Fragment synthesis: pharmacophore and diversity-oriented approaches

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Department of Chemistry

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Abstract

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By Andrew James Peter North

This thesis explores two approaches to fragment-based drug discovery. First, protein target CK2 was chosen due to its importance in the cancer phenotype. A literature fragment, NMR154L, proved to be a promising compound for fragment development, due to its binding at the interface site of the protein rather than the highly conserved ATP pocket. Analogue synthesis led to a candidate with a better IC₅₀. Additionally, computer modelling of the interface site suggested that a series of spirocyclic compounds would inhibit this protein. These were synthesised and tested in vitro. Results from these tests were analysed and informed the synthesis of new inhibitors with the aid of crystal structures and computer modelling.

Secondly, to address the lack of spirocyclic scaffolds in fragment screening libraries a number of diversity-orientated synthetic campaigns were undertaken. The first of these utilised glycine as starting material. Two terminal alkenes were installed. The alkenes were linked and the amino and acidic residues cyclised. This allowed for the formation of a diverse range of spirocyclic scaffolds from this one starting material.

Having established chemistry for linking amino and acidic residues a campaign with dehydroalanine was undertaken. This would allow for the installation of the second ring by pericyclic chemistry as well as using chemistry previously established.

This pericyclic chemistry was also applied to synthesising spirocycles from rings with exocyclic double bonds. These being readily installed from Wittig chemistry, this allowed utilisation of starting materials which contained a cyclic ketone. Of these azetidinone was a good candidate due to the fact it was a commercially available building block and allowed access to spirocycles containing a 4-membered ring; an underrepresented ring size.

Finally, computation analysis was carried out on the library to assess its diversity and any potential biological targets which these fragments may inhibit.
For Cordelia
Declaration of Authorship

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit for the Physics and Chemistry Degree Committee.

Andrew North

Gonville and Caius College
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<th>Definition</th>
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<tr>
<td>%v/v</td>
<td>per cent by volume</td>
</tr>
<tr>
<td>$^1\text{H}$</td>
<td>NMR spectrum acquired with proton decoupling</td>
</tr>
<tr>
<td>°C</td>
<td>degree(s) Celsius</td>
</tr>
<tr>
<td>ΔG</td>
<td>change in Gibbs free energy</td>
</tr>
<tr>
<td>ΔH</td>
<td>change in enthalpy</td>
</tr>
<tr>
<td>ΔS</td>
<td>change in entropy</td>
</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>Å</td>
<td>ångström(s), $10^{-10}$ m</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ADME</td>
<td>absorption, distribution, metabolism, and excretion</td>
</tr>
<tr>
<td>anh.</td>
<td>anhydrous</td>
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<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>B</td>
<td>magnetic field</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
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<tr>
<td>Boc</td>
<td>tert-butyl oxycarbonyl</td>
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<td>b.p.</td>
<td>boiling point</td>
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<td>br. s</td>
<td>broad singlet</td>
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<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>CC</td>
<td>chiral centres</td>
</tr>
<tr>
<td>CK2</td>
<td>casein kinase 2</td>
</tr>
<tr>
<td>clogP</td>
<td>calculated log of the partition coefficient</td>
</tr>
<tr>
<td>cm(^{-1})</td>
<td>wavenumber(s), inverse centimetre(s)</td>
</tr>
<tr>
<td>dd</td>
<td>double doublet</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DOS</td>
<td>diversity-oriented synthesis</td>
</tr>
<tr>
<td>dr</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>FBDD</td>
<td>fragment-based drug discovery</td>
</tr>
<tr>
<td>FP</td>
<td>fluorescence polarisation</td>
</tr>
<tr>
<td>Fsp(^3)</td>
<td>fraction of the C sp(^3) centres</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HBA</td>
<td>hydrogen bond acceptor</td>
</tr>
<tr>
<td>HBD</td>
<td>hydrogen bond donor</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>HTS</td>
<td>high-throughput screening</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz, s⁻¹</td>
</tr>
<tr>
<td>I</td>
<td>moment of inertia</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IR</td>
<td>infrared spectroscopy</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal titration calorimetry</td>
</tr>
<tr>
<td>K_d</td>
<td>dissociation constant</td>
</tr>
<tr>
<td>K_i</td>
<td>inhibition constant</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>LHMDS</td>
<td>lithium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>m</td>
<td>metre(s)</td>
</tr>
<tr>
<td>M</td>
<td>molarity, mol dm⁻³</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre(s)</td>
</tr>
<tr>
<td>MOE</td>
<td>molecular operating environment</td>
</tr>
<tr>
<td>mol</td>
<td>mole(s)</td>
</tr>
<tr>
<td>mol%</td>
<td>mole per cent</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>n</td>
<td>primary, unbranched alkyl chain</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>N.R.</td>
<td>no reaction</td>
</tr>
<tr>
<td>o/n</td>
<td>overnight</td>
</tr>
<tr>
<td>PCA</td>
<td>principle component analysis</td>
</tr>
<tr>
<td>pet.</td>
<td>petroleum (ether)</td>
</tr>
<tr>
<td>Pg</td>
<td>protecting group</td>
</tr>
<tr>
<td>PMB</td>
<td>para-methoxy benzyl</td>
</tr>
<tr>
<td>PMI</td>
<td>principle moment of inertia</td>
</tr>
<tr>
<td>PPI</td>
<td>protein-protein interaction</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PSA</td>
<td>polar surface area</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>quin</td>
<td>quintet</td>
</tr>
<tr>
<td>RBC</td>
<td>rotatable bond count</td>
</tr>
<tr>
<td>RCM</td>
<td>ring-closing metathesis</td>
</tr>
<tr>
<td>RO3</td>
<td>rule of three</td>
</tr>
<tr>
<td>RO5</td>
<td>rule of five</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>SAR</td>
<td>structure-activity relationship</td>
</tr>
<tr>
<td>sat.</td>
<td>saturated</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>SB</td>
<td>SmithKline Beecham</td>
</tr>
<tr>
<td>s</td>
<td>second(s)</td>
</tr>
<tr>
<td>singlet</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>tertiary alkyl group, tert-alkyl</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>TBDD</td>
<td>target-based drug discovery</td>
</tr>
<tr>
<td>TEA</td>
<td>triethyl amine</td>
</tr>
<tr>
<td>TGI</td>
<td>tumour growth inhibition</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl, Me₃Si—</td>
</tr>
<tr>
<td></td>
<td>tetramethylsilane, Me₄Si</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>δ</td>
<td>NMR chemical shift</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>ŷ</td>
<td>wavenumber, 1/λ</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1. Drug Discovery

1.1.1. The drug discovery pipeline

Of marketable products, pharmaceuticals are one of the most expensive to produce, time consuming to make, and heavily regulated. There is a huge burden on the drug discovery process to ensure that the drugs which are brought to market are safe and effective.\(^1\) To this end, the drug discovery process is broken down into multiple stages: a pre-clinical stage, which sees the creation of biological agents (small molecules, proteins, antibodies, etc.) to affect a medical condition, and a clinical stage where these agents are tested in man to assess their safety and efficacy (Figure 1.1).

![Figure 1.1 A graphic representing the drug discovery process separating out both the pre-clinical and clinical stages of the process.](image)

Pre-clinical stage:

*Unmet need:* The identification of a disease for which there is little or no treatment or it is believed the treatment could be improved.

*Target identification and validation:* Research is carried out to identify which macromolecules are involved in a particular disease process such that their modulation will cause the desired therapeutic response. For validation, different strategies involving a wide variety of areas can be applied such as genetics, cellular or *in vivo* and *in vitro* modelling.\(^2\)
**Introduction**

*Hit identification:* A range of compound screening assays are used to identify compounds with the desired activity. This can cover a wide range of techniques from the modification of natural products with known biological effects, to screening of compound libraries to discover novel biological antagonists (this usually takes the form of high throughput screening or fragment-based drug discover, discussed later on in this introduction).

*Hit development and lead optimisation:* Intensive Structure-Activity Relationship (SAR) studies are conducted around each core structure. Potency and selectivity are assessed as well as detailed profiling of physico-chemical and *in vitro* absorption, distribution, metabolism, and excretion (ADME) properties.³

**Clinical stage:**

*Pre-clinical trials:* The clinical candidate is first tested in animals to collect information pertinent to the safety of the compound. The species chosen will have the most similar traits to humans in the part of the body which the drug is affecting.

*Phase I, II & III:* During these stages, the clinical candidate is tested in humans, with testing split into three phases. Phase I is the ‘first-in-man’ study. Usually, healthy volunteers are given the compound in increasing doses to assess the appropriate dose-range before adverse side-effects are seen. Phase II is the first time the compound is tested in humans with the disease, and as such provides the first proof-of-concept, demonstrating whether the desired clinical outcome has been achieved, whilst also continuing to monitor safety and side effects. Phase III tests the drug therapeutic effect on a larger cohort of patients over a significantly longer time frame, often years.⁴

*Drug approval:* The gathered data is presented to a country’s licencing authority which determines whether the compound can be marketed and sold as a drug for the treatment of a specified illness.

*Market:* This time is also known as Phase IV as the long-term health impacts of the drug are continually monitored and assessed. A drug can be withdrawn from the market by the licencing authority if there are questions about its safety.

This process is protracted and costly. From a compound being identified as a potential therapeutic for a disease (hit identification in Figure 1.1) to a marketable drug, 10–15 years usually elapse.⁵ Additionally, while it is difficult to provide a reliable estimate of the costs due
to differences in accounting practices and industry confidentiality, recent research has put the cost of bringing a drug through the discovery pipeline at $1.4 billion.

This astronomical figure can be mostly ascribed to the expense of carrying out Phase I, II, and III clinical trials. Failed drugs also contribute to the large cost of drug discovery, with no possibility of recouping the cost of development upon failure. Studies have suggested that fewer than one in five drugs progress from Phase II to Phase III, and those in Phase III only have a 50% probability of making it to the market. Reasons for these failures are mostly attributed to the lack of safety and lack of efficacy of clinical candidates. About a third of drugs fail at Phase I for lack of safety and Phase II and III failure rates are 42% and 54% respectively for lack of efficacy.

Therefore, there is a huge pressure on licensed and marketed pharmaceuticals to recoup the development costs of both successful and failed drugs. This is difficult for several reasons. The purchasers of the drugs, in most cases national governments, insurance companies, and clinics, have scarce resources. On top of this is the limited life span of patents which give the producer a monopoly on the exploitation of their invention. To prohibit competitors from operating in the same areas, most compounds are patented early in the drug discovery pipeline (usually around the ‘hit-to-lead’ stage). However, given the life span of a patent is typically 20 years, this limits the length of time for which the patent remains in place once the compound reaches the market. This significantly diminishes the time the producer has to recoup the approximately $1 billion expenditure from the drug’s development. Once the patent expires, generic pharmaceutical companies can produce the same compound at a fraction of the price as they do not have to cover the large research and development costs.

Consequently, pharmaceutical companies, and researchers more generally, are searching for ways to cut down on the time and money in drug development.

1.1.2. Attrition rates and new chemical entities

The high attrition rates in the drug discovery process are an obvious place for researchers to look for a way to cut costs. If the compounds entering the clinic are more likely to succeed, costly and unsuccessful clinical trials can be avoided.
Introduction

Despite advances in research methods and technology,\textsuperscript{17} attrition rates across drug discovery have remained high,\textsuperscript{11-14,18} and as a result, research has been carried out looking to link chemical properties with the success (or otherwise) of potential lead compounds in the clinic.

One of the first and most famous of these studies was by Lipinski \textit{et al.} who performed a detailed study into the physico-chemical properties of orally bioavailable drugs. Their research found that:

Poor absorption or permeation are more likely when: There are more than 5 H-bond donors (expressed as the sum of OHs and NHs); The MWT is over 500; The Log P is over 5 (or MLogP is over 4.15); There are more than 10 H-bond acceptors (expressed as the sum of Ns and Os) Compound classes that are substrates for biological transporters are exceptions to the rule.\textsuperscript{19}

This was the first statement of Lipinski’s ‘Rule of Five’ (RO5), and it has since developed as a ubiquitous rule-of-thumb in drug development and discovery.\textsuperscript{20} Additionally, others have added to the list of physico-chemical properties which should be considered when assessing the likely oral bioavailability of a compound.

The number of rotatable bonds and the polar surface area (the surface area of all the polar atoms) were found to also be strong indicators of the oral bioavailability in rats. A rotatable bond count of less than 10 and a polar surface area of less than 140 Å\textsuperscript{2} were more reliable than the molecular weight cut-off of 500 gmol\textsuperscript{-1} as a predictor of this property.\textsuperscript{21} Consequently, these two metrics have been added to the list of properties considered within the Rule of Five (Table 1.1).\textsuperscript{22}

\textbf{Table 1.1} Lipinski’s Rule of Five.

<table>
<thead>
<tr>
<th>Property</th>
<th>Rule of Five</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (gmol\textsuperscript{-1})</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 5</td>
</tr>
<tr>
<td>Polar Surface Area (Å\textsuperscript{2})</td>
<td>≤ 140</td>
</tr>
<tr>
<td>H-Bond Acceptors</td>
<td>≤ 10</td>
</tr>
<tr>
<td>H-Bond Donors</td>
<td>≤ 5</td>
</tr>
<tr>
<td>Rotatable Bond Count</td>
<td>≤ 10</td>
</tr>
</tbody>
</table>
The advantage of physico-chemical descriptors as a metric for assessing the likely attrition of a compound is that they are easily measurable before synthesis of the compound has even taken place. Thus, chemists can plan and direct their effort to maximise the chance of compounds succeeding in the clinic. In addition, physico-chemical properties are easy to analyse en masse from drugs and failed clinical candidates to produce helpful predictive data.

Analysis of compounds at Pfizer found that compounds with a $\text{clogP} < 3$ and a $\text{PSA} > 75 \, \text{Å}^2$ were 2.5 times less likely to be toxic at 10 μM. This is known as the ‘3/75 rule’. An additional Pfizer study has suggested that a compound with a predicted human efficacious total plasma concentration of $< 250 \, \text{nM}$ was more likely to pass toxicology screening.

One interesting result from meta-analysis of attrition in four major pharmaceutical companies is that molecules with a high fraction of sp$^3$ ($\text{Fsp}^3$) atoms are more likely to become marketed drugs. This tallies with a corresponding decrease in the aromatic ring count also giving a compound greater success in making it to market.

Indeed, one failing of the Rule of Five is that, although it adequately acknowledges the physico-chemical characteristics of a molecule, it makes no reference to its spatial qualities. In this way a set of compounds can obey the RO5 with a diverse set of data, but all have a very similar shape. Thus, the chance that they are going to probe a large area of chemical space is small. Research by Lovering et al. agrees with the above observation. They showed there was a statistically significant increase in the Fsp$^3$ from lead candidates entering the clinic to the marketed drug coming out (0.36 to 0.47). In addition, the number of compounds with stereocentres increased from 53% entering the clinic to 64% coming out. They hypothesise that these 3D molecules are more adapted to the inherently 3D shape of biological targets as well as conveying beneficial physical properties like increased solubility and decreased melting point, which have been shown to aid oral bioavailability.

Physico-chemical descriptors are only one factor which determines a drug’s success in the clinic. In fact, the early drug discovery process is similarly afflicted with challenges.

### 1.2. Established Methods of Drug Discovery

#### 1.2.1. High Throughput Screening

One of the most common and powerful methods in drug discovery has been High Throughput Screening (HTS). This method aimed to combine the vast improvement in biological assay technologies over the past 20 years with expansive compound libraries
accrued by the pharmaceutical industry. Robots were designed and programmed to conduct millions of experiments between these libraries and biological assays in microplates of thousands of wells. The results could be quickly analysed by heat maps of the wells (Figure 1.2). The compounds which showed promise were modified in a combinatorial-type approach to improve their properties and selectivity. When these were believed to be optimal, they would progress to clinical trials.

Figure 1.2 Diagram depicting an HTS method of testing a compound library against a target and assessing the outcome of the experiment. (Figure ref 35)

The driving force for industry to investigate HTS was the ‘brute force’ and ‘enhanced serendipity’ of this method. Many targets have unknown structures or ligands making a ‘rational’ design difficult. However, the chances that at least one molecule of the millions tested would bind to a given target was presumed reasonably high. Thus pharmaceutical companies could put their compound collection to new uses by seeing if the chemicals would bind to targets that would not have been predicted.31

This technique has not been unsuccessful. In the period 1991–2008, of 58 drugs for which the starting compound was known, 19 owe their existence to an HTS campaign (e.g. Figure 1.3).30,36
1.2 Established Methods of Drug Discovery

![Diagram of molecules 1 and 2]

**Figure 1.3** Example of a hit from HTS, UK-107,543, leading to a marketed drug, Maraviroc, used in the treatment of HIV. The red scaffold in Maraviroc shows the features maintained from the initial HTS hit.\(^{37}\)

However, HTS has faced criticism for its failure to produce more drugs from such an unprecedented number of experiments.\(^{30,31,38,39}\) This has been put down to weakness in the two most important factors of an HTS experiment: the nature of the compound library and the quality of the biological assays used.

Macarron *et al.* attributed the problems with the biological aspect of HTS to ‘poorly validated targets,… highly artificial and non-physiological assay systems,…a severe lack of appropriate animal models, [and] unpredictable toxicities.’\(^{30}\) From a chemistry perspective, libraries consisted mostly of large, impure compounds.\(^{40}\) Even when the emphasis changed to single, pure-compound screening libraries, a distinct lack chemical diversity hampered the degree of chemical space which was being probed.\(^{30,31}\)

One of the biggest wake-up calls for the effectiveness of HTS was the GlaxoSmithKline 7-year antibiotic discovery programme. In the face of the growing threat of antibiotic resistance,\(^{41}\) SmithKline Beecham (SB) and subsequently GlaxoSmithKline (GSK) embarked on a program to test their entire compound collection for novel antibiotic activity. In total, 70 HTS were carried out using over 500,000 compounds. Only 16 hits were identified and of these only 5 became leads. Synthetic modification of these leads failed to generate any compounds to meet the desired criteria. This was incredibly disappointing considering the time and money (>$70 million) spent on the project. Analysis of the program demonstrated that the lack of chemical diversity in the compound collection inhibited the power of the screen, with many of the molecules within the same or similar areas of chemical space.

GSK were not alone in their failure to detect new antimicrobials from HTS. Between 1996 and 2004 more than 125 screens were carried out, none of which resulted in drug candidates.\(^{42}\) HTS, although a powerful tool in drug discovery, still relies on serendipity and one of the best
ways to increase the chances of success is to screen as diverse a range of compounds as possible. An industry focus on combinatorial synthesis is partly to blame in this regard.\textsuperscript{43}

### 1.2.2. Target-based drug discovery

Another prominent paradigm in drug discovery since the 90s has been target based drug discovery (TBDD).\textsuperscript{17,44,45} It was hypothesised that with sufficient information regarding a disease and its mode of action, databases could be searched or drugs could be rationally designed which block its mode of action and therefore treat the disease.\textsuperscript{17,46}

Although superficially very rational, several real-world limitations have shown this method to be less successful than first hoped. This treatment of biological systems as simple flowcharts may aid understanding in some respects, however much of the complex reality is missed. Ignoring these secondary effects at an early stage in the drug discovery process can lead to off-target activity further down the line and hence attrition.\textsuperscript{45}

Additionally, understanding of underlying biological systems is no guarantee of success or failure in drug discovery. Effective drugs have reached the market with little understanding about their mode of action (e.g. clozapine);\textsuperscript{17} for others, understanding has been gained during development (e.g. lyrica).\textsuperscript{47} Conversely, despite some understanding of their mode of action, many drugs have failed to reach or pass clinical trials (e.g. beta-amyloid targeting in the treatment for Alzheimer’s disease).\textsuperscript{17} Overemphasis on this bottom-up approach has not lead to a breakthrough in drug discovery nor a lowering in attrition.\textsuperscript{17,45}

### 1.3. Fragment-based drug discovery

#### 1.3.1. Chemical space

The main challenge facing medicinal chemists is the identification of chemical entities as potential hits and developing those compounds to form the best clinical candidates. This is a vast and non-trivial problem, a contributing factor of which is the extent of chemical space (the total possible number of molecules), which is so large as to be infinite for practical purposes.

Given that medicinal chemists focus on organic ‘drug-like’ molecules, the size of chemical space can be limited and therefore estimated. Most commonly, medicinally-relevant chemical space is restricted to thirty non-H atoms chosen from the most common elements found in organic chemistry (C, N, O, S) which puts the estimate at $10^{63}$ molecules.\textsuperscript{48}
It is not possible to synthesise even a meaningful percentage of this space to assess their pharmaceutical potential. Therefore, a strategy must be developed to effectively probe those areas of chemical space which are most likely to yield useful drugs.

Chemical space increases exponentially as the number of atoms in the molecule increases; therefore, decreasing the complexity of molecules reduces chemical space to a more manageable size. Computational research into the enumeration of smaller molecules (up to seventeen atoms of C, N, O, S, and halogens) has found the chemical space much more manageable at 166.4 billion molecules. Therefore, restricting synthesis and screening to smaller molecules allows a greater area of chemical space to be explored more efficiently. These small molecules could be regarded as ‘fragments’ of drugs which could be combined to form a potent lead ‘drug-like’ molecule.

1.3.2. What is fragment based drug discovery?

Fragment based drug discovery (FBDD) is a technique used to aid the discovery of potential drugs. It capitalises on restricting screening libraries to fragments (discussed in section 1.3.3) and screening these fragments against biological targets. The result of these screens is both physical measurements of how well the fragment inhibits the protein (usually expressed as the half maximal inhibitory concentration, IC$_{50}$ or dissociation constant, K$_d$) and crystal structures to assess the structure-activity relationship (SAR). From these results, fragments are modified, grown, and linked to produce a more drug-like molecule (Figure 1.4). These fragment modifications use the best available interactions with the protein as determined by SAR data, and the success of the modifications are measured by falling IC$_{50}$s.

*The term fragment-based lead discovery (FBLD) has also been used for the same process. This is because the output of this process is a lead compound for inhibiting a specific biological target. The final ‘drug’ being discovered after lengthy optimisation to a pre-clinical candidate, followed by clinical trials. However, because this technique is used primarily as an aid to the discovery of new drugs, the term FBDD is used in this thesis.
Figure 1.4 A cartoon representation of the fragment-based drug discovery process. a) A library of fragments is screened against a biological target or targets (usually a protein). b) Data is obtained from the screen of those compounds which bind. This includes physical measurement of the $K_d$ and $IC_{50}$, binding modes and structure-activity relationships. c) Fragments are linked, grown, and merged to create larger molecules which should have superior biological activity and improved substrate specificity. These are then screened against the target. d) A lead compound is found and can be further optimised or taken through to the next stages of the drug discovery process.

As summarised in Figure 1.4, FBDD follows a rational framework. Firstly, a fragment library is assembled or designed (this process will be discussed in more detail in section 1.3.3). This library, typically in the region of $10^2$–$10^3$ molecules, is then screened against a biological target. Unlike HTS, only a weak binding affinity c. 0.1–10 mM would be expected to be observed due to the small size of the fragments; however, they are found to produce better quality hits and fewer false-positive aggregations (organic molecules forming colloids in buffer solution which denature proteins and therefore causes non-specific inhibition). A variety of techniques can be employed to assess the nature and quality of any binding found during a screen: NMR spectroscopy and Mass Spectroscopy are quick qualitative methods for gaging whether there is binding between a protein and a fragment. Fluorescence Polarisation (FP) assays and Isothermal titration calorimetry (ITC) are employed to gain important quantitative data about how well a fragment binds (usually as an $IC_{50}$ or $K_d$ respectively). Finally, X-ray crystallography gives vital structural information about where on the target the fragment binds and what non-covalent interaction(s) it utilises.

Using the data gained in the screen, the most promising fragment hits are elaborated using structural data obtained from an X-ray structure. Fragment elaboration usually occurs by three methods: linking, growing, and merging. Linking is most often employed when two fragments have bound to a target at two separate sites. These fragments are then linked by a tether to produce a lead-like molecule with enhanced physico-chemical properties. Although this technique has been employed successfully in the past, it is the most difficult strategy
within fragment elaboration due to the difficulty in optimising the length and rigidity of the tether and the limited scope for further elaboration.\textsuperscript{53,63}

Fragment merging is most readily employed where two or more fragments have been found to occupy the same or proximal space, but structural analysis has found they interrogate different aspects of the target. In this case, structural features of the fragments are merged to give a new lead-like molecule which hopefully utilises all of the previous interactions.\textsuperscript{64} This has been employed successfully in several cases of drug discovery.\textsuperscript{65–67}

Finally, fragment growing is used to produce leads. This is the most common strategy and is used when only one fragment is found to have bound to a target. Using the crystal structure as a guide the fragment is grown along several vectors to explore additional interactions with the target. This gives rise to an iterative cycle where a number of compounds are screened and the resulting crystal structures used to direct further development and elaboration (Figure 1.5).\textsuperscript{53,68,69}

\textbf{Figure 1.5} Diagram visualising the three strategies of fragment elaboration in hit-to-lead optimisation in fragment-based drug discovery.

The first example of a systematic fragment-based approach to protein inhibition was reported in 1996 by Shuker \textit{et al.} They had discovered a method of determining the structure-activity relationship (SAR) between a ligand and a protein using NMR. A low molecular weight library was screened against a protein, FKBPI, to establish which molecules, if any, would bind.
These molecules were then modified to increase their binding affinity. Finally, they were linked to give a molecule which bound to FKBP with a $K_d$ of 19 nM (Figure 1.6).62

**Figure 1.6** Summary of Shuker’s work. A fragment library was screened against protein FKBP. Two sites of the protein were found where molecules bound. The molecules with the lowest dissociation constant ($K_d$) for each site were linked at various points and using a variety of tethers. This resulted in the discovery of a new compound which bound to FKBP with a $K_d$ of 19 nM.

FBDD aids drug discovery in a number of ways. Firstly, it allows the thermodynamics of the ligand-protein interaction to be broken down in several steps. The free energy of a ligand-protein interaction is determined by the Gibbs free energy equation (1.1):

$$
\Delta G = \Delta H - T \Delta S
$$

(1.1)

The dissociation constant ($K_d$) is related to the Gibbs free energy by the isotherm equation (1.2):

$$
\Delta G = RT \ln (K_d)
$$

(1.2)

Thus, a small $K_d$ and hence large negative $\Delta G$, will be determined by how exothermically the ligand binds to the protein (large negative $\Delta H$) and how much entropy is lost upon binding (small positive $\Delta S$).

Applying these principles to FBDD shows how growing, linking, and merging fragments can yield drug candidates with low $K_d$s.50
When two fragments, A and B, bind to a protein, their $K_d$s are determined independently of one another and are a function of their respective $\Delta H$ and $\Delta S$ (Figure 1.7). However, when A and B are combined the enthalpy of their binding will be approximately equal to the sum of the enthalpy of the binding of A and B respectively, as they are both utilising the same interactions to bind to the protein (1.3).

\[
\Delta H_{AB} \approx \Delta H_A + \Delta H_B
\]  

(1.3)

Yet, the entropy penalty of AB is much less than the entropy penalty of A and B binding separately. Although there will be a greater penalty for the more complex molecule in the form of restriction on rotating and bending modes, these pale in significance compared to the reduction in entropy required for two separate entities to leave solution and bind with a protein. Thus:

\[
\Delta S_{AB} \approx \Delta S_A \approx \Delta S_B \ll \Delta S_A + \Delta S_B
\]  

(1.4)

Therefore, when combining fragments to make a large drug-like molecule, the favourable enthalpic terms combine but the unfavourable entropic terms stay approximately the same. This is extremely powerful because, as detailed in equation 1.2, $\Delta G$ and $K_d$ are related exponentially. Every time $\Delta G$ doubles, $K_d$ is squared. This gives FBDD an advantage over HTS of large molecules which require nanomolar binding to be considered good enough for a lead, whereas fragments only need to exhibit a weaker millimolar binding as fragment development can yield high binding drugs.

Secondly, FBDD rigorously employs X-ray crystallography as well as several other techniques to ensure that the structure-activity relationships (SARs) between fragments and the target are understood. This gives FBDD fragment elaboration a rational aspect to the design of leads molecules, which can give greater success in the clinic.
Thirdly, fragments are less ‘complex’ than larger molecules. Complexity is desirable in drug candidates and medicines as it conveys selectivity to the target in question and minimises off-target activity which is likely to lead to increased toxicity. However, to probe biologically active chemical space it is best to have simple molecules which probe only certain interactions; an overly complex molecule is more likely to possess groups which inhibit binding (Figure 1.8). Therefore, it is possible that fragments are more promiscuous than lead-like molecules. However, the process of fragment elaboration should increase selectivity.

![Figure 1.8 A complex molecule versus simple fragments binding on a protein. The complex molecule, although it contains groups which would interact with the target, is prohibited from doing so because of its complexity. The fragments, containing only one or two functional groups are perfectly suited to probe the target’s biologic space. (Image taken from ref 53)](image)

This proposition of less complexity leading to more biological hits has been validated statistically. Hann et al. showed that the probability of a molecule matching a target decreases exponentially as complexity increases (Figure 1.9).
Figure 1.9 A graph showing the probability of a ligand matching a biological target compared with its complexity. The complexity was measured computationally from the length and pattern of the molecule’s features. (Image adapted from ref 70)

1.3.3. Identifying fragments

Limiting chemical space to fragments can only become a reality when there is an appropriate definition to direct synthetic research. Lipinski’s “Rule of Five” (RO5) (see section 1.1.2) was already well-established for highlighting the typical properties of orally bioavailable drug candidates when FBDD was first established. Since then, a number of studies have resulted in the development of similar “rules” to apply to ideal fragments.

Inspired by the RO5, Congreve et al. analysed the fragments used in FBDD and discovered that a ‘Rule of Three’ (RO3) might be a useful tool in guiding the construction of fragment libraries. Based on the criteria used by the RO5, they suggested that:

- Molecular weight is <300, the number of hydrogen bond donors is ≤3, the number of hydrogen bond acceptors is ≤3 and ClogP is ≤3. In addition, the results suggested NROT [number of rotatable bonds] (≤3) and PSA (≤60) might also be useful criteria for fragment selection.\(^{71}\)

More recently, pharmaceutical company Astex published its internal guide for fragment synthesis. These have further restrictions compared to the RO3 and focus on other details regarding the property of fragments, including spatial and synthetic properties which are an important addition to the field of fragment synthesis.\(^{72}\) Astex’s rules are compared to the RO5 and RO3 in Table 1.2:
Table 1.2 Comparison of the RO5 with the RO3 and Astex’s in-house fragment rules.

<table>
<thead>
<tr>
<th>Property</th>
<th>Rule of Five\textsuperscript{19}</th>
<th>Rule of Three\textsuperscript{71}</th>
<th>Astex’s Rules\textsuperscript{72}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (gmol\textsuperscript{-1})</td>
<td>&lt; 500</td>
<td>&lt; 300</td>
<td>140–230</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 5</td>
<td>≤ 3</td>
<td>0.0–2.0</td>
</tr>
<tr>
<td>Polar Surface Area (Å\textsuperscript{2})</td>
<td>≤ 140</td>
<td>≤ 60</td>
<td>–</td>
</tr>
<tr>
<td>H-Bond Acceptors</td>
<td>≤ 10</td>
<td>≤ 3</td>
<td>–</td>
</tr>
<tr>
<td>H-Bond Donors</td>
<td>≤ 5</td>
<td>≤ 3</td>
<td>–</td>
</tr>
<tr>
<td>Rotatable Bond Count</td>
<td>≤ 10</td>
<td>≤ 3</td>
<td>0–3</td>
</tr>
</tbody>
</table>

Astex also highlight additional properties which would be beneficial for fragments:\textsuperscript{72}

- Diverse polar groups for protein binding
- Multiple elaboration vectors for 3D growth
- Aqueous solubility, stability, and solution and avoidance of highly reactive groups or groups which cause aggregation
- Easy to synthesise 50–100 mg in ≤ 4 steps from commercial starting materials
- A variety of 3D scaffolds
- 0–2 chiral centres

All these suggestions should be taken as good rules of thumb to aid in the design and functionality of fragment libraries.

1.3.4. Success of FBDD

Clearly the FBDD process has some notable advantages over previous drug discovery paradigms including HTS and TBDD.

More efficient sampling of chemical space: both HTS and FBDD rely on serendipity to discover novel chemical leads. However, the chances of this happening are slim unless the HTS screening libraries sample an appropriately high volume of chemical space. Given HTS chemical space, this is likely to be in the order of $10^{63}$ molecules. Sampling a millionth of this space would require the synthesis and screening of $10^{57}$ molecules; an impossible task. However, fragment space is considerably smaller at around $10^{11}$ molecules. A millionth of this space is only 100,000 molecules; a very achievable target. In addition, advances in computational technology mean that \textit{in silico} screening can be readily employed to aid in the synthesis of fragments. TBDD does not rely on serendipity to find leads. However, over-
reliance on focusing on one biological system could see molecules which may inhibit other systems not being utilised to their full advantage.

*Greater chances of a compound binding:* Another advantage which the use of fragments gives FBDD is the molecules are less complex with fewer skeletal appendages. This allows functional groups to more efficiently probe biological space, with a lower chance of steric or electron clash related to more complex molecules.

*Hit-to-lead optimisation improved:* Fragments would ideally consist of multiple vectors of elaboration. This means that chemists could easily modify fragments with a hit in most spatial directions, hopefully allowing for almost any hit to be improved. The late stage nature of HTS libraries mean the number of vectors is less and elaboration would be a more laborious task.

FBDD has been successful over the past twenty years of drug discovery. Erlanson *et al.* identified 32 compounds in clinical trials which are known to have been identified from FBDD. Figure 1.10 shows two marketed drugs derived from FBDD. Due to this success, this thesis focuses on the synthesis of fragments for potential use in drug discovery.

![6, Vemurafenib](image6.png) ![7, Venetoclax](image7.png)

*Figure 1.10* Vemurafenib and Venetoclax with the initial fragment hits highlighted.
2.1. Introduction

2.1.1. FBDD in targeting PPIs

Biological interactions are largely governed by the interactions between proteins, called protein-protein interactions (PPIs). They modulate many key pathways in health and disease with an estimated 400,000 biological processes regulated by PPIs. Thus, they are an important target in drug discovery. However, the often large and shallow nature of the PPI pockets has given them the reputation of being ‘undruggable’. Recently, work has begun to overcome this challenge using small molecule approaches to drug discovery which has led to PPI being examined as a potential target for discovery programmes.

PPI inhibitors can be broadly sub-divided into two categories: orthosteric inhibitors, which block the interaction site between the two proteins, and allosteric inhibitors, which bind to a different site on the protein but in doing so change the shape of the protein binding site, thus inhibiting the PPI (Figure 2.1).
Figure 2.1 Diagram showing the mode of action for ortho- and allosteric inhibitors of PPIs. (Image take from ref 75).

Whilst traditional screening methods with larger molecules often fail to sufficiently bind these shallow pockets, the smaller nature of fragments allows them to probe these sites. This provides a way of identifying initial hits to kickstart a PPI inhibitor discovery process. Indeed, FBDD has been successful in discovering a new anticancer drug: Navitoclax was discovered from a FBDD campaign and offers potent treatment of small-cell lung cancer and chronic lymphocytic leukæmia.\textsuperscript{80} Two fragments, a biaryl and a tetrahydronaphthalene, were discovered that bound in the PPI site and a campaign of linking and growing produced Navitoclax,\textsuperscript{80,81} which entered the clinic in 2009 and was licenced in 2017.\textsuperscript{82,83} Due to the highly specific nature of PPI sites, this treatment lacked the off-target effects of the previous treatment, Obatoclax, and reduced negative side-effects (Figure 2.2).\textsuperscript{84}
2.1 Introduction

Figure 2.2 The initial fragment hits which led to the discovery of Navitoclax.

With the role FBDD can play in the discovery of PPI inhibitors in mind, the rest of this section will focus on a specific PPI interaction and how its inhibition could produce potent anti-cancer therapies.

2.1.2. Casein Kinase 2

Protein kinases were discovered serendipitously in 1954 when Kennedy and Burnett reported an enzyme catalysing the transfer of a phosphate from ATP to a protein. Although the physiological significance of the discovery was not initially realised, the following six decades brought considerable knowledge and understanding to the field of protein kinases.

Since then, discoveries have demonstrated the importance of Casein Kinase 2 (CK2) in human biology. Recent research on a database of almost 11,000 naturally-occurring phosphorylated sites revealed that the unique acidic marker that identifies CK2 has been found on 2275 (>20%) of them.

The pivotal role CK2 plays in controlling many other kinases and cell death has led to some calling CK2 the ‘master kinase’. Indeed, the vast number of targets of CK2 signifies that almost all cellular activity is regulated in some way by CK2. The most notable of these include: stress stimulus mediation, gene expression regulation, protein synthesis and degradation, regulation of the circadian rhythm, viral infection, and counteracting apoptosis.

2.1.3. Structure and activity of CK2

CK2 is a tetramer consisting of two of CK2α and CK2α’ subunits and two CK2β subunits. Thorough crystallographic and structural analysis has been carried out on CK2. This has
shown that the α subunit is the catalytic subunit, the β subunit is the regulator subunit, and these form the tetrameric holoenzyme of α₂β₂, αα’β₂, or α’₂β₂ (Figure 2.3).

