DESIGN, SYNTHESIS AND EVALUATION OF NOVEL C2-ARYL PYRROLOBENZODIAZEPINES AS POTENTIAL ANTICANCER AGENTS.

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Dr Philip W. Howard

This thesis describes research conducted at the University of Nottingham between September 2000 and August 2001 and in the School of Pharmacy, University of London between September 2001 and December 2003 under the supervision of Prof. David Thurston and Dr Philip Howard. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature  Date
In memory of my beloved grandfather

Christodoulos Pavlides

1924 - 2005
ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof. David Thurston and Dr Philip Howard for their continued support and encouragement throughout the duration of this PhD research project. I would also like to thank my family, in particular my mother Despina and my brother Christodoulos for all their support, encouragement and patience. Finally I would like to thank my colleagues and collaborators (Dr Stephen Gregson, Dr David Evans, Dr Tony Reszka, Dr Mire Zloh, Dr Angelica Burger, Arnaud Tiberghien and Emmanuel Samuel) who have helped and contributed to this research work.
ABSTRACT

The pyrrolobenzodiazepines are a family of anti-tumour antibiotics discovered in the fermentation broths of various streptomyces species. Whilst the early naturally occurring PBDs showed promise in vitro many were found to lack efficacy or give rise to cardiotoxicity in vivo.

Many potent naturally occurring PBDs possess a C-ring associated endo-exo unsaturation motif coupled with the 9-hydroxyl group linked to in vivo cardiotoxicity. In this project a set of novel, totally synthetic PBDs that retain the endo-exo unsaturation motif, in the form of a C2-aryl substituent, but which lack the 9-hydroxy group have been designed and synthesised in an attempt to provide potent non cardiotoxic PBDs for biological evaluation.

The C2-aryl substituents were successfully introduced by the Suzuki coupling or aryl boronic acids to the enol triflate derivative of an N10 protected PBD-dilactam. Reduction and simultaneous deprotection of the C2-aryl dilactams afforded the novel C2-aryl PBDs. The Suzuki coupling reactions could be conveniently carried out in parallel, using a carousel reactor, allowing the preparation of a small C2-aryl PBD library.

The novel C2-aryl compounds underwent biological evaluation at both the Institute for Experimental Oncology, Germany and the National Cancer Institute of the U.S.A. The C2-aryl compounds were found to possess nanomolar activity in cancer cell lines. The NCI study revealed that the molecules possessed preferential activity against melanoma and renal panels in particular.

A number of C2-aryl compounds were selected for in vivo testing based on the encouraging cytotoxicity data. The molecules exhibited some evidence of in vivo activity, with the 2,6-dimethyl analogue showing unexpected in vivo activity. In vivo testing of the naphthyl analogue, which performed well in both cytotoxicity assays and biophysical studies is awaited with interest.
Finally pilot studies were undertaken to investigate the feasibility of applying the Suzuki synthetic strategy to the synthesis of C2-aryl PBD dimers.
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<td>DNA melting studies</td>
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# ABBREVIATIONS

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<tr>
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<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CAM</td>
<td>Cell-cell Adhesion Molecule</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulphonic acid</td>
</tr>
<tr>
<td>Cyt C</td>
<td>Cytochrome C</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-Diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>DACA N</td>
<td>(2-(Dimethylamino)ethyl)acridine-4-carboxamide</td>
</tr>
<tr>
<td>DBU</td>
<td>7,11-Diazabicyclo[5.4.0]undec-11-ene</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>Diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DME</td>
<td>Ethyleneglycoldimethyl ether</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra cellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factors</td>
</tr>
<tr>
<td>FH4</td>
<td>Tetrahydrofolic acid</td>
</tr>
<tr>
<td>GI&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration required to inhibit growth of the cells by 50%</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papilloma virus</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Herpes simplex virus - type 2</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration required to kill 50% of the cells</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>LHMDS</td>
<td>Lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl ether</td>
</tr>
<tr>
<td>MOMCl</td>
<td>Methoxymethyl Chloride</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MsCl</td>
<td>Methanesulfonyl chloride</td>
</tr>
<tr>
<td>MTIC</td>
<td>Monomethyl triazenoimidazolecarboxamide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NNK</td>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone</td>
</tr>
<tr>
<td>BF3.OEt</td>
<td>Boron trifluoride etherate</td>
</tr>
<tr>
<td>P&amp;S</td>
<td>Peña and Stille</td>
</tr>
<tr>
<td>PB</td>
<td>Poisson-Boltzmann</td>
</tr>
<tr>
<td>PBD</td>
<td>Pyrrolo[1,4][2,1-c]benzodiazepine</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium dichromate</td>
</tr>
<tr>
<td>PPA</td>
<td>2-Amino-1-phenyl-propan-1-ol</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PTK</td>
<td>Protein tyrosine kinase</td>
</tr>
<tr>
<td>Py</td>
<td>Pyridine</td>
</tr>
<tr>
<td>QSAR</td>
<td>Quantitative structure activity relationship</td>
</tr>
<tr>
<td>Rb</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>RGP</td>
<td>Radial growth phase</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor tyrosine kinase</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
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<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SEM</td>
<td>Trimethylsilylethoxymethyl</td>
</tr>
<tr>
<td>SEM-Cl</td>
<td>Trimethylsilylethoxymethyl chloride</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor – β</td>
</tr>
<tr>
<td>TGI</td>
<td>Total Growth Inhibition</td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TPAP</td>
<td>Tetrapropylammonium perruthenate</td>
</tr>
<tr>
<td>Troc</td>
<td>Trichloroethoxy carbonyl</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>UVR</td>
<td>Ultraviolet radiation</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vascular endothelial growth factor receptor</td>
</tr>
<tr>
<td>VGP</td>
<td>Vertical growth phase</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>WWW</td>
<td>World Wide Web</td>
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INTRODUCTION

Cancer

In the winter of 1872, whilst excavating in the necropolis of Thebes, a wealthy Egyptian approached the German Egyptologist George Ebers, presenting him with a metal case that contained an immaculately preserved papyrus. Little did Ebers realise that this papyrus, which had a length of 20.23 metres, was to shine a new light on our understanding of the medical practices of the ancient Egyptians. This medical ‘textbook’ is the oldest to have been discovered to date* and the papyrus itself dates from the time of Rameses I (around 1700-1352 B.C.) whilst the wealth of information contained within it is believed to be from sources as far back as 4000 B.C.¹

The Ebers papyrus gives us a remarkable insight into ancient Egyptian medicine and from this we see how related their practices are to modern medicine. There are many references, as we would expect, to the woman-doctor and Goddess Isis, yet their understanding of tumours and their approach to treating the various cancers was astoundingly advanced.

* Other equally important medical/pharmaceutical papyruses include the Berlin/Brugsch Major papyrus (1350 BC), the Kennen papyrus and the Edwin Smith papyrus (1600 BC).
Although the papyrus has yet to be fully transliterated, references are found to the use of over 700 herbs. Many of these herbs and plants, such as aloe, cannabis and poppy plant extracts, are familiar to modern medicine. With regards to cancer, which they had identified and characterised, surgery was widely used in the removal of solid tumours, the blood vessels feeding the tumours were burnt and some very interesting concoctions, ingredients of which included copper-shavings, lead and sea-salt were used to treat other tumours not warranting surgery.

By the time of Hippocrates (circa 430 B.C.), ancient Greeks had dispensed with the belief that disease was an action of the Gods. Hippocrates had identified four humours—blood, phlegm, yellow bile and black bile, disease was believed to be an imbalance of these humours; cancer was a result of excess black bile. Although Hippocrates and later Galen described the various cancers in vivid detail, they both believed that tumours in the later stages were best left alone. Only cancers in the early stages were deemed to be curable and in such cases surgical intervention was the treatment of choice.

The works of Hippocrates, his students and Galen would form the basis of medicine and remained unchallenged for over 1500 years. However, during the Renaissance, Paracelsus burned the works of Galen. Despite this, Cancer was still considered to arise...
from an excess of black bile until the 17th century, when William Harvey proved that blood was circulating within our bodies and the theory of the four humours was abandoned. This discovery lead to the birth of modern medicine as we know it today.

Now in the 21st century we have a much better understanding of what causes cancers, what they are and how we could attempt to treat the various conditions that fall within this disease category.

**What is cancer?**

Cancer is a term used to describe any of a large group of neoplastic diseases characterised by the presence of malignant cells and is defined as "neoplasms characterised by the uncontrolled growth of anaplastic cells that tend to invade surrounding tissue and to metastasise to distant body sites".

**Epidemiology of Cancer**

Cancer is a major cause of mortality worldwide. In 2002, it was estimated that 12.6% of all deaths in WHO member countries worldwide were from malignant neoplasms. In Europe 19.2% of all deaths were attributed to malignant neoplasms. This makes Cancer the second major cause of death both worldwide and in Europe.

The mortality rate, however, has been falling over the past decade as can be seen in Figure 2. This may be due to improvements in management and treatment of the various cancers, improved cancer screening programs and increased patient awareness.

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1 Cardiovascular Disease was the 1st major cause of death worldwide (29.3%) and in Europe (52.0%) in 2002.
All cancers Age Standardised Rate (World) age [0-85+] 1950-1999

Figure 2: Age Standardised Rate (World) of all cancer deaths age 0-85+ from 1950 to 1999. Figure shows data for Australia, UK and USA only. Rate is per 100 000 individuals in the population of the given country. (Figure reproduced from WHO/IARC WWW database)

Aetiology of Cancer

Cancer is a multi-causal disease. Some of these include radiation, diet, chemicals, viruses and the inheritance of faulty genes.

\(^2\) A comprehensive list of carcinogens is available from IARC
Dietary and environmental factors

There are many reports of diet and links to cancer in the media; sometimes exaggerated and sometimes backed by strong evidence. For instance, animal fats have been proven to increase the risk of cancer whilst a high fibre diet has been shown to help prevent colon cancer.

There are numerous chemicals present in the environment which are carcinogenic and they include asbestos, compounds of beryllium, cadmium, chromium, nickel and lead, arsenic, aromatic amines (found in dyes), amino azo-dyes, dialkylcarbamates and alkylating agents (which, ironically, are used to treat cancers!)².

Carcinogenic chemicals can be found in cigarette smoke (Figure 3) and coal tar (Figure 4).

Another potent carcinogen found in cigarette smoke is 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). This compound is easily passed onto an unborn baby and metabolites of this compound have been found in the urine of newborn babies ¹⁰. Not only cigarette smokers are at high risk, marijuana smoke has been found to increase DNA
mutations by 300%. The carcinogen is most likely the mood-altering ingredient – THC (tetrahydrocannabinol).

Other carcinogenic chemicals are dioxin, possibly radon gas, and chloroform, that is formed when water is chlorinated. Therapeutically useful drugs can also be carcinogens. Alkylating agents have been mentioned above and oral contraceptives (e.g. norethisterone and levonorgestrel) are well known to increase the risk of breast cancer by 20% during the course of administration. Cyclosporin has been found to increase production of TGF-β, a growth factor that makes cells more likely to divide and spread. Finally, the drug Orlistat, a pancreatic lipase inhibitor used in the management of obesity, is believed to cause breast cancer.

Viruses and bacteria

Several DNA and RNA viruses have been linked to some cancers. Examples include the Epstein-Barr virus, which can cause nasopharyngeal cancer, hepatitis B, which can cause hepatocellular carcinoma, and HSV-2 and HPV, which can cause cervical cancer.

Some bacteria have also been found to cause cancer. Helicobacter pylori has been implicated in gastric cancer, sulphur bacteria in the gut can cause colon cancer and nanobacteria are thought to be carcinogenic too.

Radiation

UV radiation is a natural source of radiation that we are all exposed to, but although it is a weak source of radiation, it is very capable of producing mutations within the DNA of skin cells (such as melanocytes), especially in fair skinned subjects. Most of the DNA damage that we sustain from exposure to UVR is repaired fairly quickly (~24 hours). However, repeated high UV exposure over many years causes mutations that gradually accumulate within cells and eventually lead to the formation of tumours.
Solar UVR catalyses a number of reactions within our DNA including cyclobutane-type pyrimidine dimers (Figure 5), pyrimidine-pyrimidone photoproducts, thymine glycols, cytosine damage, purine damage, DNA strand breaks and DNA-protein cross links.\\(^{22}\)

![UV crosslink formation (UVB)](image)

Figure 5: Mechanism for the formation of UV-induced DNA cross-linking

The cross-linkage of DNA strands leads to mutations, which if not repaired, contribute to a cancerous phenotype.

X-ray/ionising radiation is powerful enough to actually break covalent bonds. The damage sustained by the DNA from such radiation occurs through strand breaks in single or double bonds. Ionising radiation has long been known to cause cancers such as leukaemia and multiple myeloma and studies have shown a correlation between the doses of radiation and deaths from these cancers\\(^{24}\). Ionising radiation can also cause a form of delayed cell damage known as genomic instability. For instance, a parent who was exposed to radiation can pass on the broken and distorted chromosomes to his or her
progeny. This renders the descendants more vulnerable to carcinogens i.e. more likely
to develop cancers such as lymphomas and leukaemia.

**Inheritance of faulty genes**

Cumulative exposure to the agents detailed above leads to alteration of key homeostatic
genes (i.e. activation of proto-oncogenes and deactivation of tumour suppressor genes). However, it is also possible to inherit faulty genes. The hereditable condition *xeroderma pigmentosum* involves the passing of defective chromosomes from one generation to another. This results in the patient developing multiple solar keratoses, amongst other symptoms, which undergo malignant change at an early age, after limited exposure to sun light.\(^\text{25}\)

**Melanoma**

Malignant cutaneous melanoma is a cancer that still claims many lives every year. The incidence and mortality rates of this aggressive cancer are on the increase throughout the world. Over the past few decades incidence has increased by 3-7% in fair-skinned subjects with mortality increasing at a slightly slower rate.\(^\text{26}\).

Ultraviolet light creates mutations where a pyrimidine base, cytosine or thymine, lies adjacent to another pyrimidine. About two thirds of these mutations are C to T substitutions, and about 10 percent of these changes occur at two adjacent cytosines, with both bases changing to thymidines.\(^\text{27}\).

Cutaneous melanomas arise from epidermal melanocytes. Melanocytes are responsible for the synthesis of melanin (a black-brown pigment that is distributed to surrounding keratinocytes in the skin via dendritic projections). Melanin exists for the purpose of protecting the skin from the damaging effects of UV radiation, which is why fair-skinned subjects are more prone to developing melanomas.

Normally, melanocytes do not readily proliferate or undergo apoptosis. Melanocytes contain a large number of anti-apoptotic proteins such as Bcl-2. This is probably Nature's
way of ensuring that these melanin cells are present even after UV-damage. However, this leads to an increased risk of DNA-mutation without destruction of the damaged cell.

![Figure 6](image-url)

Figure 6: Photograph of a woman with vitiligo, showing the photo-protective effect of epidermal melanin. This woman was exposed to midday summer sun for approximately one hour three days before this photograph was taken. Her normal skin has begun to tan and is asymptomatic. In contrast, the depigmented areas affected by vitiligo and hence lacking melanocytes are severely sunburned, with erythema and the beginning of desquamation, and are painful even to light touch.

This can cause the melanocytes to cross the epidermal basement membrane, where they normally reside, into the dermis, where they proliferate to give rise to junctional nevi. The presence of multiple nevi has been associated with an increased risk of melanoma.

The progression of melanoma involves five major steps:

1. The development of acquired and congenital nevi with structurally normal melanocytes e.g. freckles. These have a finite lifespan and generally don't carry any cytogenetic abnormalities.
2. Development of dysplastic nevi with structural and architectural atypia e.g. moles. The density of these nevi correlates with melanoma risk.
3. The development of biologically early radial growth phase (RGP) primary melanoma without any metastasis.
4. The development of advanced vertical growth phase (VGP) primary melanoma in which the cells have invaded the dermis and have the potential to metastasise.
5. Finally, metastasis of the primary melanoma to other sites (Figure 7b).
Because malignant melanoma rapidly metastasises to other areas in the body such as the liver, brain, bone, lymph nodes and lungs there is a very poor prognosis associated with malignant melanoma. This merely emphasises the need for the development of agents that can be used as adjuvants to surgery in the management and treatment of this aggressive cancer.

**Mechanism of Tumour Formation**

Three proposed mechanisms of tumour formation are:

**Mutation - the loss, substitution and/or rearrangement of DNA in a cell.**

e.g.

<table>
<thead>
<tr>
<th>mRNA code</th>
<th>Protein氨基酸 sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU CAU GAC GAG</td>
<td>Phe His Asp Glu</td>
</tr>
<tr>
<td>UUU CAG ACG AGU</td>
<td>Phe Gln Thr Ser</td>
</tr>
<tr>
<td>UUA UCA UGA CGA</td>
<td>Leu Ser STOP</td>
</tr>
</tbody>
</table>

Figure 8: Examples of how a mutation can alter the genetic code
In any of the above cases, a mutation will, as indicated in Figure 8, lead to alterations in the genetic code and consequently may affect cellular function and cause cancer.

Addition of new genetic material.

Tumour promoting viruses can introduce viral DNA into the host cell, which is then integrated into the host’s DNA, activating proto-oncogenes or deactivating tumour suppressor genes.

Altered gene expression

Unlike the previous two mechanisms, this is not an alteration of the base sequence and hence not an alteration in the genetic code. This mechanism involves a change in the way a cell expresses/uses the genetic information. For example, chromosomal translocation results in the intact c-Myc gene being placed under the control of an immunoglobulin promoter, leading to inappropriate c-Myc expression.  

In cancer cells, the general outcome of any of the above mechanisms is:

- Alteration in cellular membranes.
- The appearance of abnormal or inappropriate gene products.
- Alteration in levels of certain cellular proteins (usually enzymes).

The Six Hallmarks of Cancer

As mentioned earlier, cancer is a term that is used to describe a collection of about 200 conditions. However, these various conditions generally have six main ‘hallmarks’ in common. These are referred to as the six acquired capabilities of cancer cells and are discussed below.
The Cell Cycle

Cellular replication is controlled by the cell cycle (Figure 9). When a normal cell stops growing, it will leave the cell cycle at G1 and go into the 'resting' state - G0. Only rarely will the cells go into G0 from another part of the cycle. The anti-proliferation (cellular quiescence) of normal cells is tightly controlled by antigrowth factors which include both soluble growth inhibitors and immobilized growth inhibitors that are normally found embedded within the extracellular matrix on neighbouring cell surfaces. Extracellular growth inhibition signals act on receptors that are coupled to intra-cellular signalling circuits.

Figure 9: The Cell Cycle showing some of the factors involved in the regulation of this cycle

For a normal cell to shift from quiescence (G0) into an active proliferative state, transmembrane receptors (often a receptor tyrosine kinase (RTK) such as EGFR or IGF-IR but G-coupled receptors can also be involved) need to receive mitogenic growth signals. Growth signalling can be mediated by extracellular matrix components, diffusible growth factors or cell-cell adhesion/interaction molecules.
Acquired Capability #1 – Proliferation

Tumour cells appear to acquire the capability to short-circuit the normal homeostatic regulation of proliferative signals by producing their own growth signals, whilst the more devious of cancer cells will induce neighbouring cells to release an abundant supply of extracellular growth-stimulating signals. Figure 10 shows activation of an RTK by an extracellular growth signal/factor. Specifically, the RAS pathway is shown, but this is just one example of a number of pathways that could be activated by the abundance of growth factors (Figure 11).

Figure 10: Activation of the RAS pathway by an external growth factor
Acquired Capability # 2 – Insensitivity to antigrowth signals

Once a tumour has become self sufficient in growth factors, it still needs to circumvent the anti-proliferative signals that are also present. These tumour-suppressor proteins can be likened to quality control inspectors in the progression of a cell through its cycle (Figure 9). With respect to cancer, the most important and well documented tumour suppressor proteins are p53 and retinoblastoma (Rb). The p53 and Rb pathways (Figure 11) have been found to be inactivated in virtually all cancer cells. Over activation of the RAS pathway in normal cells will lead to the activation of both the p53 and Rb pathways which in turn leads to cell-cycle arrest. Tumour cells have overcome the problem of p53 and Rb by inactivating the proteins themselves. For instance Rb in cancer cells is hyperphosphorylated. This renders it incapable of suppressing E2F activity and allows the cell cycle to progress.

Figure 11: “Subway” diagram of the molecular circuitry of cancer. The diagram shows a selected number of pathways for clarity. Adapted from poster by Hahn, W. C. and Weinberg, R. A. (2002).
Acquired capability # 3 – Evading Apoptosis

Virtually all normal cells in the body possess an apoptotic program (Figure 12) in a dormant state. This apoptotic program is regulated through death sensors and effectors (e.g. FAS & FAS receptor and TNFα & TNF-R1)\(^3\). When the effector (e.g. TNFα) binds to the cellular death receptor (e.g. TNF-R1) the death pathway is triggered. This involves a cascade of signals that cause the release of Cytochrome C (Cyt C) which is a trigger for apoptosis\(^3\). The end result of apoptosis is:

- Disruption of cellular membranes
- Disintegration of cytoplasmic and nuclear skeletons
- Extrusion of the cytosol
- Degradation of chromosomes
- Fragmentation of the nucleus

The apoptotic process is extremely rapid, often taking less than two hours to reach completion.

---

**Figure 12: Pathways of apoptosis (Adapted from poster ‘Death of a Cell: Pathways of Apoptosis’ by Calbiochem)**
The p53 tumour suppressor protein is a significant player in pro-apoptotic regulation. It acts by upregulating the expression of Bax when DNA damage has been detected. Bax is a regulator that acts on the mitochondria causing the release of Cyt C.

In tumour cells the chances are that the DNA has sustained significant damage, a situation that would normally lead to the activation of the death pathway. These cells therefore need to protect themselves by disarming apoptosis. The most common way to achieve this is through mutation of the p53 tumour suppressor gene. The inactivation of the p53 protein has been found in over 50% of human cancers.

Acquired Capability # 4 – Angiogenesis

All living cells require a healthy supply of oxygen and nutrients. They achieve this by being in close proximity (up to 100 μM) to a blood vessel. Angiogenesis (the process of new blood vessel growth) is a normal process during development to ensure that new tissue and organs receive an appropriate blood supply.

The angiogenic process is tightly regulated and tumours in the early stages of development do not seem to possess the ability to encourage angiogenesis. For the tumour to grow larger it needs to be able to recruit a new capillary network. Established tumours secrete vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF1/2). Both these growth factors are involved in angiogenesis by binding to their receptors on nearby capillary cells which in turn activates the pathways required to begin tumour vascularization.

Acquired capability # 5 – Immortalization

Each chromosome possesses telomeres in order to prevent its ends from being damaged or engaging in inappropriate combination. Telomeres are made up of several thousand repeats of a small (6 base pair) sequence. With every cellular replication each chromosome has to "pay" with the loss of about 50-100 base pairs of this telomeric DNA. When a cell's chromosomes have lost their telomeric DNA, the chromosome ends are left
vulnerable. The consequence of this is end-to-end fusion of the chromosomes leading to karyotypic disarray and eventually cell death. This process of 'mortalization' is yet another way for nature to prevent genomic instability – as the frequency of mutations is proportional to the number of cell divisions.

The majority of tumour cells (85-90%) however, successfully use the enzyme telomerase to maintain their chromosomal telomeres thereby achieving immortality\(^1\). Telomerase counteracts the process of telomere erosion by adding hexanucleotide repeats onto the ends of telomeric DNA. The remaining 10-15% of tumour cells also maintain their telomeres, but through a mechanism called ALT that is believed to involve recombination-based inter-chromosomal exchanges of sequenced information\(^2\).

Acquired Capability # 6 – Metastasis

This is probably the most significant of the six hallmarks of cancer as far as the patient is concerned. The metastases of the primary tumour cells are the cause of 90% of cancer deaths in humans\(^3\).

The processes involved in metastases are complicated and have not been fully elucidated. Cells from the primary tumour mass break off and cross the extracellular matrix making their way to a neighbouring blood or lymphatic vessel. They then uses this fast flowing medium to travel to other distant tissues in the body, where they can settle and begin to form the metastasis.

Numerous proteins have been identified as key players in metastasis. E-cadherin, a homotypic cell-to-cell interaction molecule, is one such protein. E-Cadherin forms bridges between neighbouring cells\(^4\). However, the formation of these bridges results in antigrowth signal transmission, an undesirable event for the tumour. The alteration of this protein has been widely observed in tumour cells.

Other key proteins involved with the suppression of metastases include cell-cell adhesion molecules (CAMs)\(^5\) and integrins. These too have been found to be altered in tumour cells possessing invasive and metastatic capabilities.
Additionally extracellular proteases are thought of being key players as these enzymes are found to be active in aggressive cancers and allow them to degrade the surrounding extracellular matrix (ECM).

**Treatment of cancer**

Approaches to the treatment and management of cancer are summarised in Table 1.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TARGET</th>
<th>HOW THEY WORK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-angiogenesis factors</td>
<td>multiple</td>
<td>A growing tumour requires plenty of nutrients, and to make sure it gets them the tumour secretes substances that stimulate the growth of new blood vessels. A number of agents can block this process.</td>
</tr>
<tr>
<td>Anti-metastatic factors</td>
<td>multiple</td>
<td>Metastasis of a cancer is often fatal. A class of enzymes that enables cancer cells to enter the blood stream by dissolving tissue and boring holes through capillary walls has been identified. Drugs could therefore be targeted at these enzymes to prevent metastasis.</td>
</tr>
<tr>
<td>Anti-oncogenic factors</td>
<td>multiple</td>
<td>Tumours make growth factors by switching on oncogenes. Many cancers have a mutation in the RAS oncogene and drugs are being developed that inhibit it's growth promoting activity.</td>
</tr>
<tr>
<td>Chemoprevention therapies</td>
<td>breast, head and neck</td>
<td>Many breast cancers depend on the female sex hormone oestrogen to stimulate their growth. Tamoxifen can prevent the development of this form of cancer. Retinoids can prevent the recurrence of certain head and neck cancers.</td>
</tr>
<tr>
<td>Gene therapies</td>
<td>multiple</td>
<td>Viruses are altered to 'infect' cancer cells with healthy tumour suppressor genes.</td>
</tr>
<tr>
<td>Cytotoxics</td>
<td>multiple</td>
<td>More selective and therefore less toxic drugs are being developed. Drug delivery systems are also being treid in an attempt to decrease toxicity.</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>non-hodgkins lymphoma, breast, colon, melanoma.</td>
<td>These home in on specific proteins displayed on the surface of cancer cells. By these sites monoclonals can interfere with a tumours ability to absorb growth factors from the bloodstream.</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>multiple</td>
<td>Radiation destroys cancerous cells but can damage healthy ones aswell. Improved techniques are being developed</td>
</tr>
<tr>
<td>Surgical procedures</td>
<td>multiple</td>
<td>A common treatment but very invasive. Improvements are being looked at.</td>
</tr>
<tr>
<td>Vaccines</td>
<td>melanoma, breast, colon, ovarian, pancreatic and many others.</td>
<td>By vaccinating patients with antigens derived from tumours we can sometimes encourage white blood cells to attack cancer cells.</td>
</tr>
</tbody>
</table>
Surgery

This is often accompanied by other forms of treatment with the purpose of improving the chances of survival. It is really only effective when the tumour has not metastasised and it is not a suitable form of treatment in some cancers such as leukaemia or where the primary tumour is inoperable. The process involves physically removing the tumour and in some cases the complete organ e.g. mastectomy in the treatment of breast cancer. Following surgery (and sometimes prior to surgery) other treatments are used such as chemotherapy and photoradiation therapy in an attempt to shrink the tumour or kill any remaining cancer cells that were not successfully removed.

Radiation therapy

Radiation therapy is better than surgery in that it can effectively destroy solid tumours with minimal damage to healthy tissue. This makes it, therefore, the treatment of choice for many cancers. Radioisotopes such as cobalt-60 are used as a source of γ-rays whilst x-rays are also used in this technique.

The use of this technique is limited however, since ionising radiation is also carcinogenic. Using radiation sensitizers such as razoxone can increase the effectiveness of the treatment without increasing the dose.

Immunotherapy

Immunotherapy is used to boost the patient's immune system following surgery, radiotherapy or chemotherapy. The currently used immunotherapies are active non-specific therapies since they actively boost only general immune responses and stimulate macrophages.

Many vaccines, containing antigens derived from tumours, are currently being developed and these should prove to be more specific than older therapies.
Cancer chemotherapy

Monoclonal antibodies

These antibodies are highly specific for cancer cells. They home in on proteins displayed only on cancer cell surfaces. By attaching to these proteins, they can alter the tumour's ability to absorb growth factors from the systemic circulation therefore halting further growth of the tumour. Radioactive and chemical toxins can also be attached to these antibodies. These toxins will only be activated at the site of action and should therefore destroy only the cancer cells.

Anti-metastatic agents

As with monoclonal antibodies, these agents are still undergoing trials. These agents inhibit the enzymes that dissolve tissue and bore holes in capillary walls. This could prevent tumours from metastasising and hopefully decrease mortality rates given that two out of three cancer patients die from secondary tumours.

Antimetabolites

These compounds interfere with the formation or utilisation of an essential cellular metabolite.

There are two classes of antimetabolites, enzyme inhibitors and natural macromolecule mimics. Methotrexate is a dihydrofolate reductase (DHFR) inhibitor.

(Figure 13: methotrexate - a dihydrofolate reductase (DHFR) inhibitor)

Folic acid is reduced and then converted to tetrahydrofolic acid (FH4) by DHFR. FH4 is needed to form the formyl and methyl donors essential for many biological functions,
including the synthesis of DNA. Therefore, methotrexate prevents the formation of formyl and methyl donors and will eventually lead to apoptosis.

**Protein tyrosine kinase (PTK) inhibitors**

PTKs play a critical role in many crucial cellular processes, such as cell growth (EGFR), survival (IGF-IR) and angiogenesis (VEGFR). Consequently, the design synthesis and evaluation of PTK inhibitors has attracted a great deal of interest and a number of potential new agents are currently being evaluated with two PTK inhibitors (Gleevec and Iressa) already in the clinic.

Imatinib mesylate (Gleevec; Figure 14) binds in the ATP binding site of the Bcr-Abl tyrosine kinase. The Bcr-Abl tyrosine kinase is an enzyme found in chronic myeloid leukaemia cells and is a product of the Philadelphia chromosome abnormality. By inhibiting this PTK, imatinib mesylate effectively inhibits proliferation and induces apoptosis of the cancerous cells.

![Figure 14: The PTK inhibitor 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate (Gleevec).](image)

Gefitinib (Iressa – Figure 15), an anilinoquinazoline, is another PTK inhibitor. This inhibitor targets the EGF receptors that are, as mentioned previously (Acquired Capability # 4 – Angiogenesis), found to be over expressed in many cancer cell lines. Like Gleevec, this inhibitor competes with ATP for the ATP binding site.
Antimitotic agents

These agents are mostly naturally occurring compounds. The alkaloids vinblastine and vincristine (Figure 16) bind to tubulin in the mitotic spindle, preventing polymerisation and assembly into microtubules. Another antimitotic agent Taxol (Figure 17), however, promotes the assembly of microtubules and stabilises them against depolymerization (depolymerization following mitosis is just as important as polymerisation prior to mitosis).48

The net result of both types of antimitotic agents then, is the prevention of cell division and these agents are, therefore, cytotoxic.

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Figure 15: The PTK inhibitor 4-quinazolinamine, N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-4-morpholino]propoxy (Iressa).

Figure 16: The structure of vincristine and vinblastine.

Figure 17: The antimitotic agent Taxol.
Antisense technology

Antisense oligonucleotide drugs are designed to block the production of specific proteins at the mRNA level\(^{49}\). These drugs are oligonucleotides, resembling portions of DNA that are complementary to a specific portion of the RNA that codes for the target onco-protein. The RNaseH enzyme recognises the RNA/DNA duplex formed and degrades the RNA strand. The DNA strand is then free to target another strand of mRNA. By fooling the cell in this manner, translation of onco-proteins can be prevented\(^{50}\).

Gene therapy

Gene therapy is defined as 'the transfer of new genetic material to an individual to change that individual’s genetic make-up'\(^{51}\). Gene therapy could be particularly useful in returning functional copies of tumour suppressor genes, e.g. p53, to cancer cells that possess deactivated tumour suppressor genes.

Gene therapy and antisense-technology are very promising cancer treatments since they act at the molecular level to cure cancer. But there are still many problems associated with these therapies such as delivering the drugs to the site of action and controlling the genes when they are at the target cell.

DNA targeted agents

DNA targeting agents have been in the front line of the war on cancer since the advent of cancer chemotherapy in the late 1920's.

DNA intercalating agents and topoisomerase poisons

There are numerous groups of intercalators with a wide variety of structures but they share the following pharmacophore:

a) A planar aromatic chromophore, which slides (intercalates) between the DNA bases when the DNA is breathing.
b) Positively charged atoms such as N+. These help to stabilise the intercalator by interacting with the polyanionic backbone.

c) Hydrogen bonding sites - either donor or acceptor sites or both. Hydrogen bonds hold the DNA strands together and the intercalator hydrogen bonds with the DNA bases thereby reducing the level of disruption to the helix. Hydrogen bonds also prevent the drug from sliding out of the helix, i.e. stabilisation is increased.

Interaction occurs either in parallel mode - where the planar ring system slides into the helix parallel to the base pair axis - or perpendicular mode - where the long axis of the drug lies perpendicular to the base pair axis. The parallel mode is the more stable of the two since there is maximum overlap of the bases and the drug, but only drugs that have three rings or less can actually fit into the DNA by parallel mode.

The mechanism of their anti-tumour activity is distortion of the DNA helix thereby preventing further transcription and replication.

Examples of intercalators include doxorubicin and mitoxantrone. Doxorubicin is an anthracycline with a sugar moiety attached to ring A, which works by intercalating perpendicularly into the DNA. The quinone functions can also form free radicals and this could account for the cardiotoxicity that this drug causes. Doxorubicin is also a topoisomerase poison.

Mitoxantrone is an anthraquinone. The intercalation is stabilised by hydrogen bonds between the two hydroxyl groups and the DNA bases. The ionisable nitrogens on the side chains help to anchor the drug to the DNA by interacting with the phosphate backbone. Free radical formation is not as easy with mitoxantrone as it is with doxorubicin and this could account for the fact that mitoxantrone has less cardiotoxicity. Mitoxantrone has a slight G-C preference.

Another intercalator and topoisomerase poison is DACA (N-(2-(Dimethylamino)ethyl)acridine-4-carboxamide). This intercalates in the parallel mode. As a result, the hydrogen bonds of the base pairs are virtually undistorted. DACA is one
of the few intercalators that can interact with the major groove of B-DNA\(^2\). The ionisable nitrogen on the side chain of DACA hydrogen bonds with N7- of Guanine in the major groove while the aromatic amine nitrogen interacts with a water molecule that is hydrogen bonded to the phosphate backbone (Figure 18).

![Figure 18: DACA showing the interactions involved during intercalation\(^2\)](image)

Intercalators generally follow the neighbour exclusion principle\(^3\), where the intercalator occupies every other site on the DNA double helix. However, DACA is an exception. DACA appears to be able to occupy neighbouring sites as well\(^2\).

*Alkylating agents*

These are highly electrophilic agents and will react with nucleophilic centres such as the oxygens and nitrogens in DNA bases. They are quite often bifunctional and will therefore cross-link a DNA double helix.

They were the first cancer chemotherapy agents and they originated from mustard gas. This was too toxic to use as an anticancer agent so the sulphur was replaced with nitrogen to give a less reactive mustard.

Cyclophosphamide (Figure 19) is an example of a contemporary alkylating agent. Cyclophosphamide was designed as a pro-drug to be activated by phosphoramidase but in fact it decomposes to give two cytotoxic agents - acrolein (which carbamoylates proteins such as topoisomerase) and normustine (an alkylating agent). Nitrosoureas also decompose to give an alkylating agent (e.g. a carbocation) and an isocyanate derivative.
Cisplatin is not strictly speaking an alkylating agent but as a metalating agent. Nevertheless, it is believed to work by a similar mechanism to alkylating agents i.e. it cross-links DNA inhibiting DNA replication and hence cell proliferation ultimately resulting in apoptosis. Cisplatin is used in the clinic to treat certain tumours such as ovarian cancer and testicular teratoma.

Unfortunately, these cytotoxics have little or no selectivity for tumour cells, generally targeting any fast growing cells. As a consequence, they are toxic to bone marrow, cause immunosuppression and can be carcinogenic and mutagenic.

