200 μL per well), 10,000 cells per well (HCC1937, 200 μL per well), 1,000 cells per well (HeLa, 200 μL per well) and 2,000 cells per well (DU145, 200 μL per well) and allowed to adhere over a period of 24 hours or 48 hours in the case of the aziridine compounds. Drug solutions were applied in DMSO, ethanol or ethanol/H$_2$O (1% v/v in well). All cells were incubated at 37 °C under a humidified atmosphere containing 5% CO$_2$. 
for 24 hours or 72 hours as stated. Control cells were exposed to an equivalent concentration of vehicle control alone. MMC (Sigma) solutions were applied in DMSO solutions (1% v/v in well). Control cells were exposed to an equivalent concentration of DMSO alone. MTT (20μL, 5mg/mL solution) was added and the cells were incubated for a further 3 hours. The supernatant was then removed by careful pipetting. The resultant MTT formazan crystals were dissolved in 100 μL of DMSO and absorbance was determined using a Wallac Victor 2 1420 multilabel counter plate reader at 550 nm with a reference at 690 nm. Cell viability is expressed as a percentage of the vehicle-only treated control value. Dose-response curves were analyzed by nonlinear regression analysis and IC_{50} values were estimated by using GraphPad Prism software, v.5.02 (GraphPad Inc., San Diego, CA, USA). The in vitro activity of the drugs towards all cell lines is expressed as IC_{50} (i.e. concentration required for the reduction of the mean cell viability to 50%).
References


(18) Fracasso, P. M.; Sartorelli, A. C. *Cancer Res.* 1986, 46, 3939-3944.


(62) Hamblin, T. J. *Drugs* 2001, 61, 593-611.
(93) We thank Prof. I. D. Jenkins (Griffith University, B., Australia) for this valuable suggestion regarding the Mitsunobu reaction.


Appendix
Correlation of iminoquinone 154 to NQO1
Correlation of MMC to NQO1

![Developmental Therapeutics Program Mean Graph: Correlation of MMC to NQO1](image-url)
Correlation of compound 162 to NQO1
Correlation of compound **163** to NQO1

Developmental Therapeutics Program Mean Graph
Selected Data Vectors

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Correlation of compound 164 to NQO1
Correlation of iminoquinone 154 to compound 162

Developmental Therapeutics Program Mean Graph
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- HL-60/THP-1
- K-562
- MOLT-4
- RPMI-8226
- SR

Non-Small-Cell Lung
- A431
- A549
- HOP-92
- HOP-92
- NCI-H520
- NCI-H23
- NCI-H23
- NCI-H23
- NCI-HD366
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Colon
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- DLD1
- HCT-116
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- HT29
- KM12L5
- SW480

CNS
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- U87

Melanoma
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Ovarian
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Prostate
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Breast
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Summary
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- D1
- Range

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Correlation of iminoquinone 154 to compound 163

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164
Peer-Reviewed Publications
and
Conference Proceedings
Synthesis and toxicity towards normal and cancer cell lines of benzimidazolequinones containing fused aromatic rings and 2-aromatic ring substituents

Contribution: Aided in toxicity evaluations of compounds
One-pot double intramolecular homolytic aromatic substitution routes to dialicyclic ring fused imidazobenzimidazolequinones and preliminary analysis of anticancer activity

Vincent Fagan, Sarah Bonham, Michael P. Carty and Fawaz Aldabbagh*, Organic and Biomolecular Chemistry, 2010, 8, 3149-3156 (RSC Prospect)

Contribution: Evaluated toxicity of all compounds
First synthesis of an aziridinyl fused pyrrolo[1,2-\(a\)]benzimidazole and toxicity evaluation towards normal and breast cancer cell lines

Sarah Bonham, Liz O’ Donovan, Michael P. Carty and Fawaz Aldabbagh*

*Organic and Biomolecular Chemistry, 2011, 9, 6700-6706

Contribution: Prepared and evaluated all compounds
Conference Proceedings

“Synthesis of Benzimidazoles with Substituted/Fused Aziridines and Hypersensitive Killing of Fanconi Anemia Cells,”

“Synthesis of Benzimidazoles with Substituted/Fused Aziridines and Hypersensitive Killing of Fanconi Anemia Cells,”

“One-pot double intramolecular homolytic aromatic substitution routes to dialicyclic ring fused imidazobenzimidazolequinones and preliminary analysis of anticancer activity,”