![Figure 2.3 CK2 protein with the structure α₂β₂ (PDB: 1JWH, Image from ref 88).](image)

The three heterotetrameric forms of CK2 have displayed no differences in activity from one another. Analysis of the CK2α and CK2α’ has shown that CK2α’ lacks the C-terminal amino acids 330–391. In addition, comparison of the 1–329 sequence of CK2α and CK2α’ has shown an 86% homology. However, they exhibit the same activity.⁸⁹

CK2 is involved in many key cellular processes. Yet, there are two signalling pathways of particular interest especially if focus is concentrated on CK2 tumorogenisis. These are the Wnt pathway and the NF-B pathway, two signal transduction pathways that regulate important cell development events and play a role in some cancers.⁹⁰

The CK2α subunit is always in the active conformation and possesses the key ATP binding site which is responsible for the biological activity. The CK2β subunit is a regulatory subunit. The activity of this subunit is dependent on the substrate. CK2β is also found to increase the proteolytic and thermodynamic stability of CK2α as well as providing sites for substrates to dock.⁹¹

2.1.4. CK2 and Cancer

As a protein kinase, CK2 phosphorylates a great many substrates involved in cell growth.⁸⁹ Being a ubiquitous protein kinase, CK2 is overexpressed in a large range of cancers.⁹² Importantly, it is not mutations of CK2 which are carcinogenic, but unnaturally high quantities of CK2 and its associated activity have been observed in many tumours. In
comparison with normal cells, which sees CK2 diffused in the various cellular compartments, tumours have high levels of CK2 around the nucleus and in neoplasia and CK2 is elevated beyond what is expected for normal cells.\textsuperscript{93} CK2, as well as being involved in cell proliferation and growth, also suppresses apoptosis, linking CK2 to the cancer phenotype.\textsuperscript{94}

CK2 is up-regulated during cell proliferation however it readily returns to normal after proliferation has finished. Yet in cancerous cells it finds homoeostasis at this level and remains high.\textsuperscript{95} In addition, studies on prostate cancer—forms which are both androgen sensitive and androgen independent—showed CK2 to be functional irrespective of the growth factor.\textsuperscript{96}

Having identified CK2 as an important target, is it a viable target for cancer therapy? One reason why CK2 inhibition may be a particularly effective form of treatment is the pivotal and indispensable role it plays in the cell. If inhibited below the high critical threshold, the cancerous cell would no longer be malignant as there are no alternative signalling pathways, which could by-pass the need for CK2. Due to the role of CK2 in cell growth, proliferation, and apoptosis, its down regulation could be particularly catastrophic to cancer cells, as such down regulation of protein kinases has been researched on previous occasions.\textsuperscript{97}

2.1.5. CK2 Inhibition

Being a protein kinase, it is not surprising that initial research into inhibiting CK2 was directed at finding competitive inhibitors at the ATP binding site of the protein. One of the challenges this presented was selectivity issues when targeting the ATP pocket due to the fact that the pocket is highly conserved among kinases. There are in excess of 500 protein kinases in the human genome, with those in the same family sharing similar structural features. Thus, finding a compound which can selectively bind in the ATP site of CK2 without affecting other protein kinases would be challenging. Another issue with targeting an ATP site is that the inhibitor in question would have to compete with the high concentration (~1-10 mM) of ATP within the cell. However, this is less of an issue when the protein in question is in an inactive form.\textsuperscript{98}

This is not prohibitive, since there are some ATP inhibitors in clinical trials which are not completely selective.\textsuperscript{99} A study in 2008 involving 317 kinases (over half the human kinome) showed that of the 38 kinase inhibitors tested, none were selective within the range $K_d < 3 \mu M$. Even the most selective ATP inhibitors, GW-2580 (primary target CSF1R, not yet in clinical trial) and lapatinib (primary target EGFR and ERBB2, approved for HER breast cancer) exhibited binding to three different kinases (Figure 2.4).\textsuperscript{100} Therefore, when seeking an ATP
inhibitor for CK2, total selectivity may not be necessary (which may be impossible). Instead sufficient selectivity to avoid inhibiting other kinases and therefore avoid adverse side effects.

Figure 2.4 GW-2580 and Lapatinib, the most selective ATP inhibitors in the study by Karaman et al.

The first example of an ATP based CK2 inhibitor was found in 1986 when a compound called DRB (Figure 2.5) inhibited CK2 with an IC$_{50}$ of 15 μM.$^{101}$ However, it was also found four years later that this compound also inhibits other kinases with similar IC$_{50}$ values.$^{102}$

Figure 2.5 CK2 inhibitors, all Ki values are expressed in μM.

DRB also inspired a series of derivatives (Figure 2.5) to improve the potency of the original. To improve the hydrophobic interactions, all the positions on the benzene moiety were brominated and the appendant sugar was removed to form TBI. The triazole equivalent gave TBB and a diethyl amino substituted TBI produced DMAT. All of these were substantial improvements on DRB (DMAT being the most promising with a Ki of 0.040 μM compared to DRB’s 4.50 μM)$^{103}$
A range of natural products were also tested against this target. Emodin was found to be both unselective and lacking in potency. However, two unnatural analogues, MNX and MNA, where found to be an improvement on emodin.\textsuperscript{104} Flavonoids were found to bind better than DRB, yet they lacked selectivity.\textsuperscript{105}

The most progress made towards a drug which could inhibit CK2 through binding at the ATP site is a compound called CX4945 from Cylene Pharmaceuticals (Figure 2.6). This has been subjected to phase I clinical trials and was tested against BT-474 breast cancer cells and BxPC-3 pancreatic cells with overexpressed growth factors. It was found to suppress the PI3K/Akt signalling pathway in the cell.

When administered orally \textit{in vivo}, BT-474 cancer cells showed up to 97\% TGI, with just over 20\% showing 50\% reduction in tumour size. For BxPC-3 93\% TGI with about 30\% showing no tumour after treatment.\textsuperscript{106} However, due to CX4945’s activity on the ATP pocket, its activity against other protein kinases may lead to adverse side effects. Studies have shown CX4945 to be a promiscuous molecule, even targeting another protein, CLK2, more potently than CK2. Indeed it targets 13 other proteins with nanomolar activity, which could present a problem in its progress through the clinic.\textsuperscript{107}

Targeting the ATP site is not the only way to inhibit protein kinases. Research has also been directed to non-ATP competitive inhibitors which can overcome some of the disadvantages of the ATP inhibitors. Namely, they are more likely to be protein specific as there is more
variety in the shape of pockets outside the ATP site. Furthermore, because it does not have to compete with ATP for binding, they can be administered in concentrations closer to the $K_i$. The most commonly targeted non-ATP site in kinases is the substrate binding site.

Both small molecules and peptides have been used to bind this site. Additionally, compounds have been developed which bind to both the ATP site and the substrate site simultaneously. Unfortunately, peptide-based inhibitors come with their own set of problems: low potency, difficult uptake, and intracellular instability. However, recent developments in drug discovery are attempting to overcome these limitations. In addition, there may be other sites on the protein which can also be exploited for inhibition. As shall be seen, this is the case for CK2 at the interface of the protein-protein interaction (PPI) between the $\alpha$ and $\beta$ subunits.

Some progress has been made in finding an inhibitor of CK2 which does not act on the ATP binding site. When a crystal structure of DRB binding in CK2 was obtained, it was noted that a second molecule of DRB bound to the interface of the $\alpha$-$\beta$ subunits and that it has an inhibitory effect on CK2. However, when a peptide of the C-terminal 181-203 of CK2$\beta$ was exposed to CK2$\alpha$, it had a stimulatory effect on CK2$\alpha$ phosphorylation, yet this was less than CK2$\beta$ itself. Continued exploration of peptide-based inhibitors of CK2 found that a cyclic peptide of CK2$\beta$ 186-193 also bound in the PPI site, inhibiting the CK2 tetramer assembly. In addition to this, a molecule identified as W16—as part of wider research into podophyllotoxine indolo-analogues in CK2 PPI inhibition—was found to have an IC$_{50}$ of 20 $\mu$M (Figure 2.7).

![Figure 2.7 Compound W16.](image)

Recent research on a di(naphthalene) diazene core has identified a number of new compounds which exhibited inhibition of CK2. Of the 23 compounds based on this scaffold, five showed inhibition of CK2 (Figure 2.8). Of these compounds, compound 26 showed the lowest IC$_{50}$ of...
0.4 μM and analysis showed that it was inhibiting in both the ATP and PPI sites of CK2. It was further shown that against U373 cell line (cells from an aggressive form of brain tumour) compounds 24 and 26 both stopped the cell cycle and inhibited colony growth formation.\textsuperscript{115}

From all these examples, it seems that the field is developing for insights into inhibiting CK2; not just via the ATP pocket but also via allosteric inhibition of the PPI. Due to the higher specificity of a PPI inhibitor over an ATP inhibitor, the more the field of compounds is widened, the discovery of a molecule which could one day be used in a clinical setting becomes closer.

\section*{2.2. Outline of this work}

Capitalising on the fact that CK2 consists of a catalytic subunit, CK2α, and a regulatory subunit, CK2β, inhibition of the protein-protein interaction may lead to molecules which selectively inhibit CK2.\textsuperscript{116}

This is not a new strategy. Three molecules and a cyclic peptide have been identified as inhibiting CK2 at the α-β interface pocket.\textsuperscript{117} One of these molecules, DRB (13), a fragment, exhibited off-target activity by binding in the ATP pocket of CK2.\textsuperscript{111} The two other molecules, W16 (21) and a diazo compound (26), are large drug-like molecules with some undesirable physico-chemical properties, and lack crystal structure data to prove binding at the interface pocket (\textit{Figure 2.9}).\textsuperscript{68,114,115}
Fragment inhibitors of CK2

Figure 2.9 Structures of inhibitors of CK2α in the interface pocket.

A recent screen of a compound collection library produced by the Abell Group at the University of Cambridge found NMR154 (27), which bound in the interface pocket of CK2α (Figure 2.10).\(^{118}\)

Figure 2.10 The structure of NMR154 alongside the crystal structure of NMR154 bound at the interface pocket of CK2α (image taken from ref. 118).

NMR154 had two drawbacks. Firstly, it also bound in the ATP pocket of CK2α and secondly, it had a relatively high IC\(_{50}\) of 900 µM. The Abell Group made analogues of NMR154 to improve selectivity and inhibition. Of these, only one, NMR154L (28, Figure 2.11), showed an improved inhibition of 700 µM. However, it still bound in the ATP pocket.\(^{68}\)
Despite these failings, NMR154L has ideal features for it to be the basis of further fragment development. Firstly, its properties were well within the Rule of Three, meaning further fragment development was likely to exhibit good physico-chemical properties (Table 2.1). Secondly, NMR154L has plenty of vectors to grow and elaborate the fragment; off the aromatic ring or amine group, for example. Thirdly, the existence of a crystal structure of the molecule binding in the interface pocket allows for design of new inhibitors based on known interactions (especially that of the electrostatic interaction between the primary amine on NMR154 and Asp37, see Figure 2.10).

**Table 2.1** Table comparing the physico-chemical properties of NMR154 and NMR154L with the Rule of Three.  

<table>
<thead>
<tr>
<th>Property</th>
<th>Ideal Fragment Range (RO3)</th>
<th>NMR154</th>
<th>NMR154L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>&lt; 300</td>
<td>176.05</td>
<td>190.07</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 3</td>
<td>1.88</td>
<td>2.31</td>
</tr>
<tr>
<td>PSA</td>
<td>≤ 60</td>
<td>26.02</td>
<td>26.02</td>
</tr>
<tr>
<td>HBA</td>
<td>≤ 3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HBD</td>
<td>≤ 3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RBC</td>
<td>≤ 3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CC</td>
<td>0–1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

This chapter will explore developments of NMR154 analogues through elaboration of the aromatic ring and the generation of a new series of fragments to inhibit the interface pocket developed by computer modelling.
2.3. NMR154(L) analogues

2.3.1. Vectors of elaboration

Previous work has seen attempted optimisation of the methylene bridge and amino group on NMR154 to improve out-of-pocket binding interactions (Figure 2.12).

Unfortunately, varying the methylamine group only led to improved binding in one case, that of the ethyl amino group (discussed previously as NMR154L). The other functional groups evaluated: alcohol, amide, carboxylic acid, aldehyde, carbamate, and dihydroimidazole showed no improvement on NMR154(L) (Figure 2.12, red box).

Thus, the methylamine group was maintained (as it was synthetically easier to synthesize than the ethyl derivative and displayed interaction with Asp37) and additional groups were grown from it. A range of functionalities were explored with the view of picking up additional electrostatic interactions outside the pocket (Figure 2.12, blue box). Again, none of these showed improvement on NMR154L.

As a result, elaboration of the benzene core of NMR154 was next to be explored. The shape and structure of the α-β interaction pocket was investigated in silico using the computer program Maestro 10.2. As can be seen (Figure 2.13), the pocket is reasonably deep but narrow. The key to increasing the binding interactions into this pocket would be to utilise the two
aspartate residues at the top of the pocket. Suitable positioning of ammonium moieties could allow for hydrogen bonding. In addition, the breath of the pocket can also be explored.

Additionally, fragments could be synthesised elaborating at the ortho position of the benzene ring with regards to the ethylamino moiety on NMR154L (Figure 2.13).

![Figure 2.13](image-url) NMR154L with the elaboration vector next to the interface pocket showing the Asp residues.

The first elaboration tested was the addition of another ethyl amino group (fragment 29) to try and mimic the success of the original ethyl amino in binding to an Asp residue. Modelling predicted that this modification would show improved binding at the interface pocket. The Maestro 10.2 program assigns a ‘docking score’ to aid in the prediction of how well fragments will bind. The more negative the score, better binding is predicted. NMR154L was given a docking score of \(-6.1\) and the proposed fragment 29 was awarded a docking score of \(-7.0\), giving a good degree of confidence in its binding (Figure 2.14).

![Figure 2.14](image-url) Maestro 10.2’s prediction of how the proposed fragment (29) would bind at the interface site. This interaction was awarded a ‘docking score’ of \(-7.0\) compared to NMR154L’s \(-6.1\).
2.3.2. Synthesis of fragment 29

Due to the plane of symmetry in fragment 29 a straightforward synthesis could be proposed as all disconnections and functional group interconversions do not need to have as high a degree of regioselectivity. Looking at 29 it seemed easiest to begin from a starting material which had the dichloro functionality already present. All that would be required was an appropriate synthetic handle on the opposite side of the ring. Thus, the readily available 4,5-dichlorophthalic acid (33) was chosen as an ideal starting material.

The retrosynthetic plan firstly involved the primary amines being revealed from nitriles via reduction (Scheme 2.1). Nitriles may be added by $S_N_2$ displacement of an appropriate leaving group. This is obtained from the alcohol, itself revealed from a dicarboxylic acid.

![Scheme 2.1 Retrosynthetic analysis of 2,2'-(4,5-dichloro-1,2-phenylene)bis(ethan-1-amine) (29) from commercially available 4,5-dichlorophthalic acid (33).]

Despite having literature precedent,\textsuperscript{119} the product of the diacid reduction with borane in THF (Scheme 2.2) did not yield the desired product by NMR (only displaying one peak). One possible explanation of this could be that the anhydride was formed. Although borane should be strong enough to further reduce the anhydride, it could be possible that the reagent might have degraded. The reaction was repeated yielding the same results. The reduction was then attempted with lithium aluminium hydride (Scheme 2.2)—a much stronger reducing agent—which should have easily converted the carboxylic acid (33) to the alcohol (32). However, this also yielded the same, undesired, product by NMR. It would be extremely unlikely for the lithium aluminium hydride to stop reduction at the anhydride. As this strategy did not yield the desired diol, a different strategy was approached.
To overcome this issue, it was postulated that direct reduction of a methyl ester would not present such a problem. The diacid was methylated by treatment with thionyl chloride in methanol (Scheme 2.3). This readily produced the diester (34) in good yields which was submitted straight for reduction with lithium aluminium hydride. The pure diol (32) was afforded in good yield.

With this in hand, diol 32 was submitted for bromination under phosphorous tribromide conditions. This achieved the desired effect of producing the dibromide 31. An $S_n2$ reaction using sodium cyanide in refluxing ethanol transformed the dibromide into the dinitrile 30. Finally, all that was required was reduction of the nitriles with lithium aluminium hydride. Unfortunately, this did not produce the desired product 29. Examination of the literature showed that on a similar system (4-bromo-1,2-benzenediacetonitrile) access to the diamine was not achieved directly but via a Boc-protected intermediate. Thus, a nickel (II) and sodium borohydride mediated reduction was performed in the presence of Boc-anhydride. This successfully produced two mono-Boc protected amines 35. The product was carried
straight through to remove the Boc with 4M HCl/dioxane. This furnished 29 as the pure bis(HCl) salt (Scheme 2.3).

2.3.3. Biological testing of 29

Compound 29 was submitted to a fluorescence polarisation (FP) assay. These assays work through CK2α being soaked with a fluorescence labelled peptide which mimics the part of CK2β that binds to CK2α. If a compound binds at this interface the tumbling of the protein would be different, and this would affect the degree of polarisation of the light. In this way, the half- maximum inhibitory concentration (IC₅₀) of the compound could be measured.

The results of the experiment showed that compound 29 bound to the interface of CK2α with an IC₅₀ of 250 μM (cf NMR154L IC₅₀ of 700 μM). The crystal structure of CK2α with 29 bound at the interface was obtained to examine the nature of the binding and further inform design to improve the inhibition (Figure 2.15).

![Figure 2.15](image)

**Figure 2.15** Compound 29 in interface pocket showing two binding modes.

The crystal structure confirmed the model’s predictions about the nature of the binding in the pocket. Interestingly, one of the binding modes did show one of the ethylammonium moieties facing away from the aspartate residue.

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b All biological work was carried out by Dr Paul Brear, Department of Biochemistry, University of Cambridge.
2.4.1. Exploring the pocket width

With the improvement in binding from the ethylamine moiety in mind, it was explored whether growing the benzene core of NMR154L into a fused bicyclic core would fill more of the width of the pocket and thus aid binding by anchoring the fragment in the pocket. In this way, naphthalene-based fragment 39 was conceived (Figure 2.16).

![Figure 2.16 Growth of fragment NMR154L to incorporate a naphthalene core 39.](image-url)
2.4.3. Synthesis of 39

Synthesis of this naphthalene derivative started from commercially available 1-chloronaphthalene (40) which was subjected to a Friedel-Crafts acetylation. The electronics of the 1-chloro substituent directs the acetyl group \textit{para} to the chlorine (41). Subjecting this to the iodoform reaction converted the acetyl group into a carboxylic acid (42). Reduction then gave alcohol 43, followed by bromination which afforded a bromomethyl species, 44. This was ripe for \( S_{N}2 \) displacement with cyanide to yield nitrile 45. Reduction gave 39 as the HCl salt (Scheme 2.5).

![Scheme 2.5 Synthesis of naphthalene derivative 39 from commercially available starting material 1-chloronaphthalene 40.](image)

2.4.4. Testing of 39

Naphthalene derivative 39 was submitted to the same fluorescence polarisation assay as previous fragments. This showed an inhibition of >500 \( \mu \text{M} \), not showing an improvement.

A crystal structure was also obtained of 39 binding in the interface pocket. This was interesting as it displayed a binding mode than had not been expected (Figure 2.17).

Looking at the crystal structure of NMR154L, it was predicted that the naphthalene core would straddle the length of the pocket with the chlorine protruding into the depth of the pocket and the ethylamine making electrostatic interactions with Asp37.
However, compound 39 has turned on its side to bind into the pocket. The unsubstituted ring of the naphthalene core now explores the depth of the pocket. This leaves the ethylamine to bind with Asp37, as predicted, and the chlorine pointing into the length of the pocket (Figure 2.17). This suggests that the pocket may be able to accommodate a larger molecule into its depths as well as its length.

![Figure 2.17 Crystal structure of compound 39 in the interface pocket of CK2α.](image)

### 2.5. Expanding bis(ethylamino) fragments

#### 2.5.1. Synthesis of precursors

With the success of adding a second ethyl amino group to NMR154L, it was investigated whether adding additional alkylamine groups to other fragments, such as naphthalene 39, would also lead to improved binding.

This presented a challenge due to the lack of an appropriate synthetic handle on the naphthalene scaffold. A C-H activation paper by Giri and Yu offered a way of selectively installing a carboxylic acid group ortho to a pre-existing carboxylic acid using Pd catalysis and a CO atmosphere (Scheme 2.6).[^123]
Scheme 2.6 Summary of Giri and Yu’s work to install a carboxylic acid group ortho to a pre-existing carboxylic acid.

The advantage of using this work is that with the two carboxylic acids installed, the same chemistry to synthesise 29 can be followed.

Additionally, it was decided to pursue this methodology in tandem with further elaborations at the ortho position. If an acetylene moiety could be installed there, then its pluripotent functionality could be further transformed into a number of different groups.

It was hoped that the acetylene functionality could be easily installed through a Sonogashira cross coupling. The synthesis began with commercially available 2-bromo-4-chlorobenzoic acid (48) which was readily esterified to give methyl ester 49 for the cross-coupling. The acetylene was installed after experimenting with a few conditions. The conditions previously used by the authors did not afford the desired product (Table 2.2, Entry 1). When the reaction was repeated under reflux and double concentration, the reaction proceeded with only a 5% yield (Table 2.2, Entry 2). A literature procedure from Thorand and Krause was then tried. These conditions used similar reagents to the previous one, however less base and CuI were employed, five-times the amount of Pd(PPh3)2Cl2 used, a catalytic amount of PPh3 added, and it was performed at a slightly higher concentration. This unfortunately only returned starting material (Table 2.2, Entry 3). However, repeating the reaction under reflux gave the desired product, 50, in good yield (Table 2.2, Entry 4).
Table 2.2 Screen of conditions to install TMS acetylene on benzene core.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NEt₃ (8 eq), CuI (10 mol%), Pd(PPh₃)₂Cl₂ (1 mol%), THF (0.1M), 130 °C¹²⁴</td>
<td>SM Returned</td>
</tr>
<tr>
<td>2</td>
<td>NEt₃ (8 eq), CuI (10 mol%), Pd(PPh₃)₂Cl₂ (1 mol%), THF (0.2M), reflux¹²⁴</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>NEt₃ (1.5 eq), CuI (1.2 mol%), Pd(PPh₃)₂Cl₂ (5 mol%), PPh₃ (2.5 mol%), THF (0.25M), RT¹²⁵</td>
<td>SM Returned</td>
</tr>
<tr>
<td>4</td>
<td>NEt₃ (1.5 eq), CuI (1.2 mol%), Pd(PPh₃)₂Cl₂ (5 mol%), PPh₃ (2.5 mol%), THF (0.25M), reflux¹²⁵</td>
<td>79%</td>
</tr>
</tbody>
</table>

Ester hydrolysis and TMS deprotection were then carried out simultaneously to yield the desired 4-chloro-2-ethynylbenzoic acid (51, Scheme 2.7).

Scheme 2.7 Simultaneous ester hydrolysis and TMS deprotection to yield acid 51.

With both 42 (chloronaphtholic acid precursor of 39 in Scheme 2.5) and 51 in hand, these were subjected to Giri and Yu’s ortho carboxylation conditions (Table 2.3).
Table 2.3 Table summarising attempted ortho carboxylation of 51 and 42.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(OAc)$_2$ (10 mol%), Ag$_2$CO$_3$ (2 eq), NaOAc (2 eq), CO (1 atm), 1,4-dioxane, 130 °C, 18h</td>
<td>SM Returned</td>
</tr>
<tr>
<td>2</td>
<td>Pd(OAc)$_2$ (10 mol%), Ag$_2$CO$_3$ (2 eq), NaOAc (2 eq), CO (1 atm), 1,4-dioxane, 130 °C, 18h, sealed tube</td>
<td>Complex mixture</td>
</tr>
</tbody>
</table>

Despite several attempts to affect the conversion using the conditions outlined above, no pure product was isolated. Thinking this could be down to the difficulties with isolating vicinal diacids, the crude reaction mixture from the sealed tube experiment was submitted straight to methylation conditions in an attempt to form the di(methylester) which could be easier to separate. Unfortunately, the desired product was not isolated from the complex mixture that was returned (Scheme 2.8).

Scheme 2.8 Attempted isolation of diacid via diester.

2.5.2. Docking Studies

To assess qualitatively whether biaryl analogues of NMR154L would be good leads, a brief docking study was conducted with the structure of CK2. In this study, CK2β and residual water molecules were removed leaving just the CK2α subunit before energy minimisation was carried out.

From this result, a docking grid was placed over the interface site of the protein showing the area in which docking should take place. Into this model a variety of analogues of NMR154L and 39 were entered. These were transformed into 3D structures and their energy minimised.
With both the protein and compounds in hand, an SP docking glide was carried out and the program was asked to return the top 80% of hits. Each compound was returned with a ‘docking score’ shown in Figure 2.18. While these numbers holds little value in themselves, they can be used qualitatively against compounds known to dock to assess whether they may be worth making. As can be seen, all but compound 39 returned numbers similar to that of NMR154L.

Interestingly, compound 39 was predicted not to bind by the computer. The reason in this case for the prediction of 39 not binding could be because the model predicted all the bicycles occupying the width of the pocket and did not explore its depth. In this way, the amine on 39 would not be able to bind with Asp37. The model perhaps underestimates the protein with regards to pocket depth or its flexibility to accommodate deeper ligands (Figure 2.19).
The bicycles with two ethyl amino groups proposed present more of a synthetic challenge than 29. This is down to several factors: first of all, those compounds lack the symmetry seen in 29, thus requiring a more regioselective synthesis. Secondly, due to the unusual substitution pattern of these bicycles, finding appropriate starting materials would be difficult. Thirdly, for the sake of atom economy and efficiency, a synthesis that could feature a late stage divergence to make all the structures would be desirable. However, a shorter synthesis of one compound could be achieved to test the validity of the model before embarking on a larger synthetic effort.

For the divergent synthesis, a cross-coupling reaction was the easiest to effectively introduce pyridine (for fragments 57 and 58) and cyclopentene (for fragments 59 and 60) into the scaffold. In this way, if a common disconnection could be found between all the structures, a common synthesis may be proposed. Work by Mamane et al. showed that similar aromatic systems could be synthesised by a indium (III) mediated cyclisation. The reaction also has the benefit of positioning the chlorine at the desired position (Scheme 2.9). This worked by the indium coordinating to the alkyne, activating it for 6-endo-dig attack of the adjacent aromatic ring.
Expanding bis(ethylamino) fragments

Scheme 2.9 Example of work from Mamane. Chloroacetylene 61 is transformed into phenanthrene scaffold 62.\textsuperscript{126}

For our system, this would require synthesising an enyne with a pendant phenyl ring and groups which could be used later to produce the ethylamine functionality. There are established literature procedures for the synthesis of a chloroalkyne moiety from an aldehyde, and an $\alpha,\beta$-unsaturated aldehyde from the corresponding unsaturated TBS protected alcohol.\textsuperscript{126}

This leaves an obvious sp$^2$–sp$^2$ disconnection between the alkene and phenyl. Not only is this easily achieved through a Suzuki reaction, but it allows other rings to be added at this stage, including pyridine and furan, which form the basis for the other bicyclic systems that are to be investigated.

This leaves the key intermediate; an alkene with a bromine, methyl TBS-protected alcohol, and two groups which can be later functionalised into ethylamines (Scheme 2.10).

Scheme 2.10 Retrosynthesis of 66 to common intermediate.
The main challenge in synthesising this alkene is selectivity in the bond geometry. However, research from Wei-Jun et al. may provide an answer.\textsuperscript{127} They showed that a cyclopropyl group at one end of an alkene may be selectively opened to install both a bromine and a tosyl-protected ethylamine in a single step (Scheme 2.11). In terms of selectivity, it was found that the bromine preferentially adds to the same side as the bulkiest substituent. The methyl TBS-protected alcohol is bulkier than a nitrile and so this should give the desired selectivity.

\begin{itemize}
  \item This leaves a rather facile alkene to synthesise. The cyclopropane could be added \textit{via} a Wittig reaction. Finally, this gives a ketone for which there is literature precedence that it can be made from a molybdenum (0) catalysed opening of an isoxazole. The total forward synthesis is summarised in Scheme 2.12.
\end{itemize}
Forays were made into this synthesis to test its viability (Scheme 2.13). TBS protection of propargylic alcohol 71 is a standard procedure and proceeded without issues. 2-Oxoacetic acid (69) condensation with hydroxylamine followed by dropwise addition of elemental bromine, which led to the formation of dibromoformaldoxime, 70. In the presence of base, the formaldoxime performed a 1,3-dipolar cycloaddition with the protected of propargylic alcohol to yield the bromoisoazole, 73. Despite one example in the literature of this ring being opened with molybdenum (0), this methodology did not produce the desired result instead yielding a complex and inseparable mixture.\(^{128}\)

At this point, another strand of research in the Spring Group discovered CAM187. This was derived from NMR154 and represents the best fragment inhibitor of the CK2 interface pocket with an IC\(_{50}\) of 44 \(\mu\)M (see Appendix D for the publication regarding this discovery).\(^{68}\)
2.6. Spirocyclic fragment series

2.6.1. Computer modelling

Work in the group had also been directed towards developing a new series of fragments based upon computer models of the interface pocket of CK2. To direct the synthesis of compounds likely to bind at this site, another member of the Spring Group, Dr Qingzhong Hu, created an in silico pharmacophore model.

Hu conducted the study of the pharmacophore in Molecular Operating Environment (MOE) 2014 version. In this model, the redundant protein copies in the crystal structure of CK2 holoenzyme comprising CK2α and CK2β were removed. The CK2β subunit and water molecules were also removed. After the Amber10 force field was designated to the structure, hydrogens and partial charges were added via the Protonate3D module in MOE. A pharmacophore model was subsequently established via the structure-based approach. Employing this prepared structure followed the standard protocol with default parameters being adapted, which was followed by empirical manual modifications on the locations and confines of some pharmacophores. The virtual screening of the ZINC database\textsuperscript{129} employing this pharmacophore model was performed using MOE with pharmacophores F1 and F2 being set as essential, and a spirocyclic compound \textit{85} was identified as a hit. To further verify this hit, Hu docked compound \textit{85} back into the prepared CK2α crystal structure with GOLD after being built and energy minimized in the Amber10 force field with MOE. The function of

\textbf{Scheme 2.13} Steps towards the synthesis of \textit{74}.
automatic active site detection was switched on although amino acids composing the interface
were also manually designated as the binding site, the radius of the active site was set to 19 Å
and β4β5-loop was set to be flexible. The ligand was docked in 50 independent genetic
algorithm (GA) iterations for each of the three GOLD-docking runs. Moreover, the GOLD
score parameters were exploited, and the GA default parameters were set. The results were
subsequently ranked according to fitness and compound 85 achieve a fitness score of 38.85. It
was further evaluated with the LigX module in MOE and illustrated in contrast to the
previously-identified fragment NMR154L as shown in Figure 2.20.

![Figure 2.20](image)

**Figure 2.20** Compound 85 (in magenta) in α-β PPI pocket with previously known hit NMR154L (in cyan).

Based on the pharmacophore model producing a hit for spirocyclic compound 85, a further
library of spirocyclic compounds and fused ring compounds were proposed by Hu to see if
these compounds validated the model. Through this, an inhibitor for CK2 was found (Figure
2.21).
Figure 2.21 Products of the pharmacophore model.

2.6.2. Synthesis of compound 85

Synthesis of spirocycle 85 followed the work of Zhu. Bis(ethylchloro)ammonium chloride, 91, was initially Boc protected (Scheme 2.14) to give 92 which could be used in the synthesis of three spirocyclic compounds in the series.

Initially, 2-(2-bromophenyl)acetonitrile, 93, was reacted with sodium hydride. When compound 92 was added to the reaction this was expected to perform two S$_{N}$2 reactions at the methylene position forming a piperidine ring. Several attempts failed to yield the desired product, 94 (Scheme 2.14), returning the starting materials.

Scheme 2.14 Attempted reaction using a bromophenyl sequence.

The above procedure was repeated with the fluoro analogue, 95. Again, base was added followed by bis(chloroethyl)amine 92 (Scheme 2.15). This formed a Boc-protected piperidine ring substituted at the 4-position with a nitrile and an ortho-fluorophenyl, 96, which was deprotected to give 97. In the presence of lithium aluminium hydride, the nitrile was reduced.
to an amide anion, which performed an internal $S_N$Ar at the fluorinated position, leading in one step to the formation of the indoline system and the spirocyclic centre, 85.

![Scheme 2.15 Synthesis of compound 85.](image)

2.6.3. Synthesis of indane spirocycle 86

The synthetic techniques of 85 could be employed for the synthesis of 86. A benzyl protecting group was used on the bis(ethylchloro) amine as it was thought this could be removed in the same step as the hydrogenation of the double bond of the indene. The bis(ethylchloro)amine 91 was protected with a benzyl group to give 98 (Scheme 2.16). This was reacted with indene (99) using LHMDS in THF. The resulting spirocycle 100 was subjected to hydrogenating conditions of palladium on carbon in a hydrogen atmosphere. The alkene in the indene ring system was successfully reduced, however the benzyl remained, giving compound 101.

![Scheme 2.16 Synthesis of benzyl protected spirocycle 101.](image)

Although desirable to simultaneously remove the protecting group and reduce the alkene, Boc-removal is a reliable reaction and this extra step could easily be used to reveal the desired product. Therefore 92 was reacted with indene in the presence of LHMDS to produce the Boc-
protected piperidine-indene spirocycle 102 (Scheme 2.17). Boc deprotection, giving 103, followed in a facile and high yielding manner. Finally, 103 was hydrogenated using palladium on carbon to give 86 as the HCl salt.\textsuperscript{131}

![Scheme 2.17 Synthesis of 86.HCl.]

2.6.4. Synthesis toward spirocyclic amide 87

Compound 87 has the spirocentre α to an amide. It was thought that this position could be sufficiently acidic to broadly follow the same procedure described above using two equivalents of base with 92. To test this theory dihydroquinolone, 104, was Boc protected, giving 105, prior to being reacted with 92 and LHMDS. This reaction only returned starting material. Considering LHMDS may not be a strong enough base, the reaction was repeated with LDA made \textit{in situ}. A product was isolated from this reaction. However, it was the dihydroquinolone dimer linked by a diethylamine moiety, 106. It seems the tertiary centre was either too sterically hindered to be deprotonated or to perform the internal S\textsubscript{N}2 (Scheme 2.18).
2.6.5. Initial Biological Results

Compounds 85 and 86 were submitted to a fluorescence polarisation assay. Unfortunately, neither compounds 85 nor 86 exhibited any inhibition of CK2α.

With these results in mind, work done by Dr Hu was re-evaluated. It was noted that the known hit NMR154L had a chlorine moiety protruding into the pocket. It was postulated that
this would increase the hydrophobic interactions and therefore aid in binding. Thus, a chlorine analogue of spiroindane 86 was designed (because this core was the most convenient out of 85, 86, and 87 to make). Additionally, instead of indole 89 a chlorine analogue of that indole was be made. It was decided not to pursue compounds 88, 89, or 90.

2.6.6. Synthesis of chlorine analogue of 86

Commercially available 6-chloroindanone 114 was easily reduced to give 6-chloroindene 115 (Scheme 2.20).\(^\text{132}\) This was achieved by reacting 6-chloroindanone 114 with sodium borohydride, then taking the crude alcohol and heating it to reflux with p-toluenesulfonic acid. With chloroindene 115 in hand, the same procedure as the synthesis of 86 was used. The spirocyclic centre was formed with 92 to produce spirocycle 116. The Boc group was removed to give salt 117, and the alkene was reduced by hydrogenation, yielding the final chlorine analogue 118.

![Scheme 2.20 Synthesis of 118.](image)

2.6.7. Synthesis of chlorine analogue of 89

Finally, a tricyclic indole was synthesised. This was easily done via the Fischer indole synthesis between (4-chlorophenyl)hydrazine 119 and ethyl 4-oxopiperidine-1-carboxylate 120. This produced indole 121, which left only the removal of the ethyl ester by heating to reflux in base to give the desired product, 122 in two steps (Scheme 2.21).\(^\text{133}\)
2.7 Conclusions

2.7.1. In silico modelling

This project produced three spirocycles and one tricycle from an in silico pharmacophore model. However, none of these compounds bound to the interface pocket of CK2. The model predicted that the aromatic core would anchor in the pocket while the piperidine would form an electrostatic interaction with Asp37. This is likely due to the rigidity of the spirocycle compared with the ethyl amino group of NMR154L, which did bind. The presence of aromatic chlorides may be excluded as a factor as the chloride analogues did not bind. Due to this, it would seem that a spirocycle scaffold is unsuitable for binding in the interface pocket of CK2.

2.7.2. NMR154L analogues

Elaboration of the NMR154L fragment from the ortho position proved more successful. With a second ethyl amino group, the IC$_{50}$ was improved from 750 μM to 250 μM. Changing the benzene core of NMR154L to a naphthalene also produced a compound which bound at the interface. Attempts to find a facile way to install a vicinal di(ethyl amino) moiety on to the molecule proved unsuccessful.

2.7.3. Additional interface inhibitors

While the work in this chapter was being carried out, a series of other elaborations were made to NMR154 by substituting the para position of the benzene ring (Figure 2.22).
Of these, fragment 123 was the best binder at 150 μM incorporating an ortho-fluorophenyl moiety. Additionally, in a screen of compounds to bind in the αD pocket of CK2, the most potent inhibitor of CK2 at the interface pocket was discovered with an IC₅₀ of 44 μM. This fragment was an analogue of NMR154 with an indole elaboration from the meta position (124). This suggests that future work on small molecule inhibitors of CK2 at the interface pocket be directed towards development of this fragment.
Chapter 3

Spirocyclic fragment libraries

3.1. Introduction

3.1.1. The need for diversity

An extremely important factor when searching for new drugs is the variety, or diversity, of chemicals available to screen.\textsuperscript{43}

This Essay is a call to increase chemistry research and investment into the design and synthesis of diverse fragment molecules.\textsuperscript{72}

The concept of chemical diversity is an essential component of the solution to this problem [the optimal composition of a screening collection], as it can theoretically be shown that a diverse, high-quality collection of compounds should yield more leads than, for example, a similar-sized combinatorial library of limited structural variation.\textsuperscript{50}

There are three main considerations in assembling a fragment library. Firstly, the properties of the fragments – they... should be as diverse as possible.\textsuperscript{52}

The above quotes from the literature highlight the need for diversity in compound libraries to aid in drug discovery.

The hope was that a synthetic strategy could be devised to produce a diverse range of compounds—this strategy was developed in 2000 by Stuart Schrieber and has been termed diversity oriented synthesis (DOS).\textsuperscript{134}

This begs the questions: what is diversity? How to do we measure it? And how do we make diverse libraries? DOS is best understood when it is juxtaposed to its antithesis—target oriented synthesis (TOS). In TOS, the chemist has a specific type of compound or compounds in mind. The synthesis is usually convergent such that multiple reagents and reactants are brought together to form a single compound (or scaffold). The synthesis is usually contrived by retrosynthetic analysis with synthesis of the target as the primary goal. In DOS, a starting material is grown and modified by multiple reagents to create a collection of different molecules which are sufficiently different from one another. In this paradigm forward-
synthetic analysis is the primary driving force—there is no one target in mind but multiple. Synthetic handles and branch points are favoured to maximise the range of reactions that can be carried out (Figure 3.1).\textsuperscript{135}

**Figure 3.1** The difference between target-oriented synthesis and diversity-oriented synthesis. The grids of the right of each scheme depicting the chemical space covered by the TOS and DOS targets respectively.

TOS will often produce only one compound or a set of structurally similar compounds which only exhibit appendage diversity. DOS aims to incorporate:\textsuperscript{136}

*Structural diversity:* that is the physical shape of the molecules in space are different. This requires synthesis to connect different parts of the starting material in different ways to build a variety of skeletal frameworks.

*Stereochemical diversity:* easy synthetic access to the different stereochemical orientations a scaffold can possess.

*Functional group diversity:* the molecules which occupy a wide range of physical space must also be able to display a variety of functionality (especially if their purpose be to probe biological targets). This means that it is important to incorporate a range of heteroatoms and functional groups into the molecule and its scaffold. Synthetically, this means starting materials should be selected for their ability to undergo an array of different transformations.

*Appendage diversity:* the scaffold of the molecules must allow for the easy addition of moieties. These are often important growth vectors in the hit-to-lead optimisation of drug discovery.

Of these scaffold diversity is perhaps the most important for a DOS library to focus on as it is the best way to maximise the three other types of diversity.\textsuperscript{137-139}
3.1.2. Measuring diversity

With the principle of diversity established, the need arises for an appropriate way to measure diversity in compound libraries. Structural diversity can be most easily measured as numerical output of geometric properties. Advances in computer technology have made the measurement of spatial diversity easier.