The future of cancer chemotherapy

There has been an astounding amount of progress in the treatment of cancer over the last few decades. Clinicians are investigating and applying many more non-chemotherapy approaches to effectively treating cancer patients. However, there is still the need for chemotherapy agents in the clinic. Chemotherapy is a non-invasive treatment and the development of personal drug-delivery units allows many patients to self-administer their medicine, something that is obviously not possible where other treatment options are concerned. Additionally, chemotherapy is still a very effective treatment option. A number of cancers respond positively to the numerous cytotoxic and cytostatic agents already in the clinic. However, there still remain many cancers that are aggressive and are difficult to treat (e.g. Melanoma and Renal cancer). There is therefore a need to develop effective chemotherapeutic agents that target these aggressive cancers.

Figure 20 summarises the current targets for the development of new more effective chemotherapy agents.
Metastasis and Invasion

Although a process still under investigation, recent drug development is aimed at inhibiting matrix metalloproteases and integrin, both found in abundance in metastasising and invasive tumours.

Angiogenesis

A popular target for new drug therapies, the aim is to cut off the blood supply to tumour cells and thereby starving them of nutrients and oxygen.

Apoptosis

Re-establishing the apoptotic pathways in tumour cells would theoretically reduce the ability of the cancer to survive. New drug development studies are targeted at various effectors of the apoptotic mechanism.

Proliferation

To block growth factor induced mitogenic signals we could:
- Inhibit the growth factor receptor
- Inhibit receptor activation with ATP-mimics that bind to the intracellular domain of the receptor
- Blocking cytoplasmic proteins thereby inhibiting completion of the signalling pathway

Cell Cycle progression

Inhibition of cells at any point in the cell cycle inhibits progression and hence prevents mitosis and cell division. Many established cytotoxics cause a cell cycle block at the S or G2/M phases. New development of drugs targeting the cell cycle are targeted at more specific components of the key process.

Immortalization

Inhibition of telomerase will in theory inhibit the acquired capability of immortalization within a large number of tumour cells.

DNA Replication

Inhibition of this key step ensures that tumour cells do not complete the cell cycle. DNA Intercalators, Topoisomerase inhibitors and nucleoside analogues all act to halt this process.

Figure 20: Current chemotherapy targets
The Pyrrolobenzodiazepines

In 1965, Leimgruber and his co-workers isolated an antibiotic from a thermophilic actinomycete, *Streptomyces refuineus*. This actinomycete had previously been shown (by Tendler & Korman) to produce a fermentation broth that exhibited antitumour activity.

When Leimgruber *et al.* characterized this antibiotic, they found it to be a fused ring system consisting of a pyrrole ring, a benzene ring (bearing resemblance to anthranilic acid) and a diazepine ring with a C-ring substituent. They therefore named the molecule *anthramycin* and this new group of antitumour antibiotics, the *pyrrolobenzodiazepines* (PBDs).

Owing to the remarkable antitumour activity exhibited by anthramycin, many groups were quick to embark on the search for other PBDs both naturally occurring and synthetic.

In 1972 Arima and co-workers reported the isolation of tomaymycin from *Streptomyces achromogenes var. tomaymyceticus*. 1972 also saw the discovery of sibiromycin by Brazhnikova and co-workers from *Streptosporangium sibirium* although this work was to be challenged later by Leber *et al.* in 1988.

In 1976 the isolation and characterisation of the neothramycins (A & B) was reported by Takeuchi and co-workers. Four years later mazethramycin was discovered, a compound structurally similar to anthramycin. During the 1980s, many new PBDs came to light. Shimizu *et al.* isolated prothracarcin from *Streptomyces umbrosus*, a Japanese patent, filed by Kyowa Hakko Kogyo Co. Ltd in 1983, reported the isolation of DC-81.

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*§ Leber *et al.* argued that the structure of sibiromycin as was previously reported would not have the required right-handed twist as the C-ring was fully unsaturated. If this was the structure of sibiromycin, then biological activity would be greatly reduced and in this is not the case. They therefore proposed a new structure for this PBD which they confirmed by the total synthesis of the aglycone segment. To date, a total synthesis of sibiromycin has not been reported.*
from *Streptomyces roseiscleroticus* whilst the following year, Konishi and co-workers\textsuperscript{65} isolated and characterised chicamycin.

It was three years before the isolation of the next naturally occurring PBD, abbeymycin, by Hochlowski and co-workers\textsuperscript{66}. In 1988 Tsunakawa and co-workers\textsuperscript{67} isolated porothramycin from *Streptomyces albus* and finally, again in 1988, Hara *et al.* isolated DC-102 (sibanomycin) from a culture broth of *Streptomyces*\textsuperscript{68}.

![Chemical structures of PBDs](image)

*Figure 21: Structures of the naturally occurring PBDs*

Much of the interest in PBDs stemmed from their biological properties. PBDs are potent antitumour agents (especially the naturally occurring PBDs) yet they provoke minimal bone marrow depression\textsuperscript{57}. They are relatively small molecules that fall into the group of alkylating agents introduced in the previous chapter. They exert their antitumour activity
by covalently binding to the DNA in the minor groove and, due to their shape, they cause minimal disruption to the DNA helix. It is believed that the formation of the DNA-PBD adduct inhibits nucleic acid synthesis and causes excision-dependent single and double stranded breaks in the DNA helix. Anthramycin has been found to inhibit the action of several restriction endonucleases\(^{69}\).

**Structure and Mode of Action**

PBDs are named and numbered according to the system devised by Leimgruber in 1965. The numbering of the PBD skeleton is illustrated in Figure 22.

![Figure 22: The Leimgruber numbering system for the PBDs](image)

All PBDs exist in either an imine, carbinolamine methyl ether or a carbinolamine form at the N10-C11 position. This electrophilic moiety is essential for the antitumour activity of these molecules

![Figure 23: Diagram illustrating the interconversion of the imine (B) to the carbinolamine methyl ether (A) and the carbinolamine (C)\(^9\).](image)

The interconversion of these forms is a dynamic equilibrium, but the nature of the predominant species is dependent on the structure of the compound, the synthetic work-up or the method of isolation (in the case of the naturally occurring PBDs). The imine functionality is the most reactive of the three species as was shown in a study of anthramycin, tomaymycin and neothramycins.\(^{69}\) Neothramycins are isolated almost
exclusively as the imine forms and readily react with nucleophiles. Tomaymycin is slightly slower in its reaction with nucleophiles; it is easily converted to the imine form from the carbinolamine methyl ether\textsuperscript{70}. Tomaymycin reacts faster than anthramycin, though, which is not so easily converted from its methyl ether form.

**Binding of PBDs to DNA**

The PBDs differ from the majority of DNA binding ligands in that they form an acid labile covalent bond with the exocyclic guanine-N2 (Figure 24). Of course, this is not the only interaction that they exhibit but it is the main mode of action (Figure 27).

![Figure 24: Schematic representation of a proposed mechanism of action for the covalent linkage between the PBD-C11 and the guanine-N2 of DNA\textsuperscript{69}.](image)

The PBDs, although relatively small molecules, have a preference for PuGPu sequences in DNA\textsuperscript{71}. However, the covalent bond formation between the electrophilic N10-C11 and Gua-N2 does not account for their selectivity for this specific three base pair sequence.

The first point to note is the preferred orientation of PBDs in the minor groove (Figure 25). From the PBDs studied, the majority prefer to bind in a manner as indicated in B with the A-ring orientated towards the 3' end of the bound DNA strand and with S-stereochemistry at the C11 position that is covalently bound to DNA\textsuperscript{72-74}. 
NMR studies that investigated the reaction of a number of PBDs with the nucleophile thiophenol found that this nucleophile would initially attack from the β-face of the molecule. This gave rise to the kinetically preferred C11(S) adduct (Figure 26). Over time, the formation of the thermodynamically preferred C11(R) adduct was observed, in increasing concentrations. However, in the studies mentioned above, the C11(S) PBD-DNA adduct is the predominant species. It was therefore concluded that covalent bond formation would only occur when the molecule is in close proximity to a guanine base. The guanine – N2 may then attack from the β-face of the PBD. Once a PBD-DNA adduct is formed the various hydrogen bond and Van der Waal's interactions hold the molecule in place, discouraging epimerisation to the thermodynamically stable form.⁶⁹
A model has been proposed that helps to explain the orientation of the A-ring towards the 3'-end and at the same time partly explain the sequence selectivity of these molecules (Figure 27).

After formation of the guanine N2-C11 bond the N10 proton is in a position to form hydrogen bond with a hydrogen-bond donor such as adenine N3. The adenine nitrogen is in close proximity to the N10 proton, a situation not observed if the A-ring was orientated towards the 5'-end of the DNA strand. This model is not applicable to all PBDs of course. The stereochemistry of the guanine N10-C11 bond and the orientation of the molecules in the minor groove may be affected by the structure of the PBD itself.
It is known for instance, that guanines on the 5' side are not preferred as guanine's NH$_2$ group, that protrudes from the minor groove floor, causes steric interactions with the PBD C-ring, especially if substituents are present$^{69,74}$.

Unlike the A-ring, where substituents do not appear to have any positive or negative interactions with DNA bases, molecular modelling studies suggest that C-ring substituents may indeed interact with nearby functional groups of DNA bases$^{69}$.

**The PBD Pharmacophore**

Five major features of the PBDs have been identified as making a significant contribution to the pharmacophore of these compounds (Figure 28)$^{69}$. The most important feature is the electrophilic centre at the N10-C11 position.

The presence of an imine, carbinolamine or carbinolamine methyl ether is essential for DNA binding, as discussed previously. Dilactam PBDs are incapable of covalently binding to DNA, although they do form non-covalent interactions$^{69}$. Nevertheless, their biological activity is greatly reduced.

Equally important is the stereochemistry of the C11a-centre on the PBD skeleton. It is this S-stereochemistry that gives these molecules their right-handed twist; a characteristic that renders them isohelical with B-DNA. Synthetic PBDs with C11(R) stereochemistry exhibit a negligible DNA binding affinity and greatly reduced cytotoxicity$^{75}$. 
Another important feature of the PBD skeleton is the nature of the substituents on the aromatic A-ring. Electron donating substituents increase the electrophilicity of the N10-C11 centre\(^5\), whilst electron-withdrawing substituents attenuate biological activity arising from covalent DNA binding. Bulky substituents at C9 and N10 also eliminate biological activity and DNA-binding affinity of the PBD. Additionally, an OH group at the C9 position (as in anthramycin) has been found to be the responsible for the cardiotoxicity shown by anthramycin and related molecules.

Sibiromycin exhibits enhanced DNA binding affinity and cytotoxicity in certain cell lines compared with similar PBDs without the sugar at C7. This leads to the conclusion that a sugar at C7 enhances the biological activity of PBDs.

A significant increase in DNA-binding affinity, cytotoxicity and \textit{in vivo} antitumour activity has been observed when there is unsaturation present in the C-ring, specifically when C2 is \(sp^2\) hybridised, i.e. by the inclusion of an exocyclic double bond or a C2-C3 double bond\(^6\). On the other hand, however, complete unsaturation of the C-ring inactivates the compounds because of the involvement of the N10-C11 centre in the conjugated system, which leads to a decrease in the electrophilicity of this centre\(^5,7\). Interestingly, the recently synthesised C1-C2 \textit{endo} unsaturated C2-aryl PBD\(^8\) is less cytotoxic than the C2-C3 \textit{endo} unsaturated C2-aryl PBDs discussed in this thesis\(^9\).
PBD synthesis

A number of synthetic approaches to PBD synthesis have been developed over the last 30 years; Figure 29 shows the latter stages of some of the approaches that may be adopted for the synthesis of the PBD target molecule.

Leimgruber and co-workers reported the first total synthesis of a PBD (anthramycin) in 1968. The synthetic route was long and required 17 steps to reach the final product, significantly, eight steps were required for the installation of the C2-acrylamide substituent in its final form. The synthetic route was however successful and yielded anthramycin methyl ether as the pure C11a-(S) isomer. In 1982 Ishikura et al. reported an alternative approach to the synthesis of anthramycin. This 16 step synthesis of the key dilactam intermediate (26 steps to the final product) involved the palladium catalysed insertion of carbon monoxide, the result of which was the cyclisation of the B-ring to give a dilactam. In 1984, Reed and Snieckus also reported the total synthesis of anthramycin. Their approach involved the formation of an isatoic anhydride that was then coupled to trans-4-hydroxy-L-proline to give the dilactam PBD. The C2-alcohol, as in Ishikura’s approach, was oxidised using PCC to give the key intermediate in eleven steps.
In 1987, Peña and Stille reported an exciting new route for the synthesis of anthramycin where the acrylamide chain was attached in only three steps. Their synthetic route began by following Leimgruber’s approach until the formation of the dilactam alcohol. Swern oxidation following protection of the O9-N10 functionalities gave the C2-ketone, which was then triflated to give the key intermediate, the enol triflate. Palladium catalysed Heck coupling introduced the acrylamide side chain in one step. Finally, the C11 carbonyl was reduced and the benzal group removed to afford anthramycin.

The first formal synthesis of the neothramycins was reported in 1977 by Miyamoto et al. via a seven-step route. Mori’s approach in 1986 employed palladium catalysed carbonylation and employed the MOM protecting group. Also in 1986, Langlois’ approach introduced the use of Raney Nickel to reduce and simultaneously cyclise the B-ring, with the added bonus of deprotecting the C8-alcohol within this one step. The Fukuyama approach (1990) involved a double cyclisation step via ethyl thiol esters, to form both the B-ring and C-ring in a single step.

In 1983, Tozuka et al. reported the total synthesis of tomaymycin. The approach involved formation of the E- and Z-isomers of the C2-ethylidene side chain prior to PBD synthesis. In the same year, Kaneko et al. reported the synthesis of tomaymycin from oxatomaymycin. This approach used aluminium-amalgam reduction which is considerably milder than sodium borohydride or lithium borohydride. In 1984, Kaneko et al. applied this aluminium-amalgam reduction approach to the total synthesis of chicamycin. In 1986, Mori also reported the total synthesis of E- & Z-tomaymycin using the MOM-protecting group and via a palladium catalysed carbonylation. Unlike Tozuka, however, Mori’s approach involved synthesising Z-tomaymycin and from that, a seven-step synthesis inverted the ethylidene group to give the naturally occurring E-tomaymycin.

Again, in 1986, Mori et al. reported the total synthesis of E- & Z-prothracarcin using a similar method to that employed for tomaymycin, a logical approach considering that these PBDs are structurally similar. Thurston and Langley (1987) introduced the use of mercuric chloride mediated cyclisation to form the B-ring of the PBD and, like Tozuka’s tomaymycin approach, the E- & Z-isomers of the C-ring were formed prior to B-ring
Mazzochi’s approach in 1989 used a photochemical ring expansion of an N-pentenyl phthalimide to produce the PBD dione; a novel approach in the synthesis of PBD monomers.

In 1993, Fukuyama et al. introduced the palladium-catalysed removal of an allyl carbamate protecting group in the first reported total synthesis of porothramycin B. In the same year, Langlois et al. also synthesised porothramycin B using a methodology similar to that used in their synthesis of neothramycin.

DC-81, because of its simple structure, has been studied extensively with respect to its total synthesis. Four important reports to note are the Mazzochi, Thurston, Molina/Eguichi and Hu approaches. Mazzochi et al. (1989) employed their photochemistry approach, as with prothracarcin. Thurston et al. in 1990 used catalytic transfer hydrogenation to produce DC-81 in seven steps. In 1992 the same group reported that benzyl protection of the C8-OH group was not necessary but more importantly they reported the now widely used synthesis of DC-81 via mercuric chloride induced cyclisation. In 1995, Molina et al. and Eguichi et al., two independent groups, reported a similar total synthesis of DC-81. Both employed an intramolecular Aza-Wittig reaction in the closure of the B-ring. Molina’s Aza-Wittig cyclisation gave DC-81 in 79% and Eguichi’s Aza-Wittig gave DC-81 in 98% yield. Hu’s six step total synthesis of DC-81 in 2001 used triphosgene and a benzyl protected A-ring to produce the isatoic anhydride, which they then coupled to the C-ring (as with Peña & Stille’s approach to anthramycin). MOM protection of the N10 position followed by reduction with lithium borohydride gave the imine. The overall yield was 35% and this new synthesis is reported to be suitable for the large scale (10 g) synthesis of DC-81.

In summary, PBDs are an interesting group of alkylating agents. They are effective anti-tumour agents and their relatively simple structures and numerous synthetic approaches make them very attractive for Structure-Activity-Relationship studies of novel synthetic analogues. Additionally, when these molecules are dimerised, their ability to covalently bind to DNA means that PBD dimers are also DNA cross-linkers. One such dimer is currently in Phase I clinical trials and the PBD dimers will be discussed in detail later.
Background to the synthesis of the C2-substituted pyrrolobenzodiazepines

Synthesis of the key triflate intermediate

Leimgruber's synthesis of Anthramycin

In 1968, as previously mentioned, Leimgruber and co-workers reported the first total synthesis of anthramycin. Much of the synthetic effort in Leimgruber's approach went towards elaborating the C2 side chain (Scheme 1).

The synthesis began with the commercially available 3-hydroxy-4-methyl-2-nitrobenzoic acid, which provided the A-ring of anthramycin. The first step was to protect the C9-OH with a benzyl group. The acid chloride of the A-ring was formed using oxalyl chloride and subsequently coupled to the methyl ester of 4-hydroxy-L-proline. The use of this amino acid introduces the correct stereochemistry at the PBD C11a position. The A-ring nitro group was reduced using sodium dithionite, and exposure to acid following the formation of the amine promoted ring closure to give the dilactam C2-alcohol (4).

Leimgruber subsequently employed Jones oxidation to form the C2-ketone from the C2-alcohol. Jones oxidation is an aqueous oxidation method suited to secondary alcohols such as Leimgruber's intermediate.
Scheme 1: Total synthesis of anthramycin via the Leimgruber method.\textsuperscript{80}

This C2 ketone is employed to introduce an unsaturated ester via a Horner-Emmons olefination. Interestingly, Leimgruber obtained a $\beta,\gamma$-unsaturated ester rather than the more obvious $\alpha,\beta$-unsaturated product. Under the basic conditions employed, the double bond migrates into the C-ring to form C2-C3 \textit{endo} unsaturation (Scheme 2).
The ester was reduced with DIBAL-H to afford an aldehyde, which was then converted to a cyanohydrin. The cyanohydrin was mesylated and elimination furnished an endo-exo unsaturated nitrile. Acid mediated hydrolysis of the nitrile provided the required amide (12).

The C2 elaboration chemistry was performed on a dilactam core and required eight steps. Protection of the O9 and N10 moieties with a benzal group allowed reduction** of the dilactam to yield the protected carbinolamine. Removal of the benzal protecting group under mildly acidic conditions afforded synthetic anthramycin identical to the naturally occurring material.

*Synthesis of Prothracarcin, Neothramycin and Tomaymycin – Mori’s approach*

In 1985 & 1986, Mori reported the total synthesis of prothracarcin, tomaymycin and neothramycins A & B. Although the synthetic route is not very relevant to this project,

**Leimgruber reported the use of both LiAlH₄ and NaBH₄, however, he states that NaBH₄ is the preferred method.**
his choice of protecting N10 with MOM and subsequent reduction and MOM deprotection is important to this work.
The synthetic approaches used were very similar for all three PBDs. 4-hydroxy-\textit{L}-proline was the starting material. TFA was used to protect both the amine and the hydroxy groups. Phosphorus pentachloride was used to form the acid chloride from the carboxylic acid and this was subsequently coupled to the A-ring. TFA was removed from the C2-OH and this along with N10 was MOM protected. The TFA was then removed from the amine and insertion of carbon monoxide cyclised the B-ring to form the PBD dilactam. The C2-OH was deprotected\footnote{This method also resulted in the loss of the N10-MOM, giving a yield of 27\% of unprotected dilactam in addition to the desired N10-protected product.} and oxidation using PCC yielded the C2-ketone dilactam. The Wittig reaction was used to introduce the methyl vinyl C2-side group and hydrolysis of the tosyl group gave the free phenol. The dilactam was reduced using LiAlH\textsubscript{4}\footnote{NaBH\textsubscript{4} was used in the synthesis of prothracarcin.} and the N10 was deprotected using silica gel.

In the case of the neothramycins, the synthesis followed that of prothracarcin and tomaymycin up until the MOM protected dilactam C2-alcohol. Methylsulphonation and reduction of this intermediate yielded the olefin. CSA in methanol gave rise to the saturated C-ring intermediate. The tosyl group was removed, the lactam was reduced and MOM was removed from N10 as with the previous compounds. Finally, the methyl groups were removed from the C3-OH groups to give the final neothramycins.
Scheme 3: Mori’s total synthesis of Tomaymycin and Prothracarcin

(a) (CF₃CO)₂; (b) PCl₅; (c) 16 o-bromo-aniline; 17 2-bromo-4-methoxy-5-tosyloxyaniline; (d) aq. NaHCO₃; (e) MOMCl, i-Pr₂NEt; (f) MOMCl, NaH; (g) NH₃/MeOH; (h) 20 Pd(OAc)₂, PPh₃, CO (5 atm.), K₂CO₃, xylene; 21 Pd(PPh₃)₄, CO (10 atm.), Bu₃N, PhCH₂; (i) aq. HCl, MeOH; (j) PCC; (k) 30 Ph₃P⁺CH₂CH₂Br⁻, KO⁻Bu; 31 1. Ph₃P⁺CH₂CH₂Br⁻, KO⁻Bu; 2. aq. KOH; (l) 32 NaBH₄, EtOH, 0 °C; 33 LiAlH₄, -60 °C; (m) Silica gel.
Scheme 4: Mori's synthesis of the Neothramycins

Peña & Stille's synthesis of anthramycin

In 1989, Peña & Stille published a new concise synthesis of anthramycin based on a palladium catalysed Heck-coupling strategy (Scheme 7). In the same publication Peña & Stille reported their investigations of the Heck and Stille couplings in general PBD dilactam synthesis.
Scheme 5: Pena & Stille's synthesis of N10-Me protected, N10-MOM protected and N10-SEM protected key PBD ketone intermediates
They began the synthesis with an isatoic anhydride, which they coupled to the unprotected 4-hydroxy-L-proline (Scheme 5). Jones oxidation was applied on the N10-methyl protected molecule 46, to produce the N10-methyl C2-ketone dilactam. The unprotected dilactam was silyl protected and then N10 protected with either MOM or ethoxymethyl. Subsequent removal of the silyl group from the C2-substituent gave the N10-protected C2-OH dilactam. Oxidation with PCC/alumina or Swern oxidation yielded the C2-ketone dilactam. Triflation of all three ketones gave the enol triflates used in the Heck and Stille couplings (Scheme 6).
Scheme 6: Peña & Stille’s investigations of the Heck and Stille couplings in the synthesis of PBD dilactams.

From the results of this study, Peña & Stille concluded that the Heck coupling was more appropriate for the total synthesis of anthramycin as the modified conditions gave a
satisfactory yield. The Stille coupling had also provided satisfactory results but required the preparation of the tin reagent for the coupling.

The original Heck coupling conditions employed with the MOM protected triflate were low yielding. The triflic acid produced from the reaction resulted in the removal of the MOM group. A stronger base was therefore used to neutralise the acid and the yield, as expected, improved. As a result, these conditions were employed in the total synthesis of anthramycin itself.

Despite their thorough investigations of coupling methods, Peña & Stille sadly never reported the reduction of the model dilactams that they had synthesised.

Although this initial approach was related to Mori's strategy employing the MOM protecting group, the total synthesis of anthramycin was more closely related to Leimgruber's route and did not include the use of a MOM protecting group. Peña & Stille followed Leimgruber's route to the C2-alcohol dilactam (Scheme 7). They then removed the benzyl protecting group and protected the free C9-OH and N10 with benzaldehyde dimethyl acetal. This is an acid sensitive protecting group so Jones oxidation was not an option. Instead, they used Swern oxidation to produce the C2-ketone, another mild oxidation method suitable for secondary and primary alcohols as the absence of water limits decreases the risk of over-oxidation. Following oxidation, the ketone was then converted to the enol triflate in order to provide a substrate for the Heck reaction. Treatment of the enol triflate with acrylamide in the presence of a palladium (0) catalyst furnished the C2 side chain in one step. Peña & Stille then returned to Leimgruber's route to reduce the dilactam and remove the protecting group to obtain the target molecule, anthramycin.
Scheme 7: Total synthesis of anthramycin via the Peña & Stille method.\(^3\)
Synthesis of DC-81 – the Hu approach

In 2001, Hu reported a new concise synthesis of DC-81 (Scheme 8) employing the use of the MOM-protecting group. Since Mori’s total synthesis of tomaymycin, prothracarcin and the neothramycins, other groups have had little success with the use of MOM as a protecting group. The use of NaBH₄ on other MOM-protected PBDs failed to give the imine, but instead ring-opening products were obtained via 3-aza-Grob fragmentation. Hu et al. substituted NaBH₄ with the milder LiBH₄, and in the total synthesis of DC-81, the use of this reagent resulted in the successful synthesis of the desired imine product.

The Hu approach is concise and began with the reduction of 2-nitrobenzoic acid with tin chloride to give the amine in 92% yield. Triphosgene was used to produce the isatoic anhydride 66 in excellent yield (98%). This was coupled to L-proline in DMSO giving rise to the dilactam intermediate 67. The N10 position was protected with MOM-Cl, the
dilactam reduced with lithium borohydride§§ and finally the benzyl group was removed from the A-ring to yield the final product, DC-81 (70).

§§ Conditions for the reduction were very strict. The reaction was performed at -10 °C and the duration of the reaction was carefully controlled as longer reaction times resulted in over reduction of the imine.
AIMS AND OBJECTIVES

Malignant melanoma is one of three types of skin cancer. It has the remarkable ability to rapidly metastasise to internal organs making it the most fatal of the three. Initial treatment of this malignancy is by surgical excision but other methods, such as chemotherapy, are employed following metastasis of the primary tumour (AJCC stage IV).

Melanoma does not currently come within the top 5 cancers here in the UK\textsuperscript{101}, but it is a major killer\textsuperscript{7}. The incidence of this cancer is rapidly increasing whilst prognosis remains poor (Table 2).

Melanoma is a cancer that predominates in subjects of Celtic origin i.e. the fair skinned, fair hair subjects that burn and freckle easily when exposed to sun. Sociological and environmental factors are most likely responsible for the increase of this killer disease. Travel to hot climates has rapidly increased, due to the reduction in cost of going abroad for holidays, and together with the destruction of the protective ozone layer, this has led to an increase in the degree of damage from UV light that we subject ourselves to.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of Cases</th>
<th>Number of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>2398</td>
<td>3375</td>
</tr>
<tr>
<td>Australia and New Zealand</td>
<td>5702</td>
<td>4735</td>
</tr>
</tbody>
</table>

The standard chemotherapy for melanomas is dacarbazine (Figure 30), which is metabolised in the liver to give the active metabolite MTIC. Temozolomide (Figure 30), currently licensed for the treatment of brain tumours, is a pro-drug of MTIC and has therefore also been recommended for use in the management of melanomas (as an
unlicensed indication\textsuperscript{102}. Other chemotherapeutic agents have been investigated in the management of melanoma, but have failed to show greater benefit than dacarbazine and Temozolomide\textsuperscript{102}. Immunotherapy has been suggested as an alternative management strategy for secondary melanoma, but, to date, it has only proved useful in patients with the slow-progressing malignant melanoma subtypes\textsuperscript{102}.

\begin{figure}
\centering
\includegraphics[width=0.7\textwidth]{figure30.png}
\caption{Structure of Dacarbazine and Temozolomide}
\end{figure}

The poor prognosis for malignant melanoma, despite the fact that it is not currently one of the top five cancers in the UK, merely emphasises the need for more research to develop effective agents, whether chemotherapeutic or otherwise, for the management and treatment of this condition.

Previous studies on the PBD monomers established that the presence of a dimethoxy functionality at the 7 & 8 positions of the PBD A-ring was optimum for DNA binding affinity. Also, C2-endo & -exo unsaturation of the 2-position of the C-ring has shown to be favourable in terms of the biological activity of these compounds\textsuperscript{76}.

These previous investigations focused on developing analogues that featured an acrylyl substituent at the C2-position, to resemble the structure of naturally occurring PBDs such as anthramycin. When an aromatic substituent was attached to the C2-position (DRH417, Figure 31), however, a marked increase in the biological activity was observed (compared with similar compounds bearing an aliphatic C2-side chain). Interestingly, aromatic substituents on the C-rings of PBDs are not present in any naturally occurring PBD isolated to date.

\begin{figure}
\centering
\includegraphics[width=0.7\textwidth]{figure31.png}
\caption{DRH417 - the lead compound exhibiting the dimethoxy functionality on the A-ring and a novel aromatic substituent at the C2-position}
\end{figure}
DRH417 has shown nanomolar potency \textit{in vitro} and a preference for melanoma cell-lines in the \textit{in vitro} standard 60-cell line screen. There was therefore the need to investigate this exciting discovery further and hence this study was initiated.

![NCI Developmental Therapeutics Program Mean Graphs](image)

**DRH417**

The first aim of this project, therefore, was to design a group of C2-aryl PBD monomers with the general structure as indicated in Figure 33.

![General structure of the C2-aryl PBD monomers](image)

*Figure 33: General structure of the C2-aryl PBD monomers*

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* A guide to the NCI 60-cell line bar charts is located in Appendix A
A small focused C2-aryl substituted PBD library would be synthesised using parallel solution phase techniques. This would ensure that library members would be obtained as individual discrete molecules ready for biological testing.

Library members would be evaluated through the NCI's 60-cell line panel primary screen, hollow fibre assay progressing ultimately to \textit{in vivo} studies.

Special attention would be paid to the effect of the aryl substituent on activity.

Previous studies have shown that PBD dimers may exhibit increased biological activity\textsuperscript{103}. One such dimer (SJG136) is currently in clinical trials. These dimers have the ability to cross-link the DNA as two imine functionalities are present.

A secondary aim of this study was therefore the design, synthesis and evaluation of C2-aryl PBD dimers. These dimers would have the general structure as shown in Figure 34.

![Figure 34: General structure of the C2-aryl PBD dimers](image)

The library would be designed in the same manner as the monomers. However, a new synthetic route would be developed to allow the formation of the dimer core prior to the introduction of diversity at the C-ring, thereby allowing parallel combinatorial synthesis.

Biological evaluation of these analogues would be the same as for the monomers in order to enable comparison of the two novel groups of PBDs.

The overall objective of this work is to develop a selective anti-cancer agent for use in the clinic, specifically for the treatment of melanoma, with limited toxic side-effects.
DISCUSSION

Chemistry I

Retrosynthesis

Since 1968, when Leimgruber first reported the total synthesis of anthramycin\textsuperscript{80}, numerous other groups have reported a diverse range of synthetic approaches in the total synthesis of naturally occurring PBDs. The different synthetic approaches have been determined by the structural features of the target PBD, namely the A- & C- ring substitution patterns, the requirement for $S$-stereochemistry at C11a and the sensitivity of the N10-C11 electrophilic moiety.

The present study focused on synthesising a series of analogues that possess a common A-ring, an imine functionality at N10-C11, $S$-stereochemistry at C11a and C2-C3 \textit{endo} unsaturation as well as a diverse range of C2-aryl substituents. As this study was focused on synthesising a series of analogues, a synthetic approach that introduced diversity at the C2-position towards the latter stages of the synthesis was desirable. Secondly, the synthetic approach needed to be both cost and time efficient and allow the large-scale synthesis of a key intermediate.

In principle, the C2-aryl PBDs would be accessible from protected-carbinolamines (Fukuyama’s approach\textsuperscript{94}), a dilactam intermediate (as seen in Peña & Stille’s synthesis of anthramycin\textsuperscript{83}) or masked amino-aldehyde precursors (Thurston and Langley approach\textsuperscript{104}) (Figure 35).
The Fukuyama approach, based on palladium-mediated deprotection of an N10-allyl carbamate protected carbinolamine, has been exploited in a number of recent PBD syntheses. Since the alloc protecting group is removed using Pd(0), it is incompatible with the Suzuki-Miyaura chemistry required to introduce the C2-aryl substituent and hence can not be applied in its published form to the synthesis of the C2-aryl PBDs.

The thioacetal approach has been employed by Thurston and co-workers to the synthesis of a number of natural and synthetic PBDs. However, the route relies on the use of the stenching agent ethanethiol and requires extensive column chromatography to remove the heavy metal salts. More importantly, triflate formation reactions on uncyclised C-rings can afford mixtures of 1,2- and 2,3-unsaturated products.

On the other hand, Peña & Stille’s total synthesis of anthramycin employed a dilactam intermediate as a substrate for palladium-mediated C2-substitution. The Peña & Stille approach should, therefore, be well-suited to a synthetic strategy employing Suzuki-Miyaura chemistry.
However, Peña & Stille's report on the total synthesis of anthramycin raised a number of questions. Initially, they had explored the use of Mori's MOM-N10 and the ethoxymethyl-N10 protection strategy to prepare a series of model C2-substituted N10-protected PBD dilactams using Heck and Stille coupling reactions*. Intriguingly, Peña & Stille did not report the reduction of these novel PBD-dilactams to the corresponding imines. Additionally, other researchers^96,100 have reported problems in reducing N10-MOM protected dilactams. More importantly, Peña & Stille, themselves, reverted to the proven Leimgruber benzal protecting group strategy to actually synthesise anthramycin.

Despite these concerns over the critical reduction step, the Peña & Stille strategy has a number of desirable features:

- The triflate intermediate has been shown to support palladium mediated chemistry
- C2-diversity is introduced in the penultimate step of the synthetic sequence, making the approach suitable for parallel synthesis.
- Good regiocontrol over triflation, i.e. only the desired 2,3-unsaturated triflate is obtained.
- The robust chemistry employed to reach the C2-ketone intermediate permits large scale reactions to support a parallel synthesis campaign.

It was therefore decided to adopt the Peña & Stille strategy. The key triflate would be prepared from the C2-ketone, which would be synthesised, in bulk, by oxidation of the C2-alcohol. Ultimately, the PBD system would be assembled by coupling together the commercially available A and C-ring components.

As a potential safe-guard against problems at the critical dilactam reduction stage, it was decided to employ the SEM protecting group instead of MOM (as used by Mori^85,91 and Peña & Stille^83) at the N10 position. This protecting group could, in principle, be independently removed under PBD-compatible conditions with TBAF.

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* They investigated both the Heck and Stille couplings but used Heck in the synthesis of anthramycin as the Stille coupling required the preparation of the tin reagents.
The retrosynthesis of the C2-aryl PBDs is shown in Scheme 9. As can be seen, this approach has been adapted from Pena & Stille's approach and Mori's N10-MOM protecting strategy.

Scheme 9: Retrosynthesis of the C2-aryl PBDs
A- C- ring coupling

Synthesis commenced with the coupling of the A- and C- ring fragments. The 6-nitroveratric acid A-ring (81) is commercially available and did not require prior synthetic preparation. The chiral C-ring (82) was prepared by esterification of hydroxyl-L-proline with methanol to give the 4-trans hydroxyl-L-methyl prolinate.\(^{106}\)

The A-ring component was activated as its acid chloride and subsequently added dropwise to a solution of the C-ring (4-trans hydroxy-L-prolinate) in TEA. The reaction proceeded successfully and afforded the required amide in reasonable yield (57% yield).

The identity of the compound was confirmed by mass spectrometry (which revealed the expected mass ion - 354) and by NMR spectroscopy. Peaks in the proton NMR at 7.69 (s) and 6.85ppm (s) confirmed the presence of the A-ring H-9 and H-6 protons and at 3.75 ppm, a 3H singlet confirmed the presence of the C-ring ester methoxy group.

This reaction was successfully scaled-up (30 g) and the product obtained was of sufficient purity (confirmed by TLC) to be carried forward to the next step.
Formation of the first PBD dilactam

The next goal was to cyclise the B-ring to give rise to the first PBD product (78) of this synthetic route. The nitro group would be reduced to the amine (79) and then be encouraged to take part in an internal nucleophilic attack on the ester moiety to provide the dilactam. Three different approaches were investigated to ascertain which approach would be most suitable for the large scale synthesis of the PBD intermediate.

Leimgruber et al. had employed the sodium dithionite reduction method in their total synthesis of anthramycin. Therefore it seemed appropriate, since at this stage of the synthesis the intermediates were similar, to follow his lead. The reaction proceeded successfully up to the point of reduction of the nitro group to the amine. This was confirmed by the presence of the amine protons at 5.36 & 5.16 ppm and the shifting upfield of the A-ring aromatic protons in the proton NMR (6.69 & 6.37 ppm) spectrum. However, following successful reduction to the amine, the acid mediated cyclisation method also used by Leimgruber et al. failed to afford any of the required dilactam.

The use of tin (II) chloride as a reducing agent was also investigated. Tin (II) chloride is an acidic reagent and it was hoped that it would cause reduction and spontaneous ring closure. TLC analysis of the reaction mixture suggested that this was indeed the case. However, it proved very difficult to isolate any of the insoluble product from the tin reagent.