One leading method for measuring the spatial diversity of compound libraries is Principle Moment of Inertia (PMI) analysis.\textsuperscript{140-143} In this case the moment of inertia ($I$) is measured for a molecule about its three orthogonal principle axes. This gives each molecule three moments of inertia ($I_1$, $I_2$, and $I_3$ in ascending magnitude). Since $I_3$ is always greater than or equal to $I_1$ and $I_2$ these moments of inertia can be easily normalised by calculating $I_1/I_3$ and $I_2/I_3$. This also allows a spatial descriptor of the molecule to be displayed on a 2D cartesian plot of $I_1/I_3$ vs $I_2/I_3$.\textsuperscript{137}

By considering the three extremes of 3D space: a rod, a disk, and a sphere, we can see that a PMI forms an isosceles triangle to represent chemical space. In one corner is (0,1) which are the coordinates for a rod (or acetylene chemically speaking), (1,1) are the coordinates for a sphere (adamantane), and (0.5,0.5) represent a disk (benzene), Figure 3.2.

![Figure 3.2](image)

\textit{Figure 3.2} Graphical representation of a PMI plot showing acetylene, adamantane, and benzene on the three extremes of the triangle in which all molecular shapes fall.

In this way a compound library which is structurally diverse will seek to cover as much of the PMI plot as possible (Figure 3.3).
Another useful descriptor of chemical space is the fraction of sp³ centres (Fsp³). This is expressed as the number of sp³ carbons hybridised divided by the total carbon count of the molecule. This is a good indicator of 3-dimensionality and the prevalence of aromatic rings. However, while molecules with a high Fsp³ are likely to be more 3-dimensional, it does not follow that a low Fsp³ necessarily means a flat molecule. For example, antifungal clotrimazole has a Fsp³ count of 0.05, however because its structure is four aromatic rings around a quaternary carbon centre it is sphere-like (Figure 3.4).

**Figure 3.3** Comparison of predicted PMI plots of a non-structurally diverse TOS library and a structurally diverse DOS library.

Another method of analysing diversity (or similarity) of a library is principle component analysis (PCA). This allows selection of n molecular descriptors, which can be anything from the physico-chemical descriptors found in the RO5 to biological descriptors like data from

**Figure 3.4** Clotrimazole: low Fsp³ count but highly spherical in nature.
protein binding studies, to be plotted on a 2D graph. The power of PCA is that by taking linear combinations of the \( n \)-dimensional space, vectors of the descriptors can be projected onto a 2-dimensional cartesian axis. The axes are unitless but represent the variance in the data set. PCA results will therefore always depend on both the data set and the descriptors chosen, but the plots that are produced give useful insights as to how diverse a compound library is internally and compared to other libraries (Figure 3.5).\textsuperscript{144}

\[\text{Figure 3.5 Example of PCA plot comparing a random selection of drugs (red) with a random selection of natural products. The principle components chosen were: molecular weight, clogP, hydrogen-bond donors, hydrogen-bond acceptors, rotatable bonds, polar surface area, stereogenic centres, nitrogen atoms, and oxygen atoms. There is greater variance in the natural product library then the drug library and both libraries are different from one another. (Image taken from ref 144).}\]

3.1.3. Features of a DOS campaign

Two main synthetic strategies have been developed to enable efficient assembly of DOS libraries: a reagent-based approach and a substrate-based approach.

Reagent-based DOS sees a single starting material transformed into a diverse set of compounds by utilising both a large amount of in-built functionality, and pluripotent functional groups (i.e. a functional group that can be transformed into many others). These vectors are often folded, paired, and branched to generate the library.\textsuperscript{145-148}
In the substrate-based approach, common reaction conditions are used on a series of starting materials which contain ‘pre-encoded’ skeletal information known as σ-elements which react to form a diverse array of scaffolds upon subjugation to the common reaction conditions (Figure 3.6).145,149,150

**Figure 3.6** The two main strategies to generate DOS libraries: a) Reagent-based DOS wherein a common starting material for diverse scaffold from different reagents; and, b) Substrate-based DOS where pre-encoded starting materials form the DOS library from a common set of reaction conditions. (Image taken from ref 139).

Throughout most DOS strategies, however, a common synthetic plan of build, couple, pair (B/C/P) has emerged.148 In this, initial building blocks are synthesised from commercial reagents. These are then coupled with each other or additional reagents to give a molecule appropriately furnished with orthogonal functionality. Finally, this functionality is utilised in the pair stage where different areas of the molecule are connected and folded to yield the diverse library (Figure 3.7).139,148

**Figure 3.7** An archetypal scheme of the DOS build/couple/pair strategy (Image taken from ref 151).

3.1.4. Achievements of DOS

Despite only being conceived of around the millennium, DOS has had a number of successes in aiding drug discovery.152–158
For example, in the field of generating leads for novel antibacterial activity, DOS libraries have yielded some interesting compounds.

Starting from a reactive and pluripotent diazo ester Wyatt et al. rapidly generated a DOS library of 223 unique compounds covering 30 scaffolds.\textsuperscript{159} Phenotypic screening revealed that 64 compounds (29\% of the library) displayed growth inhibition against methicillin-resistant \textit{Staphylococcus aureus} (MRSA). One of these compounds, Emmacin, was found to be particularly potent against methicillin-susceptible \textit{Staphylococcus aureus} (MSSA) as well as to epidemic strains of MRSA, EMRSA-15 and EMRSA-16 (Scheme 3.1).\textsuperscript{160}

Likewise, another reagent-based DOS campaign discovered a novel antibacterial called, Gemmacin. This DOS strategy started from a resin supported phosphoester. Using the B/C/P technique a library of 242 compounds with 18 molecular frameworks was built. Gemmacin and Gemmacin B (a benzene analogue) were discovered to also show good activity against MSSA, EMRSA-15, and EMRSA-16 (Scheme 3.1).\textsuperscript{160,161}

\begin{scheme}
\textbf{Scheme 3.1} Summary of the reagent-based DOS approaches which lead to the discovery of three novel antibacterials: Emmacin, (−)-Gemmacin, and (±)-Gemmacin B (Image adapted from ref 151).
\end{scheme}
3.2. Gaps in fragment libraries

3.2.1. Expanding fragment space

DOS campaigns have shown the effectiveness of diversity when confronting complex biological problems (section 3.1.4). In addition, FBDD is a powerful technique to optimise the success in the hit-to-lead process (section 1.3.2). However, FBDD is only ever going to be as good as the fragments it screens. Can DOS provide FBDD with diverse fragment libraries?

Most fragment libraries lack 3D structural motifs and instead focus on sp\textsuperscript{2}-rich heteroaromatics.\textsuperscript{162} This is not surprising given their commercial ubiquity and inherent aromatic stability. In addition, rapid growth in the field of metal-mediated cross coupling reactions have allowed researchers unprecedented access to otherwise unknown unsaturated scaffolds.\textsuperscript{25,163} These flat libraries have led to advances in the field of drug discovery,\textsuperscript{164,165} however, expanding the scope of fragment libraries would allow access to otherwise uncharted areas of chemical space.

3.2.2. Unsaturated rings in drug discovery

Rings are ubiquitous in pharmaceuticals with only 5% of marketed drugs not containing any kind of ring.\textsuperscript{163}

Unsaturated ring systems are an effective way of installing complexity and 3-dimentionality into compounds. They are axiomatically more 3D than unsaturated (aromatic) ring systems and will also carry a higher Fsp\textsuperscript{3} count. Given that 60% of drugs contain at least one sp\textsuperscript{3} carbon in a ring, evidence exists that this area of chemical space is likely to contain biologically relevant compounds. Additionally, research from GSK has suggested that a high aromatic ring count negatively affects the prospects of drug-like compounds while hetero-aliphatic rings showed more desirable properties such as better solubility and reduced lipophilicity.\textsuperscript{26,166} There is also a suggestion that molecules with a greater proportion of sp\textsuperscript{3} atoms are more specific binders and so are less likely to be toxic due to off-target interactions.\textsuperscript{167,168}

However, despite the prevalence of rings in chemistry and biology the number of structures explored remains limited. For drugs brought to the market before 2013 only 351 unique ring systems were represented across 1197 unique frameworks. However, innovation continues with, on average, 28% of new drugs containing a novel ring system.\textsuperscript{74} The field is ripe for exploration especially focusing on 3-dimentionality in ring systems.
3.2.3. Spirocycles

Spirocycles, also known as spiranes, are an important and prominent class of unsaturated ring system which contain at least two rings joined at a vertex (via a single carbon atom). The presence of a spirocentre in organic compounds may give them some advantages when considering their application in pharmaceuticals. The spirocentre can introduce conformational rigidity, meaning that there would be a reduced conformational entropy penalty upon binding to a protein target. Furthermore, spirocycles have greater inherent three-dimensionality than fused (aromatic) ring systems which can prove advantageous over flat compounds that tend to have suboptimal physical properties.\(^{169}\)

It should come as no surprise that this structural motif has been utilised in drug discovery.\(^{170}\) As Figure 3.8 demonstrates, successfully marketed drugs containing spirocycles.

![Figure 3.8 Examples of marketed drugs containing spirocycles. The spirocyclic scaffold is highlighted in red.](image)

Spirocycles may be classified into three different classes.

Class 1: the isolated spirocyclic system where there is one or more spirocycle in a molecule and the molecule does not fall into the following two classes;

Class 2: the condensed spirocyclic system where by one or more rings containing a spirocentre is fused by an edge to another ring;

Class 3: the bridged spirocyclic system where the two rings in the spirocycle are additionally connected by bridging atoms.
These classes are demonstrated in Table 3.1. Each of these classes can be further subdivided by the number of spirocentres present.

**Table 3.1** The three classes of spirocycle with example. The red bonds show the spirocycles, the green bonds show the condensed cycle and the blue bonds show the bridged cycle.

<table>
<thead>
<tr>
<th>Class</th>
<th>Example skeleton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1: isolated</td>
<td><img src="image1" alt="Class 1" /></td>
</tr>
<tr>
<td>Class 2: condensed</td>
<td><img src="image2" alt="Class 2" /></td>
</tr>
<tr>
<td>Class 3: bridged</td>
<td><img src="image3" alt="Class 3" /></td>
</tr>
</tbody>
</table>

These spirocycles were analysed at their differing levels of complexity. The most basic level on which spirocycles can be analysed is their ring combination (RC). This is simply the size of the two or more rings in the spirocyclic system, ignoring all heteroatoms, multiple bonds, and appendages. The next level of complexity is the scaffold of the RC (SC[RC]), wherein the rings are furnished with their heteroatoms and multiple bonds. Above this in the hierarchy comes the scaffold of the compound as a whole (SC[CPD]) and finally the compound itself (CPD) (Table 3.2).
Recent research on the ChEMBL chemical library has shown that 47,000 spirocycles are biologically active against approximately 200 targets. Their distribution between the above three classes was assessed, alongside the number of spirocentres, and the size of the fused rings.

The researchers found that from their high confidence data series there were only 47 unique ring combinations which contained 1 spirocentre and 25 unique ring combinations which contained greater than 1 spirocentre. However, these had a combined activity against 548 targets (Table 3.3). Proving the field is ripe for research.

**Table 3.3** Number of unique ring combinations (RC) for each class of spirocycle and the number of unique biological targets in each class.

<table>
<thead>
<tr>
<th>Class</th>
<th>Unique RC (1 spiro atom)</th>
<th>Unique RC (&gt;1 spiro atom)</th>
<th>Unique targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>3</td>
<td>219</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>8</td>
<td>240</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>14</td>
<td>89</td>
</tr>
</tbody>
</table>

The number of compounds targeted by the spirocycles varied from 1 to 20. These spirocycles also possess potent biological activity with over 60% showing a potency of at least 100 nM against one or more targets.

On more drug focused discovery, a range of 3-6 sized spiro rings have proved useful. For example, compound AM-5262 a spiro variant of AM-1638, a FFA1 agonist, showed a two-fold increase in potency from an EC\textsubscript{50} of 0.162 μM to 0.081 μM. It also presented increased off-target selectivity against a panel of over 100 GPCRs, ion channels, transporters, and enzymes (Figure 3.9).
In terms of the variety of ring sizes, as to be expected, most of the spiro systems contained a six-membered ring joined to three to six-membered rings. Very few medium sized seven- or eight-membered rings were represented. This gives two interesting areas for research: one, seeing what scaffolds frequently occur in biologically active spirocycles and synthesising a diverse spirocyclic library based on these cores; and two, making entirely novel spirocyclic ring combinations in a diverse fashion.

### 3.3. Scope of this project

This project seeks to establish chemistry for the rapid assembly of diverse spirocyclic libraries using the divergent build/couple/pair (B/C/P) algorithm of DOS. The ideal synthetic route would allow for the greatest range of rings to be synthesised orthogonally from each other. In addition, the spirocycles synthesised should be as ‘fragment-like’ as possible and leave ample vectors for synthetic development.

To affect this, two synthetic routes were approached. The first installed two terminal alkene moieties onto a quaternary centre. Ring closing metathesis was used to close a ring between the alkenes and a range of other chemistry was used to produce the second ring of the spirocycle.

Secondly, cycloaddition reactions were exploited to form spirocycles. This required the synthesis of a ring wherein an exo-cyclic olefin can be easily installed. With this in place, a range of pericyclic coupling partners were used to furnish the second ring off the alkene, forming the spirocyclic library.

Finally, computational methods were explored to enumerate the library (finding the greatest number of compounds which could feasibly be made from the synthetic precedent) and decorate the library—finding which appendages and modifications would enhance the library’s properties. A collaboration was also opened up with the Bender Group to perform
an *in silico* screen of the enumerated library, aiding any future *in vitro* biological testing of the molecules.

### 3.4. Glycine-based spirocycle library

#### 3.4.1. Library strategy

The challenge in constructing a spirocyclic DOS library is finding a starting material which can be appropriately furnished to allow two separate ring forming reactions. These functional handles must be able to undergo several different reactions to form distinct scaffold ring combinations (SC[RC]), and ideally different ring combinations (RC). Hence a system which would allow easy divergence in the build stage, and a reagent-based DOS approach in the couple and pair stage, so that pluripotent functional handle can form a variety of rings.

Cheap and easy-to-handle pre-functionalised starting materials are ideal for building a DOS library. Due to this, ethyl glycinate hydrochloride (132) was initially selected as a starting material. This comes ideally equipped with an amino and ester group for use in the couple and pair stage. Additionally, the α-centre in 132 can be difunctionalised, meaning this carbon atom will be the vertex at which the two rings will join to form a spirocycle (Scheme 3.2).

![Scheme 3.2 Outline of DOS scheme to synthesise diverse spirocyclic library.](image)

With the final pair stage requiring a form of amine and ester/ester derivative coupling, orthogonal functionalities were chosen for installation in the build stage. Ring closing metathesis (RCM) is a robust catalytic technique in the forming of small rings. RCM uses a metallocarbene catalyst to connect two olefin moieties. Application to the above scheme would mean R₁ and R₂ are terminal alkenes. These were readily installed via S₂N₂ chemistry. When installed, a ring can be formed quickly by addition of a RCM catalyst, such as Grubb’s second generation catalyst (Grubb’s II). This gives scope to vary the RC by adding a range of olefin homologues. Having two olefin functionalities to form the second ring also gives orthogonal functionality between the two ring-forming steps.
With this in mind, a broad synthetic strategy was devised. Ethyl glycinate hydrochloride (132) was furnished with two terminal olefins of varying length. Once installed, the first ring was formed via RCM, known as the tail. Then a reagent-based approach was used to form the second ring between the pendent amine and ester (Scheme 3.3), known as the warhead. This strategy allowed for sufficient scope to access a number of novel RCs and SC[RC]s.

Once the spirocycles were installed, they were modified to make new SC[RC]s and explore their appendage diversity. The alkene on the bottom ring offered synthetic orthogonality to the top ring, making such processes easier.

3.4.2. Building the precursor

Established methodology allows for easy installation of the allyl moiety onto glycine.\(^{175}\) Firstly, the amino group was simultaneously protected, and the α-carbon is activated, by condensation with benzophenone (139). The α-protons of this Schiff base (140) were acidic enough to be removed by base. This anion performed an S\(_{N}2\) reaction, giving the alkylated product in good yield. It was chosen to install a but-1-enyl group and an allyl group. This formed a cyclohexene ring upon exposure to Grubbs II. But-1-enyl was installed first, leaving the more reactive allyl bromide electrophile to react with the tertiary anion formed from 141. Both reactions proceeded smoothly and the acidic workup in allyl installation readily removed the benzophenone to reveal the free amine, 142 (Scheme 3.4).
3.4 Glycine-based spirocycle library

Scheme 3.4 Synthesis of glycine derivative 142.

This strategy can be easily modified to install olefins of different length and pendent functionality onto glycinate ethyl ester.

3.4.3. Coupling and pairing to form spirocycles

With precursor 142 in hand, the warhead and tail rings were formed. As only the Grubbs II catalysed RCM is used to form the tail, whereas a variety of chemistry was to form a number of warheads, it was logical to form the tail ring first. Grubbs II, however, does not tolerate the presence of a primary amine in a reactant.\(^{176}\) Therefore 142 was protected before exposure to Grubbs II. The tert-butyloxycarbonyl (Boc) protecting group was selected due to its ease of addition and removal, and the fact that the resulting carbamate group would tolerate Grubbs II.

Therefore, amine 142 was readily Boc-protected to give diene 143, which was subjected to RCM with Grubbs II. This formed the tail ring as a cyclohexene derivative (144). While the amine was still Boc-protected, the ester was reduced to alcohol 145 for future warhead synthesis. With these reactions complete, the Boc protecting group was removed to reveal primary amine 146 as the HCl salt (Scheme 3.5).
Scheme 3.5 Scheme showing the building of the cyclohexene tail of the library.

Due to the relatively low 57% yield in the reduction of ester 144 to alcohol 145, it was seen whether reduction of diene 143 to an alcohol followed by RCM would be higher yielding. Reduction of diene 143 gave alcohol 147, however in the more disappointing yield of 27% (Scheme 3.6). Therefore, RCM followed by reduction was used as the preferred route.

Scheme 3.6 Reduction of diene 143.

3.4.4. Pairing to make the spirocycles

Before exploring the chemistry of amino alcohol 146, it was noted that exposure of the Boc-protected precursor, 145, to base caused internal displacement of the tert-butoxy group from the Boc, forming a oxazolidin-2-one spirocycle, 148 (Scheme 3.8).

Scheme 3.7 Synthesis of oxazolidine-2-one spirocycle, 148.
Examples of the nitrogen in 148 acting as a nucleophile are well precedented in the literature.\textsuperscript{177} Therefore, it was explored whether cross-coupling straight to an aromatic centre would increase the scope of 148 to be grown with a variety of aromatics. The Goldberg reaction was first tried. Although its scope was originally confined to aniline derivatives, this has recently been expanded to amides, and carbamates.\textsuperscript{178,179} Thus, it was seen whether the nitrogen carbamate on spirocycle 148 would be tolerated. Unfortunately, no product was obtained from this reaction; an inseparable complex mixture was returned. A literature procedure\textsuperscript{180} was tried to couple a meta-acetyl phenyl to spirocycle 148; this only returned starting material. Finally, conditions were tried to couple para-nitrile bromobenzene to 148 (Table 3.4).\textsuperscript{181} One possibility for the failure of these reactions could be steric hindrance due to the adjacent spirocentre. Although there is literature for these reactions to be carried out next to a quaternary spirocentre,\textsuperscript{182} there is no precedent for the reaction next to a 3–7 membered spirocycle.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Coupling partner</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Image of coupling partner" /></td>
<td>CuI (50 mol%), (N^1,N^2)-dimethylethane-1,2-diamine, (Cs_2CO_3), dioxane, 90 °C, 16 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Image of coupling partner" /></td>
<td>CuI (10 mol%), trishydroxymethyl ethane, (K_3PO_4), dioxane, DMF, 110 °C, 72 h</td>
<td>SM</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image of coupling partner" /></td>
<td>CuI (5 mol%), (N,N)-dimethylglycine, (K_2CO_3), DMF, 120 °C, 16 h</td>
<td>SM</td>
</tr>
</tbody>
</table>

Warheads derived from amino alcohol 146 were subsequently explored. Multiple reactions can be carried out with vicinal amino alcohols to form rings as well as other precursors in the synthetic steps to make 146. First of all, 146 was condensed with imidate 150 to give spirocycle
containing a dihydrooxazole ring (Scheme 3.8). This procedure allows for ready substitution of phenyl in 151 or other groups due to the ease of the imidate synthesis from nitriles.

![Scheme 3.8 Synthesis of dihydrooxazole spirocycle.](image)

In a similar manner, an amine substituted dihydrooxazole spirocycle was synthesised by exposing amino alcohol 146 to cyanogen bromide. This afforded spirocycle 152 (Scheme 3.9). This is an interesting motif containing an H-bond donor/acceptor pair similar to DNA base adenine, perhaps privileging it in biological interactions.

![Scheme 3.9 Synthesis of amino-dihydrooxazole spirocycle 152.](image)

An X-ray structure of 152 was taken to confirm the desired product was made.

![Figure 3.10 Crystal structure of the (S)–152.](image)
It was assessed whether the exocyclic \( \text{NH}_2 \) group on 152 could be readily used as a synthetic handle. A patent was adapted to see if the biologically-relevant morpholine could be installed on the amino group.\textsuperscript{110} A quick microwave experiment produced a complex mixture from which no discernible product was obtained (Scheme 3.10).

![Scheme 3.10 Attempted installation of morpholine moiety onto 152.]

Following this, 6-membered rings synthesis of morpholine-derivatives was explored. Firstly, a few microwave reactions were tested. Amino alcohol 146 was subjected to microwave reaction with ethyl benzoylformate and \( \alpha \)-chloroacetophenone to form dihydrooxazine-2-one (154) and dihydrooxazine (155) rings respectively. These would give an imine and/or ester functionality which could be a useful tool for further fragment growth. Unfortunately, neither of these techniques proved successful, even with increasing the number of molecular sieves used (Scheme 3.11).

![Scheme 3.11 Attempted microwave condensations to form spirocycles.]

A stepwise approach was then tried; either first an imine condensation followed by an \( \text{S}_2\text{N}_2 \), or an \( \text{S}_2\text{N}_2 \) followed by an internal imine condensation. Starting from Boc-protected amino alcohol 145, exposure to base and \( \alpha \)-chloroacetophenone failed to yield the desired product; the fractions isolated by column chromatography being complex mixtures. Reaction of 146 with \( \alpha \)-chloroacetophenone under Dean-Stark conditions also failed to yield the imine (Scheme 3.12).
Finally, it was postulated that synthesising the morpholin-2-one ring could be more fruitful. Following a literature procedure,\textsuperscript{183} 146 was submitted to basic conditions and phenyl 2-bromoacetate. This gave spirocycle 158 with a morpholin-2-one warhead. With 158 in hand, this could be transformed in the desired dihydrooxazine-2-one warhead as well as a morpholine \textit{via} reduction. Exposure of 158 to Pb(OAc)$_4$ readily oxidised it to dihydrooxazine-2-one spirocycle 159. This has the advantage over similar spirocycle 154 in that it lacks the phenyl group and so allows greater scope in Cu-mediated nucleophilic addition into the imine (Scheme 3.13).

Additionally, the regio-isomer of 158, a morpholin-3-one spirocycle, was prepared by reacting 146 initially with chloroacetyl chloride. The more reactive primary amine formed an amide bond with the acid chloride, making compound 160. Subsequent exposure to base allowed for an S$_2$2 reaction between the deprotonated alcohol and the chloride forming morpholin-3-one spirocycle 161 (Scheme 3.14).
Attempted reduction of the amide group in 161 to form the morpholine ring was unsuccessful, despite literature precedent. Spirocycle 161 was reacted with lithium aluminium hydride at room temperature overnight. When TLC showed only starting material, the reaction was heated under reflux for another day; starting material was returned (Scheme 3.15).

It was also explored whether the amide could be transformed into the dihydrooxazin-3-amine, 164, to imitate the same imine-amine functional motif as in 152. A literature procedure was followed, first reacting 161 with phosphorous (V) sulphide to form morpholin-3-thione intermediate (163) which, upon exposure to aqueous ammonia, should form the desired dihydrooxazin-3-amine, 164. Unfortunately, NMR after the first sulphonation step showed only starting material returned. Therefore, it was seen whether Lawesson’s Reagent would affect sulphonation. Again, this failed to produce the desired product (Scheme 3.16).
Attempted sulphonation of spirocycle 161 in order to form dihydrooxazin-3-amine spirocycle 164.

Although there was a need to protect the primary amine before RCM, this need not be done with a Boc-protecting group. A group could be installed in the amino moiety of precursor 142 which itself could be used to produce a ring. In this way, 142 was reacted with ethyl malonyl chloride to form diester 165. Exposure to base cyclised this species to form tetramic acid derivative 166, which in turn underwent ester hydrolysis-decarboxylation to give tetramic acid diene 167. With no offending primary amine present, the two terminal alkenes were readily cyclised using Grubbs II to give tetramic acid spirocycle 168 (Scheme 3.17).

Scheme 3.16 Attempted sulphonation of spirocycle 161 in order to form dihydrooxazin-3-amine spirocycle 164.

Scheme 3.17 Synthesis of tetramic acid spirocycle 168 from amino ester 142.
Tetramic acid is known to exist in two tautomeric forms: the keto form as in 168, or the enol form 168b. NMR confirmed the more common form of tetramic acid (168) was isolated due to the presence of two protons between the ketone and amide. This tetramic acid spirocycle gives several vectors for elaboration and derivation into new spirocycles.

It was investigated whether the oxo-group on the tetramic acid ring would undergo Wittig chemistry to form a substituted exocyclic olefin. Stabilised Wittig Reagent (169) was initially tried. However, no reaction took place, with the reagents re-isolated. It was examined whether the reaction would proceed if 169 were made in situ. Unfortunately, starting material was returned (Scheme 3.18).

It was postulated that 169 might be too stable to react with 168, as the literature example showed reaction with an unstabilised ylide. Therefore, the reaction was repeated with unstabilised ylide, methylenetriphenylphosphorane. The methylene group had much less steric bulk than the methylene ethyl ester of 169, also hopefully discounting any steric factors. Again, starting material was returned (Scheme 3.19).
With both these failures, it was postulated whether tetramic acid exists in the enol tautomer (168b) under the basic reaction conditions, rendering it unable to perform Wittig chemistry. This enol form of the tetramic acid was trapped out and isolated as an ethyl ether (174). This ‘protected’ version of 168 could be used to explore functionalisation on the nitrogen (Scheme 3.20).

Scheme 3.20 Synthesis of 174.

The extent to which 168 could be transformed into a γ-lactam or α,β-unsaturated-γ-lactam was then explored. First, exploiting the enol tautomer of 168, triflic anhydride formed the triflate enol 175. This triflate functionality could be exploited in cross-coupling reactions as a pseudo-halogen. This was demonstrated by Suzuki-Miyaura coupling 175 with para-methoxyphenyl boronic acid to give β-functionalised α,β-unsaturated-γ-lactam 176. This is a very useful functional handle to install a variety of sp²-sp² or sp²-sp coupled centres (Scheme 3.21).

Scheme 3.21 Transformation of 168 into β-functionalised α,β-unsaturated-γ-lactam 176.

X-ray crystallography was used to produce a crystal structure of 176 to assess its geometry (Figure 3.11). This showed that the α,β-unsaturated-γ-lactam and the aromatic ring were in the same plane and flat with respect to one another.
In addition to the substituted α,β-unsaturated-γ-lactam, it was seen whether an unsubstituted α,β-unsaturated-γ-lactam could be synthesised via elimination. Firstly, conditions were repeated that were used in the similar reduction-elimination of 6-chloroindanone (entry 1 in Table 3.5). The initial reduction to form alcohol 177 proceeded without issue however elimination was not observed. Next, a literature procedure was tried, in which the authors performed a reduction followed by elimination on a spirocyclic tetramic acid. Unfortunately, when this was tried carrying 177 through crude, only 10% of 168 was returned after purification. Therefore, the reactions were repeated step-wise as described in the literature. Reduction worked smoothly, followed by high-yielding mesylation of the alcohol to give 178. Alas, upon subjection to the final elimination conditions, 178 was consumed but no discernible product was detected. Finally, conditions were tried from a paper which performed the same reduction on a α-N-gem-dimethyl tetramic acid. After reduction trifluoroacetic anhydride was used to trifluoroacetylate both the alcohol and the amide. Exposure to TEA eliminated trifluoroacetate and reaction with KHCO₃ revealed the amide giving 179 in 30% yield (Table 3.5).

Figure 3.11 Crystal structure of (R)-176.
Table 3.5 List of conditions to transform tetramic acid spirocycle $168$ into $\alpha,\beta$-unsaturated-$\gamma$-lactam $179$.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1) NaBH$_4$, MeOH, RT, 1h. 2) TsOH, Tol, reflux, 2h</td>
<td>Alcohol returned</td>
</tr>
<tr>
<td>2</td>
<td>1) NaBH$_4$, MeOH, RT, 1h. 2) MsCl, TEA, DBU, DCM, RT, 3h</td>
<td>10% of SM returned</td>
</tr>
<tr>
<td>3</td>
<td>NaBH$_4$, MeOH, RT, 1h, gave $177$ in 86% yield; then MsCl, TEA, DCM, gave $178$ in 99% yield; then TEA, THF, reflux</td>
<td>No product</td>
</tr>
<tr>
<td>4</td>
<td>1) NaBH$_4$, MeOH, RT, 1h. 2) TFA anhydride, reflux, 12 h. 3) TEA, DCM, RT, 12 h. 4) KHCO$_3$, MeOH, RT, 2 h.</td>
<td>30%</td>
</tr>
</tbody>
</table>

Although conditions in entry 3 failed to achieve the desired $\alpha,\beta$-unsaturated-$\gamma$-lactam, $179$, it did allow access to $\gamma$-lactams $177$ and $178$.

The crystal structure of $179$ was also obtained for future comparisons with the spatial properties of derivative $176$ (Figure 3.12).
It was explored whether the amino and ester groups of precursor 142 could be linked in such a way so as to form rarer 4- and 3-membered rings. It was decided to pursue these linking strategies before RCM of the terminal alkenes, as having the cyclohexene ring may limit gyration of the amino and ester appendages in forming rings with bond angles less than the preferred 109.5°.

The synthesis of 4-membered β-lactam was explored. For this to be synthesised it would require the homologue of amine or ester to be initially synthesised. Homologation of carboxylic acids is known via the Arndt-Eistert reaction.\textsuperscript{190} This sees a carboxylic acid activated to the acid chloride before reaction with diazomethane to form a diazoketone. Exposure to silver (I) causes this to undergo the Wolff-rearrangement and form the homologous carboxylic acid (Scheme 3.22).

\begin{center}
\textbf{Scheme 3.22} General scheme for the Arndt-Eistert reaction for homologation of carboxylic acids.
\end{center}

Once the homologous carboxylic acid has been obtained, intra-molecular amide coupling reactions may couple the carboxylic acid with the amine to yield a β-lactam.

Hydrolysis of ester 143 proceeded without issue, giving carboxylic acid 184 (Scheme 3.23).

Next, it was decided not to use diazomethane as the reagent in the Arndt-Eistert reaction due to the high explosion risk. Therefore the more stable analogue, TMS-diazomethane, was used.\textsuperscript{191} The crude result of the Arndt-Eistert reaction (185) was carried through to a DCC mediated amide coupling.\textsuperscript{192} Unfortunately, no product could be isolated.
Finally, it was seen whether a 3-membered aziridine ring could be synthesised. Starting from alcohol 147, a literature sequential alcohol-activation, then internal S_N2, was tried. This activated the alcohol by tosylation, however the presence of base did not result in internal cyclisation. Unfortunately, only a crude mixture of alcohol 147 and tosylated alcohol 187 was returned (Scheme 3.24).

Scheme 3.24 Attempted synthesis of 188 from 147.

3.4.5. Summary

In summary, this strategy (to synthesise a spirocyclic library from ethyl glycinate hydrochloride (132) by furnishing it with two terminal olefins to allow orthogonal ring formation between these and the amino and ester moieties) has yielded 13 novel spirocycles comprising 2 RCs, and 10 SC[RC]s (Scheme 3.25). The longest linear sequence in making a spirocycle was 9 steps (synthesis of 161). These fragment scaffolds give ample chemical handle for further fragment growth. The warheads are appropriately furnished to allow a variety of chemistry to be installed at various points (only a fraction of the possible elaborations are shown here). In addition, the tail section of the spirocycle could be modified to allow for 5- or 7- or larger membered rings. The olefin synthetic handle in the tail is orthogonal to the functionality in the warheads allowing for easier synthetic development of this species.
3.5 Pericyclic spirocycle libraries

3.5.1 Pericyclic reactions

Pericyclic reactions proceed via a cyclic aromatic transition state and are a powerful and versatile method of forming carbon-carbon bonds. Transformations are often regio- and diastereiospecific because of the frontier orbital control of this reaction class.

The cycloaddition class of pericyclic reactions is defined as:

A reaction in which two or more unsaturated molecules (or parts of the same molecule) combine with the formation of a cyclic adduct.

For the synthesis of spirocycles, this requires unsaturation in both coupling partners to form the spirocyclic adduct. Practically, this means a ring should be furnished with an exo-cyclic double bond and the coupling partner would be a diene or 1,3-dipole. As the interaction is controlled by frontier orbitals, this means the reactions are under electronic control.

The two types of cycloaddition explored in this section will be a [4+2] Diels-Alder cycloaddition and a [3+2] 1,3-dipolar cycloaddition. Because these reactions are under orbital control, there must be the right electronic match between the coupling partners to ensure the reaction proceeds.
Spirocyclic fragment libraries

Diels-Alder reactions can be split into two categories: normal-electron-demand and inverse-electron-demand. In a normal-demand reaction, an electron-rich diene’s frontier orbital’s HOMO is the most similar in energy to a dieneophile’s LUMO. Using a frontier molecular orbital (FMO) approach, it can be seen that the molecules combine suprafacially (Figure 3.13).

Additionally, due to the orbital control of the reaction there is high regiocontrol on the arrangements of the pendent functionality in the adduct. The orbitals with the largest coefficients will have the better overlap and so will interact preferentially. Drawing the resonance structures can aid in the prediction of which orbitals are likely to interact (Figure 3.14).
Figure 3.14 Diagram showing the origin of selectivity in the Diels Alder reaction: a) the two resonance structures of electron-rich diene \(189\) (\(189a\) in blue formed from the blue curly arrows and above \(189b\) in red from the red curly arrows). The location of the negative charge shows the location of the orbitals with the greatest coefficient in the HOMO. The positive charge on the resonance structure of electron-poor dieneophile \(190\) (\(190a\)) shows the location of the largest orbital coefficient on the LUMO; b) the major product (\(193\)) of the reaction is formed when the orbitals with the most similar coefficient overlap and the minor product (\(194\)) is formed by the disfavoured dissimilar interaction.

The same theory can equally be applied to 1,3-dipolar cycloadditions. A 1,3-dipole is a functional group with a pair of delocalised electrons shared over three atoms, giving resonance structures where one atom has a formal positive charge, and another has a formal negative charge.

An FMO approach of the interaction of 1,3-dipoles with dipolarphiles can also be used to see which interactions are favoured. In this case 1,3-dipoles can be divided in three categories: Type I, Type II, and Type III (Figure 3.15).

Type I: This dipole has a high lying HOMO (as most closely resembles an electron-rich diene). This mean it will quickly react with electron-poor alkenes but react slowly or not at all with electron-rich alkene.$^{195}$
**Type II:** In this case the HOMO and LUMO of the dipole are so placed that they could readily overlap with both electron-rich and electron-poor alkenes.

**Type III:** This dipole has a low energy LUMO (just as the electron-poor diene in the Diels Alder reaction) which is positioned to react best with electron-rich alkenes.

![Diagram of the three dipole types interacting with dipolarphiles](image)

**Figure 3.15** Diagram of the three dipole types interacting with dipolarphiles. A Type I dipole interacts best with a low energy LUMO from an electron-poor dipolarphile. Type II dipoles have HOMO and LUMO so placed that interaction can be made with both electron-rich and electron-poor dipolarphile. Type III dipoles have a low lying LUMO which can best interact with a high-energy HOMO from an electron-rich dipolarphile.

### 3.5.2. Dehydroalanine derived spirocycles

In the synthesis of the ethyl glycinate series, a variety of chemistry was established to link the amine group with ester, or in its reduced form, the alcohol. It was proposed that this chemistry could be coupled with another way of making rings other than by RCM of installed terminal alkenes. A library could be built from a dehydroalanine derivative. This has the amine group and ester group to be exploited as before, but these are gem-disposed on a double bond meaning pericyclic reactions at this olefin would produce spirocycles. Thus, a general scheme was proposed where L-serine ethyl ester (195) could be transformed into dehydroalanine derivative (196) which in turn could be coupled and paired to make a spirocyclic library (Scheme 3.26). The order of the coupling and pairing is variable in this case depending on the functionality involved and the group which can be synthesised.

![Scheme 3.26](image)

**Scheme 3.26** An outline of the proposed spirocyclic library constructed from dehydroalanine.

Additionally, considering 195 has both alcoholic and ester functionalities, a ring can be constructed from this molecule before an alkene is revealed. This could then be subject to pericyclic chemistry, constructing the library (Scheme 3.27).
Starting along the dehydroalanine path, L-serinate ethyl ester hydrochloride (195) was readily Boc-protected (201) and then eliminated to form N-Boc-dehydroalanate ethyl ester (202). Reduction of 202 to alcohol 203 was explored as a potential route to 4-methyleneoxazolidin-2-one (205). With this in hand it was hoped a variety of pericyclic reactions could be explored. The reduction of 202 proved challenging, with alcohol 203 proving unstable (a $^1$H NMR of 203 was obtained however by the time $^{13}$C and 2D NMR were obtained, the sample had decomposed). Thus, alcohol 203 was reacted immediately with KOtBu in an effort to yield 205. However, the endo-cyclic alkene isomer 204 was obtained. This was even the case when base was limited (Scheme 3.28).

Although it was disappointing that 205 could not be made, the intermediate 202 could be used in pericyclic chemistry to generate a ring before the amine and ester are coupled to form the spirocycle.

With the carbamate group being an electron donor into the alkene and the ester group being an electron withdrawing from the alkene, the electronic state of the alkene in 202 was established by $^{13}$C NMR. The chemical shift of a magnetic nucleus ($\delta$) is related to the effective
magnetic field it experiences ($B_{\text{eff}}$). This is the product of the external applied field from the machine’s magnet ($B_0$) and any induced field from the electronic structure of the molecule ($B_{\text{ind}}$). The induced field is opposed to the applied field and is larger the greater the electron density around the nucleus. The induced field can be calculated around a given atom from its chemical shift.

Table 3.6 shows clearly that 202 is an electron rich alkene. The molecule was split into its two components as an ethyl acrylate moiety (206) and a tert-butyl vinylcarbamate moiety (207). The difference in the induced fields about the α and β carbons of each molecule was calculated. The results clearly show that 202 is electron-rich at the β carbon (being more similar to tert-butyl vinylcarbamate electronically).