Finally, hydrogenation of the nitro-ester in the presence of palladium on carbon was attempted. When the reaction solution was dilute, the reaction proceeded to the amine and required further acid treatment to achieve the final cyclised product. However, with a more concentrated solution, it was pleasant to discover that spontaneous cyclisation
would occur in the hydrogenation reaction vessel and hence further acid treatment was unnecessary.

On a small scale (1 g) the hydrogenation gave an excellent yield of 90% whilst on a larger scale (15 g) the yield was approximately 50%. The reason for this was that the cyclised final product was insoluble in cold ethanol and therefore precipitated out in the reaction vessel following spontaneous cyclisation. Separation from the insoluble palladium on charcoal proved difficult, but treatment with hot methanol to dissolve the product, followed by hot filtration afforded the desired dilactam on cooling. The successful synthesis of the PBD dilactam was confirmed by NMR analysis (Figure 36). As can be seen in Figure 36, the N10 proton signal is now found at 10.33 ppm (1H) indicating it is no longer an amine (signals at 5.36 & 5.16 ppm) but part of the lactam system. Additionally, the aromatic protons are further downfield at 6.70 & 7.26 ppm indicating they are less shielded as a result of the presence of the conjugated dilactam system. The broad singlet peak at 5.21 ppm is clearly the C2-OH signal whilst the C-ring protons exhibit a change in coupling patterns confirming that the PBD dilactam has been formed.

![Figure 36: The first PBD dilactam (78) of this synthetic approach. The numbers in blue indicated the $^1$H NMR peaks in ppm.](image)

The hydrogenation approach proved to be highly successful and eliminated one step from the overall synthetic route. This approach was henceforth successfully employed routinely for the large scale synthesis (15 g per vessel) of the first PBD product in this synthetic route.
The next step involved the TBDMS protection of the C2-OH alcohol. Protection of the C2-OH group allowed installation of the crucial N10-substituent, essential for the dilactam reduction approach to PBD synthesis, without involving the C2-alcohol.

The dilactam revealed a distinctive NMR spectrum, which would allow the success of subsequent chemical transformation to be readily ascertained (Figure 36).

The TBDMS ether was installed under classical conditions with TBDMS chloride and imidazole in DMF.

Mori's original approach called for the use of the methoxymethyl (MOM) protecting group. However, reports in the literature suggested that there were limitations to the use of this specific protecting group. As mentioned previously, Peña & Stille failed to report the reduction of any of their MOM-protected PBD dilactams in their investigation of the application of Stille and Heck couplings to the total synthesis of anthramycin. Instead they reverted to Leimgruber's original benzal protecting group approach, leading to the suspicion that there may have been complications with the reduction of the MOM protected analogues.
Despite these previous reports of problems with the use of MOM it was decided to investigate the use of MOM in conjunction with an alternative yet related protecting group – (2-methoxyethyl)trimethylsilane (SEM). SEM had the advantage over MOM in that it could, theoretically, be removed under mild conditions (TBAF) after reduction of the PBD dilactam, should Mori's wet silica method prove to be unsuccessful.

The N10 proton was removed with sodium hydride and the resulting anion was quenched with MOM chloride. Purification of the crude product proved difficult and the yield of protected material was only moderate (49%).

SEM protection, N10 deprotenation with sodium hydride followed by quenching with SEM chloride, afforded a 77% yield of product after column chromatography. The analogous procedure with MOM-chloride afforded a 49% yield of product after a difficult work-up. Interestingly, as in the case of the literature MOM compounds, the two hemiaminal protons were non-equivalent and were found 0.9 ppm apart. Additionally, the expected trimethylsilyl protons were observed in the 0 ppm region.

Unfortunately, one of the problems encountered with both the MOM and SEM protections was the poor solubility of the starting material in DMF and other suitable solvents. Therefore an alternative method which involved adding the base to the reaction vessel, containing a suspension of starting material in solvent, was required. It proved inconvenient to add solid sodium hydride to the reaction vessel whilst attempting to maintain an inert atmosphere. However, n-butyl lithium in hexane could be conveniently introduced to the reaction vessel, under nitrogen using standard syringe or canula techniques. Fortunately, the resulting anion was soluble in the reaction solvent and was quenched by the addition of SEM-Cl.

It was now necessary to dispense with the services of the C2-OTBDMS group. Gratifyingly, selective removal of the TBDMS group in the presence of SEM at room temperature proved successful. Although TLC analysis revealed that the reaction was very clean, column chromatography was required to remove excess TBDMS fluoride. IR
spectroscopy confirmed the removal of the TBDMS group as a broad absorption peak was observed at the 3500 region indicating a free OH group.

As mentioned above, the SEM protected intermediates exhibit interesting NMRs (Figure 37). The two methylene protons of the SEM group that are immediately adjacent to the N10 are found at considerable distance apart (greater than 0.86 ppm for compound 75). This is probably due to the fact that one of the CH$_2$-SEM protons is protruding into the shielded region of the aromatic A-ring and hence is further upfield than its partner.
A number of different oxidation methods were investigated to obtain this critical intermediate. Initially, the TPAP method was employed but this failed to yield any of the required product. The PDC method of oxidation appeared desirable as the reaction conditions did not have to be closely monitored. However, upon scale-up of the synthesis, it proved extremely difficult to isolate the product from the chromium by-products. As a result the more sensitive Swern oxidation was investigated. This gave good yields in both small scale and large scale reactions (70% yield). The successful synthesis of the ketone PBD product was confirmed by IR (ketone peak appeared at 1740 cm\(^{-1}\)) and also NMR (Figure 38) where the loss of the H2 proton peak and simplification of splitting patterns of the H3-\(\alpha\) and H3-\(\beta\) (at 3.90-3.54 ppm) protons and H1 (at 3.90-3.54 & 2.8 ppm) protons was observed.

Figure 38: The key ketone intermediate (74). The numbers in blue indicate the proton NMR signals in ppm for the corresponding protons.
A robust synthesis of the crucial C2-ketone had been achieved on a multi-gram scale. The next challenge would be to introduce the reactive enol triflate intermediate in a regioselective fashion.
DISCUSSION

Chemistry II

Before discussing the successful latter stages of the C2-aryl substituted PBD synthesis, it will be useful to review some relevant aspects of triflate and Suzuki chemistry. The use of trifluoromethanesulphonates (triflates) in organic synthetic chemistry was first introduced by P. J. Stang in 1969\textsuperscript{107}. The bond formed between the trifluoromethane sulphonate groups and the alkene stem is usually a labile bond and has proved difficult to handle in some syntheses. However, it is this lability that makes the triflate moiety an excellent leaving group and very useful as a partner in numerous, widely used cross-coupling reactions, such as the Heck, Stille and Suzuki-Miyaura procedures.

There are numerous methods of preparing triflates and the method employed is dependent on the structure of the starting material from which the triflate is to be formed as well as any regio and/or stereochemical requirements arising from the final triflated product. The most relevant methods to this project are discussed below.

Vinyl triflates are prepared by the reaction of enolates with a triflating agent* (Scheme 10). When regio/stereo specific triflates are required, the enolate generated in the initial step of the triflation reaction must exhibit the desired regio/stereo chemistry as this is conserved in the triflating step\textsuperscript{108}.

\begin{equation}
\begin{array}{c}
\text{Vinyl triflates} \\
\begin{array}{c}
R_1 \quad \text{Base} \\
\rightarrow
\end{array}
\end{array}
\end{equation}

\begin{equation}
\begin{array}{c}
\text{Triflating agent} \\
\rightarrow
\end{array}
\end{equation}

\begin{equation}
\begin{array}{c}
R_1 \quad \text{Tof} \\
\rightarrow
\end{array}
\end{equation}

Scheme 10: Triflation of vinyl triflates

* The most common triflating agents are triflic anhydride, N-phenyltriflimide and N-pyridyltriflimides
Generating regio/stereo specific enolates of uncyclised vinyl compounds presents a challenge. As a result, the use of cyclic systems is more commonly observed in the literature. In simple cyclic systems, symmetry or the substitution pattern of the cyclic system alone may mean only one enolate can possibly be generated. However, frequently in synthetic/medicinal chemistry, the organic molecule is not a simple cyclic system. In such cases, manipulation of reaction conditions and choice of base used can give almost exclusively the desired stereo/regio specific enolate. For instance, Scott & Stille successfully synthesised the kinetic enolate of their cyclic ketone by using the strong, non-nucleophilic base LDA. By merely changing this base to the Hauser base, bromomagnesium diisopropylamide, they were able to almost exclusively synthesise the thermodynamically preferred enolate as is shown in Scheme 11.

Scheme 11: The synthesis of the kinetic and the thermodynamic cyclic enolate

In a more highly substituted cyclic ketone, Baldwin et al. demonstrated the successful and exclusive synthesis of the desired "kinetic" triflate by employing the use of the strong non-nucleophilic base LHMDS at a low temperature.

Scheme 12: Baldwin's synthesis of the desired "kinetic" triflate using LHDMS

1 LDA = Lithium Diisopropyl Amine
However, Peña & Stille\(^{83}\) in their investigation of the Heck and Stille couplings in the total synthesis of anthramycin, exclusively synthesised, in good yield (70%), the thermodynamic 2,3-unsaturated C2-triflate by using the weaker pyridine base at room temperature (Scheme 13).

![Scheme 13: Mechanism of formation of the thermodynamic 2,3-unsaturated triflate PBD](image)

A recently investigated alternative synthetic route to the C2-aryl PBDs\(^ {78}\) involved the triflation of ketone 94 prior to B-ring closure. In this synthesis, pyridine proved to be a rather weak base, and using the conditions employed in the triflation of the PBD dilactam, the yield was very poor for this PBD precursor. Therefore, the stronger non-nucleophilic and sterically hindered base LDA was employed. This however, lead to a mixture of the "kinetic" 1,2-unsaturated product 96 and the thermodynamic 2,3-unsaturated triflate 95 in a 4:1 ratio (Scheme 14).
At the other extreme, the use of the thermodynamic conditions for the triflation of a carbinolamine monolactam PBD lead to the formation of aldol reaction products in addition to the desired thermodynamic triflate 104 which was obtained in modest yield (30%)\(^\text{105}\) (Scheme 15). The competing aldol reaction was also observed in the study by Lessène \textit{et al.}\(^\text{112}\) comparing the strong non-nucleophilic bases such as LDA with the milder Hauser bases for the purpose of controlling the generated “kinetic” and “thermodynamic” enolates in cyclic ketones.
Thus, it has been shown that stereo/regio chemistry of the enolate can be controlled with relative ease, even in more complex molecules such as the PBD dilactams, merely by altering the triflation reaction conditions.
Use of triflates in palladium chemistry

As previously mentioned the triflate has excellent leaving group properties, this makes it ideal for palladium catalysed cross-coupling reactions. There are numerous applications for triflates in organic and organometallic reactions but three important and widely used reactions to note are the Heck, Stille and Suzuki-Miyaura coupling reactions.

These three palladium catalysed coupling reactions are related to each other in that they share a similar reaction mechanism involving oxidative addition of the palladium to the organotriflate and finishing with reductive elimination of the palladium catalyst\textsuperscript{113}. The palladium cross-coupling mechanism is illustrated for the Suzuki reaction in Scheme 16.

The Suzuki-Miyaura coupling\textsuperscript{114,115}, first reported in 1982, involves the palladium catalysed cross-coupling of boronic acids/esters with an organic halide or triflate. Whereas the Stille and Heck reactions are more suited to the coupling of alkenes to the triflate, the Suzuki reaction is a very popular and effective reaction for introducing aromatic and heteroaromatic substituents to the organic molecule\textsuperscript{115}. This reaction will be discussed in detail below.

The Suzuki-Miyaura coupling reaction

In 1981, Suzuki & Miyaura\textsuperscript{109,113-115} discovered that aryl boronic acids undergo cross-coupling reactions with aryl halides in the presence of a palladium catalyst and a base\textsuperscript{2}. Suzuki coupling is compatible with a wide range of functional groups making it a desirable method for introducing aryl substituents to compounds such as PBDs. Aryl/vinyl triflates also undergo cross-coupling reactions with aryl boronic acids in a similar fashion to aryl halides\textsuperscript{109}. The mechanism of the cross-coupling involves oxidative addition, transmetalation and reductive elimination of the palladium catalyst as depicted in Scheme 16.\textsuperscript{113}

\textsuperscript{1} It is presumed that the base forms intermediate oxo-palladium complexes that accelerate the transmetalation step\textsuperscript{110}
The most commonly used palladium catalyst in the Suzuki coupling is tetrakis (triphenylphosphine) palladium (0) although the more expensive palladium catalysts such as PdCl₂(dppf) have also been shown to be effective catalysts for this purpose\textsuperscript{109}.

One of the major factors that has made the Suzuki coupling extremely popular in recent years is the wide range of boronic acids and esters that are commercially available, from the simple mono-substituted benzeneboronic acid to the more complex fused ring systems such as 9,9-dihexyl-9\textit{H}-fluoren-2,7-diyl-2,7-diboronic acid. This diversity, plus the ease and good yield of the coupling reaction and the selectivity for functional groups, makes the Suzuki reaction very attractive for the parallel combinatorial synthesis of large diverse libraries of C2-aryl PBD analogues.

As mentioned previously, the original Suzuki coupling involved coupling of haloaromatics, i.e. electrophilic substrates, to boronic acids. Iodine is the most electrophilic and therefore the most reactive, followed by bromine, then the triflate and finally chlorine.
as the least reactive electrophile. Aryl chlorides are still used in Suzuki couplings, however, the superior leaving group properties of the triflate group make the use of triflates far more appealing even if it may be a more expensive approach in some cases.

The Suzuki coupling has also been successfully employed in the synthesis of a short series of C7-substituted PBD molecules. Guiotto's early studies indicated the potential of Suzuki chemistry to furnish libraries of aryl substituted PBDs.

![Scheme 17: Suzuki coupling on a PBD intermediate in order to introduce aryl substituents at the C7 position of the A-ring](image)

**Completion of C2-aryl PBD Synthesis**

The synthesis of the C2-aryl PBDs was completed via a route consisting of triflation and the Suzuki coupling followed by a one step reduction/deprotection reaction (Scheme 18). A parallel combinatorial approach was employed during these later stages with diversity being introduced at the penultimate Suzuki coupling stage.
Scheme 18: Later stages of the synthesis of the C2-aryl PBDs (Suzuki coupling, where diversity is introduced, followed by reduction of the N10-C11 lactam and deprotection of N10).

**Triflation of ketone 74**

The Peña & Stille conditions were applied to the SEM protected ketone. The desired 2,3-unsaturated C2-triflate was stable enough to be purified using flash chromatography and was isolated in average yield (49%). It could be stored at low temperatures for several weeks allowing portions of a large batch to be used as required in the penultimate step, the Suzuki-Miyaura coupling reaction.

As can be seen from Table 3, the spectra of the SEM triflated compound agree very well with Peña & Stille’s MOM protected dilactam compound.
A critical feature to note is the coupling \( (J = 10.95 \text{ Hz}) \) between the H-11a and H-1 signal. This observation confirms the location of the C-ring unsaturation between C2 and C3 rather than at the alternative C1-C2 position\(^7\).

Table 3: Table comparing NMR spectra of Peña & Stille's MOM protected triflated PBD (54) and the C2-aryl SEM protected triflated PBD (73).

<table>
<thead>
<tr>
<th></th>
<th>H1</th>
<th>H3</th>
<th>H6</th>
<th>H7/7'-OME</th>
<th>H8/8'-OME</th>
<th>H9</th>
<th>N10-MOM/SEM</th>
<th>CH(_2)-CH(_2)Si(CH(_3)) \text{ (CH}(_3))(_2)O-</th>
<th>CH(_2)-Si(CH(_3))</th>
<th>CH(_2)-CH(_3)</th>
<th>Si (CH(_3))</th>
<th>H11a</th>
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<td>7.1 t</td>
<td>7.9 d</td>
<td>7.55 s</td>
<td>7.40 s</td>
<td>7.70 d</td>
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<td>5.49 d</td>
<td>3.46 s</td>
<td>4.6 ddd</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SEM</td>
<td>3.97-3.68 m</td>
<td>7.15 t</td>
<td>7.32 s</td>
<td>3.97-3.68 m</td>
<td>3.97-3.68 m</td>
<td>7.27 s</td>
<td>5.57 d</td>
<td>4.72-4.70 d</td>
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</tr>
</tbody>
</table>

\( \text{O}^\text{V}^\text{S} \text{I} \text{O}^\text{Tf} \text{H} \text{MeO} \text{MeO} \text{O}^\text{N} \text{MeO} \text{MeO} \text{O}^\text{N} \text{OTf} \)

Peña & Stille's MOM triflate PBD dilactam

SEM triflate PBD dilactam precursor to the C2-aryl PBD monomers

**Suzuki reactions on the PBD enol triflate**

Suzuki reactions typically employ anhydrous salts such as potassium carbonate in polar solvents or aqueous solutions of sodium carbonate in DME under reflux. In the Suzuki coupling between the PBD triflate and 4-methylbenzene boronic acid, classical Suzuki conditions were initially employed but the yield was not impressive (36%)\(^9\). However, alteration of the conditions from the aqueous solution of sodium carbonate in DME under reflux to the 1:1:1 ratio of water, ethanol and benzene in the presence of the base sodium carbonate and again using \textit{tetraphosphine}(triphenylphosphine) palladium (0) as catalyst lead to greatly improved yields (for the 4-methylbenzene analogue the yield was 68% and for the 4-ethylbenzene analogue 74%). Gratifyingly, this improved Suzuki coupling also proceeded at room temperature for the majority of the analogues synthesised.
$^1$H NMR confirmed the presence of the C2-aromatic substituent with the H-3 proton appearing further downfield now at 7.40 ppm as a singlet as well as new aromatic signals from the C2 aromatic ring and the presence of the ethyl signals at 2.65 and 1.25 ppm (Figure 39 & Appendix B - Fig. I).

Due to the fact that the enol triflate is synthesised in the latter stages of the synthetic approach, the introduction of diversity in a parallel synthetic manner at the penultimate step means this synthetic approach is extremely well suited for the purpose of synthesising libraries of C2-aryl PBD analogues. Large batches of ketone can be synthesised and stored for several months. Batches of the key triflate intermediate can be synthesised as required with this intermediate then being carried forward into a 2-step parallel combinatorial approach for the purpose of synthesising library members via the versatile Suzuki coupling reaction.

The library of analogues was designed based on the structural and electronic features of the C2-aromatic substituent. Candidates were selected from the following sub-groups of boronic acids:
1. Electron donating groups on the C2-benzene ring e.g. 115
2. Electron withdrawing groups on the C2-benzene ring e.g. 120
3. Fused ring systems e.g. 122
4. Bulky groups in the ortho position of the C2-benzene ring e.g. 134
5. Heteroaromatic C2-substituents e.g. 116
6. 'Extended' C2-substituents e.g. 121
7. C2-vinyl aromatics e.g. 135

Table 4: Table showing yields of selected suzuki coupling reactions

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Cpd</th>
<th>Structure</th>
<th>Yield (%)</th>
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</table>
Although it was expected that the yields of the individual Suzuki reactions would correlate with the sub-group of boronic acids used, the yields actually obtained depended on the individual analogue itself and not on the sub-group of substituents as anticipated. Overall, the Suzuki coupling yields were good to excellent and most reactions proceeded at room temperature.

Having successfully synthesised the C2-aryl N10-SEM protected dilactam PBDs in good yield, these intermediates were then carried forward to the final step involving reduction of the C11 lactam and removal of the SEM protecting group.

<table>
<thead>
<tr>
<th>Cpd</th>
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<th>Yield (%)</th>
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<th>Structure</th>
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<td>128</td>
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</table>
Reduction and SEM removal

Typical conditions for the final step in this synthetic approach involved adding sodium tetraborohydride in portions to the SEM protected dilactam (114) in anhydrous ethanol and THF. Following the reduction of the lactam, the reduced SEM protected PBD carbinolamine was deprotected using cold wet silica gel.

As can be seen in Scheme 19, the H\textsuperscript{+} from the sodium borohydride attacks the most electrophilic lactam at the C11 position. The N4-C5 lactam remains unaffected in this PBD system due to electron density being donated to this lactam from the adjacent conjugated rings. This is illustrated in Figure 40 where electrophilic regions on the molecule are shown by a red mesh surrounding the atoms. As can be seen, the N10-C11 lactam is significantly electrophilic, thereby making this lactam more prone to attack by the reducing agent sodium borohydride. Once the lactam has been reduced to the carbinolamine, the nitrogen can now be protonated under aqueous reaction conditions. Protonation of the nitrogen results in the spontaneous fragmentation of the SEM protecting group, a process assisted by mildly acidic conditions (e.g. in the presence of wet silica gel).

Figure 40: Model of PBD 142 showing electron deficient areas in red mesh and electron rich areas in blue mesh.
Scheme 19: Mechanism of reduction and deprotection of the C2-aryl SEM protected PBD dilactams.

The final product was sufficiently stable to be purified using flash column chromatography (semi-automated) with the final PBD product 2-(4-ethylbenzene)-PBD (142) being isolated in a good yield (67%).

Figure 41: $^1$H NMR shifts of the individual protons on the SEM-protected 4-ethylbenzene PBD dilactam (142).

$^1$H NMR confirmed the successful synthesis of the target molecule with the appearance of the characteristic imine signal at 7.89 ppm, the shifting of the H-9 proton upfield at 6.83 ppm and the disappearance of the distinctive SEM group signals. Additionally, the H-11a proton signal (at 4.72 ppm for compound 114) was now located at 4.41 ppm and an
alteration in coupling patterns was clearly visible indicating coupling with the two H-1 protons and the H-11 proton (Figure 41 & Appendix B - Fig. II).

The location of unsaturation at the 2,3 position was further confirmed by comparison with the 1,2-endo unsaturated C2-aryl PBD (Figure 42). In the 1,2-unsaturated molecule, complex coupling is observed between the H11a, H11 and the alkene proton at H1 (6.19 ppm, multiplet, 1H) whereas in the 2,3-unsaturated PBD, the H1 protons were located further upfield at 3.85 and 3.40 ppm and displayed a distinctive ddd coupling pattern. Finally, the alkene proton was observed as a singlet at 7.47 ppm indicating the absence of nearby protons.

Figure 42: Comparison of 1H NMR shifts of the 1,2-unsaturated C2-aryl PBDs and the 2,3-unsaturated C2-aryl PBDs
Semi-automation of both the Suzuki coupling and reduction steps allowed for the parallel synthesis of a number of analogues simultaneously, something which would have been difficult to accomplish using traditional methods. The Suzuki-coupling was performed using a 12-position solution-phase carousel followed by the use of serial flash column chromatography on a 10-position automated Flash Master. The Flash Master was also employed in the purification of the final imine PBD molecules allowing for the rapid isolation of the delicate PBD and hence reducing the risk of the PBD decomposing as has been noted by other researchers, when attempting to synthesis PBDs via the protected dilactam reduction approach.

Although the majority of reductions of the SEM-protected dilactam analogues proceeded as planned, compounds 116 and 130 were over-reduced on the first attempt to synthesise them to give the ring-opened products. This problem is not exclusive to the C2-aryl PBDs and has been reported on several occasions. The over-reduced product is clearly visible in the $^1$H NMR with the appearance of the N-10 amine signal at 5.94 ppm as a broad singlet, the C-11 OH at 4.9 ppm, two C-11 protons at 3.8 ppm and the complex coupling patterns of the C-11a and C-1 protons at 4.8, 3.3 and 2.6 ppm respectively.

Over-reduction of the PBD dilactam didn’t appear to correlate with the properties of the C2-aromatic substituent and considering that compound 116 was later successfully re-synthesised, it can be concluded that minor alterations to the reaction conditions could help to prevent the over-reduction of the analogues.

Fifteen novel C2-aryl PBDs were successfully synthesised using this approach and their biological activity will be discussed in the biology discussion chapter. Owing to the successful parallel combinatorial synthesis of a library of novel C2-aryl PBDs and their impressive biological activity as well as the pre-clinical success of the dimer SJG-136, it was decided to attempt to extend this library of C2-aryl PBD monomers with the synthesis of novel C2-aryl PBD dimers.
Table 5: Table of yields of selected C2-aryl PBDs

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<th>Compound number</th>
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DISCUSSION

Chemistry III - The C2-aryl pyrrolobenzodiazepine dimers

Overview

In 1992, the synthesis and evaluation of a novel synthetic pyrrolobenzodiazepine was reported\textsuperscript{117}. This PBD, DSB120, was different from naturally occurring PBDs in that it was not a monomer, but a C8-linked PBD dimer capable of cross-linking the DNA.

PBD dimers are capable of spanning twice as many DNA base pairs as the monomers but most importantly, they cross-link the DNA helix. The effect of not only covalently binding in the minor groove but at the same time cross-linking the DNA was very noticeable in the \textit{in vitro} cytotoxicity studies where a 300-fold increase in biological activity, compared to DSB120's monomer equivalent DC81, was observed.

DSB120 never made it to the clinic however, \textit{in vivo} activity of DC81 and DSB120 is disappointing and it was proposed that both PBDs were being sequested by sulphur nucleophiles. Interest in PBD dimers did not subside however, and a few years later, a dimer based on the naturally occurring PBD tomaymycin was synthesised and evaluated\textsuperscript{118}. This PBD dimer, SJG136, had been designed to be more resistant to attack from sulphur nucleophiles \textit{in vivo}. SJG136, also exhibited an \textit{sp}\textsuperscript{2} hybridised carbon at the C2 position, a feature we now know to increase \textit{in vitro} and \textit{in vivo} cytotoxicity.

The results of the biological studies were impressive, with SJG136 exhibiting far superior biological activity in \textit{vitro} and in \textit{vivo} compared to DSB120 and tomaymycin. Additionally, the profile of activity showed a remarkable selectivity for some of the most aggressive cancers, such as melanoma. SJG136 was adopted by the NCI in the USA for further pre-clinical development and today it is currently in phase 1 clinical trials in the UK and the USA\textsuperscript{119}.
The C2-aryl PBD dimers have all shown in vitro and in vivo biological activity that is more impressive than tomaymycin's activity. If a dimer of tomaymycin could exhibit such impressive improvements in biological activity, it is logical that the C2-aryl PBD dimers should also be investigated. The monomers on their own exhibit activity nearly as impressive as SJG136 so the synthesis and evaluation of the C2-aryl PBD dimers, that should span twice as many DNA base pairs as their monomers, would certainly be an interesting.

Synthesis of the C2-aryl PBDs

Following the successful synthesis of the C2-aryl PBD monomers, it was decided to apply the same approach to the C2-aryl PBD dimers, allowing for the introduction of diversity at the penultimate step using the versatile Suzuki coupling reaction (Scheme 20).

The initial step was to prepare the C- and A-ring fragments. The C-ring was synthesised using the same methodology as described for the monomers. The dimer core (consisting of the 2 units of the A-ring linked at the C8 position via a carbon chain) was synthesised following a published 3-step synthesis\(^{120}\).

The dimer core was prepared from methyl vanillate and 1,5-pentanediol via a Mitsonubu reaction. A 5-carbon chain linker was chosen for the C2-aryl PBD dimers as recent studies have shown optimal biological activity for PBD dimers linked via 5-carbon chains at the C8 position. The dimer ester was then nitrated and the resulting product was subjected to hydrolysis to give the final acid dimer core.
Scheme 20: Retrosynthesis of the C2-aryl PBD dimers
Initially the synthetic strategy then broadly followed that devised for the C2-aryl monomers. The dimer core was coupled to the C-ring successfully and the product was subsequently hydrogenated. Unfortunately, the amine and the spontaneously cyclised B-ring product were not observed. Leimgruber’s dithionite mediated reduction was also investigated but this also failed. Therefore, Hu’s approach to the synthesis of DC81 was employed in order to obtain the PBD dilactam dimer (Scheme 21).

The dimer core was synthesised as before except the ester was not immediately hydrolysed this time. After the Mitsonubu and the nitration steps, the dimer core was hydrogenated in order to reduce the nitro group to the amine. Hu’s triphosgene method was then employed to form the isatoic anhydride, which was stable enough to obtain a proton NMR. The two aromatic singlets (H6 and H9) were observed at 6.5 and 7.1 ppm and the CH₂ protons of the linker were clearly present at 2.3 ppm. The isatoic anhydride was then heated with DMSO and the C-ring portion to yield the desired dilactam PBD dimer. The successful synthesis of the dilactam PBD dimer was confirmed by the presence of the N10 proton at 10.3 ppm, the distinctive OH peak at 5.2 ppm, the complex coupling patterns of the H3 and H11a protons in the 4.25-3.2 ppm region and the H1 protons at 1.9 & 2.7 ppm in the ¹H NMR spectrum.

Having successfully synthesised the key dilactam PBD dimer, the synthesis proceeded as for the C2-aryl PBD monomers (scheme 2). The C2-alcohol was protected with TBDMS to prevent its involvement in the subsequent N10-SEM protection step. Successful protection was confirmed by the presence of the TBDMS signals at 0.9 and 0.1 ppm in the ¹H NMR spectrum. SEM chloride was then used to protect the N10 position. ¹H NMR analysis showed the characteristic doublets of the N10-CH₂-SEM protons at 4.6 and 5.5 ppm confirming that SEM was indeed attached to the N10 position. Following the successful SEM protection of the N10 position, TBDMS could then be removed using TBAF thereby exposing the C2 alcohol for the oxidation step.
Scheme 21: Synthesis of the C2-aryl PBD dimers via the trisphogene method

(a) Copper (II) nitrate trihydrate, acetic anhydride, 0°C (b) 20% Pd/C, EtOH, EtOAc, H₂ (c) Triphosgene, anh. THF, pyridine (d) Isatoic anhydride, trans-4-hydroxy-L-proline, DMSO, 100°C (e) TBDMS-Cl, imidazole, anhydrous DMF, RT (f) SEM-Cl, butyl lithium, anhydrous THF, 0°C, (g) TBAF, THF, RT, (h) anh. DCM, PDC, 4Å mol. sieves, N₂, RT (i) Anhydrous pyridine, anhydrous DCM, anhydrous triflic anhydride, RT (j) aryl boronic acid, benzene, EtOH, water, N₂, Na₂CO₃, tetrakis (triphenylphosphine)Pd(0), RT (k) NaBH₄, anhydrous EtOH, anhydrous THF, wet silica gel, N₂, RT.
PDC oxidation was chosen as the preferred method for oxidation due to its success with the monomer oxidation on a small scale although it is assumed that Swern oxidation would have worked just as well. The successful oxidation of the 2-OH dilactam PBD dimer (164) was confirmed by the absence of the 2-OH and C2-H peaks in the proton NMR as well as the change in coupling patterns of the H3 and H1 protons. Following the synthesis of the key PBD dilactam ketone dimer (163), the ketone was subjected to the critical triflation reaction. This proved to be highly successful with hardly any by-products being formed as was the case with the monomer. The structure of the triflate dimer was confirmed by $^1$H NMR and mass spectrometry. The H3 protons in the 4-3 ppm region were absent and a new H3 alkenic proton was clearly observed further downfield at 7 ppm. Mass spectrometry confirmed the molecular weight of the product as 1145.

For the Suzuki coupling reaction, 4-tert-Bu-benzene boronic acid was chosen as this Suzuki coupling for the monomer was high yielding and the biological activity of the 4-tert-bu benzene PBD analogue was impressive. The Suzuki coupling for the dimer was, unfortunately, low yielding and very little material was isolated for the final step. The reaction however, was successful and the product was confirmed by the presence of new aromatic peaks in the 7.1-7.8 region and the tert-Bu peak at 1.25 ppm in the $^1$H NMR. Additionally, the Suzuki coupling product, like monomers, exhibited the characteristic blue fluorescence under short wave and long wave UV in the TLC analysis. The final step was the reduction of the N10-C11 lactam and subsequent SEM deprotection. Unfortunately, this final step proved unsuccessful and no final product was isolated. This could have been due to the small quantity of starting material (as this method is known to be low-yielding for the C2-aryl PBD monomers and is expected to be low-yielding for the dimers as well) or due to the well-documented issue of over-reduction of the N10-C11 lactam.

Unfortunately, the synthesis of the C2-aryl PBD dimers could not be re-attempted as part of this research project due to time and funding restrictions. This synthetic approach is however very viable and was expected to be successful. The failure to isolate the final C2-aryl PBD dimer in the final step should therefore not be taken as a failure of the synthetic approach employed. It is likely that using a larger quantity of starting material and/or shorter reaction time that the synthesis of the C2-aryl PBD dimer would be
successful. Additionally, employing Hu’s lithium borohydride reduction instead of the sodium borohydride method used for the C2-aryl PBD monomers could also yield successful results.
BIOLOGICAL STUDIES

Biological evaluation of the C2-aryl PBDs

As discussed in the previous chapter, 18 C2-aryl PBDs were successfully synthesised and biologically evaluated in in vitro and in vivo studies in the UK (UCL and School of Pharmacy, London), Germany (Institute of Experimental Oncology, Freiburg) and in the USA (NCI, Washington DC). This chapter presents and discusses the results of the biological studies performed on these novel C2-aryl PBDs.

Cytotoxicity studies

Freiburg studies

Clonogenic assays were used for the in vitro cytotoxicity studies performed in Freiburg. Solid human tumour xenografts that were previously implanted subcutaneously in nude mice were removed, mechanically disaggregated and subsequently incubated with an enzyme cocktail \(^{122}\). These xenograft tumour cells were used for in vitro cytotoxicity studies. This contrasts with the NCI method which uses a stock supply of preserved cell lines (see below). Therefore the two data sets from Freiburg and the NCI are not directly comparable although the main purpose of the in vitro studies was to ascertain any trends in activity within the group of analogues.

The results of the Freiburg studies have been reported as T/C-values (%) (Equation 1).

\[
\frac{T}{C} = \frac{\text{colony count}_{\text{treated group}}}{\text{colony count}_{\text{control group}}} \cdot 100
\]

Equation 1: Equation for the calculation of T/C values (%)
GI_{50} and GI_{70} are the drug concentrations necessary to inhibit colony formation by 50% and 70% respectively (i.e. T/C = 50% and T/C = 30% respectively)\(^{122}\) (Equation 2).

\[
mean\_GI_{50,70} = 10^{\frac{\sum_{x=1}^{n} lg(GI_{50,70})}{n}}
\]

Equation 2: Calculation of mean GI_{50} and mean GI_{70} where x = specific tumour cell line and n = total number of tumour cell lines studied.

The results from the Freiburg clonogenic assay are presented in Table 6 and figures 43, 44 and 45. Table 6 shows the drug concentration required (in nM) to inhibit cell growth by 50% whereas figures 43, 44 and 45 display the data graphically, comparing the activity of the analogues for three selected cell lines. Two distinct groups of analogues, with regards to their *in vitro* activity in this study, can be clearly seen graphically and these have been indicated on the charts.

Table 6: GI_{50} values (nM) from the Freiburg clonogenic *in vitro* study

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<th>148</th>
<th>149</th>
<th>150</th>
<th>151</th>
<th>152</th>
<th>153</th>
<th>154</th>
<th>156</th>
<th>DRH-417</th>
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Figure 43: T/C % averages Vs Log concentration (micromolar - μM) of the C2-aryl PBDs in the renal cell line RXF486L. The chart shows, in comparison, the results of the in vitro screening experiments performed in Freiburg. The curves on the left hand side show a higher potency than those on the right, whereas compound 150 does not exhibit the same sigmoid curves (i.e. the same activity profile) as the other analogues.

Figure 44: T/C % averages Vs Log concentration (micromolar - μM) of the C2-aryl PBDs in the lung large cell cancer cell line LXFL529L
NCI 60-cell line primary screen

The NCI 60-cell line screen utilizes 60 different human tumour cell lines, representing leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The aim is to select synthetic compounds or natural product samples showing selective growth inhibition or cell killing of particular tumour cell lines for further evaluation*. In addition, following characterization of various cellular molecular targets in the 60 cell lines, it may be possible to select compounds most likely to interact with a specific molecular target.

Table 7 and figure 46 show the data obtained from the NCI 60-cell line evaluation of these novel compounds. Figure 46 shows only the LC$_{50}$ panels of the NCI bar charts (see

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* The NCI reports the data obtained as GI$_{50}$ (Equation 2), TGI (Total cell growth inhibition) and LC$_{50}$ (the drug concentration required to kill 50% of the cells).
appendix A: figs I-IV & XI for supporting information) for ease of comparison of the activity displayed by the different analogues.

### Table 7: NCI 60-cell line screening results

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<th>TGI</th>
<th>LC(_{50} )</th>
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<td>DRH417</td>
<td><img src="image" alt="Structure" /></td>
<td>&lt;10</td>
<td>64.57</td>
<td>10.47 μM</td>
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</tr>
<tr>
<td>TT9b</td>
<td><img src="image" alt="Structure" /></td>
<td>&lt;10</td>
<td>145</td>
<td>13.49 μM</td>
<td></td>
</tr>
<tr>
<td>NC085 (183)</td>
<td><img src="image" alt="Structure" /></td>
<td>12.30</td>
<td>240</td>
<td>8.13 μM</td>
<td></td>
</tr>
<tr>
<td>NC104 (185)</td>
<td><img src="image" alt="Structure" /></td>
<td>148</td>
<td>2.14 μM</td>
<td>33.11 μM</td>
<td></td>
</tr>
</tbody>
</table>
Figure 46: (a) & (b): LC_{50} 60-cell line bar charts for each analogue. Highlighted in red are the positive results for the melanoma lines (panel E) while in green are those for colon cancer (panel C), purple for non-small cell lung cancer (panel B), yellow for CNS cancer (panel D), pink for leukaemia (panel A), blue for renal (panel G) and cyan for breast cancer lines (panel I).
Figure 46 (c) & (d): LC_{50} 60-cell line bar charts for each analogue. Highlighted in red are the positive results for the melanoma lines (panel E) while in green are those for colon cancer (panel C), purple for non-small cell lung cancer (panel B), yellow for CNS cancer (panel D), pink for leukaemia (panel A), blue for renal (panel G) and cyan for breast cancer lines (panel I).
Figure 46 (e): LC50 60-cell line bar charts for each analogue. Highlighted in red are the positive results for the melanoma lines (panel E) while in green are those for colon cancer (panel C), purple for non-small cell ling cancer (panel B), yellow for CNS cancer (panel D), pink for leukaemia (panel A), blue for renal (panel G) and cyan for breast cancer lines (panel I).

Computer Aided Molecular Modelling

In *silico* molecular dynamics (MD) simulations were used to investigate interactions between the C2-aryl PBDs and DNA using the Poisson-Boltzmann (PB) method. The same DNA sequence, d(GCGCAGAGCGC)2, was used for each ligand-DNA model. The covalent bond was formed between the PBD moiety and the central guanine residue, and the ligand oriented in S3’ stereochemistry (i.e. with the PBD A ring pointing towards the 3’-terminus of the strand to which it was covalently bound and the C11-position in the S configuration). This has been shown to be the preferred configuration for most PBD monomer systems.69

Table 8 shows the data obtained from the molecular modelling studies. The data is presented with the compounds exhibiting the lower DNA binding energies (i.e. the analogues that were calculated to cause the least disruption to the DNA helix) at the top of
the table and ending with the sterically hindered analogue 157 at the bottom, which, as predicted, has the highest DNA binding energy of the group. Figure 47 shows graphical representations of the data for four of the analogues.

Table 8: Results from the molecular modelling studies showing DNA binding energies as calculated from the Poisson-Boltzmann method

<table>
<thead>
<tr>
<th>Compound</th>
<th>C2-Substituent</th>
<th>DNA Binding energies (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>151 (NC072)</td>
<td><img src="image1.png" alt="Image" /></td>
<td>-134.99</td>
</tr>
<tr>
<td>185 (NC104)</td>
<td><img src="image2.png" alt="Image" /></td>
<td>-134.28</td>
</tr>
<tr>
<td>148 (NC047)</td>
<td><img src="image3.png" alt="Image" /></td>
<td>-133.05</td>
</tr>
<tr>
<td>149 (NC048)</td>
<td><img src="image4.png" alt="Image" /></td>
<td>-130.81</td>
</tr>
<tr>
<td>142 (NC077)</td>
<td><img src="image5.png" alt="Image" /></td>
<td>-130.66</td>
</tr>
<tr>
<td>183 (NC085)</td>
<td><img src="image6.png" alt="Image" /></td>
<td>-129.62</td>
</tr>
<tr>
<td>150 (NC053)</td>
<td><img src="image7.png" alt="Image" /></td>
<td>-128.38</td>
</tr>
<tr>
<td>158 (NC081)</td>
<td><img src="image8.png" alt="Image" /></td>
<td>-128.22</td>
</tr>
<tr>
<td>147 (NC020)</td>
<td><img src="image9.png" alt="Image" /></td>
<td>-127.25</td>
</tr>
<tr>
<td>156 (NC078)</td>
<td><img src="image10.png" alt="Image" /></td>
<td>-125.24</td>
</tr>
<tr>
<td>tt9b</td>
<td><img src="image11.png" alt="Image" /></td>
<td>-124.43</td>
</tr>
<tr>
<td>157 (NC079)</td>
<td><img src="image12.png" alt="Image" /></td>
<td>-120.72</td>
</tr>
</tbody>
</table>
Figure 47: (a) NC079 (2,6-diMePh-PBD, 157); (b) NC053 (2-naphthyl-PBD, 150); (c) NC104 (4-MePh-vinyl-PBD, 185); (d) NC085 (4-phenoxybenzene-PBD, 183) covalently bound to the DNA minor groove in a minimum energy state.
DNA Melting studies

\[ \Delta T_m = T_m(DNA - PBD) - T_m(DNA) \]

Equation 3: Equation for the calculation of delta Tm values from the experimental data.

Thermal denaturation experiments (also known as DNA melting studies) aim to measure the stabilisation effect a DNA binding molecule may have on the DNA double helix. In order to translate this measurement into DNA binding affinity, known compounds are used for standardization. In the work reported here, test compounds were exposed to calf thymus DNA in a neutral buffer. Measurements were taken at 0 hours and set time points thereafter (e.g. 8, 16 and 24 hours).

At the given time points, the DNA-compound samples were measured for UV optical absorbance using a thermostatically controlled UV spectrophotometer. The temperature of the solution was gradually increased over 1 hour to obtain optical density sigmoid curves (Figure 48). These curves were then compared with the control curves (DNA in the absence of test compounds) and the ΔTm was calculated by measuring the difference between the temperature at which the DNA helix denatures and that at which the test compound + DNA helix denatures.

Table 9 shows the results of two independent DNA melting experiments. Table 9a shows an experiment with a select group of analogues, performed over an 18 hour period with readings taken at three time points. Table 9b shows the results of a larger experiment where readings were taken at one time point only (2 hours).
Figure 48: A sample DNA melting experiment graph. The red sigmoid curve shows a sample control curve, i.e. DNA without any drug added. The blue curve shows a sample curve of DNA with drug. The further to the right the blue curve is, the higher the binding affinity of the drug with the DNA. The ΔTm is difference between the two curves at 0.5 relative UV absorbance.

Table 9a: Results of DNA melting studies performed over an 18 hour time period with readings taken at 0, 4 and 18 hours.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0 h</th>
<th>4 h</th>
<th>18 h</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>δTm</td>
<td>δTm</td>
<td>δTm</td>
</tr>
<tr>
<td>Control</td>
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<td>0.00</td>
<td>0.00</td>
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</tr>
<tr>
<td>148 (NC047)</td>
<td>2.14</td>
<td>2.14</td>
<td>1.80</td>
</tr>
<tr>
<td>142 (NC077)</td>
<td>3.62</td>
<td>2.86</td>
<td>2.72</td>
</tr>
<tr>
<td>151 (NC072)</td>
<td>3.89</td>
<td>4.44</td>
<td>4.23</td>
</tr>
<tr>
<td>tt9b</td>
<td>5.42</td>
<td>5.62</td>
<td>5.63</td>
</tr>
<tr>
<td>153 (NC074)</td>
<td>7.17</td>
<td>7.43</td>
<td>7.46</td>
</tr>
<tr>
<td>158 (NC081)</td>
<td>8.84</td>
<td>9.24</td>
<td>9.26</td>
</tr>
<tr>
<td>150 (NC053)</td>
<td>11.40</td>
<td>12.27</td>
<td>12.50</td>
</tr>
<tr>
<td>Anthramycin</td>
<td>13.70</td>
<td>14.17</td>
<td>14.08</td>
</tr>
</tbody>
</table>
Table 9b: Results of the DNA melting studies (100mM CT-DNA (50mM base-pair) + 20mM drug; Incubated at 36°C for 2H prior to determination; 10mM sodium phosphate +1mM EDTA pH 7.0)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ave. Tm °C</th>
<th>Ave. Dev ±</th>
<th>ΔTm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.7</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>157 (NC079)</td>
<td>71.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>148 (NC047)</td>
<td>70.9</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>156 (NC078)</td>
<td>71.4</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>142 (NC077)</td>
<td>72.7</td>
<td>0.2</td>
<td>2.1</td>
</tr>
<tr>
<td>185 (NC104)</td>
<td>74.4</td>
<td>0.2</td>
<td>3.7</td>
</tr>
<tr>
<td>149 (NC048)</td>
<td>72.9</td>
<td>0.2</td>
<td>2.3</td>
</tr>
<tr>
<td>151 (NC072)</td>
<td>74.2</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td>147 (NC020)</td>
<td>74.6</td>
<td>0.2</td>
<td>3.9</td>
</tr>
<tr>
<td>183 (NC085)</td>
<td>75.2</td>
<td>0.2</td>
<td>4.5</td>
</tr>
<tr>
<td>152 (NC073)</td>
<td>75.9</td>
<td>0.2</td>
<td>5.2</td>
</tr>
<tr>
<td>tt9b</td>
<td>75.7</td>
<td>0.1</td>
<td>5.1</td>
</tr>
<tr>
<td>AT210</td>
<td>76.2</td>
<td>0.3</td>
<td>5.6</td>
</tr>
<tr>
<td>153 (NC074)</td>
<td>78.3</td>
<td>0.3</td>
<td>7.6</td>
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<td>DRH417</td>
<td>75.8</td>
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<td>5.1</td>
</tr>
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<td>0.1</td>
<td>7.0</td>
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<td>82.5</td>
<td>0.3</td>
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<tr>
<td>anthramycin</td>
<td>85.0</td>
<td>0.2</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Hollow fibre studies

The hollow fibre assay for preliminary in vivo testing has been adopted by the NCI to identify potential candidates suitable for further xenograft evaluation. Compounds are selected for further testing on the basis of several hollow fibre assay criteria. These include a reduction in net cell growth of 50% or greater in 5 of the 24 possible test combinations (6 cell lines x 2 sites x 2 compound doses). A reduction in net cell growth of 50% or greater in a minimum of 4 of the 24 distant site combinations (IP/SC culture) and/or a cell kill of 1 or more cell lines in either implant site.

A point system is used that allows rapid evaluation of the activity per compound. A value of 2 is given for each compound dose which results in a 50% or greater reduction in viable cell mass. The IP and SC samples are scored separately so that the criteria can be evaluated. Compounds with a combined IP and SC score of 10, a SC score of 4 or a net
cell kill of one or more cell lines are referred for further studies. The maximum possible score for an agent in this study is 48. The results of this hollow fibre assay are presented in Table 10.

It is important to note that the hollow fibre evaluation performed by the NCI on the C2-aryl PBDs was focused on cell types identified from the primary screen and hence not identical to the standard hollow fibre screening commonly performed. Therefore, the maximum theoretical and hence observed scores are lower than those expected had a full hollow fibre study been performed.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Structure</th>
<th>Hollow Fibre</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 4</td>
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<tr>
<td></td>
<td></td>
<td>SC 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 4</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>SC 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 8</td>
</tr>
<tr>
<td>150</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>IP 10</td>
</tr>
<tr>
<td>NC053</td>
<td></td>
<td>SC 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 10</td>
</tr>
<tr>
<td>142</td>
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<td>IP 0</td>
</tr>
<tr>
<td>NC077</td>
<td></td>
<td>SC 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 4</td>
</tr>
<tr>
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<td></td>
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</tr>
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<td></td>
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<td>Hollow Fibre</td>
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<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 4 SC 4 Total 8</td>
</tr>
<tr>
<td>156 (NC078)</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>IP 2 SC 2 Total 4</td>
</tr>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 2 SC 2 Total 6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 0 SC 0 Total 0</td>
</tr>
<tr>
<td>151 (NC072)</td>
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<td></td>
</tr>
<tr>
<td></td>
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<td>IP 2 SC 4 Total 6</td>
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<td>158 (NC081)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 0 SC 0 Total 4</td>
</tr>
<tr>
<td>157 (NC079)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP 0 SC 0 Total 0</td>
</tr>
</tbody>
</table>

**Xenograft studies**

Xenograft studies remain the most important means of identifying candidate anticancer agents for further pre-clinical development. The number of studies that can be performed are limited for ethical and financial reasons (hence the need for the hollow fibre assays). Xenograft studies involve the subcutaneous implantation of tumours of interest in immune-compromised nude mice. The drug is then administered intravenously whilst a number of factors, such as death, weight loss and tumour mass are monitored closely in order to establish the toxicity as well as the anti-tumour efficacy of the drug under investigation. Untreated control mice are also used in order to determine whether a
particular drug actively halts tumour growth or even reduces the tumour mass. The main results of interest are the relative tumour volume (% T/C) and the growth delay (% T-C/C), where % T/C is the mass of the treated tumour divided by the mass of the control (untreated mice) given as a percentage. This gives an indication as to the degree of tumour growth suppression, with lower percentages being favourable. % T-C/C is an expression of the time in days taken to reach a predefined mass as a percentage ratio giving the growth delay and demonstrates how effective the drug is at reducing growth rate of the tumour.

Table 11: Table summarising the data from the xenograft investigations

<table>
<thead>
<tr>
<th>Group</th>
<th>Cpd. ID</th>
<th>Dose/units</th>
<th>Route</th>
<th>Schedule</th>
<th>No. Mice</th>
<th>Drug deaths</th>
<th>% wt loss</th>
<th>Opt % T/C</th>
<th>Median Tumour weight</th>
<th>Growth Delay % T-C/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKMEL-28 melanoma xenografts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0mg/kg/dose</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
<td>12</td>
<td>0</td>
<td>No wt loss</td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>151 (NC072)</td>
<td>150</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
<td>6</td>
<td>0</td>
<td>No wt loss</td>
<td>89(24)</td>
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<td>3</td>
<td>100</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
<td>6</td>
<td>0</td>
<td>No wt loss</td>
<td>115(21)</td>
<td>113</td>
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<tr>
<td>4</td>
<td>67</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
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<td>No wt loss</td>
<td>111(24)</td>
<td>126</td>
<td>-12</td>
<td></td>
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<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
<td>12</td>
<td>0</td>
<td>No wt loss</td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>142 (NC077)</td>
<td>150</td>
<td>IV</td>
<td>Qdx5 Day 7</td>
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<td>0</td>
<td>No wt loss</td>
<td>82(17)</td>
<td>144</td>
<td>19</td>
</tr>
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<td>6</td>
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<td>IV</td>
<td>Qdx5 Day 7</td>
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<td>162</td>
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<td>7</td>
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<td>Qdx5 Day 7</td>
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</tr>
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<td>Q4Dx3 Day 14</td>
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<td>IV</td>
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<td>72(42)</td>
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<td>7.2 (32)</td>
<td>84(42)</td>
<td>156</td>
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<td></td>
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<td>4</td>
<td>112</td>
<td>IV</td>
<td>Q4Dx3 Day 14</td>
<td>6</td>
<td>0</td>
<td>12.2(56)</td>
<td>68(53)</td>
<td>108</td>
<td>-8</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Cpd. ID</td>
<td>Dose/units</td>
<td>Route</td>
<td>Schedule</td>
<td>No. Mice</td>
<td>Drug deaths</td>
<td>% wt loss (day)</td>
<td>Opt % T/C</td>
<td>Median Tumour weight</td>
<td>Growth Delay % T-C/C</td>
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<tr>
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<td>---------------------</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>IV</td>
<td>Q4Dx3 Day 14</td>
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<td>0</td>
<td>10.5(53)</td>
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<td>IV</td>
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<td>100</td>
<td></td>
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<tr>
<td>5</td>
<td>151</td>
<td>150</td>
<td>IV</td>
<td>QDx5 Day 7</td>
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<td>83(32)</td>
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<td>99(14)</td>
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<td>QDx5 Day 7</td>
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<tr>
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<td>QDx5 Day 16</td>
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<td><strong>Dose/units</strong></td>
<td><strong>Route</strong></td>
<td><strong>Schedule</strong></td>
<td><strong>No. Mice</strong></td>
<td><strong>Drug deaths</strong></td>
<td><strong>% wt loss (day)</strong></td>
<td><strong>Opt % T/C</strong></td>
<td><strong>Median Tumour weight</strong></td>
<td><strong>Growth Delay % T-C/C</strong></td>
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</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0mg/kg/dose</td>
<td>IV</td>
<td>Q4Dx3 Day 12</td>
<td>12</td>
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<td>1.1 (26)</td>
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<td>2</td>
<td>151</td>
<td>250</td>
<td>IV</td>
<td>Q4Dx3 Day 12</td>
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<td>0</td>
<td>No wt loss</td>
<td>51 (29)</td>
<td>144</td>
<td>36</td>
</tr>
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<td>168</td>
<td>IV</td>
<td>Q4Dx3 Day 12</td>
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<td>91 (29)</td>
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<td>9</td>
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<td>4</td>
<td></td>
<td>112</td>
<td>IV</td>
<td>Q4Dx3 Day 12</td>
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<td>0</td>
<td>No wt loss</td>
<td>56 (44)</td>
<td>122</td>
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SC-A498 renal tumour xenografts
Cytotoxicity studies

It has previously been established that endo and exo unsaturation at the C2-position of the core PBD molecule enhances the cytotoxicity of these antitumour agents \(^{76,77}\). In order to clarify the importance of the endo unsaturation in the absence of C2-substituents, 189 was synthesized (as part of a related project)\(^{123}\). This parent compound did show a degree of activity with an average GI\(_{50}\) of 1.3 \(\mu\)M in the standard NCI 60-cell line screen (app. A: fig. V). Addition of an aromatic ring to this baseline molecule increased the potency by more than 100-fold. However, endo unsaturation is still apparently critical to the activity of these C2-aryl PBDs as removal of the 2,3-unsaturation whilst retaining the C2-aromatic substituent molecules reduces the potency to 9.8 \(\mu\)M at the GI\(_{50}\) level\(^ {79}\) (app. A: fig X).

The position of the unsaturation in the C-ring is also important as preliminary molecular modelling studies have indicated that molecules with 1,2-endo-unsaturation\(^ {79}\) (e.g. 190 and 191) have a more planar shape and are thus less isohelical with the DNA minor groove. For example, 190 and 191, both 1,2-unsaturated C2-aryl PBDs, show a significant reduction in potency with an average GI\(_{50}\) of 85.1 and 21.9 nM respectively (app. A: figs. VI & VII). Additionally, aromatization of the C-ring results in a significant loss of activity with 192 and 193 exhibiting an average GI\(_{50}\) of 18.6 and 17.4 \(\mu\)M respectively (app. A: figs. VIII & IX). These C2-aryl PBD variants not only show decreased potency, but in the NCI 60-cell line screen their activity profile shows a loss of selectivity for particular cell line panels.
From Table 6 it is clear that both endo unsaturation and a C2-aromatic substituent are required for significant potency and selectivity. The nature of the C2-aromatic substituent also appears to have an effect on the biological activity of these molecules, although statistical analysis showed no significant correlation between Hansch constants and biological activity for either the Freiburg data or NCI data. However a pattern of activity was observed within the Freiburg data set as is shown in figures 43, 44 and 45. From visual inspection, it can be ascertained that 147, 152, 156, 151, 153 and 150 show similar biological activity and high potency in the cell lines of interest to this work, whereas 148, 149 and 154 possess lower potency in these same cell lines. An important point to note with the Freiburg data is that the most impressive and promising activity was seen in the melanoma, renal and lung cancer cell lines, with the naphthyl analogue exhibiting impressive activity in these cell lines. Therefore, although there is no statistically significant correlation in terms of QSAR, the data clearly shows a trend in biological activity within this group of analogues.

† None of the compounds indicated in Figure 49 were synthesised as part of this project but were synthesised and evaluated in parallel to this study by Spirogen. The NCI in vitro data has been provided (in Appendix A), with permission, for the purpose of comparison with the data presented in this study.
Regarding the NCI 60-cell line screening data, all of the C2-aryl PBDs tested to date show promising activity in vitro. Figure 46 shows the LC$_{50}$ portion of the NCI 60-cell line bar charts. For ease of identification, the melanoma panel has been highlighted in red. It is clear that this group of molecules exhibits selective activity for melanoma and renal cancer cell lines, with all of the molecules showing at least a 10 fold increase in activity in the melanoma lines compared to the average LC$_{50}$ across all the cell lines. The 2,6-dimethylbenzene analogue, as expected, is the least potent and the least selective in this in vitro study, whereas the naphthyl analogue shows the most impressive activity and is the most potent of the group (Table 7).

Statistical analysis

![Figure 50: Cluster analysis of the C2-aryl PBD activity profiles in the NCI 60-cell line in vitro screening of these analogues](image)

More detailed analysis of the data revealed two distinct sub-groups of compounds within the library of C2-aryl PBDs investigated in the in vitro NCI screen (table 12, figure 50). A similar clustering was observed for the Freiburg in vitro data (figure 43). In addition, a small number of outlying C2-aryl PBD analogues exhibited markedly different activity...
profiles in comparison with the other C2-aryl PBDs. However, these outlying molecules, with the exception of 152 (NC073: the 4-Cl-Ph analogue), represent significantly different structural types.

Figure 50 shows a cluster analysis of the NCI 60-cell line data. Cluster A contains the dimethylaminophenyl, the tert-butylphenyl, the naphthyl, the thiophene and the 4-methylphenyl analogues, whereas cluster B contains the biphenyl, the fluorophenyl, the methylenedioxyphenyl, the 2-methylphenyl and the ethylphenyl C2-substituted analogues (Table 12).

Table 12: Table identifying the members of clusters A and B

<table>
<thead>
<tr>
<th>Cluster A</th>
<th>Cluster B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ID</strong></td>
<td><strong>Shorthand code</strong></td>
</tr>
<tr>
<td>154 NC076</td>
<td>4-DiMeNPh</td>
</tr>
<tr>
<td>151 NC072</td>
<td>4-tBuPh</td>
</tr>
<tr>
<td>158 NC081</td>
<td>2-Thiophene</td>
</tr>
<tr>
<td>150 NC053</td>
<td>2-naphthyl</td>
</tr>
<tr>
<td>147 NC020</td>
<td>4-MePh</td>
</tr>
</tbody>
</table>

Close inspection of the GI\textsubscript{50} data confirmed that the observed clustering reflected real differences in activity profiles. In the non-small cell lung cancer panel, cluster A members exhibit better than average activity in A549 (with the exception of 147 in this line) and KM12, clusters A and B exhibit interesting potency towards the HOP92 and NCIH23 lines whereas 147 (4-MePh), 150 (2-naphthyl) and 151 (4-t-BuPh) (members of cluster A) exhibit greater than average activity in HT29.
The significant activity of these molecules in general in melanoma and renal cancer cell lines is again evident (c.f. Freiburg data) in the GI\textsubscript{50} data where the majority of cluster A and B members show better than average activity in LOXIMVI, MALME3M, MK14 and SKMEL5, with UA62 being the only cell line in the melanoma panel where the two clusters show a slight difference in activity, cluster A out-performing B.

Distinct differences in activity profiles between the two clusters are evident in the ovarian, prostate and breast cancer cell lines. In OVCAR-5 the majority of members of cluster A show greater than average activity whereas only one member of B shows similar activity. Some cluster A members show promising activity in the PC3 line whilst all members from cluster B show less than average activity in this cell line. Finally, cluster A outperforms B in the breast MDAMB-4 cell line.

The cluster analysis of the activity profiles, clearly demonstrates that C2-aryl PBDs possess differential patterns of activity. However, the challenge still remains to explain why individual C2-aryl PBDs fall into cluster A or B, or exhibit outlying profiles.

**Modelling studies**

It was expected that the steric hindrance of the ortho-methyl groups on the C2-aromatic substituent of 157 may cause disruption to the DNA helix. This proved to be the case and was confirmed in both the molecular modelling studies and the DNA melting experiments. The 2,6-dimethyl analogue, 157, was shown to have a binding energy of \(-120\) kcal/mol. In contrast, other extended planar analogues such as the fused ring naphthyl analogue 150 produced less disruption (\(-128.38\) kcal/mol) of the DNA helix when bound in the minor groove.

However, with the exception of the sterically hindered analogues, there appears to be no statistically significant correlation between DNA binding affinities and the calculated DNA binding energies. The naphthyl and thiophenyl analogues, for instance, which both exhibit high DNA binding affinity, show a higher DNA binding energy in comparison...
with the unsubstituted phenyl analogue as well as other analogues such as the methylenedioxyphenyl analogue that exhibits a poor DNA binding affinity.

This further supports the notion that no one factor is predominant in accounting for the potency and biological activity exhibited by these molecules.

**DNA melting studies**

The data from the DNA melting studies was evaluated using QSAR techniques, primarily through Topliss and Hansch analyses. However, as with the molecular modelling studies, an obvious correlation was not observed for the measured ΔTms and Hansch constants. What can be ascertained is that certain structural features in conjunction with lipophilicity may play an important role in enhancing the DNA binding affinity of this group of molecules.

From the data it is clear that steric hindrance arising from substituents on the C2-aromatic ring leads to a substantial decrease in thermal stabilisation compared with other analogues. In contrast, the highly lipophilic flat, fused ring system of the naphthyl analogue exhibits a thermal stabilisation almost comparable to that of the well known, highly potent, naturally occurring PBD, anthramycin. Nearly as impressive is the DNA binding affinity of 153, the 4-fluorophenyl analogue and DRH417, the 4-methoxyphenyl analogue (two opposites as far as the Topliss analysis is concerned) with ΔTms of 5 °C and 5.1 °C, respectively. Additionally, analogues with a high DNA binding affinity show, like anthramycin, a steady increase in ΔTm over time. Interestingly, analogues with a low DNA binding affinity exhibited no increase or even a slight decrease in ΔTm over time, possibly a reflection of dissociation from the DNA helix perhaps due to disruption of the helical structure.

Although it was not possible to draw any definitive conclusions regarding SAR for both the DNA denaturation experiments and the molecular modelling studies, the results from both studies were consistent. As can be seen from the DNA melting data, the 2-naphthyl analogue, 150, has a ΔTm value comparable to that for anthramycin. Thus, 150 has the
highest DNA binding affinity from this group of monomer analogues. Likewise, it has been shown *in silico* that 150 binds tightly in the DNA minor groove causing minimal disruption, perhaps accounting for its very high DNA binding affinity. On the other hand, sterically hindered analogues (e.g. the 2-MePh 156 and the 2,6-diMePh 159 analogues) cause disruption to the DNA helix and thus have a lower binding affinity. The lower binding affinity of the hindered 2-methylphenyl and 2,6-dimethylphenyl analogues may be due to the fact that the C2-substituent is twisted out of plane thereby causing a significant disruption to the DNA helix consistent with the molecular modelling studies.

**In vivo studies**

**Hollow-fibre studies**

The NCI selected 9 compounds for hollow fibre studies based on their performance in the 60-cell line screen. The results of the preliminary hollow fibre studies in a sample of melanoma and renal cancer cell lines are shown in Table 10. As can be seen, a number of the compounds exhibited significant activity.

In the melanoma lines, the most interesting analogues were the naphthyl and 4-ethylphenyl analogues with total scores of 8 and 10 respectively. The 4-F-Ph and 4-t-Bu both scored a total of 6, while, interestingly, the 2-methyl analogue scored a total of 8. However, lipophilic analogues appeared to score better for the intraperitoneal site rather than the subcutaneous site in the melanoma lines investigated. This suggests that an increase in lipophilicity may decrease the ability of the analogue to reach the tumour site. As was expected from the *in silico* and *in vitro* studies, the 2,6-dimethylphenyl analogue performed poorly in the melanoma hollow fibre studies.

The scores in the hollow fibre studies for the renal lines were not as impressive as for the melanoma lines. However, some analogues did show sufficient activity to warrant further investigation. The 4-t-Butyl phenyl analogue, 151, was equipotent against renal and melanoma cell lines. More surprising was the promising activity exhibited by the 2,6-
dimethylphenyl analogue, 157, at both the IP and SC sites. All prior studies had suggested that this analogue would be the least active of the group of C2-aryl PBDs.

**Xenograft studies**

Three molecules were selected from the hollow fibre studies for further investigation in classical xenograft studies. Table 11 shows the results of the xenograft studies undertaken by the NCI.

As can be seen, the 2,6-dimethylphenyl (157) and 4-t-BuPh (151) analogues have encouraging biological activity in the renal cancer CAKl-1 tumour models with optimal relative tumour volumes (%T/C) of 26 and 36%, respectively, and an equally encouraging growth delay (%T-C/C) of 84% and 86% respectively. Additionally, the 4-ethylphenyl analogue, 142, showed activity in the melanoma cell line, UACC-257, with optimal tumour volume (%T/C) of 52.5% at a dose of 112 µg/kg. Worthy of note is the activity of 151 in the renal SC-A498 line with a % T/C of 51% at the highest dosage (250 µg/kg), without any weight loss in the animals being noted.

An important observation was the unexpected activity of the sterically hindered analogue 157. This analogue, although significantly more active than related PBDs without C2-aryl substituents, was less potent than the other C2-aryl PBD analogues in the NCI 60-cell line screen. This lack of *in vitro* activity was consistent with the *in silico* studies which suggested the molecule causes significant disruption to the DNA helix and binds poorly to DNA. However, the activity exhibited by this analogue *in vivo* was a pleasant surprise. In the renal CAKl-1 line, at the lowest dose (67 µg/kg), the relative tumour volume was 26 % and the growth delay 84 %. With an increase in dose, relative tumour volume increased slightly to 32 % and 59% whilst growth delay decreased to 70 % and 21 %, an observation warranting further investigation.

In summary, the 2,6-diMePh (157) and the 4-t-BuPh (151) analogues show encouraging *in vivo* activity in the renal, CAKI-1 model. These molecules will progress to further studies in the future.
CONCLUSION – BIOLOGICAL STUDIES

From the data presented above, it can be seen that these novel C2-aryl PBDs are potent cytotoxic agents. Their biological profiles indicate that they exhibit a significant degree of selectivity towards certain tumour cell lines.

Although quantitative SAR studies have not found a correlation between Hansch constants and in vitro biological activity, lipophilicity of the C2-substituents on the PBD core may play a role in influencing the biological activity of these molecules.

It can be hypothesised that, in terms of a pharmacophore, for these PBD monomers, a lipophilic C2-substituent may be a key feature. Additionally, as has already been reported\textsuperscript{76} C2-C3 endo unsaturation plays a key role in enhancing activity and improving the selectivity of these molecules. Looking at the data for compound 190\textsuperscript{79}(app. A: fig. VI), it appears that 1-2 endo unsaturation also increases the potency of PBD monomers compared to the C-ring saturated analogue 194 (app. A: fig. X). However, the activity of this molecule is considerably lower than that of the related C2-C3 endo unsaturated analogue, DRH417.

The 2-naphthyl analogue, 150, has consistently shown throughout its progress through in vitro and in vivo evaluations, that it is one of the most promising C2-aryl PBD analogues. It exhibits both high potency and an impressive degree of selectivity for melanoma and renal cell cancers. Additionally, as can be seen from Figure 46(e), its activity in leukaemia cell lines is comparatively low, suggesting that it may have minimal myelotoxicity.

A second key feature that appears to enhance potency is the presence of a fused ring system as the C2-substituent. For example, both 149 and 150 are similar in terms of lipophilicity, but 149, the biphenyl analogue, has a considerably lower potency and DNA binding affinity compared to 150. This leads to the conclusion that lipophilic, planar fused ring systems appear to be preferred for enhancing potency and these are features that could be investigated further.
CONCLUSIONS AND FUTURE WORK

A group of fifteen novel synthetic C2-aryl PBD monomers were successfully designed, synthesized and biologically evaluated as potential anti-cancer cytotoxic agents. The study has shown that members of this novel group, which target the DNA minor groove, can be efficiently synthesised via a parallel combinatorial solution phase approach in sufficient quantities for extensive biological studies.

The nine-step synthetic route has been successfully optimised to improve overall yield and decrease costs. The parallel combinatorial steps of the synthesis have been semi-automated to increase ease of synthesis and to reduce the time required to synthesise a library of analogues in acceptable quantities.

Synthesis of the key triflate-PBD intermediate has been successfully achieved on a large scale. However, at the stage of C2-ketone formation, although the PDC approach was preferred in a medium scale synthesis, use of large quantities of PDC resulted in equally large quantities of chromium waste that were not desirable and also led to a reduced yield of the C2-ketone PBD product 74. Future synthetic work should therefore employ the equivalent Swern oxidation procedure which affords the ketone in similar yield but without producing metallic waste.

The final reduction and SEM-deprotection step, however, still remains a low-yielding and occasionally problematic synthetic step. Some of the more susceptible analogues were unfortunately over-reduced in this critical final step. Future investigations of alternative reduction approaches could be performed in order to achieve higher yields at this critical step and/or to overcome the risk of over reduction of the final PBD compound. One such alternative would be to use lithium borohydride as the reducing agent as employed by Hu et al. in the synthesis of DC81 (Figure 51). Should lithium borohydride prove to be too mild a reducing agent for some of the analogues (although this is not expected to be a likely situation), cheminformatics could be employed in order to identify susceptible
compounds and an appropriate reducing agent chosen on the basis of the cheminformatics study. An additional alternative, albeit a considerably different approach, would be to attempt the synthesis via a protected carbinolamine triflate (Figure 52) as opposed to a dilactam. Whilst the approach avoids the need for reduction, the triflation reaction may need to be re-optimised for the carbinolamine-ketone substrates.

![Figure 51: Adoption of the Hu approach to the reduction of the dilactam as applied to the synthesis of the C2-aryl PBDs.](image1)

![Figure 52: Troc protected carbinolamine triflate PBD intermediate.](image2)

The monomer synthetic route was subsequently adapted for the synthesis of the novel synthetic C2-aryl PBD dimers (160). The development of this synthetic route initially posed some challenges which were overcome by employing the triphosgene methodology, as used by Hu et al., in the first part of the synthetic route. Following successful synthesis of the PBD core dilactam (167) the synthesis proceeded along the route established for the monomers. This approach proved generally quite successful, although the overall yield at the penultimate step did not allow, unfortunately, for the synthesis of sufficient material to bring this aspect of the project to completion. However, this project could be repeated on a larger scale now that the synthetic route has been developed. Hence sufficient material could be synthesised at a later date on which biological studies could be performed to establish the effect of A-A ring dimerisation of the C2-aryl PBD molecules.
The success of the existing C2-aryl PBD monomer analogues in biological studies (both *in vitro* and *in vivo*) does not, however, mean an end to the medicinal chemistry phase of the project. The Suzuki-Miyaura coupling reaction has become increasingly popular over the last few years both in academic and industrial settings. This popularity has led to the development of an increasing number of commercially available boronic acids and esters, an increase in the chemical diversity of these boronic acids/esters and a decrease in the cost of reagents. This allows for the design and synthesis of a considerably larger library of C2-aryl PBD analogues with a greater degree of diversity in the library members than was possible at the beginning of the current project. One particular analogue of interest would be the C2-phenol PBD compound. As well as being a compound worthy of biological evaluation, this analogue could be synthesised on a large scale as the OH group would allow for triflation and further Suzuki coupling and hence a large number of 'extended' analogues could be synthesised that would have the potential to interact better with the floor of the minor groove. An alternative Suzuki coupling strategy, that could not be attempted during the course of the current project, would be to convert the PBD moiety to the boronic ester and couple this to commercially available haloaromatics (Figure 53). This would not only be interesting and novel synthetic chemistry but would also further increase the range of analogues that could be synthesised. With this increase in the diversity of possible analogues, the previously limiting factor of whether a designed analogue could be effectively synthesised would no longer be an issue, allowing for a greater involvement of *in silico* molecular modelling studies prior to synthesis.

![Figure 53: Expanding the diversity of the library members via a PBD boronic ester approach.](image)

Of the C2-aryl PBDs successfully synthesised during the course of this project, the C2-naphthyl-PBD analogue, NC053 (150), was found to be particularly active in both *in vitro*
and in vivo studies. As such, a separate study focused specifically on naphthyl analogues could possibly yield some very interesting and informative results. Therefore, future work should involve the synthesis of C2-naphthyl-PBD analogues such as C2-1-naphthyl-PBD (174) and substituted C2-2-naphthyl-PBD analogues (175) (Figure 54).

![Chemical structures](image)

**Figure 54:** The 2-naphthyl-PBD analogue NC053 and two possible analogues of this important molecule.

Finally, it is worth noting that although these analogues exhibit promising cytotoxic activity, their pharmacophore does not include one particular feature previously established to be beneficial to the overall activity of PBDs, i.e. the sugar moiety at the 7 position on the A-ring (c.f. sibiromycin). Future work could include the synthesis of C2-aryl PBDs exhibiting a sugar moiety at the 7 position (Figure 55). This modification should improve their solubility and could well improve their in vivo biological profiles.