Table 3.6 Table calculating the difference in the induced magnetic field ($B_{\text{ind}}$) between the α and β carbons on ethyl acrylate, tert-butyl vinyl carbamate and 202. $\delta$ = chemical shift, $\nu_{\text{nucleus}}$ = resonance frequency of the $^{13}$C nucleus, $B_{\text{eff}}$ = effective magnetic field experienced by the nucleus, $B_{\text{ind}}$ = magnitude of the induced magnetic field around the nucleus, $C_{\alpha} - C_{\beta}$ = the difference in the induced magnetic field between the α and β nucleus.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nucleus</th>
<th>$\delta$/ppm</th>
<th>$\nu_{\text{nucleus}}$/MHz</th>
<th>$B_{\text{eff}}$/T</th>
<th>$B_{\text{ind}}$/T</th>
<th>$C_{\alpha}-C_{\beta}$/μT</th>
</tr>
</thead>
<tbody>
<tr>
<td>206</td>
<td>$C_{\alpha}$</td>
<td>128.8</td>
<td>100.6257</td>
<td>9.39680</td>
<td>0.034957</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$C_{\beta}$</td>
<td>131.3</td>
<td>100.6260</td>
<td>9.39692</td>
<td>0.034928</td>
<td>29.13</td>
</tr>
<tr>
<td>207</td>
<td>$C_{\alpha}$</td>
<td>128.6</td>
<td>100.6257</td>
<td>9.39689</td>
<td>0.034953</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$C_{\beta}$</td>
<td>95.5</td>
<td>100.6224</td>
<td>9.39658</td>
<td>0.035264</td>
<td>-311.00</td>
</tr>
<tr>
<td>202</td>
<td>$C_{\alpha}$</td>
<td>131.5</td>
<td>100.6260</td>
<td>9.39692</td>
<td>0.034926</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$C_{\beta}$</td>
<td>104.8</td>
<td>100.6233</td>
<td>9.39667</td>
<td>0.035177</td>
<td>-250.86</td>
</tr>
</tbody>
</table>

Knowing that 202 is an electron rich alkene, it would most likely perform cycloadditions with Type II and Type III dipoles as well as inverse-demand dienes.

Compound 202 was first reacted with a nitrile oxide dipole formed in situ from hydroxylimidoyl chloride. Because of the electron-rich nature of the alkene in 202, the
reaction was expected to proceed via transition state $\text{TS}_210$, forming the regioisomer $\text{210}$. This was confirmed by NOESY NMR (Figure 3.16) showing spatial proximity between the ortho-aromatic protons (H11) and the methylene protons in the isooxazole ring (H8). Compound was reduced, giving alcohol which was cyclised to give spirocyle $\text{212}$ (Scheme 3.29). A crystal structure was taken of to confirm the regiochemical assignment of (Figure 3.17).

Scheme 3.29 Synthesis of spirocyle $\text{212}$. 

Figure 3.16 NOESY NMR of compound $\text{210}$ showing correlation between protons 11 and 8.
Benzyl azide was also reacted with 202 in an attempt to form a dihydro-1,2,3-triazole spirocycle. Initially, following a literature procedure,\textsuperscript{198} heating the reaction under reflux for 6 h only gave a mixture of benzyl azide and 202. Repeating the reaction by heating under reflux overnight seemed to produce the desired product; however, despite the NMR losing the olefin peaks of 202 and gaining aromatic peaks to integral 5, the benzyl methylene peak was absent and a valid structure could not be proposed to support the data. Finally, a more forcing condition of heating to 100 °C in a sealed vessel for 48 h was tried yet no identifiable product was isolated from this reaction (Scheme 3.30).

![Scheme 3.30 Attempted synthesis of dihydro-1,2,3-triazole 213.](image)

The second proposed strategy was then explored: forming a ring, revealing the alkene, and then performing the cycloaddition. To minimise the use of protecting groups, it was first tried to construct the ring using the already present amine and alcohol functionality in L-serinate ethyl ester 195.

Firstly, a morpholin-3-one was the synthetic target, using similar chemistry as in the synthesis of 161. Amino alcohol 195 was reacted with chloroacetyl chloride giving amide 214. Only a small amount of the difunctionalised by-product 215 was observed. It was hoped that the facile S$_N$2 ring closing between the alcohol and the chloride could produce morpholin-3-one 216, yet unfortunately this was not the case. Reaction with KOtBu in THF gave an insoluble

\[ \text{Scheme 3.30 Attempted synthesis of dihydro-1,2,3-triazole 213.} \]

\[ \text{213} \]
jelly, suggesting polymerisation had taken place. Switching the solvent to \(\text{HO}^+\text{Bu}\) produced the same product, as did IPA. However, this product was submitted for reduction in the hope that some of alcohol 217 could be recovered post-purification. No reaction took place. NMR analysis of the jelly seemed to suggest alkene 218 had been the product of the reaction (Scheme 3.31).

Scheme 3.31 Attempted synthesis of morpholin-3-one precursor for cycloadditions.

The pre-furnished amino alcohol in 195 allows quicker synthesis of the oxazolidin-2-one ring than previously. Reacting 195 with triphosgene joins the amine and the alcohol to form oxazolidin-2-one 219. However, despite literature precedent, the reduction to 220 could not be affected. Despite multiple attempts and the TLC in each case showing a spot-to-spot reaction, no material was left post work-up. Thus, this synthetic strategy was abandoned (Scheme 3.32).

Scheme 3.32 Synthesis of oxazolidin-2-one ring and attempted reduction.

Finally, reaction of 195 with ethyl benzimidate (150) yielded dihydrooxazole 221 which was subsequently reduced to alcohol 222. In spite of several attempts to eliminate this alcohol it was returned by the reaction (Scheme 3.33).
Scheme 3.33 Formation of dihydrooxazole ring but failed elimination.

A second approach to forming the ring and revealing the alkene was tried. This time, the free alcohol on 195 would be TBS-protected before ring forming reactions then a deprotection-elimination could reveal the olefin.

Compound 195 was readily TBS protected (224). Firstly, synthesis of the morpholin-3-one ring was re-examined. Chloroacetyl chloride acetylated the free amine giving compound 225 which was reduced to give alcohol 226 (Scheme 3.34). Again a 6-exo-tet ring closure was attempted using the previous conditions to produce morpholin-3-one 227. This produced a clean product by NMR, however this was not the desired product (Figure 3.18). The TBS group was present however it was one CH₂ group short of 227. No structure or reaction mechanism could be proposed which satisfied the given NMR.

Scheme 3.34 Synthesis of morpholin-3-one precursor 226.
Further, the synthesis of the tetramic acid warhead was explored. The same process in the synthesis of 168 was repeated. Reaction of TBS-protected serine ethyl ester 224 with ethyl malonyl chloride gave compound 228. However, several attempts at repeating the ring closure-hydrolysis-decarboxylation to afford tetramic acid derivative 229 failed (Scheme 3.35).

With this in mind, a different approach was taken. The literature showed that reaction of compound 224 with Bestmann’s ylide formed the enol ether of tetramic acid via internal Wittig reaction. When this reaction was performed, the expected tetramic acid enol ether with TBS protected alcohol was not observed. Instead the more desirable elimination product, 230, was isolated. This avoided additional steps to reveal the olefin via elimination. The presence of the catalytic amount benzoic acid aided in the deprotection-elimination. The eliminated enol was observed as a by-product previously, however in this case it was major product of the reaction (Scheme 3.36).
Due to the presence of two alkene moieties in 230, it was decided that deprotection of the enol ether to reveal the tetramic acid in ketone form was necessary before the pericyclic reaction to ensure selectivity on the olefin. This was initially tried with conc. HCl in THF but the reaction returned a complex mixture. However, due to literature precedent of this transformation, it is expected to work in slightly different solvents e.g. ether or dioxane.\textsuperscript{190}

In summary, despite difficulty in forming the exo-cyclic olefin from elimination, a spirocycle was formed by pericyclic reaction with \textit{N}-Boc-dehydroalanine ethyl ester and forays have been made toward synthesising tetramic acid with an exocyclic double bond.

### 3.5.3. 3-Azetidinone derived spirocycles

Alongside the synthesis of exo-cyclic olefins from \textit{L}-serinate ethyl ester hydrochloride, \textsuperscript{195}, pericyclic chemistry was also explored to investigate its application to a range of other exo-cyclic olefins.

In this case, the ease of transforming ketones into alkenes \textit{via} the Wittig reaction was used. The ‘build’ stage would see the construction of a ring which possesses a ketone. The ‘couple’ stage reacted this ketone with a Wittig reagent to form an alkene. Depending on the Wittig reagent used, the subsequent alkene can be furnished with various groups which may change its reactivity in the ‘pair’ step. In pairing, various pericyclic coupling partners could be used to form the spirocycle.
Due to the wide commercial availability of rings possessing ketone functionality, it was decided that the ‘build’ stage could be by-passed by purchasing the desired ring. N-Boc-azetidin-3-one (235) was selected as a starting material. Firstly, due to its commercial availability, and secondly, despite effort being directed towards their synthesis, four membered aza-spiro rings are under-represented in spirocyclic space. Thirdly, the nitrogen in the ring is a useful vector for further elaboration. Therefore, a general scheme was proposed; N-Boc-azetidin-3-one (235) would be furnished with an exo-cyclic olefin via the Wittig reaction, and then the spirocycle would be formed via a pericyclic reaction using a similar method as outlined in section 3.5.2 (Scheme 3.37).

N-Boc-azetidin-3-one (235) was appropriately furnished with an ethyl acetate substituted olefin by Wittig reaction to give compound 236. The ethyl acetate substitution was chosen as a versatile synthetic handle for future modification and to ensure the olefin was electron-deficient to allow for a greater range of pericyclic reactions (Scheme 3.38).

Compound 236 was first reacted with previously synthesised nitrile oxide precursor 209 and gave spirocycle 237. In contrast to the synthesis of 210, which took an hour to complete, this reaction was complete within minutes and the yield of the product diminished if the reaction was left for a long time (Scheme 3.39).
Spirocyclic fragment libraries

Scheme 3.39 Cycloaddition with nitrile oxide forming isoazole 237.

Compound 236 was also reacted with benzyl azide, a reaction which 202 was unable to perform. An initial attempt following a literature procedure by stirring at 45 °C for 12 h without solvent simply returned 236. However, the procedure was adapted, and the mixture was heated to 100 °C in a sealed vessel for 48 h. This did produce the desired dihydro-1,2,3-triazole spirocycle product, 238 (Scheme 3.40).

Scheme 3.40 Cycloaddition with azide forming 1,2,3-triazole 238.

Next, being an electron deficient alkene, 236 should also be able to perform cycloaddition reactions with Type I dipoles and ‘normal demand’ electron-rich dienes. An attempt was made to form some bridged cyclic structures using the Diels-Alder reaction. Firstly, it was attempted to react 236 with furan using a Lewis acid catalyst of zinc iodide. After refluxing overnight at 40 °C, all of the starting material was returned unreacted. The same result occurred when the reaction was repeated in a sealed tube at 50 °C. Therefore, another set of conditions was tried. These saw the reaction repeated with Lewis acid catalysis from niobium (V) chloride and the reaction carried out ~20 °C. Again, the starting material was returned and spirocycle 239 was not isolated (Scheme 3.41).
Another attempt was made at a Diels-Alder reaction using 2-(trimethylsiloxy)-1,3-cyclohexadiene with stoichiometric amounts of titanium tetrachloride. Following the literature, this was performed at \(-40 \, ^\circ C\).\textsuperscript{205} Despite the TLC indicating a product had been formed, nothing could be isolated from column chromatography (Scheme 3.42).

Moving to Type I dipoles, it was decided to move beyond standard 1,3-dipoles and try an oxypyrylium zwitterion which could undergo [5+2] cycloaddition with a dipolarphile. The precursor to this zwitterion was readily synthesised using literature procedure.\textsuperscript{206} Furan 241 was expanded into six-membered hemi-acetal 242 which was acetylated to give oxypyrylium zwitterion precursor 243 (Scheme 3.43).

This was submitted to cycloaddition with 236. Unfortunately, in this case only the oxypyrylium dimer (244) was observed (Scheme 3.44).
Spirocyclic fragment libraries

Scheme 3.44 Reaction of oxopyrylium zwitterion with 236.

3.5.4. 1,4-Oxazepane based library

7-Membered rings are underrepresented in spirocyclic libraries. Therefore increasing their representation in these libraries would hopefully allow a greater probing of biologically relevant chemical space. 1,4-Oxazepanes were chosen as a 7-membered ring to build a library around because of their potential to be synthesised from amino acids. Additionally, they are a privileged structure, having many biological effects including antifungal, anti-viral, anti-congestive heart failure, and acting as apoptotic agents (Figure 3.19).

Figure 3.19 A selection of biologically active compounds containing a 1,4-oxazepane ring (highlighted in red).

Amino alcohols were chosen as a good starting material for 1,4-oxazepane synthesis as they are easily derived from the large amino acid pool and can carry intrinsic stereochemistry. The 1,4-oxazepane could then be built by reacting an amino alcohol with a three-carbon linker. This could then be furnished with an exo-cyclic olefin on which a range of pericyclic reactions could be paired to create the spirocycle library (Scheme 3.45).
Scheme 3.45 Outline of DOS 1,4-oxazepane spirocyclic library. \( R_1 \) represents a range of functionality from commercially available amino acids and amino alcohols. \( X \) is a functionality that can be manipulated during the couple stage to reveal an exo-cyclic olefin. Finally, the range of dienes and dipoles can be used to form the spirocycles in the pair stage.

Initially \( N\)-Boc-ethanolamine (250) was chosen as a starting material as it lacked the \( \alpha \)-pendent group found in all other amino alcohols derived from the naturally occurring amino acids. This was pre-treated with exactly two equivalents of sodium hydride before reaction to 1,3-dichloroacetone (251); however, NMR of the crude reaction mixture did not show the desired product (252, Scheme 3.46).

Scheme 3.46 Attempted formation of 1,4-oxazepane ring with 1,3-dichloroacetone.

The same reaction was repeated with \( L \)-prolinol (254) to form a condensed spirocycle and to explore the amino acid pool as a source for 1,4-oxazepane formation. The prolinol (254) was easily synthesised from \( L \)-proline (253) via literature reduction.\(^{210}\) Again the 1,4-oxapene synthesis was repeated, however this failed to yield the desired product in either DMF or THF as solvents (Scheme 3.47).

Scheme 3.47 Repeat of 1,4-oxazepane synthesis with prolinol.
Wondering whether the 1,3-dichloroacetone was active enough to react with the amino alcohol, it was iodinated to 1,3-diiodoacetone (256) via the Finkelstein reaction. This was then reacted with prolinol and N-benzyl-ethanolamine, as the fluorescent benzene ring may aid in detection during reaction monitoring and purification. In this case a weaker base, potassium carbonate, was used as it was thought that sodium hydride may have been too strong and causing enolization of 1,3-dichloroacetone. The reaction with prolinol did not produce the desired product (257). However, the reaction with N-benzyl-ethanolamine produced an NMR spectrum of the crude product seemed to suggest 1,4-oxazepane 258 had been produced. Attempted purification could not get the small quantity of material clean enough to confirm its synthesis (Scheme 3.48).

![Scheme 3.48 1,4-Oxazepane synthesis with 1,3-diiodoacetone, showing the attempted reaction been prolinol and N-Boc-ethanolamine.](image)

Removal of the ketone from the coupling partner was considered for two reasons. One, the presence of base and/or deprotonated alcohols and amines may cause competing enolization; two, the ketone itself it susceptible to nucleophilic attack. Therefore, the haloacetones were replaced with 1,3-dichloropropanol (259). Upon reaction with N-benzyl-ethanolamine no discernible product (260) was seen. However, a small amount (<5%) of 1,4-oxazepane 261 was believed to have been identified by NMR spectroscopy; however, like before, this couldn’t be isolated cleanly enough or in high enough yield to progress further in the chemistry (Scheme 3.49).
3.5 Pericyclic spirocycle libraries

Scheme 3.49 1,4-Oxazepane synthesis with 1,3-dichloroisopropanol, showing the attempted reaction between prolinol and N-Boc-ethanolamine.

Additionally, a TBS-protected analogue of 1,3-dichloropropanol, compound \(262\), was submitted to the same coupling conditions with \(N\)-benzyl-ethanolamine in case the presence of the free alcohol in \(259\) prevented coupling. However, this did not yield \(263\) (Scheme 3.50).

Scheme 3.50 TBS-protection of 1,3-dichloroisopropanol before attempted 1,4-oxazepane.

It was then examined whether a functional group other than a ketone or alcohol could be used in the coupling partners. A terminal alkene could also be used. This might be transformed into a ketone via the Lemieux–Johnson oxidation or submitted for pericyclic reactions itself.

Therefore, 1,3-dichloroisobutene (\(264\)) was selected as a coupling partner. This was reacted with \(N\)-Boc-ethanolamine (\(250\)) under sodium hydride as a base and DMF solvent. This did produce a 1,4-oxazepane ring however, in the process, the nitrogen had its Boc-group removed and the olefin isomerised to an endo position within the ring (\(265\), Scheme 3.51).

Scheme 3.51 Synthesis of 1,4-oxazepane with endo-cyclic olefin.

The reaction was repeated with prolinol under similar conditions with THF as solvent. This time a 1,4-oxazepane ring was formed with the desired exo-cyclic olefin (\(266\)) albeit in a disappointing 38% yield (Scheme 3.52).
Scheme 3.52 Synthesis of 1,4-oxazepane, 266, from prolinol and 1,3-dichloroisobutene.

With the first 1,4-oxazepane in hand, its oxidation was explored to transform the alkene into a ketone so that the Wittig reaction could be exploited to allow functionalised alkenes. Initially a Lemieux-Johnson oxidation was tried. Unfortunately, this reaction only returned 15\% of the starting material and no product (Entry 1, Table 3.7). Therefore, the reaction was repeated with 20\% more catalyst and at a slightly lower concentration. Again, none of the desired product (267) was produced (Entry 2, Table 3.7). Finally, the sodium metaperiodate equivalents were doubled so that this key oxidising agent was in excess. This did not yield the product either (Entry 3, Table 3.7). As can be seen from the \textsuperscript{1}H NMRs (Figure 3.20), entries 2 and 3 gave the same product. In both cases, the alkene CH\textsubscript{2} peaks were still present as well as the other peaks found in the starting material. The new peaks could not be assigned, but all appeared in the region of the peaks for the 1,4-oxazepane ring.

Therefore, a different metallic oxidising agent was tried. A literature-based ruthenium (III) chloride oxidation was tried with Oxone acting the co-oxidising agent.\textsuperscript{211} Finally, the ruthenium (III) chloride oxidation was repeated with sodium metaperiodate as the co-oxidant, but to no avail.
### Table 3.7 Conditions for attempted oxidation of terminal alkene to ketone.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OsO₄ (2.5% in tBuOH, 0.5 mol%), NaIO₄ (2.0 eq), Dioxane (0.3 M), H₂O (0.3 M), 18 h, RT</td>
<td>15% SM retuned</td>
</tr>
<tr>
<td>2</td>
<td>OsO₄ (2.5% in tBuOH, 0.6 mol%), NaIO₄ (2.0 eq), Dioxane (0.25 M), H₂O (0.25 M), 18 h, RT</td>
<td>21% by mass of by-product</td>
</tr>
<tr>
<td>3</td>
<td>OsO₄ (2.5% in tBuOH, 0.6 mol%), NaIO₄ (4.0 eq), Dioxane (0.25 M), H₂O (0.25 M), 18 h, RT</td>
<td>27% by mass of by-product</td>
</tr>
<tr>
<td>4</td>
<td>RuCl₃ (3.5 mol%), Oxone (2.5 eq), NaHCO₃ (7.8 eq), MeCN/H₂O (5:3, 0.02 M), 30 min, RT</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>5</td>
<td>RuCl₃ (3.5 mol%), NaIO₄ (2.0 eq), DCE/H₂O (5:4, 0.1 M), 2H, RT</td>
<td>Complex mixture</td>
</tr>
</tbody>
</table>

### Figure 3.20

$^1$H NMR from attempted Lemieux-Johnson oxidation of 266. The red spectrum is the $^1$H NMR of purified 266. The green, blue, and purple spectra are the purified product of entries 1, 2, and 3 respectively in Table 3.7.
Finally, in an attempt to form a spirocycle with 1,4-oxazepane 266, and questioning the reactivity of the alkene through the attempted oxidations, it was decided to react 266 with a carbene to undergo a [1+2] carbene insertion into a double bond. Thus, a literature procedure of rhodium (II) acetate mediated ethyl diazo acetate carbene generation was used to cyclopropanate the olefin in 266. Despite a couple of attempts, only the carbene dimer (diethyl maleate) was isolated cleanly enough.

![Scheme 3.53 Reaction between 266 and ethyl diazo acetate.](image)

Due to the difficulties in constructing the 1,4-oxazepane ring and the problems in functionalising the ring when constructed, it was decided to abandon this line of research and focus on analysing the libraries already constructed.

### 3.6. Expansion and analysis of libraries

#### 3.6.1. Enumeration and decoration libraries

The synthesis of the libraries above only represents a proportion of the scope of the chemical space the chemistry could probe. The full extent of this space can be appreciated by enumerating and decorating the libraries synthesised. In this context, enumeration means the combination of chemistry to form different scaffolds. This would be regarded as the SC[CPD] in Table 3.2. Decoration is the addition of appendages onto the SC[CPD] after it has been synthesised.

In the glycine-based spirocyclic library, only the six-membered cyclohexene tail ring was used. However, work by Attila Sveiczer also synthesised cyclopentene and cycloheptene tail rings. Additionally, he performed some double bond modifications (Figure 3.21).
Figure 3.21 Summary of the tails and modifications synthesised by Sveiczer.

It is not necessary to synthesise each of these tails with each of the warheads as the modular nature of the chemistry means each can be made independently of one another. Therefore, a library can be constructed wherein all the spirocycles could be synthesised.

A first-generation library (1GLY) was computationally enumerated combining all the synthesised warheads (as detailed in section 3.4), with all the tails synthesised by Sveiczer (Figure 3.21). This is the library with the highest synthetic confidence.

A second-generation library was also enumerated (2GLY). This extended the scope of the tails by predicting that all the alkene modifications made to the cyclohexene ring would also be successful on the cyclopentene and cycloheptene rings. Additionally, a cycloheptene isomer with modification was also included in this library (Figure 3.22).

Figure 3.22 A diagram showing the component parts of libraries 1GLY and 2GLY. 1GLY is necessarily a sub-library of 2GLY.
The properties of these two libraries were analysed and compared to indicators of fragment-likeness (RO3) and likely success in biological screening.

Table 3.8 Comparison of the two enumerated libraries, 1GLY and 2GLY, showing the average value of the properties with the standard deviation in parenthesis. The 3/75 Rule finds that a compound with a clogP < 3, and a PSA of < 75 Å² is 2.5 times less likely to be toxic at 10 μM. The value in the table is the percentage of the library which obey the rule.

<table>
<thead>
<tr>
<th>Property</th>
<th>Rule of Three²¹</th>
<th>1GLY</th>
<th>2GLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (gmol⁻¹)</td>
<td>&lt; 300</td>
<td>231.42 (73)</td>
<td>242.77 (75.6)</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 3</td>
<td>0.53 (1.0)</td>
<td>0.52 (1.1)</td>
</tr>
<tr>
<td>Polar Surface Area (Å²)</td>
<td>≤ 60</td>
<td>57.7 (23)</td>
<td>60.7 (23)</td>
</tr>
<tr>
<td>H-Bond Acceptors</td>
<td>≤ 3</td>
<td>3.9 (1.2)</td>
<td>4.1 (1.3)</td>
</tr>
<tr>
<td>H-Bond Donors</td>
<td>≤ 3</td>
<td>1.3 (0.8)</td>
<td>1.3 (0.8)</td>
</tr>
<tr>
<td>Rotatable Bond Count</td>
<td>≤ 3</td>
<td>0.80 (1.1)</td>
<td>0.81 (1.1)</td>
</tr>
<tr>
<td>3/75 Rule²³</td>
<td>—</td>
<td>74%</td>
<td>70%</td>
</tr>
</tbody>
</table>

As can be seen from Table 3.8, the mean properties of the libraries broadly fall within the RO3. As expected, the mean value of the molecular weight is higher for 2GLY because two types of cycloheptene ring were allowed in the enumeration. The two properties which may cause some concern are the PSA and H-bond acceptors. These are only just in range or slightly violate the RO3. The high PSA is due to the presence of bromides and tosyl groups in some compounds. The presence of the tosyl groups also explains the high mean of H-bond acceptors. However, as can be seen from Figure 3.23, most of the compound which contributes to the high PSA fall outside the RO3 limits on molecular weight and clogP. This indicates that the compounds in the libraries either have properties which all generally fall into the RO3 or all fall outside the RO3.
Figure 3.23 Plot of clogP against molecular weight for libraries 1GLY and 2GLY. The shading of the points also indicates the value of the PSA.
For the next level of enumeration of the glycine-based library, different reagents can be explored at key points in the synthesis. This will have a lower confidence level as the proposed reactions will not have been tried on the substrates in the laboratory, however, use of the literature can guide the viability of the reactions. Examination of the synthesis of glycine library can show where enumeration points occur.

The advantages of this synthetic approach are evident in the multiple vectors of elaboration. The modular installation of the terminal alkene moieties allows early substitution on the tail ring (Figure 3.24). Consideration here would have to be given to the compatibility of the groups with the S\text{N}2 installation, acidic removal the benzophenone, and Grubbs II RCM.

![Figure 3.24](image)

*Figure 3.24* Installation of five possible vectors for further fragment development by modular installation of the alkene moieties.

Additionally, the warheads leave ample vectors for decoration and elaboration (Figure 3.25).

![Figure 3.25](image)

*Figure 3.25* Elaboration vectors on the warheads. The colours represent the possible reactions to elaborate the vectors. Red: alkylation/acylation by nucleophilic N. Blue: Addition from nucleophilic enolate formation. Green: Metal-mediated cross coupling. Purple: S\text{N}2. Yellow: Grignard-type nucleophilic-reduction of ester to alcohol. Orange: Imidate condensation.

A sample of the possible decoration vectors can be explored and assessed computationally. Using the open access LLAMA programme,\textsuperscript{212} the synthesised warheads with a cyclohexene tail were decorated with a variety of reliable reactions (see appendix). This gave a decorated library GLY-DEC1. This can give an indication about the properties of the chemical space around 1GLY and 2GLY.
Figure 3.26 Plot of clogP against molecular weight GLY-DEC1 library. The shading of the points also indicated the value of the PSA.

As can be seen from Figure 3.26, the library still has favourable physico-chemical properties and most of the molecules are either in RO3 limits or are moving more into lead-like RO5 space. This is promising for further development of the library.

Next, the dehydroalanine library was enumerated. This involved the synthesis of intermediate 202. This was successfully reacted with a nitrile oxide to form a dihydroisoxazole ring, and the Boc-protected N and ester were closed to give a carbamate warhead (Scheme 3.54).
This allows for two areas of elaboration: the amino and ester closure, and the pendent group on the nitrile oxide. The existence in the literature of the dihydroisoxazole ring with a geminally disposed primary amine and ester (270) means that there is reasonable confidence that the warheads enumerated in the 1GLY library could also be enumerated here (Figure 3.27).

To complement this, a small range of imidates were chosen as nitrile oxide precursors for dihydroisoxazole ring formation. These were selected to represent a range of functionalities: aromatic, heterocyclic, cyclic, and aliphatic.

Therefore, a library was enumerated combining the warheads with an isoazole ring substituted with the four groups in Figure 3.28. This gave library DEHAL (Figure 3.29).
The physico-chemical properties of this library are summarised in Table 3.9 and Figure 3.30. Pleasingly, this library seems to be reasonably fragment-like. Unfortunately, this library suffers from high PSA and H-bond donors for a fragment collection. This is down to the isoazole ring containing two heteroatoms which increases both metrics.

**Table 3.9** Comparison of DEHAL library, showing the average value of the properties with the standard deviation in parenthesis.

<table>
<thead>
<tr>
<th>Property</th>
<th>Rule of Three$^{71}$</th>
<th>DEHAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (gmol$^{-1}$)</td>
<td>&lt; 300</td>
<td>233.88 (54)</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 3</td>
<td>1.20 (0.9)</td>
</tr>
<tr>
<td>Polar Surface Area (Å$^2$)</td>
<td>≤ 60</td>
<td>69.7 (17.7)</td>
</tr>
<tr>
<td>H-Bond Acceptors</td>
<td>≤ 3</td>
<td>5.4 (1.0)</td>
</tr>
<tr>
<td>H-Bond Donors</td>
<td>≤ 3</td>
<td>1.2 (0.6)</td>
</tr>
<tr>
<td>Rotatable Bond Count</td>
<td>≤ 3</td>
<td>1.5 (1.1)</td>
</tr>
<tr>
<td>3/75 Rule$^{23}$</td>
<td>—</td>
<td>75%</td>
</tr>
</tbody>
</table>
Finally, a small compound library was constructed from the azetidinone series. This took the two key reactions established in the research (the nitrile oxide cycloaddition and the azide cycloaddition) and extended them for four nitrile oxide (the ones given in Figure 3.28) and four azides (again the same appendages as in Figure 3.28 but a benzyl group in lieu of the phenyl one). These were computationally-coupled with two azetidinone derivatives: the \( \alpha,\beta \)-unsaturated ethyl ester as used in the chemistry, above and the electronically similar \( \alpha,\beta \)-unsaturated nitrile. This gave the AZE library.

As with the DEHAL library, most of the properties are fragment-like yet there is the slight problem of the high PSA and number of H-bond acceptors. Perhaps a way to minimise this in
these libraries would be to use 1,3-dipoles or dienes with fewer heteroatoms (Table 3.10 and Figure 3.32).

**Table 3.10** Comparison of AZE library, showing the average value of the properties with the standard deviation in parenthesis.

<table>
<thead>
<tr>
<th>Property</th>
<th>Rule of Three$^{71}$</th>
<th>DEHAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (gmol⁻¹)</td>
<td>&lt; 300</td>
<td>214.00 (38)</td>
</tr>
<tr>
<td>clogP</td>
<td>≤ 3</td>
<td>-0.03 (1.1)</td>
</tr>
<tr>
<td>Polar Surface Area (Å²)</td>
<td>≤ 60</td>
<td>64.86 (6.4)</td>
</tr>
<tr>
<td>H-Bond Acceptors</td>
<td>≤ 3</td>
<td>5.3 (1.0)</td>
</tr>
<tr>
<td>H-Bond Donors</td>
<td>≤ 3</td>
<td>1.3 (0.9)</td>
</tr>
<tr>
<td>Rotatable Bond Count</td>
<td>≤ 3</td>
<td>2.3 (1.6)</td>
</tr>
<tr>
<td>3/75 Rule$^{23}$</td>
<td>−</td>
<td>88%</td>
</tr>
</tbody>
</table>

**Figure 3.32** Plot of clogP against molecular weight GLY-DEC1 library. The shading of the points also indicates the value of the PSA.
3.6.2. Spatial diversity of libraries

As well as analysing the raw physico-chemical properties of these libraries, the spatial diversity can also be measured by plotting PMI graphs and the diversity of the physico-chemical data can be measured by PCA graphs.

Computational analysis of the above libraries was performed using Molecular Operating Environment (MOE) software version 2016.0802 from the Chemical Computing Group.

The computational parameters of the PMI analysis and PCA are given in Appendix C.\(^c\)

The above libraries (1GLY, 2GLY, GLY-DEC1, DEHAL, and AZE) were evaluated and compared to small molecules in the top 50 selling pharmaceuticals of 2013\(^{214}\) (Structures in Appendix C).

Merck molecular force field 94X (MMFF94x), an all-atom force field parameterised for small organic molecules with the Generalised Born solvation model, was used to minimise the energy potential of the library members. A Low Mode MD search was employed for the conformation generation.

Since crystal structures were obtained for four of the spirocycles, a visual comparison was first performed. The structures are shown in Table 3.11 and are seen to be visually similar. The conformationally constrained nature of spirocycles means that computational models are more likely to be reliable in predicting conformation.

\(^c\) The procedure followed to carry out PMI analysis and PCA is based on the procedure developed by Dr Feilin Nie
3.6 Expansion and analysis of libraries

Table 3.11 Comparison of the computational lowest energy conformation and crystal structures.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Lowest Energy Confirmation</th>
<th>Crystal structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Conformation" /></td>
<td><img src="image3" alt="Crystal" /></td>
</tr>
<tr>
<td><img src="image4" alt="Structure" /></td>
<td><img src="image5" alt="Conformation" /></td>
<td><img src="image6" alt="Crystal" /></td>
</tr>
<tr>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Conformation" /></td>
<td><img src="image9" alt="Crystal" /></td>
</tr>
<tr>
<td><img src="image10" alt="Structure" /></td>
<td><img src="image11" alt="Conformation" /></td>
<td><img src="image12" alt="Crystal" /></td>
</tr>
</tbody>
</table>

The conformers of each library member were calculated, with the lowest energy conformer assigned a relative energy value of 0 kJmol\(^{-1}\). For each conformer, the principle moments of inertia (\(I_1, I_2, I_3\)) were calculated and then normalised to (\(I_1/I_3\)) and (\(I_2/I_3\)). These values were then plotted on a triangle graph, with the three vertices at (0,1), (0.5,0.5), and (1,1) representing the three 3D extremes of rod-like, disk-like, and sphere-like respectively. The lowest energy conformation is plotted in Figure 3.33.
Figure 3.33 PMI plot showing the spatial diversity of compound libraries. Each point on the plot represents a compound in the library at its lowest energy conformation as calculated by MOE. 30 top selling drugs (purple), 168 1GLY library (light blue), 408 2GLY library (red), 61 GLY-DEC1 library (orange), 52 DEHAL library (green), 16 AZE library (dark blue). x-axis $I_1/I_3$, y-axis $I_2/I_3$.

As can be expected, the spirocycles predominantly possess rod-like features, with most clustering around the top-left of the PMI plot. Pleasingly, the range of compounds seem to have escaped ‘flat-land’, the rod-disk line. By inspection of the plot it seems that the appendage decorated library, GLY-1DEC, increased the spatial spread of the spirocycles incorporating more sphere properties.

Fortunately, the cumulative frequency of the compounds can be measured as a function of their distance from the rod-disk axis. This allows for easier inspection of how ‘flat’ the library is and how much of the chemical space it covers (Figure 3.34).
3.6 Expansion and analysis of libraries

Figure 3.34 Cumulative frequency graph of compound distance from rod-disk axis. This allows easy visualisation of the diversity of compound libraries on the PMI plot. The steeper the gradient of the lines, the less chemical space the library explores. The maximum distance a compound can be form the rod-disk axis is $1/\sqrt{2} \approx 0.71$.

The least spatially diverse library is AZE, probably due to the second ring being limited to either an isoazole to 1,2,3-triazole. Despite allowing for more possible ring combinations between 1GLY and 2GLY, the libraries occupy a broadly similar chemical space. The AZE library is slightly further from flat land, however the steeper gradient of the line indicates it displays less spatial diversity. Finally, and most interestingly, there is a strong similarity between the chemical space occupied by GLY-DEC1 and the marketed drugs. The core fragment scaffolds being small, appendages can make a large difference to the spatial diversity of the library. This simple modification of the library is likely to bring it into biologically relevant space.

The lowest energy conformer is only part of the picture because a molecule’s conformation can change in solution or find favourable interactions on a biologically target. Therefore, the energy window was expanded to 3 kJmol$^{-1}$. Since $\Delta G = -RT\ln(k)$ with an energy window of $\Delta G = 3$ kJmol$^{-1}$ k $\approx 0.3$, so the top 70% lowest energy conformers are included.
Figure 3.35 PMI plots of the conformers of the five compound libraries compared to the drugs reference set. a) Energy level cut of at 0 kJmol⁻¹ (lowest energy conformer); b) energy level cut of at 1 kJmol⁻¹; c) energy level cut of at 2 kJmol⁻¹; d) energy level cut of at 3 kJmol⁻¹.

As can be seen in Figure 3.35, as the energy threshold is increased the marketed drugs start to cluster along the rod-disk axis, whereas the GLY-DEC1 library pleasingly spreads out and occupies more chemical space. The DEHAL and AZE libraries begin to cluster; DEHAL into the top left rod-like area and AZE trending more towards disk-like features. The 1GLY and 2GLY library spread out a reasonable distance from the rod-disk axis. A clearer illustration of this comparison can be seen in Figure 3.36 where each component of Figure 3.35d is separated.
3.6 Expansion and analysis of libraries

Figure 3.36 PMI plot of each DOS library at 3 kJmol$^{-1}$ threshold.
Overall, the different spirocyclic libraries integrated a large range of chemical space. Most interesting was the GLY-DEC1 library which showed that appendage growth from these scaffolds creates spatially diverse compounds. All the libraries avoided ‘flat land’, the region of chemical space which seems to be exhausted for novel chemical leads. This gives confidence that these libraries possess the necessary spatial qualities for further exploration.

3.6.3. Principle component analysis of libraries

For each compound based on its lowest energy conformation, seventeen structural and physico-chemical descriptors were selected. These ranged from solubility, weight, H-bond acceptors, PSA, etc. Due to the impossibility of visualising these data in 17-dimensions, principle component analysis was employed. Each of the 17-dimensional vectors were reduced to 2-dimensional vectors by linear transformation. A linear combination of the descriptors was calculated to represent as much of the variance in the data as possible. This gave seventeen unitless principle components. Each compound represented by a 2-dimensional vector was plotted on a scatter chart to visualise the extent of chemical space spanned by the libraries. To keep 95% of the variance original data set, seven principle components were retained (Table 3.12).

<table>
<thead>
<tr>
<th>PC#</th>
<th>Deviation(^a)</th>
<th>Condition(^b)</th>
<th>Proportion of Variance</th>
<th>% Variance(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>2.798</td>
<td>1.000</td>
<td>46.037</td>
<td>46.037</td>
</tr>
<tr>
<td>PC2</td>
<td>1.899</td>
<td>2.170</td>
<td>21.214</td>
<td>67.251</td>
</tr>
<tr>
<td>PC3</td>
<td>1.300</td>
<td>4.629</td>
<td>9.944</td>
<td>77.195</td>
</tr>
<tr>
<td>PC4</td>
<td>1.049</td>
<td>7.116</td>
<td>6.47</td>
<td>83.665</td>
</tr>
<tr>
<td>PC5</td>
<td>0.914</td>
<td>9.378</td>
<td>4.909</td>
<td>88.574</td>
</tr>
<tr>
<td>PC6</td>
<td>0.888</td>
<td>9.916</td>
<td>4.643</td>
<td>93.217</td>
</tr>
<tr>
<td>PC7</td>
<td>0.682</td>
<td>16.810</td>
<td>2.738</td>
<td>95.955</td>
</tr>
</tbody>
</table>

The first three principle components, accounting for 77% of the variance of the data set, were plotted onto three scatter graphs (Figure 3.37).
3.6 Expansion and analysis of libraries

Figure 3.37 PCA plots of each spirocyclic library against the drugs data set. a) PC1 v PC2, b) PC1 v PC3, c) PC2 v PC3.

Figure 3.37a shows the ‘Drugs’ library occupying a very broad and different range of chemical space compared to the spirocyclic libraries. The difference in breadth is likely down to the fact that the spirocyclic libraries were restricted to a spirocyclic core and were only fragment-like. Again, the differences are highlighted between the 1GLY, 2GLY, and GLY-DEC1 with different areas of the grid occupied.
In Figure 3.37b, the 1GLY and 2GLY libraries seem to cover approximately the same chemical space as in Figure 3.37a. However, this time the libraries are more differentiated. Moving from left to right along the x-axis 2GLY gives way to DEHAL and AZE, with AZE further up the y-axis. Next comes GLY-DEC1, most of whose members are in the positive region of the x-axis. Finally, the drugs represent their diversity by scattering about the first quadrant.