![Chemical structure](image)

**Figure 55:** C2-aryl substituted glycosilated PBD analogue.

Biological evaluation of these novel C2-aryl PBD monomers involved a number of in vitro and in vivo studies including DNA-melting investigations, DNA-footprinting studies, in vitro clonogenic assays, the NCI 60 cell line panel screen, in vivo hollow-fibre studies and classic xenograft investigation. All biological studies performed showed these agents to be potent cytotoxic molecules. The DNA-melting and DNA-footprinting studies showed that these molecules do indeed bind to DNA. With our existing
knowledge of the mode of action of PBDs we can conclude that these agents are most likely targeting the DNA minor groove and covalently binding to the DNA as their main mode of action.

The *in vitro* cytotoxicity studies performed at the NCI and the clonogenic assays performed at Freiburg, show these molecules to posses cytotoxic properties against a number of cancer cell lines. Interestingly, all the synthesised C2-aryl PBD compounds exhibited above average potency against renal and melanoma cell lines in the NCI panel. Therefore it can be concluded from these data that the C2-aryl PBDs are potent cytotoxic agents with a degree of selectivity towards renal cancer and melanoma cell lines. Additionally some of the analogues showed promising activity against some lung cancers. All three cancers fall in the category of aggressive tumours with a low survival rate at the 5 year level and hence the activity of these novel agents against these cancers warrants considerable further investigation.

The preliminary *in vivo* studies performed yielded interesting data. Surprisingly, the sterically hindered analogue NC079 (157) exhibited unexpected activity *in vivo*. This analogue was thought to be the least active of the library based on *in silico* and *in vitro* studies. However, this agent showed promising activity in both the hollow-fibre studies and the xenograft evaluation. As a consequence, further investigation is required to explain the activity of this compound. It may be possible that this molecule is undergoing *in vivo* metabolism leading to a more active structure.

A point of importance is that the most promising analogue of this current library, the C2-naphthyl-PBD (150), was not included in the preliminary xenograft studies. The NCI, as previously mentioned, are currently continuing the investigation of this novel group of compounds and are performing a more in depth study of these analogues to establish a more efficient dosing schedule in preparation for more complete xenograft studies. These new studies will include the naphthyl analogue.
Synthesis of toluene-4-sulphonate-4-hydroxy-2-methoxycarbonyl-pyrrolidinium (4-hydroxy-L-proline methyl ester p-toluenesulphonate)

To a suspension of trans-4-hydroxy-L-proline (100 g, 763 mmol) in anhydrous methanol (333 mL) and benzene (667 mL) was added p-toluenesulphonic acid (159.6 g, 838.9 mmol). The mixture was heated at reflux while azeotropically removing water. After 4.5 hours the solution was cooled to room temperature and diethyl ether (1.5 L) was slowly added. The resulting crystals were collected and rinsed with diethyl ether (3 x 100 mL). The crude product was recrystalised from ethanol/diethyl ether in a cold room overnight and the crystals were collected and rinsed with diethyl ether. Further drying gave the known methyl ester 82 as a white powder (185.83 g, 586.21 mmol, 77 % yield). IR (nujol) ν: 3418.31, 3080.10, 2754.52, 2620.15, 2480.62, 2360.33, 2330.74, 1750.09, 1617.53, 1491.14, 1275.67, 1230.23, 1191.59, 1126.00, 1057.19, 1065.99, 1012.05, 966.78, 907.38, 873.80, 818.16, 693.49 cm⁻¹.

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1 This method has been adapted from the literature method: Williams, M. A.; Rapoport, H. Journal of Organic Chemistry 1994, 59, 3616-3625.
Synthesis of 1-(4,5-dimethoxy-2-nitro-benzoyl)-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester

Oxalyl chloride (6.14 g, 4.22 mL, 48.4 mmol) was added in one portion to a stirred suspension of 4,5-dimethoxy-2-nitro-benzoic acid (10.0 g, 44.0 mmol) in anhydrous DCM (80 mL) at room temperature. A catalytic amount of DMF (2 drops) was added, gas was evolved and the reaction mixture was left stirring for 20 hours under an inert atmosphere. The acid chloride solution was added dropwise to a vigorously stirred solution of trans-4-hydroxy-L-proline methyl ester-p-toluene sulphonate (13.95 g, 44.0 mmol) and TEA (13.36 g, 18.38 mL, 132 mmol) in anhydrous DCM (80 mL) at -20°C and stirred for a further 16 hours at room temperature. The reaction mixture was washed with saturated NaHCO₃ (2 x 160 mL), saturated NH₄Cl (2 x 160 mL), water (2 x 160 mL), brine (2 x 160 mL) and dried over anhydrous MgSO₄. Filtration and evaporation of the solvent in vacuo afforded the crude product, which was purified by flash column chromatography using a gradient system of 80 to 90% ethyl acetate in petroleum ether as eluent. Pure fractions were combined and evaporation of the solvent in vacuo afforded the pure product 80 as a yellow foam (8.82 g, 24.92 mmol, 57% yield). ¹H (250 MHz, CDCl₃) NMR: δ 7.69 (s, 1H, H-9), 6.85 (s, 1H, H-6), 4.79 (t, 1H, J = 8.10 Hz, H-11a), 4.5 (Broad s, -OH), 4.10-3.96 (m, 7H, MeO-7 & -8 and H-2), 3.75 (s, 3H, CO₂CH₃), 3.53-3.37 (m, 1H, H-3), 3.15 (d, 1H, J = 11.09 Hz), 2.45-2.32 (m, 1H, H-1), 2.19-2.05 (m, 1H, H-1). ¹³C (62.9 MHz, CDCl₃) NMR: δ 173 (O=C-O), 166.9 (C=O), 153.9 (C-8), 149.7 (C-7), 137.5 (C-10), 126.9 (C-5), 109.8 (C-6), 107.5 (C-9), 70.2 (C-2), 57.8 (C-11a), 57.3 (CH₃O -7/8), 56.9 (CH₃O -7/8), 56.8 (C-3), 52.9 (CH₃O), 38.3 (C-1). IR (neat) ν: 3435.97, 2947.87, 1735.17, 1723.61, 1654.35, 1638.02, 1578.21, 1527.56, 1499.93, 1438.19, 1331.84, 1274.08, 1223.32, 1178.59, 1073.89, 1002.95, 868.47, 785.98, 618.08 cm⁻¹. [α]D (CHCl₃) 39.474 °, c = 0.076 g/100mL.
Synthesis of 2-hydroxy-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione

Method 1

10% Pd/C catalyst (400 mg) was added to a solution of the substrate 80 (4.11 g, 11.61 mmol) in absolute ethanol (80 mL). The reaction mixture was hydrogenated under pressure using a Parr hydrogenator at 45 psi hydrogen for 24 hours. At this point a grey/white precipitate was observed in the reaction mixture. The precipitate and palladium on charcoal was collected by filtration through celite serif. The product was separated from the Pd/C by dissolving it in hot ethanol followed by hot methanol and filtering it through the celite pad, taking care not to allow the filter cake to dry out. The filtrate (lime yellow) was allowed to stand overnight. A white precipitate (1.89 g) formed and the resulting suspension was filtered. The white precipitate was collected and dried. The remaining filtrate was evaporated to allow further precipitation to occur and again the precipitate (0.68 g) was collected and dried. The filtrate was treated with 1N HCl (65 mL) and chloroform (65 mL). The organic layer was washed with 1N HCl (30 mL) and the aqueous layers combined, neutralised to pH7 with NaHCO₃ and left to stand overnight. No further product was collected. The final pure product 78 was a foamy white solid (2.57 g, 8.80 mmol, 75 % yield). ¹H (250 MHz, DMSO) NMR: δ 10.33 (s, 1H, H-10), 7.26 (s, 1H, H-6), 6.7 (s, 1H, H-9), 5.21 (d, 1H, J = 3.74 Hz, OH), 4.40 - 4.27 (m, 1H, H-2), 4.25 - 4.10 (m, 1H, H-11a), 3.79 (s, 6H, MeO-7 & -8), 3.64 (dd, 1H, J = 3.3, 11.9 Hz, H-3), 3.47-3.39 (m, 1H, H-3), 2.7 - 2.5 (m, 1H, H-1), 2.0 - 1.8 (m, 1H, H-1).

¹³C (62.9 MHz, DMSO) NMR: δ 170.9 (C=O), 165.9 (C=O), 152.5 (C-8), 146.1 (C-7), 131.5 (Ar-C-10), 118.6 (C-5), 112.7 (C-6), 105.2 (C-9), 68.3 (C-2), 56.5 (CH₃O -7 & -8),

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On large scale synthesis of the dilactam via hydrogenation, celite was NOT used during filtration due to the product precipitating within the celite cake. Instead plain filter paper with hot buchner funnels were used.
56.4 (C-11a), 54.8 (C-3), 35.2 (C-1). IR (neat) v: 2926.00, 2854.27, 1675.91, 1649.04, 1607.56, 1520.60, 1491.75, 1459.42, 1377.56, 1269.94, 1235.60, 1117.97, 1088.80, 1011.89, 995.44, 866.31, 832.44, 722.69 cm\(^{-1}\). HRMS (FAB) exact mass calculated for C\(_{14}H_{16}N_2O_5\)(M\(^+\)): m/z 292.1059, observed 292.1979. Melting Range: 280–282 °C. \([\alpha]_D\) (DMF) 408.33 °, c = 0.06 g/100mL.

**Method 2**

10% Pd/C catalyst (880 mg) was added to a solution of the substrate 80 (8.82 g, 24.92 mmol) in absolute ethanol (100 mL). The reaction mixture was hydrogenated under pressure at 45 psi H\(_2\) for 26 hours and then filtered through celite. The celite was washed with hot ethanol taking care not to allow the cake to dry out. Removal of excess solvent from the filtrate afforded the amino intermediate product 79. NC/24/1 \(^1\)H (250 MHz, DMSO) NMR: 6.69 (s, 1H, H-6), 6.37 (s, 1H, H-9), 5.36 (Broad s, 1H, NH), 5.16 (s, 1H, NH), 4.58 (t, 1H, J = 8.5 Hz, H-11a), 4.3 (Broad s, 1H, OH), 3.79-3.54 (m, 2H, H-3 & H-2 & CO\(_2\)CH\(_3\) & MeO -7 & -8), 3.51-3.30 (m, 1H, H-3), 2.23-2.15 (m, 1H, H-1), 2.0-1.87 (m, 1H, H-1). NC/24/1 \(^{13}\)C (62.9 MHz, DMSO) NMR: 173.7 (O=C-O), 170.1 (C=O), 152.6 (C-8), 143.9 (C-7), 140.0 (C-10), 113.4 (C-6), 109.4 (C-5), 100.8 (C-9), 69.7 (C-2), 58.4 (C-11a), 58.3 (C-3), 57.3 (CH\(_3\)O -7/8), 56.0 (CH\(_3\)O -7/8), 52.8 (CH\(_3\)O -11a), 37.9 (C-1). IR (neat) v: 2362.90, 1654.28, 1560.19, 1544.48, 1508.37 cm\(^{-1}\). \([\alpha]_D\) (THF) 5.000 °, c = 0.034 g/100mL.

79 in THF (22 mL) and water (155 mL) was treated with conc. HCl (0.5 mL) and allowed to stir for 48 hours at room temperature. A white precipitate was formed which was collected, washed with water and dried to give the product 78 as a foam-like white solid (4.53 g, 15.51 mmol, 62 % yield).

**Method 3**

Tin (II) Chloride was added to a solution of 80 (1.5 g, 8.48 mmol) in methanol (100 mL) and heated at reflux for 2 hours until TLC showed complete consumption of the starting material 80. The reaction mixture was then allowed to cool to room temperature and stand overnight at 0-5°C. The reaction mixture was filtered and the precipitate collected and dried to give the product 78 as a brittle white solid (217 mg, 0.74 mmol, 18 % yield).
Method 4

Sodium bisulphite (48.75 g, 280 mmol) was added to a solution of 80 (19.8 g, 55.93 mmol) in THF (1.2 L) and water (0.8 L) and the reaction mixture was allowed to stir at room temperature for 48 hours until no further change was observed by TLC. The product was extracted with chloroform (3 x 500 mL). The organic layers were combined, evaporated in vacuo and the residue treated with a solution of THF (40 mL), water (280 mL) and HCl (0.8 mL) for a further 48 hours. The precipitate formed was collected and dried to give the product 78 as a white powder (1.56 g, 5.34 mmol, 10 % yield).

Method 5

Tin (II) Chloride was added to a solution of 80 (1.5 g, 8.48 mmol) in ethanol (100 mL) and heated at reflux for 2 hours until TLC showed complete consumption of the starting material 80. The reaction mixture was then allowed to cool to room temperature and allowed to stand overnight at a low temperature. No precipitate was formed.

Method 6

Tin (II) Chloride was added to a solution of 80 (1.5 g, 8.48 mmol) in methanol (20 mL) and heated at reflux for 2 hours until TLC showed complete consumption of the starting material 80. The reaction mixture was then allowed to cool to room temperature and allowed to stand at a low temperature. No precipitate was formed.

Synthesis of 2-(tert-butyl-dimethyl-silanyloxy)-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione

TBDMS chloride (40.66 g, 269.75 mmol) was added in one portion to a solution of the 2-hydroxy substituted dilactam 78 (31.50 g, 107.88 mmol) and imidazole (44.02 g, 646.59 mmol) in anhydrous DMF (495 mL) and allowed to stir at room temperature for 20 hours. The reaction mixture was poured into water (500 mL) and the resulting suspension filtered to afford the product, which was collected and dried to give the product, 77, as a fine rose-white powder (47.6 g, 114.11 mmol, 106 % yield). $^1$H (250 MHz, CDCl$_3$)
NMR: δ 9.42 (S, 1H, H-10), 7.42 (S, 1H, H-6), 6.54 (S, 1H, H-9), 4.5 (p, 1H, J = 5.7 Hz, H-2), 4.19 (dd, 1H, J = 4.10, 8.13 Hz, H-11a), 3.9 (s, 1H, MeO-7/8), 3.87 (s, 1H, MeO-7/8), 3.72 (dd, 1H, J = 5.51, 11.90 Hz, H-3), 3.61 (dd, 1H, J = 5.48, 11.93 Hz, H-3), 2.95 - 2.75 (m, 1H, H-1), 2.09-1.98 (m, 1H, H-1), 0.85 (s, 9H, TBDMS-C(CH₃)₃), 0.02 (s, 6H, TBDMS-Si(CH₃)₂). ¹³C (62.9 MHz, CDCl₃) NMR: δ 171 (C=O), 167 (C=O), 153 (C-8), 147 (C-7), 130.5 (N10-ArC), 119 (C-5), 112 (C-6), 104 (C-9), 69.9 (C-2), 57 (C-11), 56.6 (CH₂O -7/8), 56.5 (CH₂O -7/8), 54 (C-3), 35.5 (C-1), 26 (TBDMS-(CH₃)₃), 18 (TBDMS -C(Me)₃), -5 (TBDMS -Si(CH₃)₂). IR (nujol) v: 3277.04, 2933.76, 2857.72, 2710.20, 2631.07, 2041.54, 1701.16, 1654.07, 1622.84, 1578.19, 1560.03, 1522.04, 1497.47, 1474.18, 1457.96, 1388.46, 1374.04, 1313.85, 1286.76, 1263.14, 1234.14, 1221.34, 1191.31, 1127.67, 1102.09, 1075.94, 1027.84, 1010.10, 940.07, 902.51, 881.87, 870.06, 847.87, 805.58, 779.54, 657.70 cm⁻¹. Melting range: 264 - 266 °C. [α]D (CHCl₃) 589.3°, c = 0.028 g/100mL.

**Synthesis of (11a-S)-1,2,3,11a-tetrahydro-10-(2-trimethylsilyl ethoxymethyl)-7,8-dimethoxy-2-(tert-butyl-dimethylsilanyloxy)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione**

![Chemical Structure](image)

**Method 1:**

A solution of 77 (3.41 g, 8.40 mmol) in anhydrous DMF (15 mL) was added dropwise to a stirred suspension of sodium hydride (0.40 g of 60 % dispersion in mineral oil, 10.08 mmol) in anhydrous DMF (5 mL) at 0°C and the reaction mixture stirred for 30 minutes. SEM Chloride (1.64 mL, 1.54 g, 9.24 mmol) in anhydrous DMF (2.5 mL) was added dropwise to the stirred reaction mixture at 0 – (-5) °C and left to stir at room temperature for 20 hours. The reaction mixture was diluted with water (150 mL) to form a beige emulsion. The product was extracted with diethyl ether (1 x 120 mL then 3 x 100 mL) in the presence of brine. The organic layer was washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the crude product, which was purified by flash column...
chromatography using a gradient system, 60 to 100% mixture of ethyl acetate in petroleum ether as eluent. Pure fractions were combined and evaporated \textit{in vacuo} to afford the product 76 as an orange oily foam (3.45 g, 6.34 mmol, 77 % yield). \textsuperscript{1}H (250 MHz, CDCl\textsubscript{3}) NMR: \textdelta 7.33 (s, 1H, H-6), 7.23 (s, 1H, H-9), 5.52 (d, 1H, J = 9.87 Hz, N-CH\textsubscript{2}-SEM), 4.66 - 4.57 (m, 2H, N-CH\textsubscript{2}-SEM and H-2), 4.23 (dd, 1H, J = 3.72, 8.13 Hz, H-11a), 3.94 (s, 1H, MeO-7/8), 3.91 (s, 1H, MeO-7/8), 3.81 - 3.55 (m, 4H, O-CH-2-SEM and H-3), 2.85 (ddd, 1H, J = 3.78, 5.62, 12.8 Hz, H-1), 2.08 - 1.97 (m, 1H, H-1), 1.04 - 0.86 (m, 11H, CH\textsubscript{2}-SEM and TBDMS-(CH\textsubscript{3})\textsubscript{3}), 0.04 - 0.02 (m, 15H, SEM-Si(CH\textsubscript{3})\textsubscript{3} and TBDMS-Si(CH\textsubscript{3})\textsubscript{2}). \textsuperscript{13}C (62.9 MHz, CDCl\textsubscript{3}) NMR: \textdelta 170.4 (C=O), 166.1 (C=O), 152.2 (C-8), 147.5 (C-7), 134.3 (C-10), 121.8 (C-5), 111.6 (C-6), 105.9 (C-9), 78.5 (NCH\textsubscript{2}-SEM), 69.9 (C-2), 67.4 (OCH\textsubscript{2}-SEM), 56.9 (C-11a), 56.6. (CH\textsubscript{3}O -7/8), 56.5 (CH\textsubscript{3}O -7/8), 54.0 (C-3), 35.9 (C-1), 26.1 (TBDMS-(CH\textsubscript{3})\textsubscript{3}), 18.8 (CH\textsubscript{2}-SEM), 18.4 (TBDMS-C(Me)\textsubscript{3}), -3.2 (SEM-Si-(CH\textsubscript{3})\textsubscript{3}), -4.4 (TBDMS-Si-(CH\textsubscript{3})\textsubscript{2}). IR (nujol) v: 3354.00, 3271.31, 3090.43, 2961.24, 2480.62, 2480.62, 2041.34, 1943.17, 1695.20, 1653.87, 1609.96, 1576.38, 1522.14, 1473.06, 1364.57, 1279.33, 1217.34, 1188.92, 1137.26, 1085.60, 1036.53, 1013.28, 871.21, 788.56, 695.57, 667.15 cm\textsuperscript{-1}. [\alpha]\textsubscript{D}(CHCl\textsubscript{3}) 3.82 °, c = 0.45 g/100mL.

\textbf{Method 2a:}
Butyl lithium (1.7 mL, 2.716 mmol) was added dropwise to a solution of 77 (1 g, 2.47 mmol) in dioxane (75 mL) at 0 °C and the reaction mixture stirred for 30 mins\textsuperscript{iii}. SEM chloride (0.52 mL, 0.50 g, 2.96 mmol) in dioxane (25 mL) was added to the stirred reaction mixture at room temperature and allowed to stir for 18 hours. Additional SEM chloride (0.2 mL) in dioxane (5 mL) was added and the reaction mixture was allowed to stir for a further 6 hours after which the reaction mixture was poured into water (75 mL) to form a creamy emulsion. The product was extracted with diethyl ether (3 x 75 mL) and the organic layers were combined, washed with water (50 mL), brine (50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent \textit{in vacuo} afforded the crude product which was purified using semi-automated flash column chromatography to give the pure product 76 (648.71 mg, 1.21 mmol, 49 % yield).

\textsuperscript{iii} At the low temperatures required by this reaction, the dioxane froze. The reaction mixture was allowed to thaw at room temperature before proceeding with the reaction.
Method 2b:
Butyl lithium (2.3 mL, 3.70 mmol) was added dropwise to a solution of 77 (1 g, 2.47 mmol) in anhydrous THF (75 mL) at 0 °C and the reaction mixture stirred for 30 mins. SEM chloride (0.52 mL, 0.50 g, 2.96 mmol) in anhydrous THF (25 mL) was added to the stirred reaction mixture at 0–(-5) °C and allowed to stir for 20 hours. The reaction mixture was poured into water (75 mL) to form a creamy emulsion. The product was extracted with diethyl ether (3 x 75 mL) and the organic layers were combined, washed with water (50 mL), brine (50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the crude product which was purified using semi-automated flash column chromatography to give the pure product 76 (1.15 g, 2.15 mmol, 87 % yield).

Method 2c:
Butyl lithium (2.32 mL, 3.71 mmol) in anhydrous THF (10 mL) was added dropwise to a solution of 77 (1 g, 2.47 mmol) in anhydrous THF (75 mL) over 20 mins at 0 °C and allowed to stir for a further 60 mins. SEM chloride (0.52 mL, 0.50 g, 2.96 mmol) in anhydrous THF (25 mL) was added to the stirred reaction mixture at 0 °C and allowed to stir for 24 hours. The reaction mixture was poured into water (100 mL) to form a creamy emulsion. The product was extracted with diethyl ether (3 x 75 mL) and the organic layers combined, washed with water (50 mL), brine (50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the crude product which was purified using semi-automated flash column chromatography to give the pure product 76 (1.42 g, 2.64 mmol, 107 % yield).

Synthesis of (11a-S)-1,2,3,11a-tetrahydro-10-methoxymethyl-7,8-dimethoxy-2-(tert-butyl-dimethyl-silanyloxy)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione

![Chemical Structures](image-url)
A solution of 77 (5.0 g, 12.32 mmol) in anhydrous DMF (22 mL) was added dropwise to a stirred suspension of sodium hydride (0.61 g of a 60% dispersion in mineral oil, 15.20 mmol) in anhydrous DMF (7.33 mL) at 0°C and the reaction mixture stirred for 30 minutes. MOM chloride (1.03 mL, 1.09 g, 13.55 mmol) in anhydrous DMF (3.75 mL) was added dropwise to the stirred reaction mixture at 0°C and left to stir at 0-5°C for 20 hours. The crude reaction mixture was evaporated in vacuo and then added to water (100 mL) to afford a milky emulsion. The product was extracted with diethyl ether (3 x 80mL). The organic layer was washed with water (2 x 80 mL), brine (2 x 80 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the crude product, which was purified by flash column chromatography using 10% methanol in DCM as eluent. Pure fractions were combined and evaporated in vacuo to afford the product 176 as a white foam (2.71 g, 6.01 mmol, 49% yield). \(^{1}\)H (250 MHz, CDCl\(_3\)) NMR: \(\delta\) 7.35 (S, 1H, H-6), 7.16 (S, 1H, H-9), 5.48 (d, 1H, J = 9.8 Hz, NCH-MOM), 4.67-4.53 (m, 2H, NCH-MOM and H-2), 4.25 (dd, 1H, J = 3.9 & 8.2 Hz, H-11a), 3.95 (S, 3H, MeO-7/8), 3.92 (S, 3H, MeO-7/8), 3.7 (dd, 1H, H-3), 3.59-3.50 (m, 4H, H-3 and MOM-CH\(_3\)), 2.86 (ddd, 1H, J = 4.0, 5.45, 12.83 Hz, H-1), 2.09-1.98 (m, 1H, H-1), 0.87 (S, 9H, TBDMS-C(CH\(_3\))\(_3\)), 0.09 (S, 6H, TBDMS-Si(CH\(_3\))\(_2\)). \(^{13}\)C (62.9 MHz, CDCl\(_3\)) NMR: \(\delta\) 170.6 (C=O), 166 (C=O), 152.3 (C-8), 147.6 (C-7), 134.2 (N10-ArC), 121.8 (C-5), 111.6 (C-6), 105.7 (C-9), 80.3 (N10-C-MOM), 69.9 (C-2), 57.6 (MOM-C-O), 56.9 (C-11a), 56.6 (CH\(_3\)O-7/8), 56.5 (CH\(_3\)O-7/8), 54.0 (C-3), 35.9 (C-1), 26.1 (TBDMS-(CH\(_3\))\(_3\)), 18 (TBDMS-C-Me\(_3\)), -4.4 (TBDMS-Si-(CH\(_3\))\(_2\)).

**Synthesis of (11a-S)-1,2,3,11a-tetrahydro-10-(2-trimethylsilanyloxyethyl)-7,8-dimethoxy-2-hydroxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione**

A solution of 1N TBAF in THF (9.51 mL, 9.51 mmol) was added to a stirred solution of 76 (3.4 g, 6.34 mmol) in THF (25 mL). The reaction mixture was stirred at room temperature for 2 hours and diluted with DCM (100 mL), washed with water (2 x 50 mL),
brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent \textit{in vacuo} afforded the product, which was purified by flash column chromatography using a gradient system of 50\% to 80\% ethyl acetate in petroleum ether as the eluent. Evaporation of the pure fractions \textit{in vacuo} afforded the pure product 75 as an orange oil (1.88 g, 4.45 mmol, 70\% yield). \textsuperscript{1}H (250 MHz, CDCl\textsubscript{3}) NMR: \(\delta\) 7.5 (s, 1H, H-9), 7.27 (s, 1H, H-6), 5.5 (d, 1H, \(J = 9.88\) Hz, N-CH-SEM), 4.64 - 4.6 (m, 2H, N-CH-SEM & H-2), 4.28 (d, 1H, \(J = 5.7\) 8.1 Hz, H-11a), 3.9 - 3.51 (m, 1H, MeO -7 & -8 and O-CH\textsubscript{2}-SEM and H-3 and OH), 2.91 (dt, 1H, \(J = 5.49, 2.5, 10.97\)Hz, H-1), 2.15 - 2.01 (m, 1H, H-1), 0.96 (t, 2H, \(J = 8.25\) Hz, CH\textsubscript{2}-SEM), 0.01 (s, 9H, SEM-Si(CH\textsubscript{3})\textsubscript{3}). \textsuperscript{13}C (62.9 MHz, CDCl\textsubscript{3}) NMR: \(\delta\) 170.1 (C=O), 166.3 (C=O), 152.3 (C-8), 147.5 (C-7), 134.4 (C-10), 121.5 (C-5), 111.6 (C-6), 105.8 (C-9), 78.6 (NCH\textsubscript{2}-SEM), 69.4 (C-2), 67.5 (O-CH\textsubscript{2}-SEM), 56.9 (C-11a), 56.5 (CH\textsubscript{3}O -7/8), 54.3 (C-3), 35.3 (C-1), 18.8 (CH\textsubscript{2}-SEM), -1.4 (SEM-(CH\textsubscript{3})\textsubscript{3}). IR (nujol) v: 3433.81, 2963.09, 2637.25, 2039.97, 1700.30, 1653.79, 1560.01, 1525.49, 1478.27, 1443.06, 1376.28, 1286.27, 1107.46, 870.13, 791.84, 770.81, 696.16, 668.31, 633.40 cm\textsuperscript{-1}. [\(\alpha\)]\textsubscript{D} (CHCl\textsubscript{3}) 301.67 \textdegree, c = 0.30 g/100mL.

**Synthesis of (11a,S)-1,2,3,11a-tetrahydro-10-methoxymethyl-7,8-dimethoxy-2-hydroxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione**

A solution of 1N TBAF in THF (6.02 mL, 6.02 mmol) was added to a stirred solution of 176 (2.71 g, 6.02 mmol) in THF (24 mL). The reaction mixture was stirred at room temperature for two hours and diluted with DCM (90 mL), washed with water (2 x 90 mL), brine (2 x 90 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent \textit{in vacuo} afforded the crude product 177 as an orange oil (1.91 g, 5.67 mmol, 94\% yield). \textsuperscript{1}H (250 MHz) NMR: 7.34 (s, 1H, H-6), 7.16 (s, 1H, H-9), 5.48 (d, 1H, NCH-MOM), 4.66 (d, 1H, NCH-MOM), 4.22 (m, 1H, H-11a), 3.95 (s, 3H, MeO-7/8), 3.94 (s, 3H, MeO -7/8), (\textit{data incomplete-signals weak}).
Synthesis of (11a-S)-11a-dihydro-10-(2-trimethylsilanyloxy methyl)-7,8-dimethoxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-2,5,11-trione

Method 1
Anhydrous DCM (60 mL) was added to a mixture of 75 (2.96 g, 7.02 mmol), PDC (3.57 g, 9.48 mmol) and 4A molecular sieves (2.97 g). The reaction mixture was stirred at room temperature, under nitrogen, for 18 hours. The reaction mixture was quenched with ethanol and filtered through celite. The filtrate was evaporated in vacuo to give the crude product which was then purified using flash column chromatography to yield the pure product as a yellow-white foam (2.63 g, 6.25 mmol, 89.1%).

Method 2
Anhydrous DMSO (1.67 mL, 1.84 g, 23.5 mmol) in anhydrous DCM (20 mL) was added dropwise over 5 minutes to a stirred solution of oxalyl chloride (5.9 mL of a 2N solution in DCM, 11.8 mmol) under a nitrogen atmosphere at -50 - -55°C. A solution of 75 (3.31 g, 7.84 mmol) was stirred for 10-15 minutes in dry DCM (45 mL) and was added dropwise over 45 minutes at -50°C. A solution of TEA (7.65 mL, 5.55 g, 54.9 mmol) in dry DCM (20 mL) was added dropwise to the mixture over 20 minutes and stirred for a further 15 minutes. The reaction mixture was left to warm to room temperature and diluted with water (80 mL) and DCM (70 mL). The organic phase was washed with 1N HCl (2 x 50 mL), water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the crude product, which was purified by flash column chromatography using 50:50 → 80:20 ethyl acetate: petroleum ether as eluent. Evaporation of the pure fractions in vacuo afforded the product 74 as an orange oil/foam (1.63 g, 3.82 mmol, 49% yield). $^1$H (250 MHz, CDCl$_3$) NMR: δ 7.34 (s, 1H, H-6), 7.27 (s, 1H, H-9), 5.56 (d, 1H, N-CH-SEM), 4.72 - 4.63 (m, 2H, N-CH-SEM and H-11a), 3.96 (s, 3H, MeO -7/8), 3.94 (s, 3H, MeO -7/8), 3.9 - 3.54 (m, 5H, H-1 and O-CH$_2$-SEM and H-3), 2.8 (dd, 1H, $J = 9.87, 19.38$ Hz,
H-1), 1.1 - 0.9 (m, 2H, CH₂-SEM), 0.04 (s, 9H, SEM-Si(CH₃)₃). ¹³C (62.9 MHz, CDCl₃) NMR: δ 207.2 (O=C-2), 169.2 (C=O), 166.3 (C=O), 152.8 (C-8), 147.9 (C-7), 134.4 (C-10), 120.8 (C-5), 111.5 (C-6), 106.0 (C-9), 78.6 (NCH₂-SEM), 67.6 (O-CH₂-SEM), 56.6 (CH₃O -7 & -8), 55.2 (C-11a), 52.7 (C-3), 37.7 (C-1), 18.8 (CH₂-SEM), -0.9 (SEM-Si(CH₃)₃). IR (neat) ν: 3374.04, 3095.35, 2953.97, 1765.25, 1735.71, 1689.61, 1643.97, 1607.68, 1519.52, 1456.17, 1433.80, 1361.85, 1250.32, 1214.30, 1144.78, 1071.83, 938.92, 917.40, 860.71, 836.99, 789.57, 767.77, 694.42, 666.86, 611.85 cm⁻¹. HRMS (FAB) exact mass calculated for C₂₀H₂₈N₂O₆Si (M⁺): m/z 420.1717, observed 420.2148. [α]D(CHCl₃) 288.46°, c = 0.078 g/100mL.

Method 3
A solution of 75 (1.04 g, 2.54 mmol) in DCM (12 mL) was treated with acetonitrile (1.4 mL) and NMO (0.43 g, 3.64 mmol) in the presence of 4 Å powdered molecular sieves (0.64 g). The reaction mixture was allowed to stir at room temperature for 15 minutes and then TPAP (44 mg, 0.12 mmol) was added in one portion to the reaction mixture. A colour change to indigo was observed. The reaction mixture was allowed to stir for a further 2.5 hours. Silica gel was added to the reaction mixture, which was then evaporated in vacuo. An attempt to purify the product using flash column chromatography using 50:50 hexane: ethyl acetate as the eluent was unsuccessful.

Synthesis of (11a-S)- 1,11a-dihydro-10-methoxymethyl-7,8-Dimethoxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-2,5,11-trione

Anhydrous DMSO (1.19 mL, 1.31 g, 16.74 mmol) in anhydrous DCM (20 mL) was added dropwise over 5 minutes to a stirred solution of oxalyl chloride (4.18 mL of a 2N solution in DCM, 8.37 mmol) under a nitrogen atmosphere at -50 to -55°C. 177 (1.88 g, 5.58 mmol) was stirred for 10-15 minutes in dry DCM (40 mL) and was added dropwise over 45 minutes at -50°C. TEA (5.44 mL, 3.95 g, 39.05 mmol) in dry DCM (20 mL) was
added dropwise to the mixture over 20 minutes and stirred for a further 15 minutes.
The reaction mixture was allowed to warm to room temperature and diluted with water
(80 mL) and DCM (70 mL). The organic phase was washed with 1N HCl (2 x 50 mL),
water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate.
Filtration and evaporation of the solvent in vacuo afforded the crude product, which was
purified by flash column chromatography using 10:90 ethyl acetate: petroleum ether as
eluent to give pure product 178 as a yellow foamy solid (802 mg, 2.40 mmol, 43 % yield).

\[
\begin{align*}
\text{H} & \quad (400 \text{ MHz, CDCl}_3) \text{ NMR: } \delta 7.36 (s, 1\text{H}, \text{H}6), 7.16 (s, 1\text{H}, \text{H}9), 5.48 (d, 1\text{H}, J = 9.89 \\
& \quad \text{Hz, N-CH}_2\text{-MOM x 1}, 4.66 (d, 1\text{H}, J = 9.89 \text{ Hz, N-CH}_2\text{-MOM x 1}), 4.32 (dd, 1\text{H}, J = \\
& \quad 5.86, 7.96 \text{ Hz, H11a}), 3.95-9.85 (m, 7\text{H, MeO-7 & -8, H3 x 1}), 3.67 (dd, 1\text{H}, J = 4.76, \\
& \quad 12.63 \text{ Hz, H3 x 1}), 3.50 (s, 3\text{H, MOM-CH}_2), 2.98 (dt, 1\text{H}, J = 5.45, 13.61 \text{ Hz, H1 x 1}, \\
& \quad 2.19-2.09 (m, 1\text{H, H1 x 1}). \quad \text{C} (100 \text{ MHz, CDCl}_3) \text{ NMR: } \delta 169.9 (\text{O=C11}), 165.7 \\
& \quad (\text{O=C4}), 152.0 (\text{C8}), 147.3 (\text{C7}), 133.8 (\text{C10}), 121.4 (\text{C5}), 111.4 (\text{C6}), 105.4 (\text{C9}), 80.0 \\
& \quad (\text{N-CH}_2\text{-MOM}), 69.4 (\text{C11a}), 57.2 (\text{MOM-OCH}_3), 56.4 & 56.2 (\text{MeO-7 & -8}), 53.9 (\text{C3}), \\
& \quad 35.1 (\text{C1}). \quad \text{C} (100 \text{ MHz}) \text{ NMR: } \delta 111.4, 105.4, 80.0, 69.4, 57.2, 56.4, 56.2, 53.9, 35.1.
\end{align*}
\]

**Synthesis trifluoromethanesulphonic acid (11a-S)- 1,11a-dihydro-5,11-
dioxo-10-(2-trimethylsilanyloxyethylmethyl)-7,8-dimethoxy-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-2-yl-ester**

\[
\begin{align*}
\text{SEM} & \quad \text{SEM} \\
\text{74} & \quad \rightarrow \quad \text{73}
\end{align*}
\]

Anhydrous pyridine (0.12 mL, 0.11 g, 1.43 mmol) was added in one portion to a
vigorously stirred solution of 74 (500 mg, 1.19 mmol) in anhydrous DCM (25 mL) and
the mixture allowed to stir for 10 minutes at room temperature. Anhydrous triflic
anhydride (0.22 mL, 0.37 g, 1.31 mmol) was added quickly in one portion and the
reaction mixture was allowed to stir at room temperature for 5 hours. Over this time the
colour of the reaction mixture changed from a light red to a burgundy red colour. The
homogenous reaction mixture was diluted with cold saturated sodium hydrogen carbonate
(50 mL) and the mixture was extracted with DCM (3 x 10 mL). The organic layers were
combined and washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over
anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified by flash column chromatography using 60:40 ethyl acetate: petroleum ether as eluent. Evaporation of the pure fractions in vacuo afforded the pure product 73 as a yellow oil/foam (384 mg, 0.7 mmol, 49 % yield). \(^\text{1}^\text{H}\) (250 MHz, CDCl\(_3\)) NMR: \(\delta\) 7.32 (s, 1H, H-6), 7.27 (s, 1H, H-9), 7.15 (t, 1H, \(J = 2.0\) Hz, H-3), 5.57 (d, 1H, N-CH-SEM), 4.72 - 4.70 (d, 1H, \(J = 9.92\) Hz, N-CH-SEM), 4.65 (dd, 1H, \(J = 3.6, 10.95\) Hz, H-11a), 3.97 - 3.68 (m, 10H, MeO -7 & -8 and O-CH\(_2\)-SEM and H-1), 3.17 (ddd, 1H, \(J = 2.37, 11.02, 16.34\) Hz, H-1), 0.99 (t, 2H, \(J = 8.3\) Hz, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\))\(_3\)). \(^{13}\text{C}\) (62.9 MHz, CDCl\(_3\)) NMR: \(\delta\) 167.4 (C=O), 163.1 (C=O), 152.9 (C-8), 147.9 (C-7), 138.7 (C-3), 134.1 (C-10), 120.1 (C-5), 119.2 (C-2), 111.8 (C-6), 106.3 (C-9), 78.9 (NCH\(_2\)-SEM), 67.7 (OCH\(_2\)-SEM), 57.0 (C-11a), 56.6 (CH\(_3\)O -7 & -8), 31.0 (C-1), 18.8 (CH\(_2\) -SEM), 1.4 (CF\(_3\)), -1.0 (SEM-Si (CH\(_3\))\(_3\)). IR (neat) v: 313.80, 3011.12, 2957.25, 2041.61, 1769.21, 1693.34, 1646.32, 1607.77, 1519.57, 1454.98, 1430.77, 1360.84, 1327.77, 1250.92, 1213.74, 1139.04, 1097.98, 1071.36, 1010.71, 939.07, 911.42, 858.30, 835.55, 788.77, 760.71, 693.93, 665.91, 640.03, 609.65 cm\(^{-1}\). [\(\alpha\)]\(_D\) (CHCl\(_3\)) 301.02 °, c = 0.098 g/100mL.

**Synthesis trifluoromethanesulphonic acid (11a-S)- 1,11a-dihydro-5,11-dioxo-10-methoxymethyl-7,8-dimethoxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl-ester**

\[
\begin{align*}
\text{178} & \quad \rightarrow \quad \text{179}
\end{align*}
\]

Anhydrous pyridine (0.22 mL, 0.21 g, 2.66 mmol) was added in one portion to a vigorously stirred solution of the MOM protected ketone dilactam PBD (741 mg, 2.22 mmol) in anhydrous DCM (46 mL) and the mixture allowed to stir for 10 minutes at room temperature. Anhydrous triflic anhydride (0.41 mL, 0.69 g, 2.44 mmol) was added quickly in one portion and the reaction mixture was allowed to stir at room temperature for 6 hours. The homogenous reaction mixture was diluted with cold saturated sodium
hydrogen carbonate (50 mL) and the mixture was extracted with DCM (3 x 15 mL). The organic layers were combined and washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified by flash column chromatography using 60:40 ethyl acetate: petroleum ether as eluent. Evaporation of the pure fractions in vacuo afforded the pure product 179 as a white brittle foam solid (900 mg, 1.93 mmol, 87 % yield). 

\[ ^1H \text{ (400 MHz, CDCl}_3 \text{) NMR: } \delta 7.35 \text{ (s, 1H, H6), 7.16 (s, 1H, H9), 7.10 (m, 1H, H3), 5.49 (m, 1H, N-CH}_2\text{-MOM x 1), 4.67 (d, 1H, } J = 9.89 \text{ Hz, N-CH}_2\text{-MOM x 1), 4.32 (m, 1H, H1a), 3.97-9.64 (m, 7H, MeO -7 & -8, H1 x 1), 3.49 (s, 3H, MOM-CH}_3\text{), 3.21 (m, 1H, H1 x 1).} \]

\[ ^13C \text{ (100 MHz, CDCl}_3 \text{) NMR: } \delta 171.9 \text{ (O=C11), 160.5 (O=C4), 152.7 (C8), 146.3 (C7), 133.1 (C10), 119.0 (Tf-CF}_3\text{), 117.5 (C5), 112.4 (C6), 105.4 (C9), 78.5 (N-CH}_2\text{-MOM), 68.5 (C11a), 57.0 (MOM-OCH}_3\text{), 56.4 & 56.2 (MeO -7 & -8), 50.3 (C3), 35.5 (C1).} \]

**Synthesis of (11a-S)-11a-dihydro-10-(2-trimethylsilanyloethy methyl)-7,8-dimethoxy-2-p-tolyl-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione**

Method 1

Sodium carbonate (600 mg, 5.66 mmol), 4-methylbenzeneboronic acid (236 mg, 1.74 mmol) and tetrakis (triphenylphosphine) palladium (0) (53 mg) were added to a solution of 73 (873 mg, 1.58 mmol) in ethanol/water/benzene (4/4/12 mL) and allowed to stir at room temperature for 43 hours. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 115 as a fine yellow powder (513 mg, 1.04 mmol, 68 % yield).
Method 2
A solution of 4-methylbenzeneboronic acid (124 mg, 0.92 mmol) in DME (5 mL) was added to a stirred solution of 73 (338 mg, 0.610 mmol) in DME (5 mL) under a nitrogen atmosphere. An aqueous solution of sodium carbonate (2N, 5mL) was added followed by lithium chloride (84.11 mg, 1.98 mmol) and tetrakis (triphenylphosphine) palladium(0) (5 mol%, 39.3 mg) and the mixture was stirred for 1 hour at room temperature followed by heating at reflux for 1 hour. DCM (25 mL) was added to the reaction mixture followed by aqueous 2N sodium carbonate (25 mL) and concentrated ammonium hydroxide solution (1.5 mL). The aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic extracts were dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded a residue, which was purified by flash column chromatography using 60:40 ethyl acetate: petroleum ether as the eluent. Pure fractions were combined and evaporated in vacuo to afford the product 115 as an orange oil (110 mg, 0.22 mmol, 36 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: δ 7.42 - 7.38 (m, 2H, H-3 & H-9), 7.33 (d, 2H, $J$ = 8.16 Hz, Ar-Tolyl), 7.28 (s, 1H, H-6), 7.15 (d, 2H, $J$ = 8.01 Hz, Ar-Tolyl), 5.56 (d, 1H, $J$ = 9.93 Hz, N-CH-SEM), 4.72 - 4.62 (m, 2H, N-CH-SEM & H-1 la), 3.99 - 3.67 (m, 10H, H-1 and H-3 and O-CH$_2$-SEM and MeO -7 & -8), 3.15 (ddd, $J$ = 2.24, 10.62, 16.50 Hz, H-1), 2.35 (s, 3H, tolyl-CH$_3$), 1.02 (t, 2H, $J$ = 8.32 Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$). $^{13}$C (62.9 MHz, CDCl$_3$) NMR: δ 169 (C=O), 163 (C=O), 153 (C-8), 148 (C-7), 135 (C-2), 134 (C-10), 131 - 128 (tolyl-ArH), 122 (C-5), 112 (C-6), 106 (C-9), 78 (NCH$_2$-SEM), 68 (O-CH$_2$-SEM), 58 (C-11a), 57 (CH$_3$O -7 & -8), 21 (tolyI-CH$_3$), 19.2 (CH$_2$-SEM), -0.5 (SEM-Si(CH$_3$)$_3$). IR (neat) v: 3268.29, 1805.91, 1637.68, 1542.91, 1361.99, 970.80, 841.63, 783.58 cm$^{-1}$.

Synthesis of 2-(4-chlorophenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

![Chemical structure](image)
Sodium carbonate (300 mg, 2.83 mmol), chlorobenzeneboronic acid (155 mg, 1.00 mmol) and \textit{tetrais} (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 46 hours at which point additional chlorobenzeneboronic acid (155 mg, 0.99 mmol) and \textit{tetrais} (triphenylphosphine) palladium(0) (30 mg) was added. The solution was stirred for a further 44 hours and then quenched with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL). The solution was dried over magnesium sulphate and purified with flash column chromatography using 70% ethyl acetate in hexane as eluent to give the pure product 117 as an orange-brown bristly solid (411 mg, 0.86 mmol, 95% yield). $^1$H (250 MHz, CDCl$_3$) NMR : $\delta$ 7.46-7.22 (m, 7H, H$_3$ & H$_6$ & Ar-H x 4 & H9), 5.55 (d, 1H, J = 9.92 Hz, N-CH$_2$-OSEM x 1), 4.77-4.61 (m, 2H, N-CH$_2$-OSEM x 1 & H11a), 4.01-3.87 (m, 7H, H1 x 1 and 7- & 8- MeO), 3.87-3.63 (m, 2H, O-CH$_2$-SEM), 3.13 (ddd, 1H, J = 2.09, 10.555, 16.148 Hz, H1 x 1), 0.98 (t, 2H, J = 8.203 Hz, CH2-SEM), 0.03 (s, 9H, SEM-Si(CH$_3$)$_3$). MS (FAB) m/z (relative intensity): 537(29), 514(100), 457(29), 397(66), 369(62), 329(14), 307(26), 289(22), 280(18), 250(13), 226(36). HRMS (FAB) exact mass calculated for C$_{26}$H$_{31}$N$_2$O$_5$SiCl (M$^+$): m/z 537.1588, observed 537.1599.

**Synthesis of 2-(3,4-methylenedioxyphenyl)-7,8-dimethoxy-10-(2-trimethylsilanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one**

![Reaction Scheme](image)

Sodium carbonate (300 mg, 2.83 mmol), 3,4-methylenedioxybenzene boronic acid (165 mg, 0.99 mmol) and \textit{tetrais} (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 96 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by flash column chromatography
using 70% ethyl acetate in hexane as eluent to give the pure product 119 as a yellow oil (494 mg, 0.94 mmol, 104 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.39 (s, 1H, H6), 7.30 (Broad s, 1H, H3), 7.27 (s, 1H, H9), 6.94 (d, 1H, $J = 1.61$ Hz, Ar-H x 1), 6.88 (dd, 1H, $J = 1.61, 8.03$ Hz, Ar-H x 1), 6.78 (d, 1H, $J = 8.03$ Hz, Ar-H x 1), 5.96 (s, 2H, methylenedioxy O-CH2), 5.56 (d, 1H, $J = 9.87$ Hz, N-CH$_2$OSEM x 1), 4.71 (d, 1H, $J = 9.98$ Hz, N-CH$_2$OSEM x 1), 4.62 (dd, 1H, $J = 3.33, 10.56$ Hz, H11a), 4.00-3.63 (m, 9H, 7- & 8- MeO & O-CH$_2$-SEM & H1 x 1), 0.99 (t, 2H, $J = 8.32$ Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$). $^{13}$C (62 MHz, CDCl$_3$) NMR: $\delta$ 169.4, 162.7, 153.2, 149.1, 148.5, 148.4, 134.8, 128.8, 126.6, 122.4, 121.8, 120.2, 112.6, 109.4, 107.0, 106.5, 102.2, 68.2, 58.6, 57.3, 32.7, 19.5. MS (FAB) m/z (relative intensity): 547(35), 524(100), 407(20), 379(35), 246(17), 226(20). HRMS (FAB) exact mass calculated for C$_{27}$H$_{32}$N$_2$O$_7$Si (M+): m/z 524.1979, observed 524.1964.

Synthesis of 2-(4-biphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.83 mmol), 4-biphenyl boronic acid (203.2 mg, 1.03 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 96 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by flash column chromatography using 50% ethyl acetate in hexane as eluent to give the pure product 121 as a yellow oil (475 mg, 0.85 mmol, 94 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.65-7.27 (m, 12 H, H6, H3, H9 Ar-H x 9), 5.56 (d, 1H, $J = 9.87$ Hz, N-CH$_2$-OSEM x 1), 4.76-4.63 (m, 2H, N-CH$_2$-OSEM x 1 & H11a), 4.09- 3.90 (m, 7H, 7- & 8- MeO and H1 x 1), 3.90-3.63 (m, 2H, O-CH$_2$-SEM), 3.20 (ddd, 1H, $J = 1.69, 10.87, 15.95$ Hz, H1 x 1), 1.00 (t, 2H, $J = 8.20$ Hz, CH$_2$-SEM),
160

Synthesis of 2-(2-naphthyl)-7,8-dimethoxy-10-(2-trimethylsilanyl ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.83 mmol), 2-naphthalene boronic acid (171 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (517 mg, 0.93 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 96 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by flash column chromatography using 70% ethyl acetate in hexane as eluent to give the pure product 122 as a yellow oil (233 mg, 0.44 mmol, 47 % yield). 

\(^1\)H (250 MHz, CDCl\(_3\)) NMR : \(\delta\) 7.85-7.71 (m, 4H, Ar-H x 4), 7.67-7.54 (m, 2H, H3, Ar-H x 1), 7.52-7.39 (m, 3H, H6, Ar-H x 2), 7.33-7.27 (s, 1H, H9), 5.58 (d, 1H, \(J = 9.93\) Hz, N-CH\(_2\)-OSEM x 1), 4.79-4.66 (m, 2H, N-CH\(_2\)-OSEM x 1, H11a), 4.11 (d, 1H, \(J = 16.28\) Hz, H1 x 1), 3.97 & 3.95 (2s, 6H, 7- & 8- MeO), 3.90-3.65 (m, 2H, O-CH\(_2\)-SEM), 3.28 (dd, 1H, \(J = 10.92, 16.23\) Hz, H1 x 1), 1.00 (t, 2H, \(J = 8.23\) Hz, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\))\(_3\)). 

\(^{13}\)C (62 MHz, CDCl\(_3\)) NMR: \(\delta\) 169.1, 162.6, 148.3, 134.6, 134.4, 133.6, 131.8, 129.0, 128.7, 128.4, 127.2, 126.7, 126.3, 124.8, 124.1, 123.3, 122.2, 112.6, 106.9, 79.2, 68.0, 58.5, 57.0, 32.2, 19.2. MS (FAB) m/z (relative intensity): 553(21), 530(100), 473(22), 413(30), 266(16), 226(22). HRMS (FAB) exact mass calculated for C\(_{30}\)H\(_{34}\)N\(_2\)O\(_5\)Si (M+): m/z 530.2237, observed 530.2246.
Synthesis of 2-(4-dimethylaminophenyl)-7,8-dimethoxy-10-(2-trimethyl silanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one

Sodium carbonate (300 mg, 2.83 mmol), 4-dimethylaminobenzeneboronic acid (163.9 mg, 0.99 mmol) and tetakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 29 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the product 124 as a grey-green powdery solid (627 mg, 1.19 mmol, 132 % yield).

1H (250 MHz, CDCl₃) NMR : δ 7.48-7.20 (m, 5H, H3 & H6 & H9 & Ar-H x 2), 6.70 & 6.67 (2s, 2H, Ar-H x 2), 5.55 (d, 1H, J = 10.04 Hz, N-CH₂-OSEM x 1), 4.70 (d, 1H, J = 9.98 Hz, N-CH₂-OSEM x 1), 4.60 (dd, 1H, J = 3.17, 10.40 Hz, H11a), 4.04-3.61 (m, 9H, 7- & 8- MeO, O-CH₂-SEM, H1 x 1), 2.968 & 2.965 (2s, 6H, Ar-N(CH₃)₂), 3.11 (dd, 1H, J = 10.30, 16.24 Hz, H1 x 1), 0.99 (t, 2H, J = 8.27 Hz, CH₂-SEM), 0.03 (s, 9H, SEM-Si(CH₃)₃).

Synthesis of 2-(3,4-dimethoxyphenyl)-7,8-dimethoxy-10-(2-trimethyl silanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one
Sodium carbonate (310 mg, 2.92 mmol), 3,4-dimethoxybenzeneboronic acid (180 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 93 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 126 (511 mg, 0.95 mmol, 105 % yield). \( ^1H \) (250 MHz, CDCl\(_3\)) NMR : \( \delta 7.51-7.23 \) (m, 3H, H9 & H6 & H3), \( 7.09-6.79 \) (m, 3H, Ar-H x 2), 5.57 (d, 1H, \( J = 9.91 \) Hz, N-CH\(_2\)-OSEM X 1), 4.79-4.58 (m, 2H, N-CH\(_2\)-OSEM x 1 & HI la), 4.16-3.59 (m, 15H, 7- & 8-MeO, Ar-MeO, H1 x 1, O-CH\(_2\)-SEM), 3.15 (ddd, 1H, \( J = 1.89, 10.51, 13.00 \) Hz, H1 x 1), 1.00 (t, 2H, \( J = 8.22 \) Hz, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\))\(_3\)).

**Synthesis of 2-(2-thiophene)-7,8-dimethoxy-10-(2-trimethylsilanyl ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one**

Sodium carbonate (305 mg, 2.88 mmol), 2-thiopheneboronic acid (127 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.903 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 164.5 hours and heated at reflux at 80°C for a further 4 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 116 as a yellow foamy solid (362 mg, 0.75 mmol, 82 % yield). \( ^1H \) (250 MHz, CDCl\(_3\)) NMR : \( \delta 7.39 \) (s, 1H, H6), 7.32-7.17 (m, 3H, H3, H9, Ar-H x 1), 7.06-6.94 (m, 2H, Ar-H x 2), 5.56 (d, 1H, \( J = 9.967 \) Hz, N-CH\(_2\)-OSEM X 1), 4.71 (d, 1H, \( J = 9.910 \) Hz, N-CH\(_2\)-OSEM X 1), 4.64 (dd, 1H, \( J = 3.514, 10.555 \) Hz, H11a), 4.02-3.88 (m, 7H, 7- & 8- MeO, H1 x 1), 3.88-3.63 (m, 2H, O-CH\(_2\)-...
SEM), 3.18 (ddd, 1H, J = 2.384, 10.756, 16.105 Hz, H1 x 1), 0.99 (t, 2H, J = 8.24 Hz, CH2-SEM), 0.04 (s, 9H, SEM-Si(CH3)3).

Synthesis of 2-(2-benzo(b)thiophene)-7,8-dimethoxy-10-(2-trimethyl silanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one

Sodium carbonate (305 mg, 2.88 mmol), 2-benzo(b)thiophene boronic acid (177 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.903 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 48 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 118 (179 mg, 0.334 mmol, 38 % yield). 1H (250 MHz, CDCl3) NMR: δ 7.73 (dd, 2H, J = 7.75, 15.60 Hz, ArH x 2), 7.60-7.08 (m, 6H, H6, H3, H9, Ar-H x 3), 5.57 (d, 1H, J = 9.91 Hz, N-CH2-OSEM x 1), 4.82-4.60 (m, 2H, N-CH2-OSEM x 1, H11a), 4.15-3.60 (m, 9H, 7- & 8- MeO, H1 x 1, O-CH2-SEM), 3.26 (dd, 1H, J = 11.29, 14.51 Hz, H1 x 1), 1.01 (dt, 2H, J = 0.53, 7.85 Hz, CH2-SEM), 0.13(-0.08) (m, 9H, SEM-Si(CH3)3).
Synthesis of 2-(4-fluorophenyl)-7,8-dimethoxy-10-(2-trimethylsilyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (308 mg, 2.91 mmol), 4-fluorobenzeneboronic acid (139 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 93 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 120 as a white foamy solid (441 mg, 0.88 mmol, 99 % yield). \(^1\)H (250 MHz, CDCl\(_3\)) NMR : \(\delta\) 7.45-7.32 (m, 5H, H\(_6\) & H\(_3\) & Ar-H x 2), 7.27 (s, 1H, H9), 7.04 (t, 2H, Ar-H x 2), 5.56 (d, 1H, \(J = 9.80\) Hz, N-CH\(_2\)-OSEM x 1), 4.72 (d, 1H, \(J = 9.49\) Hz, N-CH\(_2\)-OSEM x 1), 4.65 (dd, 1H, \(J = 3.28, 10.17\) Hz, H11a), 4.04-3.88 (m, 7H, 7- & 8-MeO and H1 x 1), 3.88- 3.64 (m, 2H, O-CH\(_2\)-SEM), 3.14 (ddd, 1H, \(J = 2.26, 10.57, 16.21\) Hz, H1 x 1), 0.99 (t, 2H, \(J = 8.33\) Hz, CH\(_2\)-SEM), 0.03 (s, 9H, SEM-Si(CH\(_3\))\(_3\)).

Synthesis of 2-(4-ethylphenyl)-7,8-dimethoxy-10-(2-trimethylsilyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (302 mg, 2.85 mmol), 4-ethylbenzeneboronic acid (149 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution
of 73 (501 mg, 0.91 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 41 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 114 as a flaky white solid (342 mg, 0.67 mmol, 74 % yield). $^1$H (250 MHz, CDCl$_3$) NMR : $\delta$ 7.46-7.15 (m, 7H, H$_6$ & H$_3$ & H$_9$ & Ar-H x4), 5.57 (d, 1H, $J$ = 9.88 Hz, N-CH$_2$-OSEM x 1), 4.72 (d, 1H, $J$ = 9.95 Hz, N-CH$_2$-OSEM x 1), 4.65 (dd, 1H, $J$ = 3.53, 10.86 Hz, H11a), 4.03-3.88 (m, 7H, 7- & 8- MeO and H1 x 1), 3.88- 3.64 (m, 2H, O-CH$_2$-SEM), 3.16 (dd, 1H, $J$ = 10.84, 16.51 Hz, H1 x 1), 2.66 (q, 2H, $J$ = 7.68 Hz, Ar-CH$_2$-Me x 2), 1.24 (t, 3H, $J$ = 7.57 Hz, ArCH$_2$-CH$_3$ x 3), 0.99 (t, 2H, $J$ = 8.29 Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(4-tert-butylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyloxyethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (302 mg, 2.85 mmol), 4-tert-butylbenzeneboronic acid (177 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (502 mg, 0.91 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 41 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 123 (408 mg, 0.76 mmol, 84 % yield). $^1$H (250 MHz, CDCl$_3$) NMR : $\delta$ 7.45-7.29 (m, 7H, H$_6$ & H$_3$ & H$_9$ & Ar-H x4), 5.56 (d, 1H, $J$ = 9.98 Hz, N-CH$_2$-OSEM x 1), 4.78-4.55 (m, 2H, N-CH$_2$-OSEM x 1, H11a), 4.04-3.60 (m, 9H, 7- & 8- MeO and H1 x 1 & O-CH$_2$-SEM), 3.16 (dd, 1H, $J$ = 10.35, 15.52 Hz, H1 x 1 ), 1.38-1.21 (multiple singlets not
all Me groups equivalent, 9H, tert-Bu (CH$_3$)$_3$ x 9), 1.04-0.80 (m, 2H, CH$_2$-SEM), 0.09-(-0.04) (multiple singlets not all Me groups equivalent, 9H, SEM-Si(CH$_3$)$_3$).

**Synthesis of 2-(3-methylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5$H$-pyrrolo[2,1-c][1,4]benzodiazepine-5-one**

![Chemical structure](image)

Sodium carbonate (300 mg, 2.83 mmol), 3-methylbenzeneboronic acid (135 mg, 0.99 mmol) and *tetrakis* (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 20 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 125 as a yellow oil/foam (387 mg, 0.78 mmol, 87 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: 7.51-7.33 (m, 2H, H$_6$ & H$_3$), 7.33-7.12 (m, 4H, H$_9$ & Ar-H x 3), 7.04 (d, 1H, $J$ = 3.25 Hz, Ar-H x 1), 5.53 (d, 1H, $J$ = 9.94 Hz, N-CH$_2$-OSEM x 1), 4.77-4.54 (m, 2H, N-CH$_2$-OSEM x 1 & H$_{11}$a), 4.03-3.86 (m, 7H, 7- & 8- MeO & H$_1$ x 1), 3.86-3.59 (dq, 2H, $J$ = 9.53, 17.80 Hz, O-CH$_2$-SEM), 3.12 (ddd, 1H, $J$ = 1.98, 10.47, 16.01 Hz, H$_1$ x 1), 2.32 (s, 3H, Ar-CH$_3$), 1.02-0.89 (m, 2H, CH$_2$-SEM), 0.00 (s, 9H, SEM-Si(CH$_3$)$_3$)
Synthesis of 2-(2-methylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.83 mmol), 2-methylbenzeneboronic acid (135 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 20 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 127 as a yellow oil (439 mg, 0.89 mmol, 98 % yield). (250 MHz, CDCl$_3$) NMR: $\delta$ 7.44-7.09 (m, 7H, H$_6$ & H$_3$ & H$_9$ & Ar-H x 4), 5.59 (d, 1H, $J = 9.97$ Hz, N-CH$_2$-SEM x 1), 4.78-4.50 (m, 2H, N-CH$_2$-SEM x 1, H$_{11a}$), 4.06-3.60 (m, 9H, 7- & 8- MeO, O-CH$_2$-SEM, H$_1$ x 1), 3.22 (dd, 1H, $J = 10.233$, 16.313 Hz, H$_1$ x 1), 2.48 (Broad s, 3H, Ar-2-Me x 3), 1.06-0.79 (m, 2H, CH$_2$-SEM), 0.03 (broad s, 9H, SEM-Si(CH$_3$)$_3$).

Attempted synthesis of 2-(3-acetamidophenyI)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one

Sodium carbonate (264 mg, mmol), 3-acetamidobenzeneboronic acid (142 mg, 0.79 mmol) and tetrakis (triphenylphosphine) palladium(0) (26 mg) were added to a solution of 73 (440 mg, 0.79 mmol) in ethanol/water/benzene (18/18/18 mL) and allowed to stir at
room temperature for 8 days. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent. Several products were collected but none were positively identified as the desired pure product. $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.6-6.7 (m, 5H, H3 & H6 & H9), 5.7-5.2 (m, 2H, NCH$_2$SEM x 1, Ar-NH x 1), 4.67 (d, 1H, $J = 9.86$ Hz, NCH$_2$SEM x 1), 4.46 (d, 1H, $J = 10.55$ Hz, H11a), 4.0-3.4 (m, 10H, 7- & 8- MeO, O-CH2-SEM, H1 x 1), 3.0-2.6 (m, 1H, H1 x 1), 2.3-0.5 (m, 29H, CH$_2$SEM x 2), 0.02 (s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(4-trifluoromethylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 4-trifluoromethylbenzeneboronic acid (189 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 43.5 hours. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with water (25 mL) and brine (25 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the reaction product as a flaky white solid (322 mg, 0.59 mmol, 65 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.70-7.46 (m, 6H), 7.39 (s, 1H), 7.29 (s, 1H), 5.56 (d, 1H, $J = 9.95$ Hz), 4.82-4.65 (m, 2H), 4.19-3.61 (m, 11H), 3.18 (ddd, 1H, $J = 2.02, 10.69, 16.11$ Hz), 1.13-0.78 (m, 2H), 0.04 (s, 9H).
Synthesis of 2-(4-vinylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 4-vinylbenzeneboronic acid (147 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 8.8 days. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50% hexane in ethyl acetate gradient system as eluent to give the pure product 132 (299 mg, 0.59 mmol, 66% yield). \(^1\)H (250 MHz, CDCl\(_3\)) NMR: \(\delta\) 7.76-7.23 (m, 7H, H\(_3\) & H\(_6\) & H\(_9\) & Ar-H), 6.87-6.63 (m, 1H, vinyl-H x 1), 5.76 (d, 1H, \(J = 17.64\) Hz, vinyl-H x 1), 5.56 (d, 1H, \(J = 9.91\) Hz, N-CH\(_2\)-OSEM x 1), 5.25 (d, 1H, \(J = 10.90\) Hz, vinyl-H x 1), 4.86-4.57 (m, 2H, N-CH2-osem x 1 & H11a), 4.07-3.61 (m, 9H, 7- & 8-MeO, O-CH2-SEM, H1 x 1), 3.38-3.08 (m, 1H, H1 x 1), 1.11-0.80 (m, 2H, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\))\(_3\)).

Synthesis of 2-(2,6-dimethylphenyl)-7,8-dimethoxy-10-(2-trimethyl silanyl ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 2,6-dimethylbenzeneboronic acid (149 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution
of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 306.5 hours. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 134 (218 mg, 0.43 mmol, 47 % yield). $^1$H (250 MHz, CDCl$_3$) NMR : δ 7.42 (s, 1H, H6), 7.28 (s, 1H, H9), 7.22-7.02 (m, 3H, Ar-H x 3), 6.82 (broad s, 1H, H3), 5.62 (d, 1H, $J = 9.852$ Hz, N-CH$_2$-OSM x 1), 4.74 (d, 1H, $J = 9.72$ Hz, N-CH$_2$-OSM x 1), 4.64 (dd, 1H, $J = 3.076$, 10.505 Hz, H11a), 3.96 & 3.94 (2s, 6H, 7- & 8-MeO), 3.89-3.64 (m, 3H, O-CH$_2$-SEM, HI x 1), 3.02 (ddd, 1H, $J = 2.388$, 10.584, 16.894 Hz, H1 x 1), 2.32 (s, 6H, Ar-2,6-diMe x 6), 1.00 (t, 2H, $J = 8.268$ Hz, CH$_2$-SEM), 0.03 (s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(4-methylthiophenyl)-7,8-dimethoxy-10-(2-trimethylsilylenoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 4-methylthiobenzeneboronic acid (167 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium (0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 12.8 days. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 136 (293 mg, 0.56 mmol, 62 % yield). $^1$H (250 MHz, CDCl$_3$) NMR : δ 7.51-7.17 (multiple singlets, 7H, H3 & H6 & H9 & Ar-H), 5.56 (d, 1H, $J = 9.91$ Hz, N-CH$_2$-OSM x 1), 4.78-4.60 (m, 2H, N-CH$_2$-OSM x 1 & H11a), 4.05-3.89 (m, 7H, 7- & 8- MeO & H1 x 1) 3.76 (ddd, 2H, $J = 7.35$, 9.54, 17.78 Hz, O-CH$_2$-SEM), 3.14 (ddd, 1H, $J = 1.87$, $J = 9.91$, $J = 17.78$ Hz, 1H, O-CH$_2$-SEM).
10.67, 15.97 Hz, H1 x 1), 2.49 (s, 3H, Ar-S-CH3), 1.08-0.92 (m, 2H, CH2-SEM), 0.04 (s, 9H, SEM-Si(CH3)3).

**Attempted synthesis of 2-(2,6-dimethoxyphenyl)-7,8-dimethoxy-10-(2-trimethylsilanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one**

![Chemical structure](image1)

Sodium carbonate (300 mg, 2.85 mmol), 2,6-dimethoxybenzeneboronic acid (181 mg, 0.99 mmol) and *tetrakis* (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 12.8 days. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent but the pure desired product 138 was not isolated.

**Synthesis of 2-(4-formylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one**

![Chemical structure](image2)

Sodium carbonate (300 mg, 2.85 mmol), 4-formylbenzeneboronic acid (149 mg, 0.99 mmol) and *tetrakis* (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 12.8 days. The reaction mixture was diluted with ethyl acetate (50
mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the product 140 (293 mg, 0.56 mmol, 62 % yield). \(^1\)H (250 MHz, CDCl\(_3\)) NMR : \(\delta\) 9.95 (s, 1H, COH), 7.83 (d, 2H, \(J = 8.03\) Hz, Ar-H x 2), 7.69-7.47 (m, 3H, Ar-H x 2 & H3), 7.47-7.24 (m, 2H, H6 & H9), 5.56 (d, 1H, \(J = 9.94\) Hz, N-CH2-OSEM x 1), 4.86-4.65 (m, 2H, N-CH2-OSEM x 1 & H1a), 4.19-3.61 (m, 9H, 7- & 8- MeO & O-CH\(_2\)-SEM & H1 x 1), 3.20 (dd, 1H, \(J = 10.85, 15.54\) Hz, H1 x 1), 0.99 (t, 2H, \(J = 8.16\) Hz, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\)\(_3\))).

**Synthesis of 2-(2,6-difluorophenyl)-7,8-dimethoxy-10-(2-trimethylsilanylethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one**

[Sodium carbonate (300 mg, 2.85 mmol), 2,6-difluorobenzeneboronic acid (157 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 307 hours. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 129 (380 mg, 0.73 mmol, 81 % yield). \(^1\)H (250 MHz, CDCl\(_3\)) NMR : \(\delta\) 7.78-7.08 (m, 4H, H6 & H9 & H3 & Ar-H x 1), 7.05-6.79 (m, 2H, Ar-H x 2), 5.57 (d, 1H, \(J = 10.20\) Hz, N-CH\(_2\)-OSEM x 1), 4.73 (d, 1H, \(J = 9.84\) Hz, N-CH\(_2\)-OSEM x 1), 4.60 (dd, 1H, \(J = 10.85\) Hz, H11a), 4.20-3.85 (m, 7H, 7- & 8- MeO, H1 x 1), 3.85-3.57 (m, 2H, OCH\(_2\)-SEM), 3.53-3.28 (m, 1H, H1 x 1), 0.99 (t, 2H, \(J = 8.53\) Hz, CH\(_2\)-SEM), 0.03 (s, 9H, SEM-Si(CH\(_3\)\(_3\))).
Synthesis of 2-(4-phenoxyphenyl)-7,8-dimethoxy-10-(2-trimethylsilyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 4-phenoxybenzeneboronic acid (213 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg) were added to a solution of 73 (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 13 days. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 131 (334 mg, 0.58 mmol, 64 % yield). \(^1\)H-NMR (250 MHz) 7.52-6.92 (m, 12H, H6, H9, H3, Ar-H), 5.53 (d, 1H, 9.83 Hz, N-CH\(_2\)-OSEM x 1), 4.81-4.59 (m, 2H, N-CH\(_2\)-OSEM & HI la), 4.21-3.61 (m, 9H, 7- & 8- MeO & HI x 1 & O-CH\(_2\)-SEM), 3.11 (ddd, 1H, J = 1.6, 10.4, 12.6 Hz, H1 x 1), 0.96 (m, 2H, CH\(_2\)-SEM), 0.04 (s, 9H, SEM-Si(CH\(_3\))\(_3\)).