Figure 3.37c again shows the range of chemical space explored by the spirocycles with each library integrating different areas of space. 2GLY also demonstrates its larger physcio-chemical diversity by being represented below the x-axis. Interestingly the DEHAL library is quite diverse under these conditions, having components in quadrants I, II, and IV.

The transformation matrix of the first seven principle components (95% of the total variance) is shown in Table 3.13. This suggests that increasing the number of rings and the number of nitrogens the molecular flexibility would shift the molecules in a positive direction along the x-axis (more into the chemical space occupied by the drugs).

The most important contributors to PC2 were the number of H-bond donors and acceptors and the number of oxygen atoms. Increasingly these should shift the libraries positively along the PC2 axis, whereas increasing the number of rings would have the opposite effect.

Finally, PC3 indicates that the number of nitrogen atoms, the solubility in water, and the number of H-bond acceptors would move the spirocyclic libraries up along the PC3 axis.
3.7 Conclusions

Table 3.13 Component loadings for the PCA of the six compound libraries. The top six contributing parameters to each principle component are highlighted in grey. All values are normalised.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>PC6</th>
<th>PC7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA_H</td>
<td>0.0011</td>
<td>-0.0011</td>
<td>0.0020</td>
<td>-0.0020</td>
<td>0.0015</td>
<td>0.0015</td>
<td>0.0030</td>
</tr>
<tr>
<td>ASA_P</td>
<td>0.0004</td>
<td>0.0033</td>
<td>-0.0059</td>
<td>0.0048</td>
<td>0.0059</td>
<td>-0.0051</td>
<td>-0.0076</td>
</tr>
<tr>
<td>KierFlex</td>
<td>0.0708</td>
<td>0.0568</td>
<td>-0.1725</td>
<td>0.1722</td>
<td>-0.0880</td>
<td>0.1523</td>
<td>0.4722</td>
</tr>
<tr>
<td>SlogP</td>
<td>0.0680</td>
<td>-0.1347</td>
<td>-0.1280</td>
<td>0.1404</td>
<td>-0.0639</td>
<td>0.0846</td>
<td>-0.0863</td>
</tr>
<tr>
<td>TPSA</td>
<td>0.0023</td>
<td>0.0108</td>
<td>0.0079</td>
<td>-0.0003</td>
<td>-0.0001</td>
<td>-0.0044</td>
<td>-0.0113</td>
</tr>
<tr>
<td>Weight</td>
<td>0.0015</td>
<td>0.0005</td>
<td>-0.0024</td>
<td>0.0006</td>
<td>0.0005</td>
<td>-0.0009</td>
<td>0.0004</td>
</tr>
<tr>
<td>a_acc</td>
<td>0.0523</td>
<td>0.1495</td>
<td>0.1231</td>
<td>-0.0949</td>
<td>-0.0891</td>
<td>-0.1786</td>
<td>0.1970</td>
</tr>
<tr>
<td>a_aro</td>
<td>0.0280</td>
<td>-0.0229</td>
<td>0.0413</td>
<td>-0.0104</td>
<td>-0.0260</td>
<td>0.0332</td>
<td>-0.1695</td>
</tr>
<tr>
<td>a_don</td>
<td>-0.0092</td>
<td>0.1924</td>
<td>0.1552</td>
<td>0.0816</td>
<td>0.1762</td>
<td>0.9748</td>
<td>-0.5035</td>
</tr>
<tr>
<td>a_nN</td>
<td>0.0712</td>
<td>-0.0065</td>
<td>0.3321</td>
<td>0.3013</td>
<td>0.7741</td>
<td>-0.2422</td>
<td>0.2015</td>
</tr>
<tr>
<td>a_nO</td>
<td>0.0326</td>
<td>0.1818</td>
<td>0.0188</td>
<td>-0.1776</td>
<td>-0.3656</td>
<td>-0.1868</td>
<td>-0.1056</td>
</tr>
<tr>
<td>b_rotN</td>
<td>0.0523</td>
<td>0.0192</td>
<td>0.0522</td>
<td>0.1314</td>
<td>-0.0406</td>
<td>0.0575</td>
<td>0.1366</td>
</tr>
<tr>
<td>chiral</td>
<td>0.0261</td>
<td>0.0624</td>
<td>-0.2553</td>
<td>-0.5431</td>
<td>0.4069</td>
<td>0.2576</td>
<td>0.2794</td>
</tr>
<tr>
<td>logS</td>
<td>-0.0752</td>
<td>0.0440</td>
<td>0.1579</td>
<td>-0.0122</td>
<td>0.0867</td>
<td>0.0335</td>
<td>0.4598</td>
</tr>
<tr>
<td>mr</td>
<td>0.0633</td>
<td>-0.0119</td>
<td>0.0080</td>
<td>-0.0067</td>
<td>-0.0079</td>
<td>0.0329</td>
<td>0.0467</td>
</tr>
<tr>
<td>rings</td>
<td>0.1077</td>
<td>-0.1095</td>
<td>0.1183</td>
<td>-0.4540</td>
<td>0.3349</td>
<td>-0.3067</td>
<td>-0.3722</td>
</tr>
<tr>
<td>vol</td>
<td>0.0019</td>
<td>-0.0002</td>
<td>0.0000</td>
<td>-0.0002</td>
<td>0.0002</td>
<td>0.0012</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

Principle component analysis has shown that the different libraries integrate different areas of physico-chemical space, with the 2GLY library having the broadest reach. This analysis can also aid in the further refinement of the libraries to ensure the greatest amount of diversity.

3.7. Conclusions

Herein, four synthetic strategies have been employed to synthesise diverse fragment libraries based on spirocyclic compounds. The first of these was based upon a tetra-functionalised glycine derivative which was subjected to the B/C/P paradigm. A range of chemistry was employed to join the amino and ester functionalities to form a ‘warhead’ ring, while RCM formed a cyclohexene tail. This approach yielded thirteen novel spirocyclic scaffolds.

Next, pericyclic chemistry was explored to form spirocycles. Trying to utilise the same amino-ester couplings as previously, a dehydroalanine derivative was synthesised. A range of approaches were used in trying to synthesise spirocycles from this compound. Forming the warhead first and then using pericyclic chemistry proved unsuccessful, but direct formation of the pericyclic ring on the dehydroalanine did work. This establishing the possibility of
using electron deficient 1,3-dipoles or dienes to form the ‘tail’ and using the established amino-ester couplings to form the warhead.

The use of pericyclic reactions was further explored by attempted Wittig installation of exo-cyclic olefins onto rare or privileged scaffolds. Azetidinone proved a good starting material for the installation of an electron deficient alkene which performed pericyclic reactions. On the other hand, 1,4-oxazepanes proved a synthetic challenge to make.

These libraries were enumerated computationally giving five libraries. The spatical and physico-chemical diversity of these libraries were measured and pleasingly they were found to possess descriptors which favour successful clinical candidates.
Chapter 4

Conclusions and future work

4.1. Conclusions

This project has focused on two key areas of the fragment-based drug discovery (FBDD) process: the design of fragments, and the elaboration of fragments to increase potency of potential inhibitors of CK2.

As part of the ongoing research to discover novel non-ATP competitive inhibitors of CK2, a lead fragment NMR154L emerged in the literature. Using NMR154L as a lead, this project has utilised novel chemistry, X-ray crystallography and computational analysis to improve the binding and specificity of the fragment which, in turn, could lead to improved CK2 inhibitors.

Screening libraries of fragments have historically failed to incorporate sufficient spatial diversity in their collections and this could be leading to higher attrition rates along the drug-discovery process. Methods were developed for the rapid assembly of novel spirocyclic fragments. Spirocycles—being two rings joined at a quaternary carbon—are inherently more 3-dimensional when compared to sp	extsuperscript{3}-rich aromatic ring dominated libraries.

In the first line of investigation, two terminal alkenes were installed on to glycine ethyl ester. Combining ring-closing metathesis and a range of amine and ester coupling, it was demonstrated that an initial library of 14 novel spirocycles could be synthesised. Expanding this methodology to different ring sizes could lead to a spirocyclic library of 168 novel compounds. A second approach demonstrated how pericyclic reactions could be used to make spirocycles. This used both amino acids as building blocks as well as rare and privileged heterocycles to expand the chemical space. Computational analysis of these libraries showed that they possessed the required spatial and physico-chemical properties to make them promising fragment libraries for future drug discovery.

Overall, this work has expanded novel fragment space by demonstrating how fragment hits can be improved by analysis of structure activity relationships, and how novel sp	extsuperscript{3}-rich diverse spirocyclic libraries can be quickly assembled with promising structural and physical properties.
Conclusions and future work

4.2. Future work

4.2.1. CK2 inhibition

Work in this project and in the wider Spring Group has led to the discovery of the led to the discovery CAM187, currently the most active fragment-like interface specific CK2 inhibitor. Due to the fragment nature of this molecule, there is ample room for development to improve on the compound’s 44 μM IC\textsubscript{50}.

4.2.2. Progressing spirocycle libraries

The novel spirocyclic libraries resulting from these investigations will be screened to determine whether there are any promising leads against biological targets.

Hence, a collaboration has been initiated with the Bender Group at the University of Cambridge. They have developed computational methods for screening large libraries against a myriad of known biological targets \textit{in silico}. The libraries 1GLY and 2GLY have already been submitted for computational analysis. It is hoped that any leads from this can be tested against the biological targets \textit{in vitro} to establish any tractable leads. These could be developed into potent inhibitors.

In addition to the \textit{in silico} analysis, the synthesised compounds may be screened against a large number of protein crystals at the Diamond synchrotron. This would give crystal structures of the spirocycles binding in protein pockets. These data could be applied to the identification of new active compounds with the potential of being developed into new medicines.

4.2.3. α-Allyl serine ethyl ester spirocycles

There is excellent scope to apply synthetic methods employed in the synthesis of the glycine-based cyclohexene spirocycles and the dehydroalanine spirocycles. Again, this would start from the serine ethyl ester. However, instead of using elimination chemistry to install an exocyclic double bond, a fourth orthogonal functionality can be added onto the α-carbon; in this case an allyl group. There is established literature chemistry for the diastereoselective insertion of the allyl group onto serine (Scheme 4.1).215
4.2 Future work

Scheme 4.1 Previous work installing an allyl group onto L-serine.

This work could be repeated to form compound 278, or the pivaldehyde could be substituted with acetaldehyde to decrease the selectivity of the allyl addition so that both enantiomers can be synthesised simultaneously.

With compound 278 in hand, there are three different pairs of combinations of functional groups which could be used to make spirocycles (Figure 4.1).

Figure 4.1 The functional groups in 278 may be combined in three pairs to produce novel spirocycles.

This could lead to the synthesis of a new spirocyclic library exploring more coupling chemistry, different ring sizes, and diverse functionality.
Chapter 5

Experimental

Reactions For reactions requiring anhydrous conditions, experiments were carried out in oven-dried glassware. Unless otherwise stated, all reactions were carried out under nitrogen atmosphere. Room temperature (RT) refers to ambient temperature. All temperatures below 0 °C are that of the external bath. Temperatures of 0 °C were maintained using an ice-water bath. Temperatures of −15 °C were maintained using a salt and ice-water bath. Temperatures of −78 °C were maintained using an acetone-cardice bath.

Solvents and reagents Solvents and commercially available reagents were dried and purified before use, where appropriate using standard procedures. Toluene, hexane, diethyl ether, ethyl acetate, methanol, THF, and dichloromethane were dried and distilled using standard methods from oxygen free from solvent dispenser units under an argon atmosphere.

Chromatography Analytical thin layer chromatography (TLC) was performed using pre-coated Merck glass backed silica gel plates (Silica gel 60 F254). Flash column chromatography was undertaken on Fluka or Material Harvest silica gel (230–400 mesh) under a positive pressure of nitrogen unless otherwise stated. Visualization was achieved using ultraviolet light (254 nm) and chemical staining with basic potassium permanganate solution as appropriate. Retention factors (R_f) are quoted to 0.01.

Infrared spectra Infrared (IR) spectra were recorded on a Perkin Elmer 1FT-IR Spectrometer fitted with an ATR sampling accessory as either solids or neat films, either through direct application or deposited in CDCl_3. Absorption maxima (ν_max) are reported in wavenumbers (cm⁻¹) with the following abbreviations: w, weak; m, medium; s, strong; br, broad.

NMR spectra Magnetic resonance spectra were processed using TopSpin v. 3.5 (Bruker). An aryl, quaternary, or two or more possible assignments were given when signals could not be distinguished by any means. Measured coupling constants (J) are reported for mutually coupled signals; coupling constants are labelled apparent in the absence of an observed mutual coupling, or multiplet when none can be determined.
Proton magnetic resonance spectra were recorded using an internal deuterium lock (at 298 K unless stated otherwise) on Bruker DPX (400 MHz; 1H-13C DUL probe), Bruker Avance III HD (400 MHz; Smart probe), Bruker Avance III HD (500 MHz; Smart probe) and Bruker Avance III HD (500 MHz; DCH Cryoprobe) spectrometers. Proton assignments are supported by \(^1\)H–\(^1\)H COSY, \(^1\)H–\(^{13}\)C HSQC or \(^1\)H–\(^{13}\)C HMBC spectra, or by analogy. Chemical shifts (\(\delta_H\)) are quoted in ppm to the nearest 0.01 ppm and are referenced to the residual non-deuterated solvent peak. Discernible coupling constants for mutually coupled protons are reported as measured values in Hertz, rounded to the nearest 0.5 Hz. Data are reported as: chemical shift, multiplicity (br = broad; s = singlet; d = doublet; t = triplet; q = quartet; qn = quintet; sp = septet; m = multiplet; or a combination thereof), coupling constants, number of nuclei, and assignment. Diastereotopic protons are assigned as X\(_a\) and X\(_b\), where X\(_b\) designates the lower-field proton.

Carbon magnetic resonance spectra were recorded using an internal deuterium lock (at 298 K unless stated otherwise) on Bruker DPX (101 MHz), Bruker Avance III HD (101 MHz) and Bruker Avance III HD (126 MHz) spectrometers with broadband proton decoupling. Carbon spectra assignments are supported by DEPT editing, \(^1\)H–\(^{13}\)C HSQC or \(^1\)H–\(^{13}\)C HMBC spectra, or by analogy. Chemical shifts (\(\delta_C\)) are quoted in ppm to the nearest 0.1 ppm and are referenced to the deuterated solvent peak. Data are reported as: chemical shift, number of nuclei (if not one), multiplicity (if not a singlet), coupling constants, and assignment.

Fluorine magnetic resonance spectra were recorded on Bruker Avance III (376 MHz; QNP Cryoprobe) or Bruker Avance III HD (376 MHz; Smart probe) spectrometers. Chemical shifts (\(\delta_F\)) are quoted in ppm to the nearest 0.1 ppm. Data are reported as: chemical shift, number of nuclei (if not one), multiplicity (if not a singlet), coupling constants and assignment.

The numbering of molecules used for \(^{13}\)C and \(^1\)H NMR assignments does not conform to IUPAC standards.

**Mass spectra** High-resolution mass spectra (HRMS) were measured on a Micromass LCT Premier spectrometer using electron spray ionization (ESI) techniques. Masses are quoted within the 5 ppm error limit.
**Melting points** Melting points were obtained on a Buchi B-545 melting point apparatus and are uncorrected.

**Optical rotations** Chiral products had their optical rotation recorded on an Anton-Paar MCP 100 polarimeter. $[\alpha]_D^{20}$ values are reported in $^\circ$g$^{-1}$cm$^{-2}$10$^{-1}$ at the sodium D-line of 598 nm, concentration (c) is given in g(100 mL)$^{-1}$ in the solvent stated.
Experimental

2,2′-(4,5-Dichloro-1,2-phenylene)bis(ethan-1-aminium) dichloride, 29

Compound 35 (60.6 mg, 0.14 mmol) was stirred in HCl/dioxane (4M, 5 mL) for 1 h. The mixture was filtered and the filtrate was washed with cold diethyl ether (5 mL). The isolated white crystals were placed in a desiccator overnight to yield the pure white crystalline product (39.0 mg, 0.13 mmol, 14% over two steps); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2936 (C-H, m), 1760 (C=O, s), 1499 (C=C, m). $^1$H NMR (400MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 8.26 (s, 6H, NH$_3$) 7.57 (s, 2H, H1) 3.00 (s, 8H, H4,5). $^{13}$C NMR (101 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 137.3 (C1 $\times$ 2) 131.8 (C2 $\times$ 2) 129.5 (C3$\times$2) 66.4 (C5$\times$2) 29.2 (C4$\times$2). HRMS (ESI) C$_{10}$H$_{15}$N$_2$Cl$_2$ m/z: [M+H]$^+$ 233.0600 (calc. 233.0607). m.p. 349-350 °C.

2,2′-(4,5-dichloro-1,2-phenylene)diacetonitrile, 30

To a solution of dibromide 31 (1.08 g, 3.24 mmol, 1.0 eq) in ethanol (21 mL) was added sodium cyanide (3.98 mg, 8.11 mmol, 2.5 eq) in water (7 mL) at 0 °C. The reaction was refluxed overnight. The excess solvent was removed in vacuo before being diluted with ethyl acetate (20 mL) and washed with water (20 mL x 2). The aqueous layer was extracted with ethyl acetate (30 mL $\times$ 3) and the combined organics were washed with brine (50 mL). This was dried over MgSO$_4$, filtered, concentrate and purified by column chromatography (25% EtOAc in Hexane) to give the pure product as a colourless oil (631 mg, 2.80 mmol, 86%); $R_f$ 0.50 (40% EtOAc in Hexane); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3029 (C-H, w), 2859 (C-H, w), 2252 (C≡N, m), 1481 (C=C, s), 682 (C-Cl, s). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 7.58 (s, 2H, H2), 3.74 (s, 4H, H4). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 133.8 (C1$\times$2), 131.7 (C2$\times$2), 128.1 (C3$\times$2), 115.5 (C5$\times$2), 21.1 (C4$\times$2). HRMS (ESI) C$_{10}$H$_{16}$N$_2$Cl$_2$Na m/z: [M+Na]$^+$ 246.9793 (calc. 246.9800).

The physical and spectroscopic data was found to be in agreement with Rosowsky et al.$^{216}$
1,2-Bis(bromomethyl)-4,5-dichlorobenzene, 31

Dialcohol 32 (550 mg, 2.65 mmol, 1.0 eq) was dissolved in diethyl ether (5 mL). Phosphorous tribromide (0.6 mL, 6.36 mmol, 2.4 eq) was added dropwise at 0 °C and the reaction was stirred overnight at RT. The reaction was poured into ice water (5 mL), the organic phase was separated and washed with brine (5 mL). This was dried over MgSO₄, filtered, concentrated and purified by column chromatography (10% CH₂Cl₂ in Hexane) to give the pure product as off white crystals (761 mg, 2.29 mmol, 86%); Rf 0.39 (10% CH₂Cl₂ in Hexane); IR (thin film) ν/cm⁻¹: 1588 (C=C, w), 1473 (C-H, m), 951 (C-Br, s), 683 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.82 (s, 2H, H2), 4.80 (s, 4H, H4). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 138.0 (C1 × 2), 133.3 (C2 × 2), 131.8 (C3 × 2), 29.6 (C4 × 2). HRMS (ESI) C₈H₆Cl₂Br₂Na m/z: [M+Na]+ 354.8099 (calc. 354.8088). m.p. 65–66 °C.

The physical and spectroscopic data was found to be in agreement with Levy et al.²¹⁷

(4,5-Dichloro-1,2-phenylene)dimethanol, 32

To a suspension of lithium aluminium hydride (600 mg, 15.7 mmol, 2.0 eq) in THF (10 mL) was added 34 (2.07 g, 7.9 mmol, 1.0 eq) dissolved in THF at 0 °C. The reaction was warmed to RT and stirred for 24 h. Following this, the reaction was cooled to 0 °C and water (0.6 mL), 15% NaOH (0.6 mL), and water (1.8 mL) were added and the mixture was stirred for 30 min. This mixture was filtered and washed with THF (10 mL). The excess solvent of the filtrate was removed in vacuo to yield the pure product (1.5043 g, 7.25 mmol, 92%); Rf 0.21 (20% diethyl ether/pet. ether); IR (thin film) ν/cm⁻¹: 3265 (O-H, br s), 2901 (C-H, w), 1483 (C=H, m), 1050 (C-O, s), 732 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.46 (s, 2H, H2), 4.68 (s, 4H, H4), 2.65 (s, 2H, H5). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 139.0 (C1 × 2), 132.0 (C3 × 2), 131.1 (C2 × 2), 62.9 (C4 × 2). HRMS (ESI) C₈H₆O₂Cl₂Na m/z: [M+Na]+ 228.9786 (calc. 228.9794).
Experimental

The physical and spectroscopic data was found to be in agreement with Di Lauro et al.\textsuperscript{218}

**Dimethyl 4,5-dichlorophthalate, 34**

To a solution of 4,5-dichlorophthalic acid (3 g, 12.76 mmol, 1.0 eq) in methanol (25 mL) cooled to 0 °C thionyl chloride (2.33 mL, 31.90, 2.5 eq) was added dropwise. The reaction was warmed to RT and stirred for 3 days. The mixture was quenched with sat. aq. NaHCO$_3$ and extracted with CH$_2$Cl$_2$. The organic layer was dried over MgSO$_4$, filtered and concentrated \textit{in vacuo}. The crude material was purified by column chromatography (50% diethyl ether in pet. ether) to give the pure product as a translucent crystal (3.0838 g, 11.7 mmol, 92%); \textbf{IR (thin film)} $\tilde{\nu}$/cm$^{-1}$: 3097 (C-H, w), 2957 (C-H, w), 1719 (C=O, s), 1592 (C=C, m), 1551 (C=C, m), 1430 (C=C, m), 1290 (C-O, s), 781 (C-Cl, s). \textbf{1H NMR} (400 MHz, CDCl$_3$) $\delta$/ppm: 7.81 (s, 2H, H2) 3.90 (s, 6H, H5).

\textbf{13C NMR} (101 MHz, CDCl$_3$) $\delta$/ppm: 161.0 (C4×2), 135.8 (C1×2), 131.3 (C3×2), 131.0 (C2×2), 52.3 (C5×2). \textbf{HRMS (ESI)} C$_{10}$H$_9$O$_4$Cl$_2$ m/z: [M+H]$^+$ 262.9866 (calc. 262.9872). \textbf{m.p.} 48–49 °C.

The physical and spectroscopic data was found to be in agreement with Hennessy et al.\textsuperscript{219}

**Di-tert-butyl ((4,5-dichloro-1,2-phenylene)bis(ethane-2,1-diyl))dicarbamate, 35**

Sodium borohydride (470.6 mg, 12.44 mmol, 14.0 eq) was added portion wise at 0 °C to a mixture of 30 (200 mg, 0.89 mmol, 1.0 eq), NiCl$_2$.6H$_2$O (42.2 mg, 0.18 mmol, 0.2 eq), and di-tert-butyl dicarbonate (0.82 mL, 3.55 mmol, 4.0 eq) in methanol (10 mL). The reaction was stirred at RT for 6 h. The reaction mixture was poured into ice water and the excess solvent
was removed in vacuo. This was diluted with ethyl acetate (20 mL) and washed with NaHCO₃ (20 mL). The aqueous was extracted with ethyl acetate (20 mL × 3). The combined organics were washed with brine, dried over MgSO₄, filtered, concentrated and purified by column chromatography (15% ethyl acetate in hexane) to give the pure product which was carried straight over to the next reaction; Rf 0.21 (50% Et₂O in pet. ether); IR (thin film) ν/cm⁻¹: 3326 (N-H, m), 2978 (C-H, w), 1678 (C=O, s), 1524 (C=C, s), 1136 (C-O, s), 780 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.24 (s, 2H, H2) 3.30 (q, J = 7.0 Hz, 4H, H5) 2.80 (t, J = 7.0 Hz, 4H, H4) 1.43 (s, 18H, H8). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 155.9 (C6 × 2), 137.6 (C1 × 2), 131.6 (C2×2), 130.3 (C3×2), 79.5 (C7×6), 41.1 (C5×2), 32.7 (C4×2), 28.3 (C8×2). HRMS (ESI) C₂₀H₃₁N₂O₄Cl₂ m/z: [M+H]⁺ 433.1644 (calc. 433.1661).

(4,5-Dichloro-1,2-phenylene)dimethanaminium dichloride, 38

Sodium borohydride (1.34 g, 35.353 mmol, 14.0 eq) was added portion wise at 0 °C to a mixture of dichlorodicyanobenzene (500 mg, 2.54 mmol, 1.0 eq), NiCl₂.6H₂O (120.6 mg, 0.51 mmol, 0.2 eq), and di-tert-butyl dicarbonate (2.33 mL, 10.15 mmol, 4.0 eq) in methanol (25 mL). The reaction was stirred at RT for 6h before the reaction was poured into ice. The mixture was extracted with ethyl acetate (3 × 20 mL) and washed with sat. aq. NaHCO₃ (20 mL) and brine (20 mL). This was dried over anh. MgSO₄, filtered and concentrated in vacuo. The crude oil was dissolved in 4M HCl/Dioxane (5 mL) and stirred for 1h. The reaction was filtered and the residue was washed with cold acetone to give the product as white crystals (241.3 mg, 0.87 mmol, 34%); IR (thin film) ν/cm⁻¹: 2847 (N-H, br s), 1498 (C=C, s), 1116 (C-N, s), 683 (C-Cl, m). ¹H NMR (400 MHz, (CD₃)₂SO) δ/ppm: 8.45 (br s, 6H, NH), 8.08–8.07 (m, 2H, H2), 4.21 (s, 4H, H4). ¹³C NMR (101 MHz, (CD₃)₂SO) δ/ppm: 136.7 (C1), 134.6 (C3), 132.0 (C2), 39.9 (C4). HRMS (ESI) C₈H₁₅Cl₂ m/z: [M+H]⁺ 205.0290 (calc. 205.0294). m.p. 277–278 °C.
2-(4-Chloronaphthalen-1-yl)ethan-1-aminium chloride, 39

To a suspension of lithium aluminium hydride (11 mg, 0.25 mmol, 2.0 eq) in diethyl ether (3 mL) under N₂ was added 45 (25 mg, 0.12 mmol, 1.0 eq) dissolved in diethyl ether (2 mL) dropwise. The reaction was stirred overnight. Water (25 μL) was added dropwise at 0 °C followed by 15% aq. NaOH (25 μL) and water (75 μL). This was warmed to RT and stirred for 15 min. The resulting mixture was diluted with diethyl ether (5 mL) and washed with brine (10 mL). The organics were dried over anh. MgSO₄, filtered and concentrated in vacuo to give the crude oil. This was stirred with 4M HCl in dioxane (5 mL) for 15 min and then filtered giving the residue as pure white-yellow crystalline product (20.1 mg, 0.08 mmol, 69%); IR (thin film) ν/cm⁻¹: 3382 (N-H, m), 3029 (C-H, m), 1500 (C=C, m), 758 (C-Cl, s).

¹H NMR (400 MHz, (CD₃)₂SO) δ/ppm: 8.27-8.21 (m, 2H, H₆&₉), 7.95 (br s, 3H, NH₃⁺), 7.76-7.71 (m, 2H, H₇&₈), 7.68 (d, J = 7.5 Hz, 1H, H₂), 7.43 (d, J = 7.5 Hz, 1H, H₃), 3.38-3.34 (m, 2H, H₁₁), 3.10 (br s, 2H, H₁₂). ¹³C NMR (101 MHz, (CD₃)₂SO) δ/ppm: 133.4 (C₄), 132.6 (Cl), 130.3 (C₅), 130.0 (C₁₀), 127.6 (C₇/₈), 127.5 (C₇/₈), 127.4 (C₃), 126.2 (C₂), 124.6 (C₆/₉), 124.4 (C₆/₉), 39.5 (C₁₂), 30.1 (C₁₁). HRMS (ESI) C₁₂H₁₃ClN m/z: [M]+ 206.0735 (calc. 206.0737). m.p. 270–271 °C.

1-Acetyl-4-chloronaphthalene, 41

Acetyl chloride (2.80 mL, 40.4 mmol, 1.1 eq) was dissolved in CH₂Cl₂ (150 mL) under N₂. Aluminium trichloride (5.68 g, 42.6 mmol, 1.16 eq) was added and stirred for 5 min before 1-chloronaphthalene (5 mL, 36.7 mmol, 1.0 eq) was added dropwise over 10 min. The reaction was refluxed for 1 h. The reaction was poured into a mixture of ice and conc. HCl. This was
extracted with diethyl ether (3 × 50 mL) and the ethereal layer was washed with brine. The combined organics were dried over MgSO₄. The mixture was filtered and concentrated in vacuo. The crude was purified by column chromatography (10% diethyl ether in pet. ether) to give the pure product as a yellow oil (6.0849 g, 29.73 mmol, 81%); R_s 0.28 (20% diethyl ether in pet. ether); IR (thin film) ν/cm⁻¹: 3005 (C-H, w), 1674 (C=O, s), 1565 (C=C, s), 1505 (C=C, s) 758 (C-Cl, s). ^1H NMR (400 MHz, CDCl₃) δ/ppm: 8.78–8.75 (m, 1H, H₆), 8.36–8.33 (m, 1H, H₉), 7.64 (d, J = 8.0 Hz, 1H, H₂), 7.68–7.63 (m, 2H, H₇&8), 7.59 (d, J = 8.0 Hz, 1H, H₃), 2.73 (s, 3H, H₁₂).

The physical and spectroscopic data was found to be in agreement with Wiley et al.¹²¹

4-Chloro-1-naphthoic acid, 42

Compound 41 (6.00 g, 29.32 mmol, 1.0 eq) was dissolved in pyridine (25 mL). To this iodine (4.09 g, 32.25 mmol, 1.1 eq) dissolved in pyridine (25 mL) was added dropwise and the reaction was refluxed for 40 min. The mixture was cooled to RT and diluted with diethyl ether until a brown precipitate formed. The precipitate was filtered and suspended in 6M NaOH. The mixture was refluxed for 2 h. Afterwards, it was cooled to RT and acidified with 3M HCl. This was extracted with diethyl ether (3 × 30 mL) and the combined organics were washed with brine (30 mL). The organics were dried over anh. MgSO₄ and the mixture was filtered and concentrated in vacuo. This give the pure product as white crystals (3.08 g, 14.90 mmol, 51%); IR (thin film) ν/cm⁻¹: 2926 (O-H, br s), 1682 (C=O, s), 1509 (C=C, m), 1249 (C-O, s) 761 (C-Cl, s). ^1H NMR (400 MHz, (CD₃)₂SO) δ/ppm: 13.40 (br s, 1H, OH), 8.97–8.93 (m, 1H, H₆), 8.36–8.28 (m, 1H, H₉), 8.11 (d, J = 8.0 Hz, 1H, H₂), 7.89 (d, J = 8.0 Hz, 1H, H₃), 7.78 (m, 2H, H₇&8). ^13C NMR (101 MHz, (CD₃)₂SO) δ/ppm: 168.1 (C11), 135.3 (C1), 131.9 (C4), 130.2 (C3), 129.9 (C2), 128.6 (C8), 127.9 (C7), 127.8 (C5), 126.4 (C6), 125.7 (C3), 124.3 (C9). HRMS (ESI) C₁₁H₁₀ClO m/z: [M+H]^+ 205.0412 (calc. 205.0415).

m.p. 220-221 °C.
The physical and spectroscopic data was found to be in agreement with Wiley et al.\textsuperscript{121}

\textbf{(4-Chloronaphthalen-1-yl)methanol, 43}

\begin{center}
\begin{tabular}{c}
\includegraphics[width=0.5\textwidth]{4-chloronaphthalen-1-ylmethanol}
\end{tabular}
\end{center}

Lithium Aluminium Hydride (264 mg, 6.97 mmol, 7.2 eq) was suspended in diethyl ether (2 mL) under nitrogen. Compound 42 (200 mg, 0.97 mmol, 1.0 eq) dissolved in diethyl ether (4 mL) was added dropwise. The reaction was refluxed for 3 h then returned to RT and stirred for 14 h. Following this water (0.26 mL), 15% aq. NaOH (0.26 mL), and water (0.78 mL) was added dropwise. The resulting suspension was filtered and the filtrate was extracted with diethyl ether (3 × 10 mL). This was dried over anh. MgSO\textsubscript{4} and the mixture was filtered and concentrated in \textit{vacuo}. This give the pure product as white crystals (114.5 g, 0.59 mmol, 61\%); \textit{R}\textsubscript{f} 0.24 (50\% diethyl ether in pet. ether); \textbf{IR (thin film)} \textit{v}/cm\textsuperscript{-1}: 3261 (O-H, br s), 2971 (C-H, w), 2860 (C-H, w), 1508 (C=C, m), 1076 (C-O, m), 752 (C-Cl, m). \textbf{\textit{H} NMR (400 MHz, CDCl\textsubscript{3}) \textit{δ}/ppm:} 8.36–8.32 (m, 1H, H6), 8.16–8.12 (m, 1H, H9), 7.65–7.60 (m, 2H, H7&8), 7.55 (d, \textit{J} = 7.5 Hz, 1H, H2), 7.45 (d, \textit{J} = 7.5 Hz, 1H, H3), 5.14 (s, 2H, H11). \textbf{\textit{C} NMR (101 MHz, CDCl\textsubscript{3}) \textit{δ}/ppm:} 135.6 (C1), 132.3 (C4), 131.0 (C10), 128.7 (C5), 127.1 (C7/8), 127.0 (C7/8), 125.6 (C2), 125.2 (C3&6), 124.0 (C9), 63.3 (C11). \textbf{HRMS (ESI)} \textit{C\textsubscript{11}H\textsubscript{10}ClO} \textit{m/z}: [M+H]\textsuperscript{+} 193.0420 (calc. 193.0415). \textbf{m.p.} 71–72 °C.

The physical and spectroscopic data was found to be in agreement with Wiley et al.\textsuperscript{122}
1-(Bromomethyl)-4-chloronaphthalene, 44

Phosphorus tribromide (58.5 μL, 0.62 mmol, 1.2 eq) was added dropwise at 0 °C to a solution of 43 (100 mg, 0.52 mmol, 1.0 eq) in diethyl ether (1 mL). The reaction was stirred overnight at RT, before being poured into ice water. The organic layer was separated and washed with brine (10 mL), then dried over anh. MgSO₄. This was filtered and the filtrate was concentrated in vacuo to give a crude oil. This was purified by column chromatography (10% diethyl ether in pet. ether) to yield the product as white crystals (93.0 mg, 0.36 mmol, 70%); Rf 0.63 (20% diethyl ether in pet. ether); IR (thin film) v/cm⁻¹: 2987 (C-H, s), 2901 (C-H, s), 1568 (C=C, m).

¹H NMR (400 MHz, CDCl₃) δ/ppm: 8.36–8.34 (m, 1H, H6), 8.18–8.16 (m, 1H, H9), 7.71–7.64 (m, 2H, H7&8), 7.52 (d, J = 7.5 Hz, 1H, H2), 7.47 (d, J = 7.5 Hz, 1H, H3), 4.93 (s, 2H, H11).

¹³C NMR (101 MHz, CDCl₃) δ/ppm: 133.5 (C4), 132.6 (C1), 132.1 (C5), 131.3 (C10), 127.5 (C3), 127.4 (C7/8), 127.3 (C7/8), 125.7 (C2), 125.4 (C6), 124.2 (C9), 31.0 (C11). HRMS (ESI) C₁₁H₉BrCl m/z: [M+H]⁺ 254.9559 (calc. 254.9571). m.p. 90–91 °C.

The physical and spectroscopic data was found to be in agreement with Dixon et al.¹²²

2-(4-Chloronaphthalen-1-yl)acetonitrile, 45

Sodium cyanide (23 mg, 0.47 mmol, 1.3 eq) was dissolved in water (0.7 mL) and added dropwise at 0 °C to a solution of 44 (92 mg, 0.36 mmol, 1.0 eq) in ethanol (2.4 mL). The reaction mixture was refluxed overnight. It was then concentrated in vacuo and diluted with ethyl acetate (10 mL). This was washed with water (3 × 10 mL), the aqueous was extracted with ethyl acetate (2 × 10 mL), and the combined organics were washed with brine (30 mL). The organic layer was dried over anh. MgSO₄ filtered and concentrated in vacuo to give the crude
Experimental

product. This was purified by column chromatography (20% diethyl ether in pet. ether) to yield the pure product as white crystals (26.7 mg, 0.13 mmol, 37%); Rf 0.21 (20% diethyl ether in pet. ether); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2247 (C≡N, w), 1511 (C=C, m), 751 (C-Cl, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 8.40–8.37 (m, 1H, H6), 7.91–7.88 (m, 1H, H9), 7.71–7.67 (m, 2H, H7&8), 7.59 (d, \( J = 7.5 \text{ Hz} \), 1H, H3), 7.53 (d, \( J = 7.5 \text{ Hz} \), 1H, H2), 4.13 (s, 2H, H11). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 133.1 (C4), 131.8 (C10), 131.0 (C5), 127.9 (C7/8), 127.5 (C7/8), 126.4 (C2), 125.7 (C3), 125.7 (C6), 125.1 (C1), 122.9 (C9), 117.2 (C12), 21.7 (C11). HRMS (ESI) C\(_{12}\)H\(_{7}\)ClN \( m/z \): [M-H\(^-\)] 200.0275 (calc. 200.0272).

Methyl 2-bromo-4-chlorobenzoate, 49

\[
\text{\begin{array}{c}
\text{Cl} & 4 \\
\text{\textcdot} & 2 \\
\text{Br} & 1 \\
\text{O} & 8 \\
\end{array}}
\]

2-Bromo-4-chlorobenzoic acid (2 g, 8.49 mmol, 1.0 eq) was dissolved in methanol (25 mL) and cooled to 0 °C before thionyl chloride (2.30 mL, 32.82 mmol, 3.8 eq) was added dropwise. The reaction was refluxed for 4 h. The mixture was concentrated \( \text{in vacuo} \) and was diluted with dichloromethane (25 mL). This was washed with water (20 mL), sat. aq. NaHCO\(_3\) (20 mL), and brine (20 mL). The organic layer was dried over anh. Na\(_2\)SO\(_4\), filtered and concentrated \( \text{in vacuo} \) to give the crude product. This was purified by column chromatography (15% diethyl ether in pet. ether) to give the pure product as a colourless oil (1.46 g, 5.87 mmol, 69%); Rf 0.50 (15% diethyl ether in pet. ether) IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2952 (C-H, w), 1732 (C=O, s), 1581 (C=O, s), 1100 (C-O, s), 763 (C-Cl, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 7.76 (d, \( J = 8.5 \text{ Hz} \), 1H, H6), 7.67 (d, \( J = 2.0 \text{ Hz} \), 1H, H3), 7.33 (dd, \( J = 2.0, 8.5 \text{ Hz} \), 1H, H5), 3.91 (s, 3H, H8). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 165.6 (C7), 138.3 (C4), 134.2 (C6), 132.4 (C3), 130.2 (C1), 127.5 (C5), 122.6 (C2), 52.6 (C8). HRMS (ESI) C\(_{12}\)H\(_{7}\)BrClO\(_2\) \( m/z \): [M+H\(^+\)] 248.9315 (calc. 248.9318).