Synthesis of 2-(3,4,5-trimethoxyphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one

Sodium carbonate (300 mg, 2.85 mmol), 3,4,5-trimethoxybenzeneboronic acid (211 mg, 0.99 mmol) and tetrakis (triphenylphosphine) palladium(0) (30 mg at t = 0 hrs and 30 mg at t = 117 hrs) was added to a solution of 73 (500 mg, 0.90 mmol) in
ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 336 hours. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 133 (334 mg, 0.58 mmol, 64 % yield). $^1$H-NMR (250 MHz) 7.51-7.24 (m, 3H, H6, H9, H3), 6.65 (s, 2H, Ar-H), 5.57 (d, 1H, J = 9.91 Hz, N-CH$_2$-OSEM x 1), 4.81-4.60 (m, 2H, N-CH$_2$-OSEM x 1 & H11a), 4.20-3.56 (m, 18H, 7- & 8-MeO & Ar-MeO & H1 x 1 & O-CH$_2$-SEM), 3.14 (ddd, 1H, J = 1.97, 10.53, 16.09 Hz, H1 x 1), 1.10-0.93 (m, 2H, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(1-vinyl-2(4-methylphenyl))-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (270 mg, 2.57 mmol), 1-vinyl-4-methylbenzeneboronic acid (144 mg, 0.89 mmol) and tetrakis (triphenylphosphine) palladium(0) (27 mg) were added to a solution of 73 (449 mg, 0.81 mmol) in ethanol/water/benzene (2/3/6 mL) and allowed to stir at room temperature for 9 days. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 135 as a dark orange foamy solid (214 mg, 0.41 mmol, 51 % yield). $^1$H (400 MHz, CDCl$_3$) NMR : δ 7.58-6.84 (m, 8H, H6 & H9 & H3 & Ar-H & vinyl-H x 1), 6.49 (d, 1H, J = 15.99 Hz, vinyl-H x 1), 5.57 (d, 1H, J = 10.00 Hz, N-CH$_2$-OSEM x 1), 4.77-4.58 (m, 2H, N-CH$_2$-OSEM x 1 & H11a), 4.19-3.56 (m, 9H, 7- & 8-MeO & O-CH$_2$-SEM & H1 x 1), 3.02 (ddd, 1H, J = 11.24, 15.31 Hz, H1 x 1), 2.34 (s, 3H, Ar-CH$_3$), 1.07-0.92 (m, 2H, CH$_2$-SEM), 0.04 (broad s, 9H, SEM-Si(CH$_3$)$_3$).
Synthesis of 2-(t-vinylphenyl)-7,8-dimethoxy-10-(2-trimethylsilyl ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (270 mg, 2.57 mmol), t-vinylbenzeneboronic acid (132 mg, 0.89 mmol) and tetrakis (triphenylphosphine) palladium(0) (27 mg) were added to a solution of 73 (449 mg, 0.81 mmol) in ethanol/water/benzene (2/2/6 mL) and allowed to stir at room temperature for 9 days. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 137 as a yellow solid (196 mg, 0.39 mmol, 48 % yield). $^1$H (400 MHz, CDCl$_3$) NMR: $\delta$ 7.58-7.39 (m, 5H, Ar-H) 7.35-6.87 (m, 4H, H$_6$ & H$_9$ & H$_3$ & vinyl-H x 1), 6.49 (d, 1H, $J=15.99$ Hz, vinyl-H x 1), 5.56 (d, 1H, $J=10.00$ Hz, N-CH$_2$-OSEM x 1), 4.79-4.59 (m, 2H, N-CH$_2$-OSEM x 1 & HI la), 3.98-3.56 (m, 9H, 7- & 8- MeO & O-CH$_2$-SEM & HI X1), 3.02 (dd, 1H, $J=11.24$, 15.31 Hz, H1 x 1), 1.05-0.92 (m, 2H, CH$_2$-SEM), 0.03 (s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(1-vinyl-2-(4-trifluoromethylphenyl))-7,8-dimethoxy-10-(2-trimethylsilyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

Sodium carbonate (270 mg, 2.57 mmol), 1-vinyl-4-trifluoromethylbenzeneboronic acid (192 mg, 0.89 mmol) and tetrakis (triphenylphosphine) palladium (0) (27 mg) were added to a solution of 73 (450 mg, 0.81 mmol) in ethanol/water/benzene (2/3/6 mL) and allowed...
to stir at room temperature for 9 days. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 139 as an orange foam (261 mg, 0.46 mmol, 57 % yield). $^1$H (400 MHz, CDCl$_3$) NMR : δ 7.57 (d, 2H, $J = 8.3$ Hz, Ar-H x 2), 7.50 (d, 2H, $J = 8.2$ Hz, Ar-H x 2), 7.37 (s, 1H, H9), 7.32-7.24 (m, 1H, H3), 7.16 (s, 1H, H6), 7.05 (d, 1H, $J = 16.0$ Hz, vinyl-H x 1), 6.51 (d, 1H, $J = 16.0$ Hz, vinyl-H x 1), 5.57 (d, 1H, $J = 10.0$ Hz, N-CH$_2$-OSEM x 1), 4.77-4.61 (m, 2H, N-CH$_2$-OSEM x 1 & H11a), 3.96 & 3.95 (2s, 6H, 7- & 8-MeO), 3.89-3.61 (m, 3H, O-CH$_2$-SEM & H1 x 1), 3.04 (dd, 1H, $J = 10.79$, 15.64 Hz, H1 x 1), 1.07-0.92 (m, 2H, CH$_2$-SEM), 0.04 (broad s, 9H, SEM-Si(CH$_3$)$_3$).

Synthesis of 2-(4-biphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one dimer

Sodium carbonate (300 mg, mmol), biphenyl-4,4'-diyldiboronic acid (182 mg, 0.75 mmol) and tetrakis (triphenylphosphine) palladium (0) (40 mg) were added to a solution of 73 (730 mg, 1.37 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 4 days. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (75 mL) and brine (75 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product 141 as an orange foam (589 mg, 0.52 mmol, 38 % yield). $^1$H NMR signals were weak and data was inconclusive. The molecule was taken forward to the next step without further purification.
Attempted synthesis of 2-(2-thiophene)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one dimer

Sodium carbonate (300 mg, mmol), thiophene-2,5-diylboronic acid (130 mg, 0.75 mmol) and tetrakis (triphenylphosphine) palladium (0) (40 mg) were added to a solution of 73 (730 mg, 1.37 mmol) in ethanol/water/benzene (20/20/20 mL) and allowed to stir at room temperature for 4 days. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (75 mL) and brine (75 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent but a pure product was not successfully isolated.

Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-methylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (60 mg, 1.60 mmol) was added in one portion to a stirred solution of 115 (85 mg, 0.17 mmol) in a mixture of anhydrous ethanol (1.7 mL) and anhydrous THF (3.4 mL) and left to stir at room temperature for 5 hours. The reaction mixture was diluted with water (8.5 mL) and stirred for 30 mins with silica gel (1.7g). The mixture was extracted with ethyl acetate (3 x 10 mL). The organic layers were combined and washed with brine (5 mL) then dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 50:50 mixture of hexane: ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 147 as a
yellow powder (22.3 mg, 0.06 mmol, 38 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: δ 7.90 (d, 1H, $J = 3.9$ Hz, H11), 7.53 (s, 1H, H9), 7.47 (broad s, 1H, H3), 7.31 (d, 2H, $J = 8.15$ Hz, Ar-tolyl), 7.18 (d, 2H, $J = 8.04$ Hz, Ar-tolyl), 6.84 (s, 1H, H6), 4.42 (ddd, 1H, $J = 4.1$, 5.2, 11.4 Hz, H11a), 3.98 & 3.95 (2s, 6H, 7- & 8- MeO), 3.59 (ddd, 1H, $J = 1.9$, 11.5, 16.3 Hz, H1), 3.39 (ddd, 1H, $J = 1.9$, 5.3, 16.6 Hz, H1), 2.36 (s, 3H, Ar-Me). $^{13}$C (150 MHz) NMR: δ 162.7 (C=O), 161.5 (C=N-Ar), 151.9 (C-8), 147.8 (C-7), 140.4 (C-10), 137.6 (C-3), 130.5 (C-2), 129.5 - 124.8 (tolyl- ArH), 122.9 (C-5), 111.6 (C-6), 109.9 (C-9), 56.3 (CH3O -7/8), 56.2 (CH3O -7/8), 53.9 (C-11a), 35.5 (C-1), 21.3 (tolyl-CH3). IR (neat) ν: 3321.71, 3103.05, 2926.30, 2857.88, 1724.22, 1626.00, 1595.65, 1560.88, 1508.53, 1451.31, 1430.06, 1382.02, 1262.81, 1218.38, 1207.71, 1100.45, 1075.82, 1009.31, 965.54, 893.90, 859.47, 810.56, 780.56, 753.80, 650.50 cm$^{-1}$. MS (FAB) m/z (relative intensity): 349 (M$^+$, 76), 348 (38), 347 (99), 333 (13), 206 (13), 192 (100), 185 (33), 180 (44). HRMS (FAB) exact mass calculated for C$_{21}$H$_{20}$N$_2$O$_3$ (M): m/z 349.1552, observed 349.1559. [α]$_D$ (CHCl$_3$) 395.4 °, C = 0.07 g/100ml

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(3,4-methylene-dioxyphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

![SEM](image)

Solid sodium tetraborohydride (413 mg, 10.87 mmol) was added in three portions at half hour intervals to a stirred solution of 119 (494 mg, 0.94 mmol) in a mixture of anhydrous ethanol (9.5 mL) and anhydrous THF (19 mL) and left to stir at room temperature for 28 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (20 mL), water (40 mL) and silica gel (9.5 g) and left to stir at room temperature for 18 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (2 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography.
with a 70-50 % hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 148 as a yellow solid (27 mg, 0.07 mmol, 8 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.87 (d, 1H, J = 3.9 Hz, H11), 7.70-7.30 (m, 3H, H9, 2 x Ar-H), 5.97 (s, 2H, Methyldioxy-CH2), 4.39 (dt, 1H, J = 4.6, 11.3 Hz, H11a), 3.96 & 3.93 (2s, 6H, 7- & 8-MeO), 3.62- 3.47 (m, 1H, H1), 3.34 (ddd, 1H, J = 1.6, 5.3, 16.4 Hz, H1). $^{13}$C (150 MHz) NMR: $\delta$ 162.6, 161.4, 151.9, 148.2, 147.9, 147.4, 140.4, 128.9, 127.7, 127.5, 123.3, 122.6, 119.1, 118.7, 111.7, 109.9, 108.5, 105.2, 101.3, 56.3, 56.2, 53.9, 35.8. MS (FAB) m/z (relative intensity): 439(29), 419(33), 393(100)379(17), 329(37). MS (EI) m/z (relative intensity): 408(17), 376(65), 219(11), 177(21), 147(15), 133(100), 125(12), 117(34). HRMS (EI) exact mass calculated for C$_{21}$H$_{19}$N$_2$O$_5$ (M): m/z 376.1059, observed 376.1072. HRMS (FAB) exact mass calculated for C$_{21}$H$_{19}$N$_2$O$_5$ (M): m/z 379.1294, observed 379.1300. $\left[\alpha\right]_D$ (CHCl$_3$) 987.5 $^\circ$, C = 0.05 g/100ml

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-biphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (400 mg, 10.53 mmol) was added in three portions at two hourly intervals to a stirred solution of 121 (450 mg, 0.81 mmol) in a mixture of anhydrous ethanol (8.2 mL) and anhydrous THF (16.5 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (8.1 g) and left to stir at room temperature for 18 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 149 as a yellow powder.
Solid sodium tetraborohydride (654 mg, 17.21 mmol) was added in three portions at two hourly intervals to a stirred solution of 122 (971 mg, 1.83 mmol) in a mixture of anhydrous ethanol (19 mL) and anhydrous THF (37 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated *in vacuo*. To the crude product was added ethanol (50 mL), water (30 mL) and silica gel (18.3 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70-50 % hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product 150 as a yellow powder (151 mg, 0.39 mmol, 22 % yield).  $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.92 (d, 1H, J = 4.0 Hz, H11), 7.86-7.40 (m, 8H, H3, 6 x Ar-H), 6.85 (s, 1H, H9), 4.45 (dt, 1H, J = 4.8, 11.5 Hz, H11a), 3.98 & 3.95 (2s, 6H, 7- & 8-MeO), 3.65 (ddd, 1H, J = 1.6, 11.2, 16.4 Hz, H1), 3.45 (ddd, 1H, J = 1.5, 5.0, 16.4 Hz, H1). $^{13}$C (150 MHz) NMR: $\delta$ 162.6, 161.5, 152.0, 147.9, 140.5, 140.4, 140.3, 132.4, 128.9, 127.4, 127.4, 126.9, 125.3, 123.8, 123.0, 119.1, 111.7, 109.9, 56.3, 56.2, 54.0, 35.5. MS (FAB) m/z (relative intensity): 564(42), 411(100), 397(22), 381(14), 343(20), 329(44). MS (El) m/z (relative intensity): 408(100), 392(14), 219(31). HRMS (El) exact mass calculated for C$_{26}$H$_{23}$N$_2$O$_3$ (M): m/z 408.1474, observed 408.1481. HRMS (FAB) exact mass calculated for C$_{26}$H$_{23}$N$_2$O$_3$ (M): m/z 411.1709, observed 411.1690. $[\alpha]_D$ (CHCl$_3$) 22.17°, C = 0.20 g/100 ml.
(125 MHz) NMR: 6 162.7 (C-11), 161.6 (C=O), 152.0 (C-8), 147.9 (C-7), 140.5 (naphthyl-C), 133.5 (C-10), 132.8 & 130.9 (naphthyl-C), 128.5 – 123.51 (naphthyl-CH), 123.46 (C-2), 123.1 (C-3), 119.1 (C-5), 111.7 (C-6), 109.9 (C-9), 56.3 & 56.2 (7/8-OMe), 54.0 (C11a), 35.5 (Cl). IR (neat) v: 3391.71, 3017.98, 2933.28, 1623.90, 1509.47, 1451.28, 1431.21, 1380.97, 1265.52, 1217.22, 1101.79, 1071.74, 1041.19, 1010.58, 962.74, 847.40, 815.20, 751.81, 665.67 cm\(^{-1}\). MS (FAB) m/z (relative intensity): 538(14), 399(50), 385(100), 377(23), 371(15), 359(29), 340(11), 327(12). MS (EI) m/z (relative intensity): 382(100). HRMS (EI) exact mass calculated for C\(_{24}\)H\(_{21}\)N\(_2\)O\(_3\) (M): m/z 382.1317, observed 382.1314. HRMS (FAB) exact mass calculated for C\(_{24}\)H\(_{21}\)N\(_2\)O\(_3\) (M): m/z 385.1552, observed 385.1539. [\(\alpha\)]\(_D\) (CHCl\(_3\)) 806.62 \(^\circ\), C = 0.19 g /100 ml.

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-tert-butylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (531 mg, 13.97 mmol) was added in three portions at 0, 5 and 30 hours to a stirred solution of 123 (398 mg, 0.74 mmol) in a mixture of anhydrous ethanol (7.5 mL) and anhydrous THF (15 mL) and left to stir at room temperature for 44 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (7.5 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 151 as yellow glassy solid (193 mg, 0.49 mmol, 67 % yield). \(^1\)H (250 MHz, CDCl\(_3\)) NMR: 6 7.89 (d, 1H, J = 3.94 Hz, H11), 7.58-7.17 (m, 6H, H3,H6, 4 x Ar-H), 6.84 (s, 1H, H9), 4.49-4.35 (m, 1H, H11a), 4.05- 3.51 (m, 7H, 7- & 8-MeO, H1), 3.49- 3.33 (m, 1H, H1), 1.34 (s, 9H, Ar-tBu). \(^13\)C (150 MHz)
NMR: δ 162.6, 161.4, 151.8, 150.8, 147.8, 140.4, 130.4, 125.7, 125.6, 124.6, 124.3, 123.3, 123.0, 119.1, 111.6, 109.8, 57.2, 56.2, 56.1, 55.8, 53.9, 35.5, 34.6, 31.2, 30.4. IR (neat) v: 3351.65, 2963.15, 1625.46, 1597.95, 1511.56, 1453.81, 1433.51, 1391.97, 1265.23, 1216.91, 1138.19, 1079.82, 871.78, 827.92, 753.88, 665.79 cm⁻¹. MS (FAB) m/z (relative intensity): 424(18), 391(38), 307(67), 289(7), 200(30), 194(100), 176(35). HRMS (FAB) exact mass calculated for C_{24}H_{27}N_{2}O_{3} (M+H): m/z 391.2022, observed 391.2014. [α]D (CHCl₃) 575.13 °, C = 0.19 g /100 ml.

Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-chlorophenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (531 mg, 13.97 mmol) was added in three portions at 0, 5 and 12 hours to a stirred solution of 117 (386 mg, 0.75 mmol) in a mixture of anhydrous ethanol (7.5 mL) and anhydrous THF (15 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (7.5 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 152 as a yellow solid (97.5 mg, 0.26 mmol, 35 % yield). ^1H (250 MHz, CDCl₃) NMR: δ 7.90 (d, 1H, J = 3.9 Hz, H11), 7.53 (s, 1H, H6), 7.50 (broad s, 1H, H3), 7.32 (s, 4H, 4 x Ar-H), 6.84 (s, 1H H9), 4.44 (ddd, 1H, J = 4.1, 5.3, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-MeO), 3.58 (ddd, 1H, J = 1.98, 11.6, 16.3 Hz, H1), 3.4 (ddd, 1H, J = 1.7, 5.4, 16.3 Hz, H1). C (150 MHz) NMR: δ 162.4, 161.6, 152.0, 147.8, 140.4, 133.1, 131.9, 128.9, 126.0, 124.2, 122.1, 118.8, 111.6, 109.9, 56.2, 56.2, 53.9, 35.4. IR (neat) v: 3334.50, 3110.33, 3016.37, 2935.93, 2842.84,
Solid sodium tetraborohydride (201 mg, 5.26 mmol) was added in two portions at 0 and 5 hours to a stirred solution of 120 (429 mg, 0.86 mmol) in a mixture of anhydrous ethanol (8.6 mL) and anhydrous THF (17.2 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated *in vacuo*. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (8.6 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product 153 as a yellow glassy solid (139 mg, 0.39 mmol, 46 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: δ 7.90 (d, 1H, J = 3.9 Hz, H11), 7.53 (s, 1H, H6), 7.45 (s, 1H, H3), 7.40-7.20 (m, 1H, 1 x Ar-H), 7.10-6.90 (m, 3H, 3 x Ar-H), 6.84 (s, 1H, H9), 4.43 (ddd, 1H, J = 4.1, 5.4, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-MeO), 3.69- 3.50 (m, 1H, H1), 3.39 (ddd, 1H, J = 1.5, 5.3, 16.3 Hz, H1). $^{13}$C (150 MHz) NMR: δ 162.4, 161.5, 152.0, 147.8, 140.4, 129.5, 126.4, 126.4, 123.4, 122.3, 118.9, 115.9, 115.7, 111.6, 109.9, 56.2, 56.2, 53.9, 35.6. IR (neat) v: 3322.99, 3111.11, 3012.91, 2930.23, 2847.54, 1599.40, 1511.04, 1430.69, 1382.65, 1219.59, 1157.93, 1101.10,
828.06, 754.11 cm\(^{-1}\). MS (FAB) m/z (relative intensity): 353(24), 289(18), 242(15), 223(26), 208(36), 192(100), 180(54), 167(65). HRMS (FAB) exact mass calculated for C\(_{20}\)H\(_{18}\)FN\(_2\)O\(_3\) (M+H): m/z 353.1301, observed 353.1305. \([\alpha]\)_D (CHCl\(_3\)) 924.37 °, C = 0.12 g/100 ml.

Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-trifluoromethylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

![Chemical Structure](image)

Solid sodium tetraborohydride (198 mg, 5.21 mmol) was added in two portions at 0 and 5 hours to a stirred solution of 130 (322 mg, 0.59 mmol) in a mixture of anhydrous ethanol (5.8 mL) and anhydrous THF (11.6 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (8.6 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 155 as a dark yellow solid. MS (FAB) m/z (relative intensity): 422(100), 409(21), 307(22), 289(20), 257(52), 243(22), 228(46), 212(53), 206(24).
Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-dimethylaminophenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (562 mg, 14.79 mmol) was added in three portions at 0, 5 and 12 hours to a stirred solution of 124 (607 mg, 1.16 mmol) in a mixture of anhydrous ethanol (12 mL) and anhydrous THF (23 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (12 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the product 154 as a dark yellow powder (41 mg, 0.11 mmol, 9 % yield).

$^1$H (250 MHz, CDCl$_3$) NMR: δ 7.88 (d, 1H, J = 4.1 Hz H11), 7.79 – 7.07 (m, 4H, H3, H6, o-Ar-H), 6.83 (s, 1H, H9), 6.72 (d, 2H, m-Ar-H), 4.51- 4.27 (m, 1H, H11a), 4.21- 3.23 (m, 8H, 7- & 8-MeO, H1 x 2), 2.99 (s, 6H, Ar-N(Me)$_2$).

$^{13}$C (150 MHz) NMR: δ 124.9, 119.3, 111.3, 108.7, 59.3, 55.2, 55.1, 39.4, 28.7, 13.2. MS (FAB) m/z (relative intensity): 460(22), 424(24), 390(27), 378(41), 353(19), 307(36), 289(17), 206(26), 192(100), 176(38). HRMS (FAB) exact mass calculated for C$_{22}$H$_{24}$N$_3$O$_3$ (M+H): m/z 378.1818, observed 378.1821. [α]$_D$(CHCl$_3$) 401.24 °, C = 0.22 g /100 ml.
Solid sodium tetraborohydride (160 mg, 4.21 mmol) was added in one portion to a stirred solution of 114 (332 mg, 0.65 mmol) in a mixture of anhydrous ethanol (6.5 mL) and anhydrous THF (13 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (6.5 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70 % - 50 % hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 142 as a yellow solid (160 mg, 0.45 mmol, 67 % yield).

$^1$H (250 MHZ, CDC$_3$I) NMR: $\delta$ 7.88 (d, 1H, $J = 3.9$ Hz, H11), 7.53 (s, 1H, H6), 7.46 (s, 1H, H3), 7.39- 7.08 (m, 4H, 4 x Ar-H), 6.83 (s, 1H, H9), 4.46- 4.34 (m, 1H, H11a), 3.96 & 3.94 (2s, 6H, 7- & 8-MeO), 3.89- 3.48 (m, 1H, H1), 3.38 (dd, 1H, $J = 3.8$, 16.6 Hz, H1), 2.73- 2.51 (m, 2H, Ar-CH$_2$-R), 1.35- 1.12 (m, 3H, Ar-R-CH$_3$). $^{13}$C (150 MHz) NMR: $\delta$ 162.6, 161.4, 151.8, 147.8, 143.9, 140.4, 130.7, 128.3, 124.9, 123.5, 122.9, 119.1, 111.6, 109.8, 56.2, 56.1, 53.9, 35.5, 28.6, 15.5. IR (neat) v: 3312.66, 2961.24, 2929.81, 2847.54, 1731.36, 1599.90, 1509.29, 1430.29, 1380.07, 1265.87, 1218.29, 1101.10, 1005.53, 825.04, 755.43 cm$^{-1}$. MS (FAB) m/z (relative intensity): 409(13), 396(33), 381(55), 367(89), 363(100), 351(17), 307(14), 289(12), 236(15), 208(59). HRMS (FAB) exact mass calculated for C$_{22}$H$_{23}$N$_2$O$_3$ (M+H): m/z 363.1709, observed 363.1720. [\(\alpha\)]$_D$ (CHCl$_3$) 380.12 °, C = 0.34 g /100 ml.
Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (500 mg, 13.16 mmol) was added in three portions at 0, 5 and 12 hours to a stirred solution of 127 (429 mg, 0.87 mmol) in a mixture of anhydrous ethanol (8.5 mL) and anhydrous THF (17.5 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), ice water (25 mL) and silica gel (8.5 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 156 as a yellow solid (74 mg, 0.21 mmol, 25 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: $\delta$ 7.92 (d, 1H, $J = 3.9$ Hz, H11), 7.55 (s, 1H, H6), 7.34-7.14 (m, 5H, H3, 4 x Ar-H), 6.85 (s, 1H, H9), 4.39 (dt, 1H, $J = 4.7$, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-MeO), 3.78-3.59 (m, 1H, H1), 3.47 (dd, 1H, $J = 5.2$, 16.2 Hz, H1), 2.49 (s, 3H, Ar-CH3). $^{13}$C (150 MHz) NMR: $\delta$ 162.7, 161.6, 151.9, 147.8, 140.4, 136.1, 132.7, 131.3, 127.3, 126.2, 126.1, 122.7, 119.1, 111.6, 109.8, 56.2, 56.2, 53.0, 38.0, 38.0. IR (neat) v: 3322.99, 3012.91, 2934.79, 1595.99, 1509.75, 1425.25, 1263.68, 1218.30, 755.51 cm$^{-1}$. MS (FAB) m/z (relative intensity): 367(15), 349(70), 347(27), 343(24), 329(14), 307(35), 289(26), 249(21), 236(20), 221(25), 208(100). HRMS (FAB) exact mass calculated for C$_{21}$H$_{21}$N$_2$O$_3$ (M+H): m/z 349.1552, observed 349.1540. [a]$^\text{o}$(CHCl$_3$) 393.87 °, C = 0.20 g /100 ml.
Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2,6-dimethylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (276 mg, 7.26 mmol) was added in two portions at 0 and 24 hours to a stirred solution of 134 (208 mg, 0.41 mmol) in a mixture of anhydrous ethanol (4.1 mL) and anhydrous THF (8.2 mL) and left to stir at room temperature for 47 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), ice water (25 mL) and silica gel (8.2 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70 % - 50 % hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 157 as a yellow solid (43.3 mg, 0.12 mmol, 29 % yield). $^1$H (250 MHz, CDCl$_3$) NMR: δ 7.93 (d, 1H, J = 3.7 Hz, H11), 7.55 (s, 1H, H6), 7.20- 7.04 (m, 3H, 3 x Ar-H), 6.89 (s, 1H H3), 6.85 (s, 1H, H9), 4.49- 4.38 (m, 1H, H11a), 3.98 & 3.96 (2s, 6H, 7- & 8-MeO), 3.53- 3.35 (m, 1H, H1), 3.27- 3.12 (m, 1H, H1), 2.31 (s, 6H, Ar-CH$_3$ x 2). $^{13}$C (150 MHz) NMR: δ 162.5, 161.5, 151.8, 147.8, 140.4, 136.9, 132.9, 127.8, 127.5, 126.0, 123.1, 119.3, 111.6, 109.7, 56.2, 53.7, 38.4, 20.2. IR (neat) v: 3327.35, 3028.63, 2928.63, 2854.51, 1735.88, 1601.74, 1509.37, 1453.83, 1435.41, 1382.03, 1343.75, 1263.28, 1217.41, 1135.39, 1101.75, 1069.17, 1039.97, 955.23, 873.40, 756.87, 665.75 cm$^{-1}$. MS (FAB) m/z (relative intensity): 381(23), 363(50), 343(47), 301(12), 251(13), 223(16). HRMS (FAB) exact mass calculated for C$_{22}$H$_{23}$N$_3$O$_3$ (M+H): m/z 363.1709, observed 363.1720. [α]$_D$ (CHCl$_3$) 492.37 °, C = 0.27 g / 100 ml.
Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-ethenylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (161 mg, 4.24 mmol) was added to a stirred solution of 132 (288 mg, 0.57 mmol) in a mixture of anhydrous ethanol (5.7 mL) and anhydrous THF (11.5 mL) and left to stir at room temperature for 50 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (5.7 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, 180, which was purified using flash column chromatography with a 70 % - 50 % hexane in ethyl acetate as eluent. Insufficient pure product was isolated for further investigations. MS (FAB) m/z (relative intensity): 382(77), 380(43), 363(18), 325(24), 301(23), 292(75), 279(31), 270 (23), 263(27), 255(34), 242(29), 232(38), 221(87), 209(50).

Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-thiophenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one

Solid sodium tetraborohydride (520 mg, 13.68 mmol) was added in three portions at 0, 7 and 14 hours to a stirred solution of 116 (333 mg, 0.685 mmol) in a mixture of anhydrous ethanol (6.9 mL) and anhydrous THF (13.8 mL) and left to stir at room temperature for 30 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl
acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (6.9 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 158 as a yellow solid (49.6 mg, 0.146 mmol, 21 % yield). $^1$H-NMR (500 MHz) $\delta$ 7.90 (d, 1H, $J = 4.0$ Hz), 7.52 (s, 1H), 7.37 (s, 1H), 7.26 (m, 1H), 7.18 (dd, 1H, $J = 4.7$, 43.0 Hz), 7.01 (dd, 1H, $J = 3.6$, 5.1 Hz), 6.96 (dd, 1H, $J = 3.7$, 5.1 Hz), 6.85 (m, 1H), 6.24 (s, 1H), 4.96 (m, 1H), 4.83 (m, 1H), 4.42 (ddd, 1H, $J = 4.1$, 5.1, 11.5 Hz), 3.65 (m, 1H), 2.86 (s, 1H), 2.61 (ddd, 1H, $J = 1.2$, 4.6, 16.0 Hz). $^{13}$C (150 MHz) NMR: $\delta$ 169.0, 162.3, 153.5, 146.5, 140.3, 139.1, 137.4, 127.5, 125.2, 124.5, 123.8, 123.8, 123.6, 123.0, 118.9, 118.3, 118.1, 113.9, 111.6, 109.8, 106.7, 95.4, 67.3, 62.3, 57.0, 56.2, 56.1, 55.8, 53.8, 34.4, 30.3. MS (ES) m/z (relative intensity): 389 (22), 373 (14), 341 (8), 194 (100). [a]$_D$ (CHCl$_3$) 772.73 $^\circ$, C = 0.11 g/100 ml.

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-benzo(b) thiophenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (126 mg, 3.32 mmol) was added to a stirred solution of 118 (179 mg, 0.33 mmol) in a mixture of ethanol (3.4 mL) and THF (7.8 mL) and left to stir at room temperature for 36 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (3.4 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate.
Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 181 (85 mg, 0.218 mmol, 66 % yield). $^1$H-NMR (500 MHz) δ 7.91 (d, 1H), 7.72-7.05 (m, 8H, Ar-H x 5, H6, H3, H9), 4.42 (ddd, 1H, J = 4.1, 5.1, 11.5 Hz, H11a), 3.98-3.95 (m, 6H, 7/8-OMe), 2.56-2.31 (m, 2H, H1 x2). $^{13}$C (150 MHz) NMR: δ 162.6, 161.3, 151.9, 149.2, 149.0, 147.8, 140.4, 132.0, 126.3, 123.4, 122.2, 119.1, 117.7, 111.6, 111.2, 109.8, 107.8, 60.4, 56.2, 56.1, 56.0, 53.9, 35.6, 21.0, 14.2. [α]D(CHCl3) 875.45 °, C = 0.12 g/100 ml.

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(3,4-dimethoxy phenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

![Chemical Structure](image)

Solid sodium tetraborohydride (196 mg, 5.16 mmol) was added to a stirred solution of 126 (434 mg, 0.80 mmol) in a mixture of ethanol (8.1 mL) and THF (16.2 mL) and left to stir at room temperature for 26 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (8.1 g) and left to stir at room temperature for 44 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 182 as a yellow glassy solid (75 mg, 0.192 mmol, 24 % yield). $^1$H-NMR (500 MHz) δ 7.92 (d, 1H, J = 3.9 Hz, H11), 7.62-6.54 (m, 5H, H9 & H6 & H3, Ar-H x 2), 4.59-4.28 (m, 1H, H11a), 4.02-3.46 (m, 13H, 7- & 8-MeO, Ar-MeO, H1 x 1), 3.20-3.12 (m, 1H, H1 x 1). $^{13}$C (150 MHz) NMR: δ 146.6, 140.0, 139.2, 137.2, 127.6, 124.6, 123.2, 123.1, 122.2, 122.1, 122.0, 120.3, 118.2, 116.7, 114.9, 113.8, 111.6, 109.9, 108.0, 103.7, 95.4, 82.7, 67.2, 67.1, 62.5, 57.0, 56.4, 56.3, 56.2, 56.2, 56.1, 56.0, 55.8, 54.0, 41.1, 34.0, 30.3, 29.7.
Solid sodium tetraborohydride (28 mg, 0.74 mmol) was added in two portions at 0 and 23 hours to a stirred solution of 129 (304 mg, 0.586 mmol) in a mixture of anhydrous ethanol (5.8 mL) and anhydrous THF (11.6 mL) and left to stir at room temperature for 45 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (5.8 g) and left to stir at room temperature for 20.5 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 159 as a yellow solid (47.7 mg, 0.129 mmol, 22% yield). 

$^1$H (250 MHz, CDCl$_3$) NMR: δ 7.89 (d, 1H, J = 4.08 Hz, H11) 7.75-6.77 (m, 6H, H6 & H9 & H3 & Ar-H x 3), 4.60 (dd, 1H, J = 3.18, 10.90 Hz, H11a), 4.25-3.63 (m, 2H, 7- & 8- MeO, H1 x 1), 3.62-3.27 (m, 1H, H1 x 1). $^{13}$C (150 MHz) NMR: δ 169.6, 162.8, 161.8, 161.5, 161.4, 159.5, 159.4, 153.7, 152.0, 147.8, 146.7, 140.5, 139.1, 132.4, 132.3, 132.3, 128.0, 127.7, 127.6, 127.5, 118.9, 114.0, 112.0, 112.0, 111.9, 111.9, 111.8, 111.8, 111.7, 111.7, 111.6, 111.6, 111.4, 109.8, 106.5, 95.3, 67.3, 61.8, 56.9, 56.2, 56.2, 55.7, 35.5, 30.3. MS (ES) m/z (relative intensity): 419.3 (42), 403.3 (17), 375.3 (100), 371.2 (2), 194.3 (100). [α]D (CHCl$_3$) 437.5°, C = 0.16 g/100 ml.
**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-phenoxyphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (141 mg, 3.71 mmol) was added to a stirred solution of 131 (334 mg, 0.58 mmol) in a mixture of anhydrous ethanol (5.8 mL) and anhydrous THF (11.6 mL) and left to stir at room temperature for 39 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated *in vacuo*. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (5.8 g) and left to stir at room temperature for 20.5 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product 183 as an orange solid (101 mg, 0.24 mmol, 41 % yield). 

\[
\begin{align*}
^{1}H-NMR & \ (250 \text{ MHz}) \ 7.89 \ (d, \ 1H, \ H11), \ 7.62-6.98 \ (m, \ 12H, \ H6, \ H9, \ H3, \ Ar-H), \ 4.63-4.59 \ (m, \ 1H, \ H11a), \ 3.98 & 3.96 (2s, 6H, 7- & 8- MeO) \ 3.94-3.10 \ (m, \ 2H, \ H1x2). \\
^{13}C \ (150 \text{ MHz}) \ NMR: \ & \delta \ 162.5, \ 161.4, \ 156.9, \ 156.8, \ 151.9, \ 147.8, \ 140.4, \ 129.8, \ 128.4, \ 126.2, \ 123.6, \ 122.8, \ 122.8, \ 119.0, \ 118.9, \ 111.6, \ 109.8, \ 56.2, \ 56.1, \ 53.9, \ 35.6. \ HRMS \ (FAB) \ exact \ mass \ calculated \ for \ C_{26}H_{22}N_{2}O_{4} \ (M+H): \ m/z \ 426.1580, \ observed \ 426.1320. \\
[a]_{D} \ (\text{CHCl}_3) \ & \ 489.72^\circ, \ C = 0.14 \ g / 100 \ ml.
\end{align*}
\]

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-hydroxymethylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

[Diagram]
Solid sodium tetraborohydride (240 mg, 6.32 mmol) was added in two portions to a stirred solution of 140 (264 mg, 0.52 mmol) in a mixture of anhydrous ethanol (5.3 mL) and anhydrous THF (10.4 mL) and left to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated *in vacuo*. To the crude product was added ethanol (50 mL), ice water (25 mL) and silica gel (5.2 g) and left to stir at room temperature for 32 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Collected fractions were combined and evaporated *in vacuo*. A brown oil was collected. (13 mg, 0.04 mmol, 7 % yield). (*NMR signals too weak, inconclusive data*).