The physical and spectroscopic data was found to be in agreement with Sun et al.\(^{220}\)
Experimental

Methyl 4-chloro-2-(((trimethylsilyl)ethynyl)benzoate, 50

![Methyl 4-chloro-2-((trimethylsilyl)ethynyl)benzoate](image)

Trimethylsilylacetylene (0.42 mL, 3.01 mmol, 1.5 eq), 49 (500 mg, 2.00 mmol, 1.0 eq), bis(triphenylphosphine)palladium(II) dichloride (70.2 mg, 0.10 mmol, 5 mol%), triphenylphosphine (13.1 mg, 0.05 mmol, 2.5 mol%), triethylamine (0.42 mL, 3.01 mmol, 1.5 eq) were dissolved in tetrahydrofuran (8mL) and stirred at RT for 20 min. Copper(I) iodide (4.6 mg, 0.024 mmol, 1.2 mol%) was added and the reaction was refluxed for a further 16 h. This was concentrated in vacuo and diluted with dichloromethane and was filtered through celite. The filtrate was concentrated in vacuo to give the crude product. This was purified by column chromatography (6.25% ethyl acetate in hexane) to give the pure product as a yellow oil (420.5 mg, 1.58 mmol, 79%); RF 0.31 (6.25% ethyl acetate in hexane); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2955 (C-H, w), 2163 (C≡C, w), 1736 (C=O, s), 1247 (C-O, s). $^1$H NMR (400 MHz, CDCl$\text{$_3$}$) $\delta$/ppm: 7.86 (d, $J = 8.5$ Hz, 1H, H6), 7.57 (d, $J = 2.0$ Hz, 1H, H3), 7.33 (dd, $J = 2.0$, 8.5 Hz, 1H, H5), 3.91 (s, 3H, H8), 0.27 (s, 9H, H11). $^{13}$C NMR (101 MHz, CDCl$\text{$_3$}$) $\delta$/ppm: 165.9 (C7), 137.8 (C2), 134.2 (C3), 131.7(C6), 130.7 (C4), 128.5 (C5), 125.0 (C1), 101.9 (C9/10), 101.5 (C9/10), 52.1 (C8), -0.2 (C11). HRMS (ESI) C$_{13}$H$_{16}$ClO$_2$Si m/z: [M+H]$^+$ 267.0619 (calc. 267.0608).

4-Chloro-2-ethynyl benzoic acid, 51

![4-Chloro-2-ethynyl benzoic acid](image)

Compound 50 (200 mg, 0.75 mmol, 1.0 eq) was dissolved 3:2 ethanol:dichloromethane (2mL) and cooled to 0 °C when 1M NaOH (3 mL, 3.00 mmol, 4.0 eq) was added dropwise over 10 min. The reaction was warmed to RT and stirred for 4h. The solution was washed with diethyl ether (5 mL) and 1M HCl was added dropwise until a precipitate appeared. The precipitate was collected by filtration, dissolved in diethyl ether (5 mL) and washed with water (5 mL). The organic layer was dried over Na$_2$SO$_4$ and the excess solvent was removed in vacuo to yield
the pure product as yellow crystals (98.6 mg, 0.55 mmol, 73%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3304 (C≡C-H, w), 3282 (O-H, m), 2952 (C-H, m), 2113 (C≡C, m), 1686 (C=O, s), 1586 (C=C, s), 1271 (C-O, s), 777 (C-Cl, s). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 13.29 (s, 1H, OH), 7.85 (d, $J$ = 8.5 Hz, 1H, H6), 7.67 (d, $J$ = 2.0 Hz, 1H, H3), 7.57 (dd, $J$ = 2.0, 8.5 Hz, 1H, H5), 4.51 (s, 1H, H9). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 166.2 (C7), 136.3 (C2), 133.7 (C3), 132.6 (C4), 131.8 (C6), 129.1 (C5), 123.6 (C1), 87.0 (C9), 80.8 (C8). HRMS (ESI) C$_9$H$_4$ClO$_2$ m/z: [M-H]$^-$ 178.9903 (calc. 178.9905).

Hydroxycarbonimidic dibromide, 70

Glyoxylic acid (5.00 g, 54.32 mmol, 1.0 eq) and hydroxylamine hydrochloride (3.77 g, 54.32 mmol, 1.0 eq) were stirred in water (150mL) for 24 h. NaHCO$_3$ (9.13 g, 108.60 mmol, 2.0 eq) was added and stirred with CH$_2$Cl$_2$ (200 mL). The mixture was cooled to 6 °C and stirred vigorously. Bromine (5.43 mL, 108.60 mmol, 2.0 eq) was added dropwise over 20 min and stirred for 3 h. The organic layer was separated, the aqueous was extracted with CH$_2$Cl$_2$ (100 mL $\times$ 3), and the combined organic layers were dried over MgSO$_4$. The salt was filtered and the remaining organic layer was concentrated in vacuo to give the product as a white crystal (6.7510 g, 33.29 mmol, 61%): $R_f$ 0.45 (20% Et$_2$O in pet. ether); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3290 (O-H, br s), 1580 (C=N, m), 890 (C-Br, s). $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 12.74 (s, 1H, OH). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 96.7. HRMS (ESI) CH$_2$NOBr$_2$ m/z: [M+H]$^+$ 200.8425 (calc. 200.8433).

The physical and spectroscopic data was found to be in agreement with Soleimani et al.\textsuperscript{221}
** tert-Butyldimethyl(prop-2-yn-1-yloxy)silane, 72 **

![Diagram of tert-Butyldimethyl(prop-2-yn-1-yloxy)silane](image)

Propargylic alcohol (3.00 mL, 51.5 mmol, 1.0 eq) and imidazole (5.26 g, 77.3 mmol, 1.5 eq) were dissolved in CH$_2$Cl$_2$ (150 mL). The mixture was cooled to 0 °C TBSCl (11.7 g, 77.3 mmol, 1.5 eq) was added and stirred for 90 min. The reaction was quenched with sat. aq. NH$_4$Cl (50 mL), extracted with diethyl ether (20 mL × 3), and washed with brine (50 mL). The organic layer was dried over MgSO$_4$, the salt was filtered and the remaining organic layer was concentrated in vacuo. The crude oil was purified by column chromatography (20% diethyl ether in pet. ether) to give the product as a colourless oil (6.2657 g, 36.79 mmol, 71%); $R_f$ 0.70 (20% diethyl ether in pet. ether); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2970 (C-H, s), 2901 (C-H, s), 2125 (C≡C, w), 1253 (C-Si, m), 1087 (C-O, s). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 4.29 (d, $J$ = 2.5 Hz, 2H, H3), 2.36 (t, $J$ = 2.5 Hz, 1H, H1), 0.89 (s, 9H, H6), 0.10 (s, 6H, H4). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 82.4 (C2), 72.8 (C1), 51.5 (C3), 25.8 (C6×2), 18.3 (C5), −5.2 (C4×3). HRMS (ESI) C$_9$H$_{19}$OSi m/z: [M+H]$^+$ 171.1195 (calc. 171.1200).

The physical and spectroscopic data was found to be in agreement with Yatvin et al.$^{222}$

** 3-Bromo-5-(((tert-butyldimethylsilyl)oxy)methyl)isoxazole, 73 **

![Diagram of 3-Bromo-5-(((tert-butyldimethylsilyl)oxy)methyl)isoxazole](image)

Alkyne 72 (5.00 g, 29.4 mmol, 4.0 eq) and oxime 70 (1.49 g, 7.34 mmol, 1.0 eq) were dissolved in CH$_2$Cl$_2$ (10 mL) refluxed for 18 h. The reaction was cooled to room temperature and poured into 1M HCl (10 mL). The organic layer was separated and dried over anh. NaHSO$_4$. This mixture was filtered and the excess solvent of the filtrate was removed in vacuo. The crude was purified by column chromatography (40% diethyl ether in pet. ether) to give the pure product as a yellow oil (1.7948 g, 6.14 mmol, 84%); $R_f$ 0.28 (40% diethyl ether in pet. ether); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2955 (C-H, w), 2858 (C-H, w), 1594 (C=C, m), 1258 (N-O, m), 1124 (C-O, m). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 6.30 (t, $J$ = 1.0 Hz, 2H, H4), 4.78 (d, $J$ = 1.0 Hz, 1H, H2), 0.94 (s, 9H, H5), 0.14 (s, 6H, H7). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 174.0 (C1), 140.3
(C3), 105.1 (C2), 57.3 (C4), 25.7 (C7×3), 18.2 (C6), –5.5 (C5×2). **HRMS (ESI)** \( \text{C}_{10}\text{H}_{19}\text{O}_{2}\text{NBrSi} \) m/z: [M+H]^+ 292.0351 (calc. 292.0363).

**Spiro[indoline-3,4′-piperidin]-1′-ium chloride, 85**

A suspension of lithium aluminium hydride (749 mg, 19.7 mmol, 4.75 eq) in glyme (20 mL) was cooled to 0 °C and ethanol (1.7 mL) added. The mixture was heated to reflux and compound 96 (1.00 g, 4.15 mmol, 1.0 eq) dissolved in glyme (10 mL) was added followed by continuing reflux for 72 h. Subsequently the reaction was cooled to 0 °C and water (20 mL) was added slowly. Added to this was a conc. solution of Rochelle’s salt (20 mL) and extracted with CH\(_2\)Cl\(_2\) (50 mL). The organic layers were dried over anh. MgSO\(_4\) which was removed by filtration and the excess solvent was evaporated *in vacuo*. To the residual oil was added 4M HCl/Dioxane (5 mL). The insoluble salt produced was isolated by filtration and washed with cold acetone (10 mL) to give the product as white crystals (546 mg, 2.09 mmol, 50%); **IR (thin film)** \( \bar{\nu} / \text{cm}^{-1} \): 2923 (C-H, s), 2714 (C-H, s), 1596 (C=C, m). **\(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 9.21 (br. s, 2H, NH\(_2\)), 7.59–7.54 (m, 2H, H3&4), 7.44–7.36 (m, 2H, H2&5), 3.55 (d, \( J = 13.5 \text{ Hz} \), 2H, H10a), 3.36 (s, 2H, H7), 3.18 (t, \( J = 13.5 \text{ Hz} \), 2H, H10b), 2.56–2.51 (m, 2H, H9a), 2.40–2.33 (m, 2H, H9b). **\(^{13}\)C NMR (101 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 159.2 (C6), 131.3 (C3), 127.2 (C4), 125.5 (C2), 119.9 (C1), 117.0 (C5), 40.9 (C10×2), 38.9 (C8), 36.9 (C7), 30.2 (C9×2). **HRMS (ESI)** \( \text{C}_{12}\text{H}_{17}\text{N}_{2} \) m/z: [M+H]^+ 188.1313 (calc. 188.1319). **m.p.** 264–265 °C.
2,3-Dihydrospiro[indene-1,4′-piperidin]-1′-ium chloride, 86

The 10% Pd on C (384 mg, 0.36 mmol, 0.1 eq) was suspended in ethanol (10 mL) and hydrogen gas was bubbled through the mixture. Compound 103 (500 mg, 2.25 mmol, 1.0 eq) was added to the reaction and it was stirred for 2 h in an atmosphere of hydrogen. The catalyst was removed by filtering through celite and the ethanol was removed in vacuo. The crystals were washed with cold acetone (10 mL) to yield product as white crystals (402 mg, 1.80 mmol, 80%);

**IR (thin film)** \(\tilde{\nu}/\text{cm}^{-1}: \) 2943 (C-H, s), 2730 (C-H, s), 1595 (C=C, m).

**1H NMR** (400MHz, (CD$_3$)$_2$SO) \(\delta/\text{ppm}:\) 9.71 (d, \(J = 64.0\) Hz, 2H, NH$_2$), 7.30–7.20 (m, 4H, H$_2$–5), 3.55 (d, \(J = 12.5\) Hz, 2H, H$_{11a}$), 3.11 (q, \(J = 11.5\) Hz, 2H, H$_{11b}$), 2.97 (t, \(J = 7.5\) Hz, 2H, H$_7$), 2.35 (td, \(J = 14.0, 4.0\) Hz, 2H, H$_{10a}$), 2.07 (t, \(J = 7.5\) Hz, 2H, H$_8$), 1.75 (d, \(J = 14.0\) Hz, 2H, H$_{10b}$).

**13C NMR** (101 MHz, (CD$_3$)$_2$SO) \(\delta/\text{ppm}:\) 148.5 (C$_6$), 142.4 (C$_1$), 127.6 (C$_2$), 127.0 (C$_4$), 124.8 (C$_5$), 122.7 (C$_3$), 45.2 (C$_9$), 41.8 (C$_{11\times2}$), 34.6 (C$_8$), 33.2 (C$_{10\times2}$), 29.8 (C$_7$).

**HRMS (ESI)** C$_{13}$H$_{17}$N \(m/z: [M+H]^+ 188.1447\) (calc. 188.1439). **m.p.** 289–290 °C.

The physical and spectroscopic data was found to be in agreement with Chambers et al.$^{131}$

**tert-Butyl bis(2-chloroethyl)carbamate, 92**

Di-tert-butyl dicarbonate (4.6 mL, 20.0 mmol, 1.2 eq) and bis(2-chloroethyl)amine (3.0 g, 16.8 mmol, 1.0 eq) were dissolved in CH$_2$Cl$_2$ (20 mL). Triethylamine (2.6 mL, 20.1 mmol, 1.2 eq) was added dropwise. The mixture was left to stir for an hour before more triethylamine (0.2 mL, 1.4 mmol, 0.08 eq) was added. The reaction was stirred overnight before TLC showed it had gone to completion. Excess solvent was removed in vacuo. The residue was dissolved in
diethyl ether (30 mL) and extracted with water (30 mL). The combined ethereal layers were
dried over anh. MgSO$_4$ and the excess solvent was removed in vacuo. The residue was purified
via column chromatography (pet. ether/diethyl ether 20%) to give the product as a colourless
oil (3.10 g, 12.8 mmol, 76%); R$_f$ 0.31 (pet. ether / Et$_2$O (20%)); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2977 (C-H, m), 1693 (C=O, s), 1465 (C-H, m), 1405 (C-H, m), 775 (C-Cl, m). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 3.66–3.59 (m, 8H, H1&2), 1.47 (s, 9H, H5). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 154.9 (C3), 80.7 (C4), 51.0 (C2x2), 42.1 (C1x2), 28.3 (C5x3). HRMS (ESI) C$_{5}$H$_{10}$NO$_2$Cl$_2$ m/z: [M+H–t-butyl]$^+$ 186.0089 (calc. 186.0094).

The physical and spectroscopic data was found to be in agreement with Tantry et al.$^{223}$

### 4-Cyano-4-(2-fluorophenyl)piperidin-1-ium chloride, 95

![Chemical Structure](image)

Compound 96 (476 mg, 1.57 mmol) was stirred in HCl/dioxane (4M, 5 mL) for 1 h. The
mixture was filtered and the filtrate was washed with cold diethyl ether (10 mL). The isolated
white crystals were placed in a desiccator overnight to yield the pure white crystalline product
(183 mg, 0.76 mmol, 48%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2925 (C-H, m), 2712 (C-H, m), 1597 (C=C, m), 1597 (C=C, m). $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 9.26–9.20 (m, 2H, NH$_2$), 7.55–7.50 (m, 2H, H3&5), 7.40–7.25 (m, 2H, H2,4), 3.51 (d, $J$ = 13.5 Hz, 2H, H10), 2.90–2.85 (m, 2H, H10), 2.52–2.48 (m, 2H, H9), 2.33 (td, $J$ = 13.5, 3.5 Hz, 2H, H9). $^{13}$C NMR (101 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 161.8 (C6), 159.4 (C1), 131.7 (C3), 127.5 (C5), 125.9 (C2), 120.3 (C8), 117.3 (C4), 41.2 (C10x2), 37.3 (C7), 30.6 (C9x2). HRMS (ESI) C$_{12}$H$_{14}$FN$_2$ m/z: [M+H]$^+$ 205.1130 (calc. 205.1136). m.p. 276–277 °C.
**Experimental**

**tert-Butyl 4-cyano-4-(2-fluorophenyl)piperidine-1-carboxylate, 96**

2-(2-Fluorophenyl)acetonitrile (1.6 mL, 12.39 mmol, 1.5 eq) was added to a suspension of sodium hydride (50% in mineral oil, 3.2 g, 80.95 mmol, 9.8 eq) in dry THF (20 mL) at 5 °C. The mixture was warmed to RT and stirred for 30 min. The reaction was cooled to 0 °C and compound 92 (2.0 g, 8.26 mmol, 1.0 eq) was added before being heated to reflux for 3 h. After the reaction had finished, as shown by TLC, the excess solvent was removed in vacuo, ice water (10 mL) was added slowly to the residue before being extracted with diethyl ether (20 mL x 3). The combined organic layers were dried over MgSO₄ and the excess solvent was removed in vacuo. The resulting substance was purified by column chromatography (40% diethyl ether in pet. ether) to give the product as a white amorphous solid (2.09 g, 6.87 mmol, 83%); Rₓ 0.34 (40% diethyl ether in pet. ether); IR (thin film) 𝜈/cm⁻¹: 2979 (C-H, s), 2251 (C≡N, m), 1694 (C=O, s), 1605 (C=C, m). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.46 (td, J = 8.0, 2.0 Hz, 1H, H4), 7.37–7.34 (m, 1H, H2), 7.19 (td, J = 7.5, 1.5 Hz, 1H, H3), 7.13 (ddd, J = 12.0, 8.0, 1.0 Hz, 1H, H1), 4.28 (br. s, 2H, H10a), 3.23 (br. s, 2H, H10b), 2.21–2.06 (m, 4H, H9), 1.48 (s, 9H, H13). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 161.7 (C11), 159.7 (C5×3), 154.4 (C6), 130.4 (C4), 127.0 (C2), 124.7 (C3), 120.2 (C8), 117.0 (C1), 80.1 (C12), 41.3 (C7), 40.2 (C9×2), 33.8 (C10×2), 28.4 (C13). HRMS (ESI) C₁₇H₂₁N₂O₂FNa m/z: [M+Na]⁺ 327.1479 (calc. 327.1470).

The physical and spectroscopic data was found to be in agreement with Xie et al.¹³⁰
**Experimental**

*N-Benzyl-2-chloro-N-(2-chloroethyl)ethan-1-amine, 98*

Benzyl bromide (0.80 mL, 6.75 mmol, 1.0 eq) was added dropwise to a suspension of K₂CO₃ (0.93 g, 6.72 mmol, 1.2 eq) and bis(2-chloroethyl)ammonium chloride (1.00 g, 5.60 mmol, 1.0 eq) in acetonitrile (7 mL). The reaction was refluxed overnight. Afterwards the excess solvent was removed *in vacuo* and the remaining mixture was re-dissolved in diethyl ether (20 mL) and extracted with water (20 mL) and brine (20 mL) and the combined aqueous layers were extracted with diethyl ether (20 mL × 3). The combined ethereal layers were dried over MgSO₄ and the excess solvent was removed *in vacuo*. The product was purified via column chromatography (100% pet. ether) to give the pure product as a colourless oil (500 mg, 2.15 mmol, 38%); Rᵣ 0.17 (pet. ether); **IR (thin film)** ν/cm⁻¹: 2961 (C-H, m), 2809 (C-H, m), 1610 (C=C, w), 1453 (C-H, m), 742 (C-Cl, s). **¹H NMR (400 MHz, CDCl₃)** δ/ppm: 7.34–7.25 (m, 5H, Ar), 3.75 (s, 2H, H₃), 3.50–3.33 (m, 4H, H₁), 3.01–2.91 (m, 4H, H₂). **¹³C NMR (101 MHz, CDCl₃)** δ/ppm: 138.8 (C₄), 128.6 (C₅/₆×2), 128.4 (C₅/₆×2), 127.4 (C₇), 59.2 (C₃), 56.3 (C₂×2), 42.0 (C₁×2). **HRMS (ESI)** C₁₁H₁₆NCl₂ m/z: [M+H]⁺ 232.0668 (calc. 232.0660).

The physical and spectroscopic data was found to be in agreement with Chan *et al.*²²⁴

**1’-Benzylspiro[indene-1,4’-piperidine], 100**

Indene (0.50 mL, 4.31 mmol, 1.0 eq) was dissolved in THF (10 mL) and cooled to 0 °C and LHMDS (1M in THF, 8.62 mL, 8.62 mmol, 2.0 eq) was added over 15 min. The mixture was stirred for 30 min before compound 98 (1.00 g, 4.31 mmol, 1.0 eq) was added dropwise. The reaction was heated to RT and stirred for 2 h. When the reaction had concluded, the solvent
was removed in vacuo and the residue was diluted with diethyl ether (20 mL) and washed with sat. aq. NH₄Cl (20 mL) and brine (20 mL). The combined aqueous layers were extracted with diethyl ether (20 mL × 3). The combined ethereal layers were dried over MgSO₄ and the excess solvent was removed in vacuo. The given oil was purified via column chromatography (20% diethyl ether in pet. ether) to give the product as a golden oil (809 mg, 2.94 mmol, 68%); Rf 0.11 (20% diethyl ether in pet. ether); IR (thin film) v/cm⁻¹: 3065 (C-H, m), 2945 (C-H, m), 2804 (C-H, m), 1454 (C=C, m), 698 (C-H, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.42–7.19 (m, 9H, Ar), 6.87 (d, J = 5.5 Hz, 1H, H8), 6.75 (d, J = 5.5 Hz, 1H, H7), 3.67 (s, 2H, H12), 3.00 (dt, J = 12.0, 3.0 Hz, 2H, H10a), 2.39 (td, J = 12.0, 2.5 Hz, 2H, H10b), 2.22 (td, J = 12.5, 3.5 Hz, 2H, H11a), 1.36 (d, J = 12.0 Hz, 2H, H11b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 152.4 (C1), 142.9 (C6), 141.7 (C8), 138.3 (C13), 129.4 (C14/15/16), 129.3 (C7), 128.3 (C14/15/16), 127.1 (C14/15/16), 126.8 (C2/3/4/5), 125.2 (C2/3/4/5), 121.8 (C2/3/4/5), 121.3 (C2/3/4/5), 63.7 (C12), 52.2 (C11x2), 52.1 (C9), 33.9 (C10x2). HRMS (ESI) C₂₀H₂₂N m/z: [M+H]⁺ 276.1739 (calc. 276.1747).

1'-Benzyl-2,3-dihydrospiro[indene-1,4'-piperidine], 101

The 10% Pd on C (142 mg, 0.13 mmol, 0.1 eq) was suspended in ethanol (10 mL) and hydrogen gas was bubbled through the mixture. Compound 100 (230 mg, 0.84 mmol, 1.0 eq) was added to the reaction as a solution in ethanol (5 mL) and it was stirred for 2 h in an atmosphere of hydrogen. The catalyst was removed by filtering through celite and the ethanol was removed in vacuo. The given oil was purified via column chromatography (5 % methanol in CH₂Cl₂) to give the product as a colourless oil (110 mg, 0.40 mmol, 48%); Rf 0.26 (5 % methanol in dichloromethane); IR (thin film) v/cm⁻¹: 3021 (C-H, m), 2924 (C-H, s), 1602 (C=C, w), 1494 (C=C, w). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.38–7.13 (m, 9H, Ar), 3.57 (s, 2H, H12), 2.91–2.85 (m, 4H, H7,11a), 2.20 (t, J = 10.5 Hz, 2H, H11b), 2.02–1.92 (m, 4H, H8,10a), 1.53 (d, J = 13.0 Hz, 2H, H10b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 151.5 (C1), 143.2 (C6), 138.4 (C13), 129.4 (C2/3/4/5), 128.3 (C2/3/4/5), 127.1 (C2/3/4/5), 126.7 (C2/3/4/5), 126.5 (C14/15/16), 124.6
Experimental

(C14/15/16), 122.7 (C14/15/16), 63.7 (C12), 51.3 (C11), 46.5 (C9), 36.9 (C10), 35.1 (C8), 30.0 (C7). HRMS (ESI) C_{20}H_{24}N m/z: [M+H]^+ 278.1891 (calc. 278.1903).

tert-Butyl spiro[indene-1,4'-piperidine]-1'-carboxylate, 102

Indene (0.10 mL, 0.86 mmol, 1.0 eq) was dissolved in dry THF (2 mL) and cooled to 0 °C. LHMDS (288 mg, 1.72 mmol, 2.0 eq) was added dropwise over 15 min. This was left to stir for 30 min before compound 92 (208 mg, 0.86 mmol, 1.0 eq) was added at 0 °C and stirred for 2 h. After the reaction had gone to completion excess solvent was removed in vacuo. This was purified via column chromatography (20% diethyl ether in pet. ether) to give the product as a yellow oil (203 mg, 0.712 mmol, 83%); R_f 0.28 (20% diethyl ether in pet. ether); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1}: \) 3057 (C-H, m), 2976 (C-H, m), 2935 (C-H, m), 2865 (C-H, m), 1687 (C=O, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ/ ppm: 7.36–7.19 (m, 4H, H2–5), 6.85 (d, \( J = 5.5 \text{ Hz} \), 1H, H8), 6.79 (d, \( J = 5.5 \text{ Hz} \), 1H, H7), 4.20–4.18 (m, 2H, H11a), 3.13 (t, \( J = 12.5 \text{ Hz} \), 2H, H11b), 2.01 (td, \( J = 12.5, 4.5 \text{ Hz} \), 2H, H10a), 1.51 (s, 9H, H14), 1.34 (d, \( J = 13.0 \text{ Hz} \), 2H, H10b). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ/ ppm: 155.1 (C12), 151.7 (C1), 142.8 (C6), 140.4 (C8), 130.3 (C7), 127.0 (C2/3/4/5), 125.4 (C2/3/4/5), 121.7 (C2/3/4/5), 121.5 (C2/3/4/5), 79.6 (C13), 77.2 (C11), 52.1 (C9), 33.4 (C10), 28.5 (C14). HRMS (ESI) C_{18}H_{23}NO_2 m/z: [M+H]^+ 286.1812 (calc. 286.1811).

The physical and spectroscopic data was found to be in agreement with Tantry et al.\(^{223}\)
Experimental

**Spiro[indene-1,4'-piperidin]-1'-ium chloride, 103**

![ Chemical structure of spiro[indene-1,4'-piperidin]-1'-ium chloride. ]

Compound 102 (1.78 g, 6.24 mmol) was stirred in HCl/dioxane (4M, 5.00 mL) for 1 h. The mixture was filtered and the filtrate was washed with cold diethyl ether (10 mL). The isolated white crystals were placed in a desiccator overnight to yield the pure white crystalline product (405 mg, 5.23 mmol, 84%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3361 (N-H, m), 2936 (C-H, m), 2801 (C-H, m), 1644 (C=C, m), 1600 (C=C, m). $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 9.92 (d, $J = 33.5$ Hz, 2H, NH$_2$), 7.48 (d, $J = 7.0$ Hz, 1H, H5), 7.37–7.24 (m, 3H, H2–4), 6.87 (d, $J = 5.5$ Hz, 1H, H7), 6.74 (d, $J = 5.5$ Hz, 1H, H8), 3.69 (d, $J = 13.0$ Hz, 2H, H11a), 3.26 (q, $J = 13.0$ Hz, 2H, H11b), 2.58 (td, $J = 13.0$, 4.0 Hz, 1H, H10a) 1.60 (d, $J = 13.0$ Hz, 2H, H10b). $^{13}$C NMR (101 MHz, (CD$_3$)$_2$SO) $\delta$/ppm: 150.0 (C1), 142.3 (C6), 138.1 (C8), 132.1 (C7), 127.7 (C2/3/4), 126.1 (C2/3/4), 122.0 (C2/3/4), 121.9 (C5), 50.1 (C9), 42.81 (C11×2), 30.4 (C10×2). HRMS (ESI) C$_{13}$H$_{17}$N m/z: [M+H]$^+$ 186.1275 (calc. 186.1277). m.p. 309–310 °C.

**tert-Butyl 2-oxo-3,4-dihydroquinoline-1(2H)-carboxylate, 105**

![ Chemical structure of tert-butyl 2-oxo-3,4-dihydroquinoline-1(2H)-carboxylate. ]

3,4-Dihydroquinolin-2(1H)-one (100 mg, 0.68 mmol, 1.0 eq) was dissolved in dry CH$_2$Cl$_2$ (5 mL) before triethylamine (0.095 mL, 0.68 mmol, 1.0 eq), di-tert-butyl decarbonate (0.19 mL, 0.82 mmol, 1.2 eq), and 4-dimethylaminopyridine (8.50 mg, 0.07 mmol, 0.1 eq) were added to the mixture. The mixture was stirred for 18 h at RT. After the reaction had finished the excess solvent was removed in vacuo. To the mixture water (20 mL) was added and the mixture was extracted with diethyl ether (10 mL × 3). The combined ethereal layers were washed with 1 M KHSO$_4$ (20 mL), sat. NaHCO$_3$ (20 mL), and brine (20 mL). The organic layers were dried
Experimental

over Na₂SO₄ and the excess solvent was removed in vacuo. The resulting substance was purified by column chromatography (20% diethyl ether in pet. ether) to give the product as a white crystalline solid (154 mg, 0.62 mmol, 92%); Rₗ 0.17 (20% diethyl ether in pet. ether); IR (thin film) v/cm⁻¹: 2981 (C-H, w), 1755 (C=O, s), 1605 (C=C, m). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.23–7.17 (m, 2H, H₆,₇), 7.06 (td, J = 7.5,1.0 Hz, 1H, H₅), 6.94 (d, J = 8.0 Hz, 1H, H₈), 2.95 (t, J = 7.0 Hz, 2H, H₃), 2.68–2.64 (m, 2H, H₂), 1.60 (s, 9H, H₁₂). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 169.3 (C₁), 151.8 (C₁₀), 137.1 (C₉), 128.0 (C₇), 127.3 (C₆), 125.9 (C₄), 124.1 (C₅), 117.0 (C₈), 85.0 (C₁₁), 32.3 (C₂), 27.7 (C₁₂×3), 25.5 (C₃). HRMS (ESI) C₁₄H₁₇NO₃Na m/z: [M+Na]⁺ 270.1063 (calc. 270.1063). m.p. 68–69 °C.

The physical and spectroscopic data was found to be in agreement with Evans et al.²²⁵

1-(tert-Butyl) 4-ethyl piperidine-1,4-dicarboxylate, 109

Di-tert-butyl dicarbonate (0.17 mL, 0.76 mmol, 1.2 eq) and ethyl piperidine-4-carboxylate (0.10 mL, 0.64 mmol, 1.0 eq) were dissolved in CH₂Cl₂ (5 mL). Triethylamine (0.11 mL, 0.76 mmol, 1.2 eq) was added dropwise and the mixture was left to stir overnight. The excess solvent was removed in vacuo and the residue was dissolved in diethyl ether (20 mL) before being extracted with water (20 mL × 3). The ethereal layers were dried over MgSO₄ and the excess solvent was removed in vacuo. The remaining solid was purified via column chromatography (20% diethyl ether in pet. ether) to give the product as a colourless oil (162 mg, 0.63 mmol, 98%); Rₗ 0.18 (20% diethyl ether in pet. ether); IR (thin film) v/cm⁻¹: 2978 (C-H, m), 1732 (C=O, s), 1692 (C=O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 4.14 (q, J = 7.0 Hz, 2H, H₈), 4.01 (d, J = 13.0 Hz, 2H, H₄), 2.83 (t, J = 12.5 Hz, 2H, H₄), 2.43 (tt, J = 11.0, 4.0 Hz, 1H, H₆), 1.87 (d, J = 13.5 Hz, 2H, H₅), 1.67–1.57 (m, 2H, H₅), 1.45 (s, 9H, H₁), 1.25 (t, J = 7.0 Hz, 3H, H₉). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 174.6 (C₇), 154.7 (C₃), 79.6 (C₂), 60.5 (C₈), 43.2 (C₄×2), 41.2 (C₆), 28.4 (C₁×3), 28.0 (C₅×2), 14.2 (C₉). HRMS (ESI) C₁₃H₂₃NO₃Na m/z: [M+Na]⁺ 280.1535 (calc. 280.1525).

The physical and spectroscopic data was found to be in agreement with Ueno et al.²²⁶
1-(tert-Butyl) 4-ethyl 4-(2-nitrobenzyl)piperidine-1,4-dicarboxylate, 111

![Chemical Structure Image]

A solution of 109 (1.21 g, 4.70 mmol, 1.0 eq) in THF (10 mL) was cooled to −78 °C and NaHMDS (1M in THF, 6.58 mL, 6.58 mmol, 1.4 eq) was added dropwise. The reaction was stirred at this temperature for 1 h, before 2-nitrobenzyl bromide (1.22 g, 5.64 mmol, 1.2 eq) was added and the reaction was warmed to RT and stirred overnight. At completion, the excess solvent was removed in vacuo and the residue was extracted between water and ethyl acetate and the organic washed with brine. The organic layer was dried over MgSO₄ and the solvent was removed in vacuo. The crude oil produced was purified via column chromatography (20% ethyl acetate in hexane) to give the product as a brown oil (184.6 mg, 0.47 mmol, 10%); Rf 0.26 (20% ethyl acetate in hexane); IR (thin film) ν/cm⁻¹: 2913 (C-H, w), 2713 (C-H, w), 1652 (C=O, s), 1596 (N=O, s), 1401 (N=O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.86 (d, J = 8.0 Hz, 1H, H15), 7.48 (td, J = 1.5,11.5 Hz, 1H, H13), 7.40-7.37 (m, 1H, H14), 7.20 (dd, J = 1.5,7.5 Hz, 1H, H12) 4.07 (q, J = 7.0 Hz, 2H, H8), 3.67 (br. s, 2H, H4a), 3.31-3.29 (m, 2H, H4b), 2.69 (br. s, 2H, H10), 2.07-2.05 (m, 2H, H5a), 1.56 (s, 2H, H5b), 1.42 (s, 9H, H1), 1.18 (t, J = 7.0 Hz, 3H, H9). ¹³C NMR (101MHz, CDCl₃) δ/ppm: 174.0 (C7), 154.7 (C3), 150.4 (C16), 133.2 (C12), 132.8 (C13), 132.2 (C11), 128.0 (C14), 127.9 (C15), 79.6 (C6), 61.0 (C8), 47.6 (C2), 41.7 (C4), 38.4 (C10) 33.3 (C5), 28.4 (C1), 14.0 (C9). HRMS (ESI) C₂₀H₂₉N₂O₆ m/z: [M+H]+ 393.2042 (calc. 393.2026).

1-(tert-Butyl) 4-ethyl 4-(2-aminobenzyl)piperidine-1,4-dicarboxylate, 112

![Chemical Structure Image]

Hydrogen gas was bubbled through a suspension of palladium on carbon (10%, 112 mg, 0.10 mmol, 20 mol %) in ethanol (10 mL) to which 111 (179 mg, 0.46 mmol, 1.0 eq) was added. After completion, the reaction was filter through celite and the excess solvent was removed in vacuo. The product was purified by column chromatography (10% methanol in CH₂Cl₂) to give the...
Experimental

6-Chloro-1H-indene, 115

To a solution of 5-chloro-1-indanone (1.00 g, 6.00 mmol, 1.0 eq) in methanol (30 mL) was added sodium borohydride (613mg, 16.21mmol, 2.7 eq). The mixture was warmed to RT and stirred for 1 h. Next water (10 mL) was added and the reaction was extracted with CH$_2$Cl$_2$ (30 mL × 3). The organic layer was dried over MgSO$_4$ which was subsequently filtered off and the filtrated was concentrated in vacuo to give the crude alcohol. This alcohol was dissolved in toluene (30 mL) and p-toluenesulfonic acid monohydrate (34.2 mg, 0.18 mmol, 0.03 eq) was added before the reaction was refluxed for 2 h. The reaction was cooled to RT and sat. aq. NaHCO$_3$ (30 mL) was added before extraction with ethyl acetate (20 mL × 3). The organic layers were washed with brine (50 mL) and dried over MgSO$_4$. This was filtered off and the filtrate was concentrated in vacuo. The crude oil was purified via column chromatography (100%hexane) to yield the pure product as a colourless oil (615 mg, 4.09 mmol, 68%); R$_f$ 0.45 (100% hexane); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2988 (C-H, m), 1583 (C=C, m), 818 (C-Cl, s). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 7.47 (s, 1H, H2), 7.33 (d, J = 8.0 Hz, 1H, H5), 7.28–7.26 (m, 1H, H4), 6.87 (d, J = 5.5 Hz, 1H, H7), 6.58 (dt, J = 5.5,2.0 Hz, 1H, H8), 3.42 (s, 2H, H9). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 145.4 (C6), 143.3 (C1), 134.6 (C8), 131.4 (C7), 130.6 (C3), 126.4 (C4), 124.1 (C5), 121.6 (C2), 39.0 (C9). HRMS (ESI) C$_{26}$H$_{31}$N$_2$O$_4$ m/z: [M+H]$^+$ 363.2268 (calc. 363.2278).

The physical and spectroscopic data was found to be in agreement with Capkova et al.\textsuperscript{132}
**Experimental**

*tet*-Butyl 6-chlorospiro[indene-1,4’-piperidine]-1’-carboxylate, 116

![Chemical structure](image)

To a solution of 115 (615 mg, 4.08 mmol, 1.0 eq) dissolved in THF (10 mL) cooled to 0 °C was added LiHMDS (1M in THF, 8.16 mL, 8.16 mmol, 2.0 eq) over 15 min. The reaction was stirred for 30 min before 92 (988 mg, 4.08 mmol, 1.0 eq) was added dropwise. The mixture was stirred for 2 h at RT. The excess solvent was removed in vacuo. The residue was diluted with diethyl ether (20 mL) and washed with sat. aq. NH4Cl (20 mL). The aqueous was extracted with diethyl ether (20 mL × 3). The organic layers were dried over MgSO4 and the excess solvent was removed in vacuo to give a crude oil. This was purified by column chromatography (20% diethyl ether in pet. ether) to give the pure product as a colourless oil (1.0022 g, 3.13 mmol, 77%); Rf 0.24 (20% diethyl ether in pet. ether); IR (thin film) ν/cm⁻¹: 2974 (C-H, m), 1686 (C=O, s), 1601 C=C, m), 733 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.30–7.28 (m, 1H, H2), 7.23–7.16 (m, 1H, H4,5), 6.87 (dd, J = 22.0, 5.5 Hz, 1H, H8), 6.73 (dd, J = 5.5,4.0 Hz, 1H, H7), 4.18 (br. s, 2H, H11a), 3.10 (t, J = 12.5 Hz, 2H, H11b), 1.97 (td, J = 12.5, 4.5 Hz, 2H, H10a), 1.51 (s, 9H, H14) 1.35–1.30 (m, 2H, H10b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 154.9 (C12), 153.5 (C6), 149.9 (C3), 144.4 (C1), 141.2 (C8), 129.6 (C7), 127.2 (C5), 125.2 (C4), 122.4 (C2), 79.7 (C13), 52.4 (C9), 42.1 (C11×2), 33.3 (C10×2), 28.5 (C14). HRMS (ESI) C₁₈H₂₂NO₂ClNa m/z: [M+Na]⁺ 342.1225 (calc. 342.1231).

6-Chlorospiro[indene-1,4’-piperidin]-1’-ium chloride, 117

![Chemical structure](image)

A solution of 116 (1.0 g, 3.13 mmol, 1.0 eq) was stirred in 4M HCl/dioxane (10 mL) for 30 min. The mixture was filtered and the residue was desiccated to give the product as a white crystal.
Experimental

(552 mg, 2.15 mmol, 69%); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2944 (C-H, s), 2730 (C-H, s), 1595 (C=C, m), 1457 (C=C, m), 1428 (C-N, m), 1072 (C-Cl, m), 734 (C-Cl, s).

\(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 8.89 (br. s, 2H, NH\(_2\)), 7.45–7.27 (m, 3H, H2,4,5), 7.20 (dd, \( J = 24.0, 5.5 \) Hz, 1H, H7), 6.86 (t, \( J = 5.5 \) Hz, 1H, H8), 3.41–3.38 (m, 2H, H11a), 3.22 (td, \( J = 3.0,13.0 \) Hz, 2H, H11b), 2.33–2.22 (m, 2H, H10a), 1.36–1.30 (m, 2H, H10b).

\(^{13}\)C NMR (101 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 153.6 (C1), 149.6 (C6), 144.6 (C7), 141.9 (C3), 129.6 (C8), 127.4 (C2/4/5), 125.3 (C2/4/5), 123.0 (C2/4/5), 50.7 (C9), 41.8 (C11×2), 29.7 (C10×2).

HRMS (ESI) C\(_{13}\)H\(_{15}\)NCl \( m/z \): [M+H]\(^+\) 220.0879 (calc. 220.0888). m.p. 294–296 °C.

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6-Chloro-2,3-dihydrospiro[indene-1,4'-piperidin]-1'-ium chloride, 118

Hydrogen gas was bubbled through a suspension of Pd on C (10% w/w, 200 mg, 0.19 mmol, 0.16) in ethanol (10 mL). To this 117 was added and the reaction was stirred for 1 h. The catalyst was removed by filtration through celite. The excess solvent of the filtrate was removed \( \text{in vacuo} \) to give the product as white crystals (299 mg, 1.16 mmol, 99%); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2953 (C-H, s), 1475 (C=C, m), 1439 (C-H, m), 1073 (C-N, s), 815 (C-Cl, m).

\(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 8.83 (br. s, 2H, NH\(_2\)), 7.29–7.11 (m, 3H, H2,4,5), 3.27 (d, \( J = 13.0 \) Hz, 2H, H11a), 3.00 (t, \( J = 13.0 \) Hz, 2H, H11b), 2.87 (q, \( J = 8.0 \) Hz, 2H, H7), 2.10–1.93 (m, 4H, H8,10a), 1.62–1.60 (m, 2H, H10b).

\(^{13}\)C NMR (101 MHz, (CD\(_3\))\(_2\)SO) \( \delta / \text{ppm} \): 152.3 (C1), 141.9 (C6), 131.1 (C3), 127.2 (C2/4/5), 126.5 (C2/4/5), 122.2 (C2/4/5), 45.3 (C9), 41.0 (C11×2), 34.3 (C8), 32.6 (C10×2), 29.0 (C7).

HRMS (ESI) C\(_{13}\)H\(_{17}\)NCl \( m/z \): [M+H]\(^+\) 222.1036 (calc. 222.1044). m.p. 300–301 °C.
Experimental

8-Chloro-2-(ethoxycarbonyl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indol-5-ium chloride, 121

Ethyl 4-oxopiperidine-1-carboxylate (0.84 mL, 5.59 mmol, 1.0 eq) and (4-chlorophenyl) hydrazine (1 g, 5.59 mmol, 1.0 eq) were dissolved in ethanol (10 mL) and refluxed for 3 h before being left to stir at room temperature overnight. The residual solid was collected by filtration and washed with 1:1 mixture of H$_2$O:EtOH to give the product as a white amorphous solid (872.6 mg, 3.14 mmol, 56%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3414 (N-H, br. s), 2988 (C-H, m), 1658 (C=O, m), 1050 (C-N, s), 823 (C-Cl, m). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 11.13 (s, 2H, NH$_2$), 7.48 (d, $J$ = 2.0 Hz, 1H, H7), 7.29 (d, $J$ = 8.5 Hz, 1H, H4), 7.02 (dd, $J$ = 8.5, 2.0 Hz, 1H, H6), 4.56 (s, 2H, H1), 4.09 (q, $J$ = 7.0 Hz, 2H, H13), 3.74 (t, $J$ = 5.5 Hz, 2H, H11), 2.79 (t, $J$ = 5.5 Hz, 2H, H10), 1.21 (t, $J$ = 7.0 Hz, 3H, H14). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 155.2 (C12), 134.6 (C3), 134.3 (C8), 126.3 (C9), 123.2 (C5), 120.5 (C6), 116.8 (C7), 112.4 (C4), 105.7 (C2), 61.0 (C13), 40.9 (C1), 40.2 (C11), 23.0 (C10), 14.7 (C14). HRMS (ESI) C$_{14}$H$_{16}$ClN$_2$O$_2$ m/z: [M+H]$^+$ 279.0904 (calc. 279.0895).

The physical and spectroscopic data was found to be in agreement with Yin et al.$^{133}$

8-Chloro-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indol-2-ium chloride, 122

A mixture of 121 (340 mg, 1.22 mmol, 1.0 eq), 0.8M potassium hydroxide (5 mL, 6.10 mmol, 5.0 eq) and ethanol (10 mL) was refluxed overnight. After the reaction was cooled to RT it was stirred with 4M HCl/dioxane. The mixture was filtered and the residue dried in a desiccator to achieve the product as a white crystalline solid (207 mg, 0.85 mmol, 71%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2919 (C-H, m), 2727 (C-H, m), 1441 (C=C, m), 790 (C-Cl, s). $^1$H NMR (400 MHz,
**Experimental**

\[(\text{CD}_3)_2\text{SO}\] δ/ppm: 10.9 (s, 2H, NH₂), 7.37 (d, \(J = 2.0\) Hz, 1H, H7), 7.21 (d, \(J = 8.5\) Hz, 1H, H4), 7.08 (dd, \(J = 8.5, 2.0\) Hz, 1H, H6), 4.02 (t, \(J = 1.5\) Hz, 2H, H11), 3.22 (t, \(J = 5.5\) Hz, 2H, H1), 2.77 (t, \(J = 5.5\) Hz, 2H, H10). \(^{13}\text{C NMR (101 MHz, (CD}_3)_2\text{SO)}\) δ/ppm: 135.5 (C3), 133.9 (C8), 126.8 (C9), 122.8 (C5), 119.9 (C6), 116.5 (C7), 112.1 (C4), 108.3 (C2), 42.9 (C1), 41.5 (C11), 24.1 (C10). HRMS (ESI) C\(_{11}\)H\(_{12}\)N\(_2\)Cl m/z: [M+H]\(^+\) 207.0676 (calc. 207.0684). m.p. 277-279 °C.

The physical and spectroscopic data was found to be in agreement with Yin et al.\(^{133}\)

**Ethyl (diphenylmethylene)glycinate, 140**

![Ethyl (diphenylmethylene)glycinate](image)

Ethyl glycinate hydrochloride (20 g, 143.3 mmol, 1.0 eq), benzophenone (26.11 g, 143.3 mmol, 1.0 eq), and diisopropylethylamine (25 mL, 143.3 mmol, 1.0 eq) were dissolved in toluene (90 mL) and heated with Dean-Stark apparatus until one equivalent of water was observed in the trap. The reaction was cooled to RT and diluted with water (50 mL). The aqueous was extracted with ethyl acetate (3 × 25 mL) and the combined organics were washed with water (50 mL), sat. aq. NaHCO\(_3\) (50 mL), and brine (50 mL). The organics were dried over anh. MgSO\(_4\), filtered, and concentrated in vacuo to give the crude product. This was purified by column chromatography (10% ethyl acetate in pet. ether) to yield the pure product as white crystals (16.61 g, 62.13 mmol, 43%); \(R_f\) 0.14 (10% ethyl acetate in pet. ether); IR (thin film) \(\tilde{\nu}/\text{cm}^{-1}\): 2981 (C-H, w), 1749 (C=O, s), 1620 (C=N, m), 1188 (C-O, s). \(^1\text{H NMR (400 MHz, CDCl}_3\)) δ/ppm: 7.68–7.65 (m, 2H, Ar), 7.49–7.31 (m, 6H, Ar), 7.20–7.17 (m, 2H, Ar), 4.21 (q, \(J = 7.0\) Hz, 2H, H2), 4.20 (s, 2H, H4), 1.27 (t, \(J = 7.0\) Hz, 3H, H1). \(^{13}\text{C NMR (101 MHz, CDCl}_3\)) δ/ppm: 171.9 (C5), 170.7 (C3), 139.3 (Ar C), 136.0 (Ar C), 130.5 (Ar C), 128.8 (Ar C), 128.8 (Ar C×2), 128.7 (Ar C×2), 128.1 (Ar C×2), 127.7 (Ar C×2), 60.9 (C2), 55.7 (C4), 14.2 (C1). HRMS (ESI) C\(_{17}\)H\(_{17}\)NO\(_3\)Na m/z: [M+Na]\(^+\) 290.1160 (calc. 290.1157). m.p. 51–52 °C.

The physical and spectroscopic data were found to be in agreement with Mukherjee et al.\(^{227}\)
**Experimental**

**Ethyl 2-((diphenylmethylene)amino)hex-5-enoate, 141**

Potassium tert-butoxide (3.15 g, 28.06 mmol, 1.5 eq) was dissolved in THF (150 mL) under nitrogen at 0 °C. To the mixture was added 140 (5 g, 18.70 mmol, 1.0 eq) and this was stirred for 10 min. 4-bromo-1-butene (5.7 mL, 56.11 mmol, 3.0 eq) was added to the reaction dropwise before it was warmed to RT and left to stir overnight. The excess solvent was removed via a rotary evaporator and the residue was diluted with ethyl acetate (50 mL). This was washed with sat. aq. NaHCO₃ (50 mL). The organic layer was extracted with ethyl acetate (3 × 20 mL) before the combined organics were washed with brine (50 mL). The organic layer was dried over anh. MgSO₄. This was filtered and concentrated in vacuo to yield the pure product as a yellow oil (5.7169 g, 17.8 mmol, 95%); IR (thin film) ̃ν/cm⁻¹: 2979 (C-H, w), 1735 (C=O, s), 1622 (C=C, m), 1238 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.66–7.64 (m, 2H, Ar), 7.45–7.31 (m, 6H, Ar), 7.19–7.16 (m, 2H, Ar), 5.77–5.67 (m, 1H, H7), 4.98–4.88 (m, 2H, H8), 4.19–4.04 (m, 3H, H2&4), 2.10–1.96 (m, 4H, H5&6), 1.26 (t, J = 7.0 Hz, 3H, H1). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 172.3 (C9), 170.6 (C3), 139.6 (Ar), 137.8 (C7), 136.5 (Ar), 130.9 (Ar), 128.8 (Ar×2), 128.6 (Ar), 128.5 (Ar×2), 128.1 (Ar×2), 127.9 (Ar×2), 115.0 (C8), 64.9 (C4), 60.9 (C2), 33.0 (C5/6), 30.2 (C5/6), 14.2 (Cl). HRMS (ESI) C₂₁H₂₅NO₂Na m/z: [M+Na]⁺ 344.1624 (calc. 344.1626).

The physical and spectroscopic data were found to be in agreement with Andrei *et al.*²²⁸
Experimental

**Ethyl 2-allyl-2-aminohex-5-enoate, 142**

- Potassium tert-butoxide (6.47 g, 57.76 mmol, 1.5 eq) was dissolved in THF (100 mL) under nitrogen at 0 °C. To the mixture was added 141 (12.36 g, 38.4 mmol, 1.0 eq) and was stirred for 10 min. Allyl bromide (10.0 mL, 115.32 mmol, 3.0 eq) was added dropwise before the reaction was warmed to RT and stirred overnight. This was quenched with 3M HCl (38.4 mL, 1.0 eq) and stirred for 5 min. This mixture was diluted with dichloromethane (50 mL) and water (20 mL). This was extracted with dichloromethane (3 × 30 mL). The combined aqueous was basified with Na₂CO₃ and was extracted with ethyl acetate (3 × 30 mL). These organic extractions were dried over MgSO₄, filtered and concentrated in vacuo to yield the pure product as a yellow oil (6.5392 g, 33.15 mmol, 86%); IR (thin film) ν/cm⁻¹: 3078 (C-H, w), 1727 (C=O, s), 1640 (C=C, m), 1199 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 5.82–5.74 (m, 1H, H7), 5.73–5.64 (m, 1H, H10), 5.15–5.12 (m, 2H, H11), 5.04–4.93 (m, 2H, H8), 4.20–4.15 (m, 2H, H2), 2.58–2.54 (m, 1H, H9a), 2.28–2.23 (m, 1H, H9b), 2.17–2.09 (m, 1H, H6a), 1.99–1.91 (m, 1H, H6b), 1.88–1.82 (m, 1H, H5a), 1.67–1.61 (m, 3H, H5b&NH₂), 1.29–1.26 (m, 3H, H1). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 176.5 (C3), 137.9 (C7), 132.6 (C10), 119.5 (C11), 114.9 (C8), 61.0 (C2), 60.4 (C4), 44.3 (C9), 39.1 (C5), 28.4 (C6), 14.8 (C1). HRMS (ESI) C₁₁H₁₈NO₂Na m/z: [M+Na]⁺ 220.1313 (calc. 220.1313).

**Ethyl 2-allyl-2-((tert-butoxycarbonyl)amino)hex-5-enoate, 143**

- Compound 142 (4 g, 20.28 mmol, 1.0 eq) was dissolved in THF (100 mL). Boc₂O (7 mL, 30.41 mmol, 1.5 eq) was added dropwise and the reaction was refluxed at 70 °C for 3 hours. The excess solvent was removed in vacuo and the crude oil was purified via column
chromatography (100% dichloromethane) to yield the product as a colourless oil (5.1314 g, 17.25 mmol, 85%): IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3430 (N-H, w), 3076 (C-H, w), 1714 (C=O, s), 1642 (C=C, w). $^{1}$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 5.80–5.70 (m, 1H, H7), 5.67–5.56 (m, 1H, H10), 5.51 (br s, 1H, NH$_2$), 5.01–4.91 (m, 2H, H8), 4.20 (q, $J$ = 7.0 Hz, 2H, H11), 5.08–5.05 (m, 2H, H1, H13), 3.07 (m, 1H, H9a), 2.40 (m, 1H, H5a), 2.11–2.01 (m, 1H, H6a), 1.88–1.78 (m, 2H, H5b&H6b), 1.43 (s, 9H, H1), 1.28 (t, $J$ = 7.0 Hz, 3H, H14). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 173.3 (C12), 146.7 (C3), 137.6 (C7), 132.5 (C10), 118.8 (C11), 115.0 (C8), 85.2 (C2), 63.2 (C4), 61.7 (C13), 39.8 (C9), 34.4 (C5), 28.4 (C6), 27.4 (C1), 14.2 (C14). HRMS (ESI) $^{13}$C$_{16}$H$_{28}$NO$_4$ m/z: [M+H]$^+$ 298.2007 (calc. 298.2018).

Ethyl 1-((tert-butoxycarbonyl)amino)cyclohex-3-ene-1-carboxylate, 144

Compound **143** (1 g, 3.36 mmol, 1.0 eq) was dissolved in dichloromethane (170 mL) and Grubbs second generation catalyst (285 mg, 0.34 mmol, 10 mol%) was added. The reaction was refluxed at 50 °C for 1 hour under nitrogen. When the reaction had gone to completion the excess solvent was removed in vacuo to give the crude product. The was purified via column chromatography (50 % ethyl acetate in pet. ether) to yield the pure product as a brown oil (709.6 mg, 2.63 mmol, 78%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3379 (n-H, w), 2975 (C-H, w), 1708 (C=O, s), 1163 (C-O, s). $^{1}$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 5.74–5.71 (m, 1H, H7), 5.59–5.55 (m, 1H, H8), 4.22–4.12 (m, 2H, H11), 2.59–2.53 (m, 1H, H9a), 2.28–2.18 (m, 2H, H9b&H5a), 2.13–2.04 (m, 2H, H6), 1.93–1.86 (m, 1H, H5b), 1.42 (s, 9H, H1), 1.25 (t, $J$ = 2.0 Hz, 3H, H12). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 174.1 (C12), 154.9 (C3), 127.1 (C7), 122.5 (C8), 79.8 (C2), 61.1 (C11), 56.8 (C4), 34.1 (C9), 28.3 (C1), 27.5 (C5), 21.8 (C6), 14.2 (C14). HRMS (ESI) $^{13}$C$_{14}$H$_{23}$NO$_4$Na m/z: [M+Na]$^+$ 292.1515 (calc. 292.1519).

The physical and spectroscopic data were found to be in agreement with Fariba et al.229
**Experimental**

**tert-Butyl (1-(hydroxymethyl)cyclohex-3-en-1-yl)carbamate, 145**

![Chemical Structure](image)

Compound 144 (660 mg, 2.45 mmol, 1.0 eq) was dissolved in THF (25 mL) and cooled to 0 °C before lithium borohydride (267 mg, 12.25 mmol, 5.0 eq) was added and the reaction was stirred overnight. This was quenched with sat. aq. NH₄Cl (25 mL) and stirred for 10 min. The layers were separated and the aqueous was extracted with ethyl acetate (3 × 20 mL). The combined organics were washed with sat. aq. NaHCO₃ (30 mL) and brine (30 mL) and dried over MgSO₄ before being filtered and concentrated *in vacuo* to give a crude oil. This was purified via column chromatography (20% ethyl acetate in pet. ether) to yield the product as white crystals (319.4 mg, 1.41 mmol, 57%); Rₚ 0.18 (20% ethyl acetate in pet. ether); IR (thin film) ν/cm⁻¹: 3423 (O-H, m), 2889 (C-H, w), 1681 (C=O, s), 1528 (C=C, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 5.76–5.74 (m, 1H, H₇), 5.59–5.56 (m, 1H, H₈), 4.65 (br s, 1H, NH), 4.22 (br s, 1H, OH), 3.70 (d, J = 6.5 Hz, H₁₀), 2.17–2.00 (m, 5H, H₅a&6&9), 1.74–1.69 (m, 1H, H₅b), 1.44 (s, 9H, H₁). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 156.6 (C₃), 127.4 (C₇), 123.1 (C₈), 80.0 (C₂), 69.2 (C₁₀), 55.0 (C₄), 34.0 (C₉), 28.3 (C₁), 27.3 (C₅), 22.1 (C₆). HRMS (ESI) C₁₂H₂₁NO₃Na m/z: [M+Na]⁺ 250.1403 (calc. 250.1414). m.p. 77–78 °C.

**1-(Hydroxymethyl)cyclohex-3-en-1-aminium chloride, 146**

![Chemical Structure](image)

Compound 145 (151.6 mg, 0.67 mmol) was dissolved in hydrochloric acid (4M in dioxane, 10 mL) and was left for 1 h. The reaction was concentrated *in vacuo* to give off white crystals. These were washed with cold acetone to yield the pure product as white crystals (108.3 mg, 0.66 mmol, 99%); IR (thin film) ν/cm⁻¹: 3217 (O-H, m), 2837 (C-H, s), 1628 (C=C, m), 1048 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.95 (br s, 3H, NH₃⁺), 5.69–5.66 (m, 1H, H₆), 5.57–5.54 (m, 1H, H₅), 5.47 (t, J = 5.0 Hz, 1H, OH), 3.42 (d, J = 5.0 Hz, 2H, H₁), 2.14 (br s, 2H, H₇), 2.08–2.06 (m, 2H, H₄), 1.81–1.66 (m, 2H, H₃). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 126.2 (C₆), 162
Experimental

122.9 (C5), 62.7 (C1), 55.1 (C2), 30.3 (C7), 26.5 (C3), 21.4 (C4). **HRMS (ESI)** \( C_7H_{14}NO \) \( m/z \): [M-Cl]⁺ 128.1071 (calc. 128.1070). **m.p.** 222–223 °C.

3-Oxa-1-azaspiro[4.5]dec-7-en-2-one, 148

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Compound 145 (200 mg, 0.88 mmol, 1.0 eq) was dissolved in THF (10 mL). The mixture was cooled to 0 °C and potassium tert-butoxide (197 mg, 1.76 mmol, 2.0 eq) was added and the reaction was warmed to RT and stirred for 1 h. The reaction was then diluted with sat. aq. NH₄Cl (10 mL), extracted with dichloromethane (3 × 10 mL), dried over anh. MgSO₄, before the mixture was filtered and concentrated in vacuo. The crude was purified by column chromatography (40% ethyl acetate in pet. ether) to give the product as white crystals (108.0 mg, 0.71 mmol, 80%); **Rₙ** 0.11 (40% ethyl acetate in pet. ether); **IR (thin film)** \( \tilde{\nu} / \text{cm}^{-1} \): 3240 (N-H, w), 2924 (C-H, w), 1730 (C=O, s). **¹H NMR (400 MHz, CDCl₃)** \( \delta / \text{ppm} \): 5.76–5.71 (m, 1H, H6), 5.65–5.61 (m, 1H, H7), 5.28 (br s, 1H, NH), 4.15 (d, \( J = 8.5 \text{ Hz} \), 1H, H2a), 4.12 (d, \( J = 8.5 \text{ Hz} \), 1H, H2b), 2.32–2.21 (m, 4H, H5&8), 1.89–1.84 (m, 1H, H4a), 1.74–1.72 (m, 1H, H4b). **¹³C NMR (101 MHz, CDCl₃)** \( \delta / \text{ppm} \): 158.5 (C1), 127.0 (C6), 123.7 (C7), 75.5 (C2), 55.9 (C3), 36.8 (C5/8), 32.2 (C4), 22.5 (C5/8). **HRMS (ESI)** \( C_8H_{11}NO_2Na \) \( m/z \): [M+Na]⁺ 176.0690 (calc. 176.0682). **m.p.** 107–108 °C.

**tert-Butyl (4-(hydroxymethyl)octa-1,7-dien-4-yl)carbamate, 147**

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Compound 143 (2 g, 6.72 mmol, 1.0 eq) was dissolved in THF (50 mL) and cooled to 0 °C under nitrogen. Lithium borohydride (2M in THF, 10.1 mL, 20.17 mmol, 3eq) was added dropwise.
Experimental

The reaction was warned to RT and stirred overnight. The reaction was quenched by slow addition of sat. aq. NH₄Cl. This was extracted with ethyl acetate (3 × 25 mL). The combined organics were dried over anh. Na₂SO₄, filtered and the excess solvent removed in vacuo to give the crude product. This was purified via column chromatography (25 % ethyl acetate in pet. ether) to yield the product as a colourless oil (463.2 mg, 1.81 mmol, 27%); Rf 0.24 (25% ethyl acetate in pet. ether); IR (thin film) $\tilde{\nu}$/cm⁻¹: 3404 (O-H, br w), 2978 (C-H, w), 1683 (C=O, s), 1640 (C=C, m), 1164 (C-O, s), 1053 (C-OH, s). $^1$H NMR (400 MHz, CDCl₃) $\delta$/ppm: 5.83–5.72 (m, 2H, H7&10), 5.15–5.11 (m, 2H, H11), 5.04–4.93 (m, 2H, H8), 4.66 (br s, 1H, OH), 4.22 (br s, 1H, NH₂), 3.69–3.60 (m, 2H, H9), 2.38–2.27 (m, 2H, H6), 1.74–1.59 (m, 2H, H5), 1.41 (s, 9H, H1). $^{13}$C NMR (101 MHz, CDCl₃) $\delta$/ppm: 156.2 (C3), 138.1 (C7), 132.8 (C10), 119.4 (C11), 11408 (C8), 79.9 (C2), 67.8 (C12), 58.7 (C4), 39.0 (C9), 33.6 (C5), 28.3 (C1), 27.7 (C6). HRMS (ESI) C₁₄H₂₆NO₃ m/z: [M+H]+ 256.1908 (calc. 256.1913).

Ethyl benzimidate hydrochloride, 150

![Chemical Structure](image)

To a solution on benzonitrile (5.00 g, 48.49 mmol, 1.0 eq) in ethanol (34 mL, 581.85 mmol, 12.0 eq), acetyl chloride (27.68 mL, 387.90 mmol, 8.0 eq) was added dropwise. The reaction was stirred overnight then concentrated in vacuo to yield the product as white crystals (8.0401 g, 43.31 mmol, 89%); IR (thin film) $\tilde{\nu}$/cm⁻¹: 2828 (C-H, br s), 1626 (C=N, s), 1451 (C=C, s), 1059 (C-O, s). $^1$H NMR (400 MHz, CDCl₃) $\delta$/ppm: 12.60 (br s, 1H, NH), 11.91 (br s, 1H, NH), 8.40–8.38 (m, 2H, H3), 7.76–7.67 (m, 1H, H1), 7.58–7.54 (m, 2H, H2), 4.93 (q, $J = 7.0$ Hz, 2H, H6), 1.61 (t, $J = 7.0$ Hz, 3H, H7). $^{13}$C NMR (101 MHz, CDCl₃) $\delta$/ppm: 170.9 (C5), 135.6 (C1), 129.7 (C3), 129.3 (C2), 125.3 (C4), 71.3 (C6), 13.9 (C7). HRMS (ESI) C₉H₁₂NO m/z: [M+Cl]⁺ 150.0913 (calc. 150.0913). m.p. 120–121 °C.

The physical and spectroscopic data were found to be in agreement with Berger et al.²³⁰
Experimental

2-Phenyl-3-oxa-1-azaspiro[4.5]deca-1,7-diene, 151

![Structural formula of compound 151](image)

Compound 146 (50 mg, 0.31 mmol, 1.1 eq) and 150 (41.4 mg, 0.28 mmol, 1.0 eq) were dissolved in 1,2-dichloroethane (1 mL) and refluxed overnight. The reaction was cooled to RT and filtered. The filtrate was concentrated in vacuo and the crude was purified by column chromatography (30% ethyl acetate in hexane) to yield the product as white crystals (34.0 mg, 0.16 mmol, 57%); Rf 0.44 (30% ethyl acetate in hexane); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2905 (C-H, w), 1648 (C=N, s), 1581 (C=C, m), 1059 (C-O, m). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 7.96–7.92 (m, 2H, H3), 7.48–7.44 (m, 1H, H1), 7.41–7.37 (m, 2H, H2), 5.77–5.73 (m, 1H, H10), 5.69–5.63 (m, 1H, H11), 4.16 (d, \( J = 8.5 \) Hz, 1H, H6a), 4.11 (d, \( J = 8.5 \) Hz, 1H, H6b), 2.48–2.43 (m, 1H, H12a), 2.36–2.29 (m, 1H, H9a), 2.17–2.08 (m, 2H, H9b&12b), 2.05–1.98 (m, 1H, H8a), 1.75–1.69 (m, 1H, H8b). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 162.5 (C5), 131.2 (C1), 128.28 (C3), 128.26 (C2), 128.1 (C4), 127.2 (C10), 124.4 (C11), 77.6 (C6), 69.4 (C7), 37.3 (C12), 33.0 (C8), 22.8 (C9). HRMS (ESI) C\(_{14}\)H\(_{16}\)NO \( m/z \): [M+H]\(^+\) 214.1222 (calc. 214.1226).

3-Oxa-1-azaspiro[4.5]deca-1,7-dien-2-amine, 152

![Structural formula of compound 152](image)

Cyanobromide (175 mg, 1.65 mmol, 1.2 eq) was added to a solution of 146 (175 mg, 1.38 mmol, 1.0 eq) as a free base in ethanol (5 mL) and refluxed overnight. This was concentrated in vacuo and dissolved in dichloromethane (10 mL). This was washed with 1M NaOH (10 mL) and the aqueous was extracted with dichloromethane (10 mL). The combined organics were concentrated in vacuo to yield the product as off-white crystals (122.2 mg, 0.80 mmol, 58%); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 3437 (N-H, m), 2903 (C-H, m), 1666 (C=N, s), 1650 (C=C, m), 1010 (C-O, s).
Experimental

\( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 5.72–5.68 (m, 1H, H6), 5.64–5.60 (m, 1H, H7), 4.01 (d, \( J = 8.0 \text{ Hz}, 1\text{H}, \text{H2a} \)), 3.97 (d, \( J = 8.0 \text{ Hz}, 1\text{H} \text{H2b} \)), 2.31–2.21 (m, 2H, H5a&8a), 2.12–2.02 (m, 2H, H5b&8b), 1.83–1.78 (m, 1H, H4a), 1.67–1.62 (m, 1H, H4b). \( ^{13}C \) NMR (101 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 159.2 (C1), 127.0 (C6), 124.6 (C7), 78.6 (C2), 66.6 (C3), 37.9 (C5/8), 33.4 (C4), 23.1 (C5/8).

HRMS (ESI) \( \text{C}_8\text{H}_{13}\text{N}_2\text{O} \, m/z: [\text{M}+\text{H}]^+ 153.1025 \) (calc. 153.1028). m.p. 198–199 °C

Crystallographic data for this compound is in Appendix B.

4-Oxa-1-azaspiro[5.5]undec-8-en-3-one, 158

![Chemical Structure](attachment:image.png)

Compound 146 as a free base (150 mg, 0.92 mmol, 1.0 eq) was dissolved in acetonitrile before phenyl bromoacetate (216.5 mg, 1.00 mmol, 1.1 eq) and diisopropylethylamine (0.4 mL, 2.29 mmol, 2.5 eq) were added. The reaction was stirred at RT for 4 h before being concentrated \textit{in vacuo} to give the crude product. This was purified by column chromatography (100% ethyl acetate) to yield the pure product as white crystals (20 mg, 0.12 mmol, 13%); IR (thin film) \( \tilde{\nu}/\text{cm}^{-1} \): 3317 (N–H, w), 2921 (C–H, w), 1730 (C=O, s), 1215 (C–N, s), 1040 (C–O, s). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 5.74–5.70 (m, 1H, H7), 5.62–5.57 (m, 1H, H8), 4.20 (d, \( J = 11.0 \text{ Hz}, 1\text{H}, \text{H3a} \)), 4.13 (d, \( J = 11.0 \text{ Hz}, 1\text{H}, \text{H3b} \)), 3.68 (s, 2H, H1), 2.17–1.97 (m, 5H, H6&9&NH), 1.80–1.74 (m, 1H, H5a), 1.67–1.61 (m, 1H, H5b). \( ^{13}C \) NMR (101 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 169.1 (C2), 126.7 (C7), 123.3 (C8), 76.2 (C3), 48.2 (C4), 44.0 (C1), 33.2 (C6/9), 28.6 (C5), 22.0 (C6/9). HRMS (ESI) \( \text{C}_9\text{H}_{14}\text{NO}_2 \, m/z: [\text{M}+\text{H}]^+ 168.1017 \) (calc. 168.1019).
Experimental

4-Oxa-1-azaspiro[5.5]undeca-1,8-dien-3-one, 159

Compound 158 (100 mg, 0.60 mmol, 1.0 eq), was dissolved in acetonitrile (6 mL) and lead (IV) acetate (345 mg, 0.78 mmol, 1.3 eq) was added before the reaction was allowed to stir for 30 min at RT. This was then diluted with ethyl acetate (10 mL) and filtered through Celite. The filtrate was washed with sat. aq. NaHCO₃ solution (20 mL) and brine (20 mL) before being dried over anh. MgSO₄, filtered and concentrated in vacuo. The crude oil was purified by column chromatography (20% ethyl acetate in hexane) to give the pure product as a yellow oil (91.1 mg, 0.55 mmol, 92%); Rf 0.25 (20% ethyl acetate in hexane); IR (thin film) ̃ν/cm⁻¹: 2923 (C-H, w), 1737 (C=O, s), 1622 (C=N, m), 1038 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.82 (s, 1H, H1), 5.83–5.77 (m, 1H, H7), 5.71–5.65 (m, 1H, H8), 4.29 (d, J = 11.5 Hz, 1H, H3a), 4.25 (d, J = 11.5 Hz, 1H, H3b), 2.31–2.21 (m, 2H, H6a&9a), 2.15–2.03 (m, 2H, H6b&9b), 2.02–1.95 (m, 1H, H5a), 1.70–1.64 (m, 1H, H5b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 154.6 (C2), 151.0 (C1), 127.0 (C7), 122.9 (C8), 72.0 (C3), 55.8 (C4), 32.6 (C6/9), 30.1 (C5), 22.0 (C6/9). HRMS (ESI) C₉H₁₁NO₂Na m/z: [M+H]⁺ 188.0681 (calc 188.0682).

2-Chloro-N-(1-(hydroxymethyl)cyclohex-3-en-1-yl)acetamide, 160

Compound 145 (1.19 g, 5.24 mmol, 1.0 eq) was dissolved in THF (50 mL). 3M HCl (17.5 mL, 0.3M) was added and the mixture was refluxed for 3 h. This was concentrated in vacuo and the residue was dissolved in dichloromethane (50 mL) before triethylamine (2.2 mL, 15.7 mmol, 3.0 eq), and chloroacetyl chloride (0.42 mL, 5.24 mmol, 1.0 eq) were added dropwise at 0 °C. This was stirred for 90 min. The reaction was then diluted with sat. aq. NH₄Cl
Experimental

(25 mL) and stirred for 10 min. This was extracted with dichloromethane (3 × 20 mL) and the organics were dried over anh. MgSO₄ before being filtered and concentrated in vacuo. This crude was purified via column chromatography (dichloromethane to 10% diethyl ether in dichloromethane) to give the pure product as white crystals (261.2 mg, 1.28 mmol, 24%); Rf 0.14 (10% diethyl ether in dichloromethane); IR (thin film) ʋ/cm⁻¹: 3352 (O-H, br m), 3271 (N-H, m), 2938 (C-H, w), 1654 (C=O, s), 1549 (C=C, s), 656 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.67 (br s, 1H, N-H), 5.81–5.77 (m, 1H, H6), 5.64–5.60 (m, 1H, H7), 4.30 (t, J = 6.5 Hz, 1H, OH), 4.04 (s, 2H, H1), 3.75 (d, J = 6.5 Hz, 2H, H9), 2.27–2.18 (m, 3H, H8&5a), 2.11–2.03 (m, 2H, H5b&4a), 1.79–1.73 (m, 1H, H4b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 166.9 (C2), 127.4 (C6), 122.8 (C7), 68.3 (C9), 57.4 (C3), 42.9 (C1), 33.4 (C8), 27.2 (C4), 21.8 (C5). HRMS (ESI) C₉H₁₅ClNO₂ m/z: [M+H]+ 204.0776 (calc. 204.0786). m.p. 86–87 °C.

4-Oxa-1-azaspiro[5.5]undec-8-en-2-one, 161

Compound 145 (260 mg, 1.28 mmol, 1.0 eq) was dissolved in tert-butanol (25 mL) and heated to 30 °C before potassium tert-butoxide (158 mg, 1.40 mmol, 1.1 eq) was added and the reaction was left to stir for 5 h. It was then diluted with sat. aq. NH₄Cl (20 mL) and stirred for 10 min. This was extracted with dichloromethane (3 × 20 mL). The organics were dried over anh. MgSO₄. This was filtered and concentrated in vacuo to yield the pure product as off-white crystals (211.9 mg, 1.27 mmol, 99%); Rf 0.28 (10% diethyl ether in dichloromethane); IR (thin film) ʋ/cm⁻¹: 3169 (N-H, m), 3070 (C-H alkene, w), 2924 (C-H alkene, w), 1661 (C=O, s), 1098 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.13 (br s, 1H, NH), 5.76–5.73 (m, 1H, H7), 5.62–5.60 (m, 1H, H8), 4.16 (d, J = 4.5 Hz, 2H, H2), 3.60 (s, 2H, H3), 2.27–2.21 (m, 1H, H9a), 2.18–2.16 (m, 2H, H6), 2.05–1.99 (m, 1H, H9b), 1.93–1.86 (m, 1H, H5a), 1.70–1.63 (m, 1H, H5b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 168.7 (C1), 126.9 (C7), 123.0 (C8), 71.8 (C3), 67.2 (C2), 52.4 (C4), 34.7 (C9), 31.0 (C5), 21.9 (C6). HRMS (ESI) C₉H₁₃NO₂Na m/z: [M+Na]+ 190.0831 (calc. 190.0839). m.p. 128–129 °C.
Ethyl 2-allyl-2-(3-ethoxy-3-oxopropanamido)hex-5-enoate, 165

Compound 142 (1.0 g, 5.07 mmol, 1.0 eq) was dissolved in dichloromethane (50 mL) at 0 °C under nitrogen. Triethylamine (1.52 mL, 10.90 mmol, 2.15 eq) and ethyl malonyl chloride (1.04 mL, 8.11 mmol, 1.6 eq) were added dropwise. The reaction was warmed to RT and stirred for 30 min. This was quench with sat. aq. NH₄Cl (25 mL) and stirred for a further 10 min. The layers were separated and the aqueous was extracted with dichloromethane (2 × 20 mL). The combined organics were dried over anh. MgSO₄, filtered and concentrated in vacuo to give the crude product. This was purified by column chromatography (20% ethyl acetate in pet. ether) to yield the pure product as a yellow oil (1.2711 g, 4.08 mmol, 81%); Rₖ 0.23 (20% ethyl acetate in pet. ether); IR (thin film) ʋ/cm⁻¹: 3337 (N-H, w), 2980 (C-H, w), 1732 (C=O ester, s), 1681 (C=O amide, m), 1650 (C=C, m), 1221 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ ppm: 7.66 (br s, 1H, NH), 5.77–5.67 (m, 1H, H₉), 5.63–5.52 (m, 1H, H₁₂), 5.10–4.90 (m, 4H, H₁₀&₁₃), 4.25–4.18 (m, 4H, H₂&₁₅), 3.28 (s, 2H, H₄), 3.24–3.18 (m, 1H, H₁₁a), 2.59–2.47 (m, 2H, H₈a&₁₁b), 2.07–1.99 (m, 1H, H₇a), 1.90–1.74 (m, 2H, H₈b&₁₅), 1.30–1.26 (m, 6H, H₁&₁₆). ¹³C NMR (101 MHz, CDCl₃) δ/ ppm: 173.1 (C₁₄), 168.8 (C₃), 163.8 (C₅), 137.3 (C₉), 132.2 (C₁₂), 118.9 (C₁₃), 115.1 (C₁₀), 64.5 (C₆), 61.9 (C₂/₅), 61.6 (C₂/₅), 42.6 (C₄), 39.4 (C₁₁), 34.0 (C₇), 28.5 (C₈), 14.2 (C₁/₁₆), 14.1 (C₁/₁₆). HRMS (ESI) C₁₆H₂₆NO₅ m/z: [M+H]⁺ 312.1820 (calc. 312.1811).
5-Allyl-5-(but-3-en-1-yl)pyrrolidine-2,4-dione, 167

Potassium tert-butoxide (691.9 mg, 6.17 mmol, 1.6 eq) was dissolved in THF (35 mL) and 165 (1.2 g, 3.85, 1.0 eq) was added dropwise. The reaction was refluxed for 2 hours. It was then cooled to RT and diluted with ethyl acetate (20 mL) and 1M HCl (20 mL) before stirred for 5 min. The layers were separated and the aqueous was washed with ethyl acetate (10 mL). The combined organics were dried over MgSO₄, filtered, and concentrated in vacuo. This crude yellow oil was dissolved in acetonitrile-water mixture (10:1, total volume 35 mL). This was refluxed at 100 °C for 1 hour. The reaction was cooled to RT, dried over MgSO₄, filtered, and concentrated in vacuo. The crude oil was purified via column chromatography (50% ethyl acetate in pet. ether) to yield the product as white crystals (147.0 mg, 0.76 mmol, 20%); Rₐ 0.28 (50% ethyl acetate in pet. ether); IR (thin film) ν/cm⁻¹: 3197 (N-H, m), 2980 (C-H, w), 1642 (C=O, s), 1574 (C=C, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.34 (br s, 1H, NH), 5.74–5.66 (m, 2H, H7&10), 5.22–5.14 (m, 2H, H11), 5.06–4.98 (m, 2H, H8), 2.87 (d, J = 3.5 Hz, 2H, H2), 2.45–2.34 (m, 2H, H9), 2.22–2.14 (m, 1H, H6a), 2.02–1.92 (m, 2H, H6b&5a), 1.75–1.66 (m, 1H, H5b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 209.5 (C3), 170.3 (C1), 136.8 (C7), 130.2 (C10), 121.5 (C11), 116.1 (C8), 71.2 (C4), 42.1 (C9), 41.5 (C2), 35.6 (C5), 28.3 (C6). HRMS (ESI) C₁₁H₁₅NO₂ m/z: [M]+ 193.1099 (calc. 193.1103). m.p. 99–100 °C.