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-(1-ethenyl-2-(4-methyl)phenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (200 mg, 5.26 mmol) was added to a stirred solution of 135 (188 mg, 0.36 mmol) in a mixture of anhydrous ethanol (3.6 mL) and anhydrous THF (7.2 mL) and left to stir at room temperature for 48 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated *in vacuo*. To the crude product was added ethanol (25 mL), water (10 mL) and silica gel (3.6 g) and left to stir at room temperature for 43 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (25 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product 185 as a yellow solid (43 mg, 0.12 mmol, 32 % yield). $^1$H (400
MHz, CDCl₃) NMR: δ 7.89 (d, 1H, J = 3.98 Hz, H11), 7.64-6.70 (m, 8H, H6, H9, H3, Ar-H x 4, 1 x vinyl-H), 6.40 (d, 1H, J = 16.02 Hz, 1 x vinyl-H), 4.39 (td, 1H, J = 4.64, 11.45, H11a), 4.03-3.71 (m, 6H, 7- & 8-MeO), 3.46 (dd, 1H, J = 11.31, 15.99 Hz, 1 x H1), 3.28 (dd, 1H, J = 5.10, 16.24 Hz, 1 x H1), 2.41-2.24 (m, 3H, Ar-CH3). HRMS (FAB) exact mass calculated for C₂₃H₂₂N₂O₃ (M+H): m/z 374.1630, observed 374.1638

**Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-(1-ethenyl-2-(4-trifluoromethyl)phenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one**

Solid sodium tetraborohydride (127 mg, 3.34 mmol) was added to a stirred solution of 139 (230 mg, 0.40 mmol) in a mixture of ethanol (4.0 mL) and THF (8.0 mL) and left to stir at room temperature for 48 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (25 mL), water (10 mL) and silica gel (4.0 g) and left to stir at room temperature for 43 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (25 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated in vacuo to afford the pure product 186 as a yellow solid (45 mg, 0.10 mmol, 26 % yield). ¹H (400 MHz, CDCl₃) NMR: δ 7.75-7.15 (m), 6.95-6.67 (m), 6.46 (s), 6.41 (s), 6.28-6.04 (m), 5.09-4.98 (m), 4.86-4.71 (m), 4.54 (s), 4.14-3.27 (m), 3.04 (d, J = 16.11 Hz), 2.90 (s), 2.82 (d, J = 10.5 Hz), 1.23 (s), 1.04-0.76 (m), 0.07-(-0.06) (multiple singlets). ¹³C (101 MHz, CDCl₃) NMR: δ 187.3, 150.0, 149.3, 145.6, 140.7, 130.1, 130.0, 129.7, 127.2, 127.1, 127.1, 127.0, 126.95, 118.1, 109.5, 105.1, 84.1, 68.5, 57.7, 57.6, 57.4, 42.6, 36.7.
Attempted synthesis of 1,1'-di((11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-methylphenyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)

Solid sodium tetraborohydride (170 mg, mmol) was added in three portions (t = 0, 23, 47 h) to a stirred solution of 141 (589 mg, 0.52 mmol) in a mixture of ethanol (10.4 mL) and THF (20.8 mL) and left to stir at room temperature for 8 days. The reaction mixture was diluted with water (75 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated in vacuo. To the crude product was added ethanol (50 mL), water (25 mL) and silica gel (10.4 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 75 mL). The organic layers were combined and washed with brine (30 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation in vacuo afforded the crude product 187. NMR analysis failed to confirm the synthesis of the desired dimer product.
EXPERIMENTAL

Synthesis of C2-aryl PBD dimers

Synthesis of the dimer core


**Synthesis of dimethyl 4,4'-(pentane-1,5-diylbis(oxy))bis(3-methoxybenzoate)**

Diisopropyl azidodicarboxylate (100 g, 494.54 mmol) was added dropwise over 30 minutes to a stirred solution of methyl vanillate (81.9 g, 449.58 mmol) and triphenyl phosphine (176.88 g, 674.37 mmol) in anhydrous THF (1.6 litres) and the reaction mixture was allowed to stir at 0 °C for 1 hour. The cold reaction mixture was treated dropwise over 20-30 minutes with a solution of 1,5-pentanediol (23.55 mL, 23.41 g, 224.79 mmol) in anhydrous THF (16 mL). The reaction mixture was allowed to stir overnight using an overhead stirrer at room temperature and the precipitated product was collected by vacuum filtration. Dilution of the filtrate with methanol precipitated further product. The precipitates were collected and air dried to give the product 171 (NC095) as a white powder (85.22 g, 197.06 mmol, 43 % yield).
Synthesis of dimethyl 4,4'-(pentane-1,5-diylbis(oxy))bis(5-methoxy-2-nitrobenzoate)

Solid copper (II) nitrate trihydrate (105 g, 434.38 mmol) was added in several portions over a period of 15 minutes to a suspension of 171 (NC095) (85 g, 196.55 mmol) in acetic anhydride (6.25) at 0 °C (ice/acetone). The reaction mixture was allowed to stir at 0 °C for 1.5 hours. The ice bath was removed and the reaction mixture was allowed to come to room temperature. The temperature rose gradually (but not exceeding 30 °C) with evolution of NO₂. The reaction was allowed to stirred for a further 3 hours once the exotherm had subsided. The reaction mixture was poured into ice/water and stirred overnight. The resulting yellow precipitate was collected by vacuum filtration to give a yellow solid which was air dried to give the product 170 (NC096) (63.88 g, 122.38 mmol, 62 % yield).

Synthesis of 4,4'-(pentane-1,5-diylbis(oxy))bis(5-methoxy-2-nitrobenzoic acid)

A suspension of 170 (NC096) (21.04 g, 40.3 mmol) in 1M NaOH (aq) (400 mL) and THF (400 mL) was allowed to stir for 72 hours at room temperature. The THF was removed in vacuo, the aqueous layer was filtered and the pH adjusted to 1 (using conc. HCl and ice). The product separated from the aqueous layer as an oil and solidified. The sticky oil solid was collected, dissolved in THF, filtered and evaporated in vacuo to give a yellow solid as the product 169 (NC097) (20.28 g, 41.02 mmol, 98 % yield).
Approach I:

Synthesis of 1-1'-[(pentane-1,3-diyldioxy]bis[(1-(4,5-dimethoxy-2-nitro-benzoyl)-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester)]

Oxalyl chloride (3.8 mL, 5.65 g, 44.50 mmol) was added in one portion to a stirred solution of 169 (10 g, 20.23 mmol) in anhydrous DCM (150 mL) at room temperature. A catalytic amount of DMF (7 drops) was added, gas was evolved and the yellow solution turned red and finally orange over 2 minutes. The reaction mixture was allowed to stir for 24 hours under a dry nitrogen atmosphere. The acid chloride solution was added dropwise to a vigorously stirred solution of trans-4-hydroxy-L-proline methyl ester-p-toluene sulphonate (12.83 g, 40.45 mmol) and TEA (16.91 mL, 12.28 g, 121.36 mmol) in anhydrous DCM (150 mL) at -40 °C and stirred for a further 24 hours at room temperature. The reaction mixture was washed with saturated NaHCO₃ (2 x 75 mL), water (2 x 75 mL), saturated ammonium chloride (2 x 75 mL) and brine (2 x 75 mL). Finally the collected organic layers were dried over anhydrous magnesium sulphate, filtered and excess solvent was removed by rotary evaporation under reduced pressure to afford the product 168 (4.42 g, 5.79 mmol, 29 % yield). ¹H (400 MHz, CDCl₃) NMR: δ 7.64 (s, 2H, H9), 6.83 (s, 2H, H6), 4.88-4.73 (m, 2H, H1 la), 4.44 (broad s, 2H, H2), 4.27-3.68 (m, 16H, linker-OCH₃, Ar-OCH₃, ester-OCH₃), 3.56-3.44 (m, 2H, H3), 3.25-3.03 (m, 2H, H3), 2.49-2.30 (m, 2H, H1), 2.30-2.08 (m, 2H, H1), 2.08-1.87 (m, 4H, linker-CH₂), 1.87-1.63 (m, 2H, linker-CH₂). ¹³C (100 MHz) NMR: δ 172.4, 166.7, 154.7, 154.6, 148.6, 137.3, 126.9, 109.5, 109.5, 108.1, 69.9, 69.2, 57.3, 56.7, 52.5, 28.2.
Synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis[(1-(4,5-dimethoxy-2-amino-benzoyl)-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester)]

A slurry of 20 % Pd/C catalyst (800 mg) in 5 mL ethyl acetate was added to a solution of the substrate 168 (4 g, 5.85 mmol) in absolute ethanol (30 mL). The reaction mixture was hydrogenated under pressure using a Parr hydrogenator at 45 psi hydrogen for 48 hours. The reaction mixture was filtered through celite and the celite washed with hot methanol. The filtrate was collected and excess solvent was removed by rotary evaporation under reduced pressure. The reaction afforded what was believed to be the crude amino ester product NC116. ¹H (400 MHz, CDCl₃) NMR: δ (provisional assignment, some signals obscured by ethanol) 7.45-7.08 (m, 2H, H9), 6.84-6.57 (m, 2H, H₆), 4.88-2.37 (m, 28H, H1, H₂, linker-OCH₂, Ar-OCH₃, ester-OCH₃, H1, H3), 1.98-1.67 (m, 6H, linker-CH₂, linker-CH₃). MS (ES) m/z (relative intensity): 485(39), 475(40), 463(81), 431(100), 399(23).

Attempted synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis [2-hydroxy-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]

NC116 in THF (10 mL) and water (80 mL) was treated with conc. HCl (0.5 mL) and allowed to stir for 72 hours at room temperature. NMR analysis of the crude product failed to show the presence of the A-ring core and hence compound 167.
Approach II:

**Synthesis of** $4,4'-(\text{pentane-1,5-diylbis(oxy)})\text{bis}(2\text{-amino-5-methoxy benzoic acid})$

A slurry of 20 % Pd/C catalyst (304 mg) was added to a solution of the substrate 170 (1.52 g, 3.07 mmol) in absolute ethanol and ethyl acetate (60 mL). The reaction mixture was hydrogenated under pressure at 45 psi H$_2$ for 10 hours and then filtered through celite. The filtrate was collected and the solvent excess solvent was removed by rotary evaporation under reduced pressure to afford the product, 172, as a glassy solid (205 mg, 0.47 mmol, 15 % yield).

**Synthesis of** $7,7'-(\text{pentane-1,5-diylbis(oxy)})\text{bis}(6\text{-methoxy-1H-benzo[d][1,3]oxazine-2,4-dione})$

Triphosgene (4.30 g, 14.40 mmol) was added in one portion to a solution of 172 (4.60 mg, 10.60 mmol) in anhydrous THF. Pyridine (3.78 ml, 47.72 mmol) was added dropwise and the reaction mixture and heated at reflux for 3 hours. The reaction mixture was left to cool to room temperature and then poured into water/ice. The resulting precipitate was filtered and collected to give the product 173 as a white solid (4.94 g, 9.87 mmol, 93 % yield). MS (ES+) m/z (relative intensity): 550(12), 528(11), 519(27), 504(100), 487(88), 469(99).
Synthesis of 1-1'-[[[(pentane-1,3-diyl)dioxy]bis [2-hydroxy-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]

A solution of the isatoic anhydride 173 (4.736 g, 9.75 mmol) and trans-4-hydroxy-L-proline (3.32 g, 25.34 mmol) in DMSO (50 mL) was heated to between 100-120 °C for 4 hours. After cooling to room temperature the solution was poured into water (300 mL) and allowed to stand in an ice bath overnight. The resulting precipitate was filtered, collected and dried to give the product 167 as a white solid (4.57 g, 6.69 mmol, 67 % yield). $^1$H (400 MHz, CDCl$_3$) NMR : δ 10.31 (s, 2H, NH), 7.32 (s, 2H, H6), 6.77 (s, 2H, H9), 5.19 (broad s, 2H, OH), 4.36 (broad s, 2H, H2), 4.27-3.94 (m, 6H, H11a, linker-OCH$_2$), 3.93-3.75 (m, 6H, ArMeO), 3.75-3.59 (m, 2H, H3), 3.59-3.25 (m, 2H, H3), 2.82-2.42 (m, 2H, H1), 2.11-1.73(m, 6H, H1, linker-CH$_2$), 1.73-1.44 (m, 2H, linker-CH$_2$). $^{13}$C (100 MHz) NMR: δ 44.2, 40.9, 36.1, 30.9, 23.4, 15.1. (Signals weak – data incomplete).

MS (ES-) m/z (relative intensity): 554(39), 485(100), 137(82), 91(61). MS (ES+) m/z (relative intensity): 578(25), (2), 556(31), 475(31), 454(100), 413(92), 391(32), 273(31), 104(38).
Synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis [2-(tert-butyl-dimethylsilanyloxy)-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]

TBDMS chloride (5.02 g, 33.32 mmol) was added in one portion to a solution of the 2-hydroxy substituted dilactam 167 (4.55 g, 6.66 mmol) and imidazole (2.73 g, 40.14 mmol) in anhydrous DMF (100 mL) and allowed to stir at room temperature for 22 hours. The reaction mixture was poured into water (100 mL) and the resulting suspension filtered to afford the product, which was collected and dried to give the product, 166 (6.79 g, 7.83 mmol, 117 % yield). $^1$H (400 MHz, CDCl$_3$) NMR: δ 8.53 (s, 2H, NH), 7.41 (s, 2H, H6), 6.47 (s, 2H, H9), 5.72 (broad s, 2H), 4.65-4.43 (m, 2H, H2), 4.28-3.05 (m, 16H, H11a, linker-OCH$_2$, ArMeO, H3), 2.85-2.75 (m, 2H, H1), 2.12-1.97 (m, 2H, H1), 1.97-1.68 (m, 4H, linker-CH$_2$), 1.68-1.48 (m, 4H, linker-CH$_2$), 0.93-0.76 (multiple singlets, 18H, TBDMS-C(CH$_3$)$_3$), 0.08 (s, 12H, TBDMS-Si(CH$_3$)$_2$). $^{13}$C (100 MHz) NMR: δ 174.8, 170.1, 166.9, 156.1, 151.0, 133.8, 122.9, 109.4, 73.6, 60.5, 40.8, 35.8, 30.1, 30.0, 30.0, 22.4, 22.3, 1.4, 0.7.
Synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis [(11a-S)-1,2,3,11a-tetrahydro-10-(2-trimethylsilanylethoxymethyl)-7,8-dimethoxy-2-(tert-butyl-dimethyl-silanyloxy)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione

Butyl lithium (10.97 mL, 17.56 mmol) in anhydrous THF (50 mL) was added dropwise to a solution of 166 (6.79 g, 7.98 mmol) in anhydrous THF (100 mL) over 20 mins at 0 °C and allowed to stir for a further 90 mins. SEM chloride (3.39 mL, 3.19 g, 19.15 mmol) in anhydrous THF (80 mL) was added to the stirred reaction mixture at 0 °C and allowed to stir for 24 hours. The reaction mixture was poured into water (100 mL) and the product was extracted with ethyl acetate (3 x 200 mL), the organic layers combined, washed with water (75 mL), brine (75 mL) and dried over anhydrous magnesium sulphate. Filtration and removal of solvent by rotary evaporation under reduced pressure afforded the crude product which was purified using semi-automated flash column chromatography to give the final product 165 (2.69 g, 2.45 mmol, 31 % yield). $^1$H (400 MHz, CDCl$_3$) NMR : $\delta$ 7.33 (s, 2H, H6), 7.20 (s, 2H, H9), 5.58 (d, 2H, NCH$_2$SEM x 2), 4.79-4.52 (m, 4H, H2, NCH$_2$SEM x 2), 4.32-3.40 (m, 18H, Ar-MeO, O-CH-2-SEM, linker-OCH$_2$ and H-3), 2.97-2.76 (m, 2H, H1), 2.10-1.83 (m, 6H, H1, linker-CH$_2$), 1.83-1.58 (m, 2H, linker-CH$_2$), 1.09-0.76 (m, 22H, CH$_2$-SEM, TBDMS-C(CH$_3$)$_3$), 0.28(-0.02) (m, 30H, TBDMS-Si(CH$_3$)$_2$, SEM-Si(CH$_3$)$_3$). $^{13}$C (100 MHz) NMR: $\delta$ 171.2, 166.9, 152.7, 148.7, 135.1, 122.6, 112.8, 107.9, 70.8, 70.1, 68.3, 57.8, 57.4, 54.9, 36.8, 30.0, 27.0, 23.9, 23.3, 19.6, 19.4, 19.3, 19.2.
Synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis [(11a-S)-1,2,3,11a-tetrahydro-10-(2-trimethylsilyl-ethoxymethyl)-7,8-dimethoxy-2-hydroxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]

A solution of 1N TBAF in THF (7.23 mL, 7.23 mmol) was added to a stirred solution of 165 (2.65 g, 2.41 mmol) in THF (25 mL). The reaction mixture was allowed to stir at room temperature for 2 hours and diluted with DCM (100 mL), washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation of the solvent in vacuo afforded the product, which was purified by flash column chromatography using a gradient system of 50% to 80% ethyl acetate in petroleum ether as the eluent. The pure fractions were combined and removal of excess solvent by rotary evaporation under reduced pressure afforded the pure product 164 (1.74 g, 2.03 mmol, 84 % yield). $^1$H (400 MHz, CDCl$_3$) NMR: δ 7.32 (s, 2H, H6), 7.18 (s, 2H, H9), 5.57-5.45 (m, 2H, NCH$_2$SEM x 2), 5.29 (s, 2H, OH), 4.70-4.58 (m, 4H, H2, NCH$_2$SEM x 2), 3.64-2.44 (m, 18H, Ar-MeO, O-CH-2-SEM, linker-CH$_2$ and H-3), 3.01-2.87 (m, 2H, H1), 2.19-1.51 (m, 8H, H1, linker-CH$_2$, linker-CH$_2$), 1.06-0.36 (m, 4H, CH$_2$-SEM), 0.08 & 0.01 (2s, 18H, SEM-Si(CH$_3$)$_3$). $^{13}$C (100 MHz) NMR: δ 171.1, 167.1, 152.6, 148.6, 135.1, 122.7, 113.0, 108.0, 70.2, 70.1, 68.3, 60.3, 57.8, 57.5, 55.3, 53.4, 36.3, 30.0, 26.5, 25.4, 21.5, 21.1, 19.6, 15.0, 14.8.
Synthesis of 1-1’-[(pentane-1,3-diyl)dioxy]bis [(11a-S)-1,11a-dihydro-10-(2-trimethylsilanyl-ethoxymethyl)-7,8-dimethoxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-2,5,11-trione]

Anhydrous DCM (50 mL) was added to a mixture of 164 (1.73 g, 2.02 mmol), PDC (1.83 g, 4.85 mmol) and 4A molecular sieves (2.02 g). The reaction mixture was stirred at room temperature, under nitrogen, for 20 hours. The reaction mixture was quenched with ethanol and filtered through celite. Removal of solvent by rotary evaporation under reduced pressure afforded the crude product which was then purified using flash column chromatography to yield the pure product 163 as a white foam (393 mg, 0.87 mmol, 44 % yield). $^1$H (400 MHz, CDCl$_3$) NMR : δ 7.30 (s, 2H, H$_6$), 7.20 (s, 2H, H$_9$), 5.50 (d, 2H, NCH$_2$SEM x 2), 4.78-4.55 (m, 4H, H$_2$, NCH$_2$SEM x 2), 4.30 (m, 20H, HI la, Ar-MeO, O-CH-2-SEM, linker-OCH$_2$, H$_1$ and H-3), 2.63-2.82 (m, 2H, H$_1$), 2.10-1.87 (m, 4H, linker-CH$_2$), 1.80-1.62 (m, 2H, linker- CH$_2$), 1.02-0.80 (m, 4H, CH$_2$-SEM), 0.02 (s, 18H, SEM-Si(CH$_3$)$_3$).

Anhydrous pyridine (0.09 mL, 83.89 mg, 1.06 mmol) was added in one portion to a vigorously stirred solution of 163 (377 mg, 0.44 mmol) in anhydrous DCM (25 mL) and...
the mixture allowed to stir for 10 minutes at room temperature. Anhydrous triflic anhydride (0.16 mL, 0.27 g, 0.97 mmol) was added quickly in one portion and the reaction mixture was allowed to stir at room temperature for 6 hours. The homogenous reaction mixture was diluted with cold saturated sodium hydrogen carbonate (50 mL) and the mixture was extracted with DCM (3 x 10 mL). The organic layers were combined and washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous magnesium sulphate. Filtration and removal of solvent by rotary evaporation under reduced pressure afforded the crude product, which was purified by flash column chromatography using 60:40 ethyl acetate: petroleum ether as eluent. Evaporation of the pure fractions by rotary evaporation under reduced pressure afforded the pure product 162 as a white bristly solid (236 mg, 0.23 mmol, 53 % yield). 

\[ \text{1H (400 MHz, CDCl}_3\text{) NMR : } \delta 7.32 (s, 2H, H_6), 7.20 (s, 2H, H_9), 5.52 (d, 2H, NCH}_2\text{SEM x 2), 4.70-4.55 (m, 4H, H}_2\text{, NCH}_2\text{SEM x 2), 4.20-4.05 (m, 2H, H}_1\text{n1a), 4.00-3.52 (m, 18H, Ar-MeO, O-CH-2-SEM, linker-OCH}_2\text{, H1 and H-3), 2.85-2.70 (m, 2H, H1), 2.10-1.92 (m, 4H, linker-CH}_2\text{), 1.81-1.68 (m, 4H, linker-CH}_2\text{), 1.07-0.90 (m, 4H, CH}_2\text{-SEM), 0.08 (s, 18H, SEM-Si(CH}_3\text{)}_3\text{).} \]

\[ \text{13C (100 MHz) NMR: } \delta 169.7, 166.2, 153.4, 149.2, 139.6, 134.9, 122.6, 121.0, 113.0, 108.3, 79.9, 70.3, 68.7, 58.0, 57.6, 32.0, 30.1, 24.0, 19.7, 15.5. \]

**Synthesis of 1-1' - [((pentane-1,3-diyl)dioxypyridox]bis [2-(4-tert-butylphenyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one]]**

Sodium carbonate (260 mg, 2.50 mmol), 4-tert-butylbenzene boronic acid (85 + 44 mg, 0.73 mmol) and tetrakis (triphenylphosphine) palladium(0) (26 + 14 mg) were added in two portions to a solution of 162 (500 mg, 0.903 mmol) in ethanol/water/benzene (10/10/10 mL) and allowed to stir at room temperature for 9 days. The reaction mixture
was diluted with ethyl acetate (200 mL) and washed with water (50 mL), brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the reaction product (427 mg, 0.38 mmol, 42 % yield). ¹H (400 MHz, CDCl₃) NMR : δ 7.72-7.17 (m, Ar-H), 7.75 (d, 2H, H3), 5.60-5.43 (m, NCH₂SEM x 2), 4.79-4.48 (m, 4H, H2, NCH₂SEM x 2), 4.25-3.41 (m, H11a, Ar-MeO, O-CH-2-SEM, linker-OCH₂, H1 and H-3), 2.48-0.75 (m, H1, linker-CH₂ x 6, CH₂-SEM), 0.02 (s, 18H, SEM-Si(CH₃)₃).
EXPERIMENTAL

Biology

NCI 60-Cell line screening*

The NCI 60 human tumour cell line screen is a standard, universally recognised, rapid screening for novel compounds as potential anti-tumour agents. The standardisation of the screen allows for comparison of novel agents with known anti-cancer drugs so as to ascertain whether these novel agents meet the predefined criteria for further investigation.

The experiment involves exposing a selection of common human tumour stock cell lines to the novel agent. After incubating the cell lines and novel agent for a predetermined time period, a pink stain is added to the well (Sulphorhodamine B (SRB)), incubated for 10 minutes and then unbound stain is removed by rinsing (if a cell has undergone apoptosis, it will not be stained by SRB). Absorbance readings are then taken (using an automated plate reader) and from these measurements net protein increase/decrease can be calculated so as to determine the dose of drug required to achieve the GI_{50} (50 % growth inhibition), TGI (100 % growth inhibition) and LC_{50} (50 % reduction in growth compared to the control) values as shown on the classic NCI 60-cell line bar charts and accompanying charts/tables.

The methodology for the NCI 60-cell line screen is provided in appendix C.

* Methodology as provided by the National Cancer Institute.
Hollow Fibre Assay

A hollow fibre assay is an *in vivo* assay that allows the investigator to use one mouse for 3 different tumour cell lines and hence shares some characteristics with *in vitro* screening. Small hollow fibres (3 per mouse) containing the different standard tumour cell lines are implanted into the mouse subcutaneously. After 3-4 days, the drug is administered to the mouse intraperitonealy at 2 dose levels. The day after the fourth treatment, the hollow fibres are collected and evaluated using spectrophotometry (optical density of the hollow fibres is measured). A positive result is a 50% or more reduction in net growth of the tumour cell lines and these data are used to provide a final score for the novel drugs.

The experimental details for this assay are provided in appendix C.

Freiburg Clonogenic Assay

This assay is similar to the NCI 60-cell line *in vitro* screen and serves a similar purpose. However, key differences include the use of cloned cell lines in this assay whereas in the NCI screen, stock cells that have been re-grown numerous times and frozen for future use are used. Additionally, tryptan blue stain is used in this assay whereas the NCI 60-cell line screen uses SRB stain.

The experimental details for this assay are provided in appendix C.

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† Methodology as provided by the National Cancer Institute.

‡ Methodology provided by Burger, A. Institute for Experimental Oncology, Freiburg.
Molecular Modelling

Simulations

Molecular dynamics (MD) simulations of each ligand covalently bound to the same DNA sequence d(GCGCAGAGCGC)₂ were used. The covalent bond was formed between the PBD moiety and the central guanine residue, and the ligand oriented in S3' stereochemistry (i.e. with the PBD A ring pointing towards the 3' terminus of the strand to which it is covalently bound), which has been shown to be generally preferred for most PBD systems.

Simulations were carried out using the AMBER8 package. The parm99 parameters were used for the DNA, TIP3P water molecules represented the solvent explicitly and Na⁺ counterions were added to neutralise the system. The force field parameters for each ligand were obtained from the gaff potential, using the antechamber program from AMBER8. Charges for each ligand were obtained using the RESP method, following a geometry optimisation in the PM3 semi-empirical force field and a single point energy and wave function calculation with the 6-31G* basis set (the GAMESS package was used for these calculations). The RESP procedure was modified to ensure the overall complex had integer charge.

Periodic boundary conditions (truncated octahedral) and the NVT ensemble were used. Particle-Mesh-Ewald with a direct space cut-off of 8 Å was used for the electrostatic energies, and van der Waals forces interactions were also truncated at 8 Å. The solvent box was constructed to lie at least 8 Å away from all non-water atoms in the system. The equilibration protocol was 1000 cycles minimisation with complex atoms restrained (500 kcal/Å) 1000 cycles minimisation with no restraints; 3 ps MD with complex atoms restrained to final geometry of previous stage in NPT ensemble, pressure set to 1 atm, T = 300K using Berendsen thermostat and barostat; further 27 ps MD with complex atoms restrained to final geometry of previous stage (in these two stages the box volume shrank

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8 Methodology provided by Evans, D, Spirogen, London, UK.
dramatically and the density increased from ~ 0.84 g/cm$^3$ to ~ 1.03 g/cm$^3$); 100 ps NVT MD under identical conditions to production run.

All production simulations were run at 300K for 1 ns, with 2 fs time steps. The SHAKE algorithm was used to constrain the lengths of bonds to hydrogen, and the Langevin thermostat used with a collision frequency of 1 ps$^{-1}$.

All calculations were done on Intel Xeon 3.2 GHz processors.

Calculating binding energies

The potential energy of binding interactions was estimated using Equation 4.

$$E_{\text{bind}} = E_{\text{comp}} - E_{\text{lig}} - E_{\text{rec}}$$

Equation 4: Estimation of the potential energy of binding interactions. $E_{\text{comp}}$, $E_{\text{lig}}$ and $E_{\text{rec}}$ are the potential energies of the complex, ligand and receptor respectively.

Entropies, and therefore free energies, were not considered in this study. It was assumed that the change in entropy of the ligand and receptor on binding was not significantly different for all these ligands, an assumption that may be justified by the fact that all the ligands are of similar size and shape.

$E_{\text{comp}}$, $E_{\text{lig}}$ and $E_{\text{rec}}$ were evaluated from snapshots of the MD trajectories. These snapshots were taken every 4 ps, based on a calculation of the statistical inefficiency for a related system, which enabled the standard error in $E_{\text{bind}}$ to be evaluated. The MM-PBSA approach$^{126}$ was used to calculate the energies for each species at each snapshot. This estimated the overall energy of a solvated species by adding the molecular-mechanics (MM) energy of the isolated molecule in vacuo (from the parm99 + gaff potential) to an estimate of the solvation energy obtained from a continuum electrostatics method. The results presented here are from the Poisson-Boltzmann (PB) method, as implemented by the DelPhi program$^{127}$ with PARSE radii$^{128}$ to define the solute boundary and the same charges as used in the MM calculation and MD simulation, and also the generalised Born
(GB) method, as implemented in AMBER8 (option igb = 5) which was shown to give good performance in comparison with more precise PB methods.

The ‘single trajectory’ approach to MM-PBSA was used; i.e. all energies were obtained from the same snapshots of a simulation of the solvated complex, with $E_{rec}$ and $E_{lig}$ calculated by cutting away the relevant part of the molecule from each snapshot. The covalent binding in these systems means the receptor and ligand are in fact non-chemical species with dangling bonds and non-integer charge. The analysis is not affected by this detail, but it does mean that there is a systematic error in the binding energies because of the unknown covalent rearrangement energy on binding. Calculating relative binding energies should largely remove this error from the final results.

**Thermal denaturation studies**

The compounds were subjected to DNA thermal denaturation (melting) experiments using calf thymus (CT) DNA [type-1, highly polymerised; 42% G + C] purchased from the Sigma-Aldrich Company Ltd and used as supplied. CT-DNA had $A_{260}/A_{280} = 1.85$ and was satisfactorily free from protein. A molar extinction value at 260 nm ($e_{260}$) of 6600 dm$^3$ mol$^{-1}$ cm$^{-1}$ was used for the CT-DNA.

Aqueous solutions of DNA were prepared in Millipore-purified water buffered at pH 7.00 ± 0.01, containing 10 mmol dm$^{-3}$ sodium phosphate and 1 mmol tetrasodium EDTA$^{-1}$. No added salt or support electrolyte were used. Working solutions containing 100 µmol dm$^{-3}$ DNA alone and in the presence of 20 µmol dm$^{-3}$ of compound were monitored at 260 nm using a Varian-Cary 300 Bio spectrophotometer fitted with a Varian-Cary temperature controller accessory. Samples were either examined immediately, or incubated at 36°C for 2, 4 or 18 hours before determination. Heating was applied at 1°C min$^{-1}$ from 40°C to 95°C. Data was processed using OriginLab Origin 7.0 and the thermal denaturation point ($Tm$) determined by fitting to a Boltzmann sigmoidal curve. Results are reported as the

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**Methodology provided by Reszka, T., School of Pharmacy, University of London, UK.**
mean of at least 4 results with an error of ± 0.1°C. The change in Tm (ΔTm) following interaction of CT-DNA with an added compound is shown in Equation 5:

\[
\Delta Tm = Tm_{DNA-drug} - Tm_{DNA}
\]

Equation 5: Calculation of the DNA melting (Tm) values

**Buffer preparation**

Two buffers were prepared, the first (a) containing 10mM NaH₂PO₄ + 1mM Na₄EDTA (measured pH 5.94), and the second (b) containing 10mM Na₂HPO₄ + 1mM Na₄EDTA (measured pH 9.84). Addition of 240ml of buffer (a) to 180ml of buffer (b) gave a buffer of pH 7.0. Final sodium ion concentration in the buffer was 18.29 mM.
Figure 1: Bar chart of the data from the NCI 60-cell line evaluation for compound 150.
Figure II: Bar chart of the data from the NCI 60-cell line evaluation for compound 158.
Figure III: Bar chart of the data from the NCI 60-cell line evaluation for compound 185.
Figure IV: Bar chart of the data from the NCI 60-cell line evaluation for compound 157.
**Figure V:** Bar chart of the data from the NCI 60-cell line evaluation for compound 189 (reproduced with permission from Springer).
### Figure VI: Bar chart of the data from the NC1 60-cell line evaluation for compound 190 (reproduced with permission from Spirogen).

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### National Cancer Institute Developmental Therapeutics Program

**Report Date:** May 13, 2003

**Test Date:** March 24, 2003

**Units:** Molar

**SSPL:** OCC

**Exp. ID:** 03090300-11
### National Cancer Institute Developmental Therapeutics Program

#### Mean Graphs

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Figure VII: Bar chart of the data from the NCI 60-cell line evaluation for compound 191 (reproduced with permission from Spirogen).
# Figure VIII: Bar chart of the data from the NCI 60-cell line evaluation for compound D2 (reproduced with permission from Spirogen).

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**Note:** The data is presented in terms of IC50 values (in nM) for each cell line, indicating the concentration at which 50% of the cell growth is inhibited. The range shown is from -3 to 3, indicating the variability in the results.
Figure IX: Bar chart of the data from the NCI 60-cell line evaluation for compound B3 (reproduced with permission from Spirogen).
Figure X: Bar chart of the data from the NCI 60-cell line evaluation for compound 194 (reproduced with permission from Spirogen).
Quick guide to interpreting NCI 60-cell line bar charts

Bars to the left of the mean show the cell lines that are less sensitive overall to the drug being tested.

Bars to the right of the mean show the cell lines that are more sensitive to the drug compared to the other cell lines.

Range

Cell line panels

Cell line 1
Cell line 2
Cell line 3
Cell line 4
Cell line 5
Cell line 6
APPENDIX B

SUPPLEMENTARY DATA: SELECTED NMR SPECTRA

Figure 1: $^1$H NMR of compound 114
Figure II: $^1$H NMR of compound 142

Figure III: $^{13}$C NMR of compound 142
APPENDIX C

SUPPLEMENTARY INFORMATION: BIOLOGICAL EVALUATION METHODS

NCI 60-Cell line screen

Experimental procedure as provided by the National Cancer Institute, Washington DC.

The human tumour cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% foetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilised in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional 4, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5% CO₂, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After
staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain is subsequently solubilised with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as shown in equation 6.

Equation 6: Equation to calculate percentage growth inhibition. Ti = test growth in the presence of the drug; Tz = time zero; C = control growth

\[
[(Ti - Tz)/(C - Tz)] \times 100
\]

for concentrations for which Ti \( \geq \) Tz

\[
[(Ti - Tz)/Tz] \times 100
\]

for concentrations for which Ti < Tz.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI\(_{50}\)) is calculated from \([(Ti-Tz)/(C-Tz)] \times 100 = 50\), which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC\(_{50}\) (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from \([(Ti-Tz)/Tz] \times 100 = -50\). Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested\(^{132}\).
Hollow Fiber Assay

*Experimental procedure as provided by the National Cancer Institute, Washington DC.*

A standard panel of 12 tumour cell lines were used for the routine hollow fiber screening of the *in vitro* actives. These included NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295. The cell lines were cultivated in RPMI-1640 containing 10% FBS and 2 mM glutamine. On the day preceding hollow fiber preparation, the cells were given a supplementation of fresh medium to maintain log phase growth. For fiber preparation, the cells were harvested by standard trypsinization technique and resuspended at the desired cell density (2-10 X 10^6 cells/ml). The cell suspension is flushed into 1 mm (internal diameter) polyvinylidene fluoride hollow fibers with a molecular weight exclusion of 500,000 Da. The hollow fibers were heat-sealed at 2 cm intervals and the samples generated from these seals were placed into tissue culture medium and incubated at 37 °C in 5% CO_2 for 24 to 48 hours prior to implantation. A total of 3 different tumour lines were prepared for each experiment so that each mouse receives 3 intraperitoneal implants (1 of each tumour line) and 3 subcutaneous implants (1 of each tumour line). On the day of implantation, samples of each tumour cell line preparation were quantitated for viable cell mass by a stable endpoint MTT assay so that the time zero cell mass is known. Mice were treated with experimental agents starting on day 3 or 4 following fiber implantation and continuing daily for 4 days. Each agent is administered by intraperitoneal injection at 2 dose levels. The fibers were collected from the mice on the day following the fourth compound treatment and subjected to the stable endpoint MTT assay. The optical density of each sample is determined spectrophotometrically at 540 nm and the mean of each treatment group is calculated. The percent net growth for each cell line in each treatment group is calculated and compared to the percent net growth in the vehicle treated controls. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples is considered a positive result. Each positive result is given a score of 2 and all of the scores were totalled for a given compound. The maximum possible score for an agent is 96 (12 cell lines X 2 sites X 2 dose levels X 2 [score]). A compound is referred for xenograft testing if it has a combined ip + sc score of 20 or greater, a sc score of 8 or greater, or produces cell kill of any cell line at either dose level evaluated. This scoring system has been validated by DCTDC statisticians in CTEP to represent a level of detection expected to score current "standard" agents as active.133
**Freiburg Clonogenic Assay**

Preparation of Single cell suspensions from human tumour xenografts

Solid human tumour xenografts growing subcutaneously in serial passages in thymus aplastic nude mice (NMRI nu/nu strain) were removed under sterile conditions, mechanically disaggregated and subsequently incubated with an enzyme cocktail consisting of collagenase (41 μL, Sigma), DNAse I (125 U/mL, Roche), hyaluronidase (100 U/mL, Sigma) and dispase 11 (1.0 U/mL, Roche) in RPM I 1640-Medium (Life Technologies) at 37 °C for 30 minutes. Cells were passed through sieves of 200 μm and 50 μm mesh size and washed twice with sterile PBS-buffer. The percentage of viable cells was determined in a Neubauer-hemocytometer using trypan blue exclusion.

**Culture methods**

The clonogenic assay was performed in a 24-well format according to a modified two-layer soft agar assay introduced by Hamburger & Salmon.

The bottom layer consisted of 0.2 ml/well of Iscove's Modified Dulbecco's Medium (supplemented with 20% foetal calf serum and 1% gentamicin) and 0.75% agar. 4·10⁴ to 8·10⁴ cells were added to 0.2 ml of the same culture medium supplemented with 0.4% agar and plated in 24-multiwell dishes onto the bottom layer. Cytostatic drugs were applied by continuous exposure (drug overlay) in 0.2 ml culture medium. Every dish included six control wells containing the vehicle and drug treated groups in triplicate at 6 concentrations.

Cultures were incubated at 37°C and 7.5% CO₂ in a humidified atmosphere for 8 - 20 days and monitored closely for colony growth using an inverted microscope. Within this period, in vitro tumour growth led to the formation of colonies with a diameter of > 50 μm. At the time of maximum colony formation, counts were performed with an automatic image analysis system (OMNICON FAS IV, Biosys GmbH). 24 hours prior to evaluation, vital colonies were stained with a sterile aqueous solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (1 mg/ml, 100 μl/well).

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* Methodology provided by Burger, A. Institute for Experimental Oncology, Freiburg.
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