1-Azaspiro[4.5]dec-7-ene-2,4-dione, 168

Compound 167 (119 mg, 0.62 mmol, 1.0 eq) was dissolved in dichloromethane (31 mL) and degassed with nitrogen for 5 min. Grubbs second generation catalyst (52.3 mg, 0.06 mmol, 10
mol %) was added and the reaction was refluxed for 1 hour. The reaction was cooled to RT and concentrated in vacuo. This was purified via column chromatography (dichloromethane then ethyl acetate) to yield the pure product as white crystals (60 mg, 0.36 mmol, 59%); Rf 0.14 (100% diethyl ether); IR (thin film) ν/cm⁻¹: 3185 (N-H, w), 3035 (C-H, w), 1651 (C=C, s), 1588 (N-H, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.27 (br s, 1H, NH), 5.81–5.78 (m, 1H, H7), 5.72–5.68 (m, 1H, H8), 3.16 (d, J = 22.0 Hz, 1H, H2a), 3.07 (d, J = 22.0 Hz, 1H, H2b), 2.62–2.54 (m, 1H, H9a), 2.34–2.29 (m, 1H, H6a), 2.21–2.09 (m, 1H, H6b), 2.06–2.00 (m, 1H, H9b), 1.93–1.85 (m, 1H, H5a), 1.74–1.68 (m, 1H, H5b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 209.0 (C3), 169.6 (C1), 126.5 (C7), 123.2 (C8), 66.0 (C4), 40.1 (C2), 33.3 (C9), 29.3 (C5), 21.3 (C6). HRMS (ESI) C₉H₁₂NO₂ m/z: [M+H]⁺ 166.0866 (calc. 166.0863). m.p. 187–188°C.

4-Ethoxy-1-azaspiro[4.5]deca-3,7-dien-2-one, 174

Compound 168 (100 mg, 0.61 mmol, 1.0 eq) was dissolved in THF (10 mL) at 0 °C. To this was added Potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.22 mL, 0.61 mmol, 1.0 eq) and the reaction was left to stir for 10 min before ethyl bromide (0.09 mL, 0.73 mmol, 1.2 eq) and 18-crown-6 (176 mg, 0.66 mmol, 1.1 eq) was added. The reaction was warmed to RT and left to stir overnight. The reaction was concentrated in vacuo and purified via column chromatography (ethyl acetate) to give the product as yellow-white crystals (64.1 mg, 0.33 mmol, 54%); Rf 0.24 (ethyl acetate); IR (thin film) ν/cm⁻¹: 3188 (N-H, w), 3060 (C=H alkene, w), 2933 (C-H alkane, w), 1672 (C=O, s), 1026 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 5.82–5.80 (m, 1H, H7), 5.72–5.68 (m, 1H, H8), 4.91 (d, J = 1.5 Hz, 1H, H2), 4.01 (q, J = 7.0 Hz, 2H, H10), 2.64 (m, 1H, H9a), 2.32–2.12 (m, 2H, H6), 1.97–1.85 (m, 2H, H9b&5a), 1.60–1.55 (m, 1H, H5b), 1.39 (t, J = 7.0 Hz, 3H, H11). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 180.4 (C1), 173.2 (C3), 126.5 (C7), 124.1 (C8), 92.1 (C2), 67.1 (C10), 59.9 (C4), 33.6 (C9), 29.2 (C5), 22.5 (C6), 14.1 (C11). HRMS (ESI) C₁₁H₁₆NO₂ m/z: [M+H]⁺ 194.1181 (calc. 194.1176). m.p. 145–146 °C.
2-Oxo-1-azaspiro[4.5]deca-3,7-dien-4-yl trifluoromethanesulfonate, 175

![Chemical Structure](image)

Compound **168** (100 mg, 0.61 mmol, 1.0 eq) was dissolved in dichloromethane (5 mL), triethylamine (0.25 mL, 1.82 mmol, 3.0 eq) and triflic anhydride (0.3 mL, 1.82 mmol, 3.0 eq) were added dropwise at 0 °C, and the reaction was stirred for 1 h. The reaction was concentrated in vacuo. This was purified by column chromatography (70% to 90% diethyl ether in pet. ether) to give the pure product as white crystals (107.0 mg, 0.36 mmol, 59%); Rf 0.18 (70% diethyl ether in pet. ether); IR (thin film) ν/cm⁻¹: 3164 (N-H, w), 2926 (C-H, w), 1698 (C=O, s), 1634 (C=C, s), 1332 (S=O, m), 1131 (C-F, s).¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.44 (br s, 1H, NH), 5.93 (d, J = 2.0 Hz, 1H, H2), 5.86–5.83 (m, 1H, H7), 5.77–5.73 (m, 1H, H8), 2.67–2.60 (m, 1H, H9a), 2.41–2.33 (m, 1H, H6a), 2.28–2.17 (m, 1H, H6b), 2.02–1.94 (m, 2H, H9a&5a), 1.71–1.67 (m, 1H, H5b).¹³C NMR (101 MHz, CDCl₃) δ/ppm: 168.5 (C1/3), 126.7 (C7), 1332 (S=O, m), 123.1 (C8), 120.0 (C10), 107.3 (C2), 60.8 (C4), 32.6 (C9), 28.6 (C5), 22.4 (C6).¹⁹F NMR (376 MHz, CDCl₃) δ/ppm: -72.5. HRMS (ESI) C₁₀H₁₁F₃NO₄S m/z: [M+H]+ 298.0361 (calc. 298.0355). m.p. 119–120 °C.

4-(4-Methoxyphenyl)-1-azaspiro[4.5]deca-3,7-dien-2-one, 176

![Chemical Structure](image)

Compound **175** (50 mg, 0.17 mmol, 1.0 eq) was dissolved in THF (1.7 mL). (4-Methoxyphenyl)boronic acid (38.3 mg, 0.25 mmol, 1.5 eq) was added and stirred until it was dissolved. Tetrakis(triphenylphosphine)palladium(0) (9.7 mg, 0.008 mmol, 5 mol%) was added as well as sodium carbonate (39.2 mg, 0.37 mmol, 2.2 eq) dissolved in water (0.2 mL). The reaction was stirred for 40 min before being refluxed for 3 h. It was then called to RT, filtered through Celite, washed with ethyl acetate, and concentrated in vacuo. The crude was
Experimental

purified via column chromatography (diethyl ether to 50% diethyl ether in ethyl acetate to ethyl acetate) to give the pure product as yellow-white crystals (31.5 mg, 0.12 mmol, 73%); \( R_f \) 0.11 (diethyl ether); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 3160 (N-H, w), 3035 (alkene C-H, w), 2924 (C-H, w), 1680 (C=O, s), 1607 (C=C aromatic, m), 1181 (C-O, s). \(^1\mathrm{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta / \text{ppm}: \)

\[ 7.43 (\text{d}, J = 9.0 \text{ Hz}, 2\text{H}, \text{H11}), \]

\[ 6.93 (\text{d}, J = 9.0 \text{ Hz}, 2\text{H}, \text{H12}), \]

\[ 6.35 (\text{br s}, 1\text{H}, \text{NH}), \]

\[ 6.17 (\text{d}, J = 2.0 \text{ Hz}, 1\text{H}, \text{H2}), \]

\[ 5.89-5.84 (\text{m}, 1\text{H}, \text{H7}), \]

\[ 5.80-5.75 (\text{m}, 1\text{H}, \text{H8}), \]

\[ 3.84 (\text{s}, 3\text{H}, \text{H14}), \]

\[ 2.35-2.18 (\text{m}, 3\text{H}, \text{H6&5a}), \]

\[ 1.98-1.94 (\text{m}, 1\text{H}, \text{H9b}), \]

\[ 1.73-1.71 (\text{m}, 1\text{H}, \text{H5b}), \]

\[ 13\mathrm{C} \text{NMR} (101 \text{ MHz, CDCl}_3) \delta / \text{ppm}: \]

\[ 171.9 (\text{C1}), \]

\[ 165.4 (\text{C3}), \]

\[ 160.6 (\text{C13}), \]

\[ 132.1 (\text{C10}), \]

\[ 129.0 (\text{C11}), \]

\[ 126.7 (\text{C7}), \]

\[ 124.7 (\text{C8}), \]

\[ 120.4 (\text{C2}), \]

\[ 63.5 (\text{C4}), \]

\[ 55.4 (\text{C14}), \]

\[ 34.8 (\text{C9}), \]

\[ 30.4 (\text{C5}), \]

\[ 34.8 (\text{C9}), \]

\[ 30.4 (\text{C5}), \]

\[ 23.1 (\text{C6}). \]

HRMS (ESI) \( \text{C}_{16}\text{H}_{18}\text{NO}_2 \) m/z: [M+H]\(^+\) 256.1331 (calcd. 256.1338). m.p. 196-197 °C.

4-Hydroxy-1-azaspiro[4.5]dec-7-en-2-one, 177

\[ \begin{align*}
\text{O} & \\
\text{NH} & \\
\text{H} & \\
\text{OH} & \\
\end{align*} \]

Compound 168 (200 mg, 1.21 mmol, 1.0 eq) was added to a suspension of sodium borohydride (77.9 mg, 2.06 mmol, 1.7 eq) in methanol (5 mL) at 0 °C. The reaction was warmed to RT and stirred for 1 h. Water (5 mL) was added dropwise and the mixture was extracted with dichloromethane (3 × 10 mL). the combined organic layers were dried over anh. MgSO\(_4\), filtered, and concentrated in vacuo. The product was purified by column chromatography (ethyl acetate) to yield the pure product as white crystals (32 mg, 0.19 mmol, 16%); \( R_f \) 0.06 (ethyl acetate); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1}: \)

\[ 3368 (\text{N-H, m}), \]

\[ 3194 (\text{O-H, br m}), \]

\[ 2950 (\text{C-H, w}), \]

\[ 1698 (\text{C=O, s}), \]

\[ 1662 (\text{C=C, s}), \]

\[ 1058 (\text{C-O, s}). \]

\(^1\mathrm{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta / \text{ppm}: \]

\[ 5.87-5.73 (\text{m}, 3\text{H}, \text{NH, OH, H7}), \]

\[ 5.64-5.60 (\text{m}, 1\text{H}, \text{H8}), \]

\[ 4.19 (\text{q}, J = 5.55 \text{ Hz}, 1\text{H}, \text{H3}), \]

\[ 2.75 (\text{dd}, J = 17.0, 7.0 \text{ Hz}, 1\text{H}, \text{H2a}), \]

\[ 2.39 (\text{dd}, 1\text{H}, J = 17.0, 5.0 \text{ Hz}, 1\text{H}, \text{H2b}), \]

\[ 2.31-2.28 (\text{m}, 1\text{H}, \text{H6a}), \]

\[ 2.23-2.09 (\text{m}, 2\text{H}, \text{H6b&9a}), \]

\[ 2.03-1.97 (\text{m}, 1\text{H}, \text{H9b}), \]

\[ 1.88-1.82 (\text{m}, 1\text{H}, \text{H5a}), \]

\[ 1.78-1.73 (\text{m}, 1\text{H}, \text{H5b}). \]

\(^1\mathrm{C} \text{NMR} (101 \text{ MHz, CDCl}_3) \delta / \text{ppm}: \]

\[ 173.9 (\text{C1}), \]

\[ 127.5 (\text{C7}), \]

\[ 123.9 (\text{C8}), \]

\[ 73.6 (\text{C3}), \]

\[ 61.0 (\text{C4}), \]

\[ 39.2 (\text{C2}), \]

\[ 36.3 (\text{C9}), \]

\[ 26.4 (\text{C5}), \]

\[ 22.5 (\text{C6}). \]

HRMS (ESI) \( \text{C}_{16}\text{H}_{18}\text{NO}_2 Na \) m/z: [M+Na]\(^+\) 190.0841 (calcd. 190.0844). m.p. 130-131 °C.
2-Oxo-1-azaspiro[4.5]dec-7-en-4-yl methanesulfonate, 178

Compound 177 (30 mg, 0.18 mmol, 1.0 eq) was dissolved in dichloromethane (1 mL) before mesyl chloride (16 μL, 0.22 mmol, 1.2 eq) and triethylamine (35 μL, 0.25 mmol, 1.4 eq) were added dropwise at 0 °C. The reaction was stirred at this temperature for 1 h, before being quenched with sat. aq. NaHCO₃ (5 mL) and extracted with dichloromethane (3 × 5 mL). The combined organics were concentrated in vacuo and the crude was purified by column chromatography (ethyl acetate) to give the pure product as a white amorphous solid (54.0 mg, 0.17 mmol, 94%); Rf 0.24 (ethyl acetate); IR (thin film) ν/cm⁻¹: 3352 (N-H, w), 2929 (C-H, w), 1692 (C=O, s), 1643 (C=C, s), 1302 (S=O, m). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 5.97 (br s, 1H, Nh), 5.80–5.77 (m, 1H, H7), 5.64–5.61 (m, 1H, H8), 5.02 (dd, J = 7.0, 5.0 Hz, 1H, H3), 3.08 (s, 3H, H10), 2.92–2.83 (m, 1H, H2a), 2.72–2.60 (m, 1H, H2b), 2.47–2.30 (m, 2H, H6a&9a), 2.20–2.04 (m, 2H, H6b&9b), 1.90–1.76 (m, 2H, H5). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 171.9 (C1), 127.5 (C7), 123.1 (C8), 79.8 (C3), 60.2 (C4), 38.7 (C2), 37.0 (C10), 36.1 (C9), 26.9 (C5), 22.1 (C6).

HRMS (ESI) C₁₀H₁₆NO₄S m/z: [M+H]⁺ 245.0732 (calc. 245.0722).

1-Azaspiro[4.5]deca-3,7-dien-2-one, 179

Compound 177 (23 mg, 0.14 mmol, 1.0 eq) was dissolved in trifluoroacetic anhydride (67 μL, 0.48 mmol, 3.5 eq) and refluxed for 12 h. Excess solvent was removed in vacuo and the residue was dissolved in dichloromethane (0.25 mL). Triethylamine (24 μL, 0.17 mmol, 1.26 eq) was added and the reaction was stirred for a further 12 h. Potassium hydrogen carbonate (36 mg, 0.36 mmol, 2.63 eq) in methanol (0.25 mL) was added to the reaction and it was allowed to stir for a further 2 h. Chloroform (5 mL) was added and the mixture was washed with 1M HCl (5
Experimental

mL), water (5 mL), and brine (5 mL). The organic layer was then dried over anhyd. MgSO₄. The mixture was filtered and the filtrate was concentrated in vacuo to give a crude product. This was then purified by column chromatography (ethyl acetate) to give the pure product as off-white crystals (6.3 mg, 0.04 mmol, 30%); Rf 0.18 (ethyl acetate); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 3170 (N-H, m), 3030 (C-H alkene, w), 2926 (C-H alkane, w), 1682 (C=O, s), 1655 (C=C, s).

\[ \text{1H NMR (400 MHz, CDCl₃)} \delta / \text{ppm: } 7.08 (d, J = 5.0 \text{ Hz, } 1H, H3), 6.15 (\text{br s, } 1H, \text{ NH}), 6.03 (d, J = 5.0 \text{ Hz, } 1H, H2), 5.84–5.79 (m, 1H, H7), 5.76–5.71 (m, 1H, H8), 2.38–2.32 (m, 1H, H9a), 2.27–2.21 (m, 2H, H6), 2.10–2.04 (m, 1H, H9b), 1.84–1.77 (m, 1H, H5a), 1.73–1.67 (m, 1H, H5b). \]

\[ \text{13C NMR (101 MHz, CDCl₃)} \delta / \text{ppm: } 154.6 (C3), 129.1 (C2), 126.7 (C7), 124.2 (C8), 62.2 (C4), 34.3 (C9), 30.9 (C5), 23.6 (C6). \]

HRMS (ESI) \( C_9H_{12}NO \) m/z: [M+H]+ 150.0918 (calc. 150.0919). m.p. 75–76 °C.

2-Allyl-2-((tert-butoxycarbonyl)amino)hex-5-enoic acid, 184

![Chemical Structure](attachment:image.png)

Compound 143 (1g, 3.36 mmol, 1.0 eq) was dissolved in methanol (67.25 mL) and 1M NaOH (67.25 mL, 67.25 mmol, 20.0 eq) was added dropwise. The reaction was refluxed at 75 °C for 24 hours. The solvent was removed in vacuo and the residue was diluted with water (20 mL). Ice and 0.5 M HCl (135 mL) was added. The precipitate was filtered and washed with cold water to yield the product as a colourless oil (383.7 mg, 1.42 mmol, 42%); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 2979 (C-H, w), 1707 (C=O, s), 1642 (C=C, s). \[ \text{1H NMR (400 MHz, CDCl₃)} \delta / \text{ppm: } 10.57 (\text{br s, } 1H, \text{ COOH}), 5.82–5.74 (m, 1H, H7), 5.72–5.64 (m, 1H, H10), 5.16–5.11 (m, 2H, H11), 5.04–4.95 (m, 2H, H8) 3.01 (br s, 1H, H9a), 2.64–2.56 (m, 1H, H9b), 2.36 (br s, 1H, H5a), 2.12–2.05 (m, 1H, H6a), 1.95–1.89 (m, 2H, H5b&6b), 1.44 (s, 9H, H1). \[ \text{13C NMR (101 MHz, CDCl₃)} \delta / \text{ppm: } 178.0 (C12), 154.2 (C3), 137.3 (C7), 132.0 (C10), 119.4 (C11), 115.2 (C8), 79.8 (C2), 63.1 (C4), 39.7 (C9), 34.3 (C5), 28.3 (C1&6). \]

HRMS (ESI) \( C_{14}H_{23}NO_4Na \) m/z: [M+Na]+ 292.1523 (calc. 292.1519).
Experimental

**Ethyl (tert-butoxycarbonyl)-L-serinate, 201**

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{N} & \quad \text{C} \\
\text{O} & \quad \text{H} \\
\text{C} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

L-serine ethyl ester hydrochloride (5 g, 29.48 mmol, 1.0 eq) and di-tert-butyl decarbonate (6.43 mL, 28.01 eq, 0.95 mmol) were dissolved in ethyl acetate (40 mL) and water (40 mL). Potassium carbonate (6.11 g, 44.22 mmol, 1.5 eq) was added to the reaction and it was left to stir overnight at RT. The layers were then separated and the aqueous extracted with ethyl acetate (2 × 30 mL) and the combined organics were washed with brine (50 mL). This was then dried over anh. MgSO₄. This mixture was filtered and the filtrate was concentrated *in vacuo* to give the product as a pure colourless oil (6.41 g, 27.49 mmol, 93%); Rₚ 0.07 (20% ethyl acetate in pet. ether); IR (thin film) \(\tilde{\nu}/\text{cm}^{-1}: 3383 (\text{O-H, br s}), 2978 (\text{C-H, w}), 1714 (\text{C=O ester, s}), 1692 (\text{C=O carbamide, s}), 1158 (\text{C=O, s})\). ¹H NMR (400 MHz, CDCl₃) \(\delta/\text{ppm}: 5.44 (\text{br s, 1H, NH}), 4.36 (\text{br s, 1H, NH}), 4.36 (\text{br s, 1H, NH}), 4.24 (\text{q, } J = 7.0 \text{ Hz, 2H, H7}), 3.98–3.89 (\text{m, 2H, H5}), 2.30 (\text{br s, 1H, O}), 1.46 (\text{s, 9H, H1}), 1.30 (\text{t, } J = 7.0 \text{ Hz, 3H, H8})\). ¹³C NMR (101 MHz, CDCl₃) \(\delta/\text{ppm}: 170.7 (\text{C6}), 155.8 (\text{C3}), 80.3 (\text{C2}), 63.8 (\text{C5}), 61.8 (\text{C7}), 55.8 (\text{C4}), 28.3 (\text{C1}), 14.1 (\text{C8})\). HRMS (ESI) C₁₀H₁₉NO₅Na \(m/z: [\text{M+Na}]^+ 256.1151 \) (calc. 256.1155). [α]₂₀D –17.2 (c = 1.0, CHCl₃).

The physical and spectroscopic data were found to be in agreement with Tang *et al.*

**Ethyl 2-((tert-butoxycarbonyl)amino)acrylate, 202**

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{N} & \quad \text{C} \\
\text{O} & \quad \text{H} \\
\text{C} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

Compound 201 (6.4 g, 27.44 mmol, 1.0 eq) was dissolved in dichloromethane (70 mL) at -15 °C. Mesyl chloride (2.65 mL, 34.30 mmol, 1.25 eq) was added dropwise followed by triethylamine (11.41 mL, 82.31 mmol, 3.0 eq). The reaction was stirred for 30 min before being warmed to RT and stirred for a further 2 h. Then one third molar HCl (30 mL) was added. The mixture was washed with water and the combined organics were dried over anh. MgSO₄. This mixture was filtered, and the filtrate was concentrated *in vacuo* to give the product as a yellow oil.
Experimental

(5.5667 g, 25.86 mmol, 94%); Rf 0.60 (20% ethyl acetate in pet. ether); IR (thin film) v/cm⁻¹: 3420 (N⁻H, w), 2980 (C−H, w), 1733 (C=O carbamate, m), 1707 (C=O ester, s), 1638 (C=C, w), 1152 (C−O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.02 (br s, 1H, NH), 6.14 (br s, 1H, H5a), 5.72 (d, J = 1.5 Hz, 1H, H5b), 4.28 (q, J = 7.0 Hz, 2H, H7), 1.48 (s, 9H, H1), 1.33 (t, J = 7.0 Hz, 3H, H8). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 164.0 (C6), 152.6 (C3), 131.5 (C4), 104.8 (C5), 80.6 (C2), 62.0 (C7), 28.2 (C1), 14.1 (C8). HRMS (ESI) C₁₀H₁₇NO₄Na m/z: [M+Na]+ 238.1046 (calc. 238.1050).

The physical and spectroscopic data were found to be in agreement with Ramesh et al.²³²

4-Methylloxazol-2(3H)-one, 204

To a solution of potassium tert-butoxide (181.4 mg, 1.62 mmol, 1.0 eq) in THF (15 mL) was added 203 (280 mg, 1.62 mmol, 1.0 eq) at 0 °C and the reaction was stirred for 1h. The reaction was diluted with sat. aq. NH₄Cl (10 mL) and extracted with dichloromethane (3 × 10 mL). The combined organics were dried over anh. MgSO₄, filtered, and concentrated in vacuo to give the pure product as a white crystalline solid (57.2 mg, 0.58 mmol, 36%); IR (thin film) v/cm⁻¹: 3163 (N⁻H, br s), 1706 (C=O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 9.14 (br s, 1H, NH), 6.53 (s, 1H, H2), 2.02 (d, J = 1.5 Hz, 3H, H4). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 157.7 (C1), 124.6 (C2), 122.7 (C3), 9.2 (C4). HRMS (ESI) C₄H₆NO₂ m/z: [M+H]+ 100.0397 (calc 100.0393). m.p. 50–51°C.
**N-Hydroxybenzimidoyl chloride, 209**

![Chemical Structure](image)

Benzaldehyde (1.5 g, 14.13 mmol, 1.0eq) was dissolved in diethyl ether (12 mL) and hydroxylamine (50% in H₂O, 1.73 mL, 28.27 mmol, 2.0 eq) was added. The reaction was stirred for 10 min. The reaction was dried with direct addition of anh. MgSO₄. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residual oil was dissolved in DMF (12 mL) and *N*-chlorosuccinimide (2.08 g, 15.55 mmol, 1.1 eq) was added and the reaction was stirred for 30 min. The reaction was diluted with water (10 mL), and extracted with ethyl acetate (2 × 10 mL). The combined organics were washed with water (20 mL) and brine (20 mL) before being dried over anh. Na₂SO₄. The mixture was filtered and the filtrate was concentrated *in vacuo* to give the crude product as a yellow oil. This was carried through crude to the following reactions. (2.17 g, 13.95 mmol, 99%); **¹H NMR (400 MHz, CDCl₃)** \( \delta \) ppm: 8.94 (br s, 1H, OH), 7.89–7.86 (m, 2H, H₃), 7.47–7.41 (m, 3H, H₄&5).

The physical and spectroscopic data were found to be in agreement with Castellano *et al.*

**Ethyl 4-((tert-butoxycarbonyl)amino)-3-phenyl-4,5-dihydroisoxazole-4-carboxylate, 210**

![Chemical Structure](image)

Triethylamine (2.2 mL, 15.80 mmol, 6.8 eq) was added to a mixture of 202 (500 mg, 2.32 mmol, 1.0 eq) an 209 (2.17 g, 13.94 mmol, 6.0 eq) in dichloromthane (40 mL) and the reaction was stirred at RT for 1 h. Water (30 mL) was added and the organic layer was washed with water (30 mL) and brine (30 mL). The combined organics were dried over anh. MgSO₄. This mixture was filtered, and the filtrate was concentrated *in vacuo* to give the crude product. This was
Experimental

purified by column chromatography (20% diethyl ether in pet. ether) to give the pure product as off-white amorphous solid (448.6 mg, 1.34 mmol, 58%); Rf 0.12 (20% diethyl ether in pet. ether); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 3410 (N-H, m), 2976 (C-H, w), 1741 (C=O, m), 1721 (C=N, s), 1484 (C=C, s), 1308 (N-O, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 7.70–7.66 (m, 2H, H11), 7.43–7.38 (m, 3H, H12&13), 6.08 (br s, 1H, NH), 4.32 (q, \( J = 7.0 \text{ Hz} \), 2H, H6), 3.91 (br s, 2H, H8), 1.40 (s, 9H, H1), 1.32 (t, \( J = 7.0 \text{ Hz} \), 3H, H7). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 167.6 (C5), 156.3 (C9), 153.3 (C3), 130.4 (C12), 129.8 (C10), 128.7 (C13), 126.9 (C11), 92.4 (C4), 81.2 (C2), 63.4 (C6), 42.7 (C8), 28.2 (C1), 14.0 (C7). HRMS (ESI) \( \text{C}_{17}\text{H}_{23}\text{N}_{2}\text{O}_5 \) \( m/z \): [M+H]\(^+\) 335.1591 (calc. 335.1607).

tert-Butyl (4-(hydroxymethyl)-3-phenyl-4,5-dihydroisoxazol-4-yl)carbamate, 211

Compound 210 (100 mg, 0.30 mmol, 1.0 eq), was dissolved in an 8:1 THF/methanol mixture (1.8 mL). This was colled to 0 °C and sodium borohydride (2.83 mg, 0.07 mmol, 0.25 eq) were added. The reaction was stirred for 4 h before further sodium borohydride (8.49 mmol, 0.21 mmol, 0.75 eq) was added. The reaction was stirred overnight. It was quenched with sat. aq. NH\(_4\)Cl (2 mL), and extracted with dichloromethane (3 × 5 mL). The combined organics were then dried over anh. MgSO\(_4\). This mixture was filtered, and the filtrate was concentrated \textit{in vacuo} to give the crude product. This was purified via column chromatography (55% ethyl acetate in hexane) to give the pure product as a white powder (54.0 mg, 0.18 mmol, 62%); Rf 0.29 (55% ethyl acetate in hexane); IR (thin film) \( \tilde{\nu} / \text{cm}^{-1} \): 3408 (N-H, w), 3278 (O-H, w), 2970 (C-H, w), 1717 (C=O, s), 1545 (C=C, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 7.66–7.64 (m, 2H, H7), 7.40–7.37 (m, 3H, H8&9), 5.74 (br s, 1H, NH), 3.90–3.86 (m, 2H, H10a&11a), 3.77–3.73 (m, 1H, H11b), 3.30 (d, \( J = 17.5 \text{ Hz} \), 1H, H10b), 3.05 (br s, 1H, OH), 1.40 (s, 9H, H1). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} \): 157.3 (C5), 154.1 (C3), 130.3 (C8), 129.6 (C6), 128.7 (C9), 126.7 (C7), 95.4 (C4), 80.9 (C2), 66.2 (C11), 40.4 (C10), 28.2 (C1). HRMS (ESI) \( \text{C}_{15}\text{H}_{21}\text{N}_{2}\text{O}_4 \) \( m/z \): [M+H]\(^+\) 293.1498 (calc. 293.1501).
To a solution of potassium tert-butoxide (30.7 mg, 0.27 mmol, 2.0 eq) in THF (1.4 mL) at 0 °C was added 211 (40 mg, 0.14 mmol, 1.0 eq) and the reaction was stirred for 1 h. This was then diluted with sat. aq. NH₄Cl (5 mL) and extracted with dichloromethane (3 × 5 mL). The combined organics were dried over anh. MgSO₄, filtered and concentrated in vacuo to give the crude product. This was purified by column chromatography (60% ethyl acetate in hexane) to give the pure product as a white powder (17 mg, 0.08 mmol, 56%); Rf 0.16 (60% ethyl acetate in hexane); IR (thin film) ν/cm⁻¹: 3226 (N-H, w), 1765 (C=O, s), 1048 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.64–7.62 (m, 2H, H7), 7.48–7.41 (m, 3H, H8&9), 5.97 (br s, 1H, NH), 4.77 (d, J = 10.0 Hz, 1H, H2a), 4.59 (d, J = 10.0 Hz, 1H, H2b), 3.59 (d, J = 18.0 Hz, 1H, H4a), 3.49 (d, J = 18.0 Hz, 1H, H4b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 156.3 (C5), 156.2 (C1), 131.1 (C8), 129.1 (C9), 128.2 (C6), 126.6 (C7), 96.1 (C3), 73.9 (C2), 42.8 (C4). HRMS (ESI) C₁₁H₁₀N₂O₃Na m/z: [M+H]⁺ 241.0576 (calc 241.0584). m.p. 178–179 °C

Crystallographic data for this compound is in Appendix B.

Ethyl (2-chloroacetyl)-L-serinate, 214

l-Serinate ethyl ester hydrochloride (1 g, 5.90 mmol, 1.0 eq), was dissolved in dichloromethane (60 mL) and triethylamine (1.73 mL, 12.38 mmol, 2.1 eq) was added to the solution. This was cooled to 0 °C before chloroacetyl chloride (0.47 mL, 5.90 mmol, 1.0 eq) was added dropwise and the reaction was stirred for 90 min. After this it was diluted with aq. sat.
NH₄Cl and stirred for 10 min. The mixture was extracted with dichloromethane. The combined organics were dried over anh. MgSO₄, filtered and concentrated in vacuo to give the crude product. This was purified via column chromatography (80% ethyl acetate in hexane) to yield the pure product as a white crystalline solid (673.8 mg, 3.21 mmol, 54%); Rf 0.31 (80% ethyl acetate in hexane); IR (thin film) v/cm⁻¹: 3500 (O-H, m), 3276 (N-H, s), 2957 (C-H, w), 1712 (C=O ester, s), 1664 (C=O amide, s), 1555 (N-H, s), 1035 (C-O, s), 781 (C-Cl, m). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.43 (br s, 1H, NH), 4.65 (dt, J = 7.5, 3.5 Hz, 1H, H₃), 4.28 (q, J = 7.0 Hz, 2H, H₆), 4.10 (s, 2H, H₁), 4.07–3.94 (m, 2H, H₄), 2.26 (t, J = 5.5 Hz, 1H, OH), 1.32 (t, J = 7.0 Hz, 3H, H₇). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 169.7 (C₅), 166.5 (C₂), 63.2 (C₄), 62.3 (C₆), 42.4 (C₁), 14.1 (C₇). HRMS (ESI) C₇H₁₃ClNO₄Si m/z: [M+H]+ 210.0524 (calc. 210.0528). [α]D²⁰ +41.3 (c = 1.00, CHCl₃).

The physical and spectroscopic data were found to be in agreement with Yoshimura et al.²³⁴

Ethyl N,O-bis(2-chloroacetyl)-L-serinate, 215

The title compound was isolated as a by-product from the reaction to synthesise 214. White crystalline solid (87.5 mg, 0.42 mmol, 5.2%); Rf 0.13 (60% ethyl acetate in hexane); IR (thin film) v/cm⁻¹: 3268 (O-H, s), 2957 (C-H, w), 1744 (C=O ester, s), 1679 (C=O amide, s), 1540 (N-H, s), 1194 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.31 (d, J = 6.5 Hz, 1H, NH), 4.86–4.82 (m, 1H, H₃), 4.60–4.53 (m, 2H, H₄), 4.27 (q, J = 7.0 Hz, 2H, H₈), 4.08 (s, 2H, H₁), 4.06 (s, 2H, H₆), 1.30 (t, J = 7.0 Hz, 3H, H₉). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 168.4 (C₇), 166.9 (C₅), 166.1 (C₂), 64.9 (C₄), 62.6 (C₈), 52.0 (C₃), 42.3 (C₁), 40.4 (C₆), 14.1 (C₉). HRMS (ESI) C₉H₁₅ClNO₅Si m/z: [M+H]+ 232.0536 (calc. 232.0535). [α]D²⁰ +45.0 (c = 1.00, CHCl₃).
**Ethyl (S)-2-oxooxazolidine-4-carboxylate, 219**

![Chemical Structure](image)

Triphosgene (2.73 g, 9.20 mmol, 0.5 eq) and L-serine ethyl ester hydrochloride (3.00 g, 17.69 mmol, 1.0 eq) were dissolved in THF (180 mL) and refluxed for 1h. The reaction was concentrated *in vacuo* and submitted to column chromatography (80% ethyl acetate in hexane) to yield the pure product as off-white crystals (2.7054 g, 17.00 mmol, 96%); *R*<sub>f</sub> 0.27 (80% ethyl acetate in hexane); IR (thin film) *v*/cm<sup>−1</sup>: 3264 (N-H, br w), 2998 (C-H, w), 1739 (C=O ester, s), 1714 (C=O carbamide, s), 1204 (C=O, s). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ/ppm: 6.03 (br s, 1H, NH), 4.64–4.59 (m, 1H, H<sub>2a</sub>), 4.54–4.51 (m, 1H, H<sub>2b</sub>), 4.41–4.38 (m, 1H, H3), 4.27 (q, *J* = 7.0 Hz, 2H, H5), 1.31 (t, *J* = 7.0 Hz, 3H, H6). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ/ppm: 170.0 (C4), 158.7 (C1), 148.2 (C6), 120.4 (C-O, s), 120.4 (C=O carbamide, s), 120.4 (C=O, s). HRMS (ESI) C<sub>6</sub>H<sub>10</sub>NO<sub>4</sub> m/z: [M+H]<sup>+</sup> 160.0603 (calc. 160.0604). [α]<sub>D</sub> <sup>20</sup> -14.8 (c = 1.00, CHCl<sub>3</sub>).

The physical and spectroscopic data were found to be in agreement with Paz *et al.*<sup>235</sup>

**Ethyl (S)-2-phenyl-4,5-dihydrooxazole-4-carboxylate, 221**

![Chemical Structure](image)

Compound 150 (1.6 g, 10.72 mmol, 1.0 eq) as a free base was dissolved in 1,2-dichloroethane (20 mL) and L-serine ethyl ester hydrochloride (2.00 g, 11.79 mmol, 1.1 eq) was added. The reaction was refluxed overnight. This was cooled to RT and filtered. The filtrate was concentrated *in vacuo* and the crude oil was purified by column chromatography (30% ethyl acetate in hexane) to yield the pure product as a white amorphous solid (1.4862 g, 6.78 mmol, 63%); *R*<sub>f</sub> 0.33 (30% ethyl acetate in hexane); IR (thin film) *v*/cm<sup>−1</sup>: 2984 (C-H, w), 1726 (C=O, s), 1631 (C=N, s), 1201 (C-O, s). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ/ppm: 7.98–7.96 (m, 2H, H<sub>3</sub>), 7.50–7.46 (m, 1H, H1), 7.41–7.37 (m, 2H, H2), 4.91 (dd, *J* = 10.5, 8.0 Hz, 1H, H7), 4.68–4.64 (m, 1H, H6a), 4.60–4.55 (m, 1H, H6b), 4.30–4.21 (m, 2H, H9), 1.31 (t, *J* = 7.0 Hz, 3H, H10). <sup>13</sup>C NMR
(101 MHz, CDCl₃) δ/ppm: 171.2 (C8), 166.2 (C5), 131.8 (C1), 128.6 (C3), 128.3 (C2), 127.0 (C4), 69.6 (C6), 68.8 (C7), 61.8 (C9), 14.2 (C10). **HRMS (ESI)** C₁₂H₁₄NO₃ m/z: [M+H]⁺ 220.0909 (calc. 220.0974). [α]_D⁰ +122.0 (c = 1.00, CHCl₃).

The physical and spectroscopic data were found to be in agreement with Robertson *et al.*²³⁶

(R)-(2-Phenyl-4,5-dihydrooxazol-4-yl)methanol, 222

![Structure](image)

Calcium chloride (3.14 g, 28.28 mmol, 4.0 eq) and 221 (1.55 g, 7.07 mmol, 1.0 eq) were dissolved in ethanol (125 mL). The mixture was cooled to 0 °C and sodium borohydride (2.14 g, 56.56 mmol, 8.0 eq) was added portion-wise. The reaction was stirred at 0 °C for a further 5 h. Water (100 mL) was added and stirred for 1 h. The resulting mixture was filtered, and the filtrate concentrated in vacuo. This was diluted with ethyl acetate (50 mL) and washed with brine (50 mL), dried over anh. Na₂SO₄, filtered, and contracted in vacuo. This gives the pure product as an amorphous white solid (882.6 mg, 4.98 mmol, 70%); **IR (thin film)** ν/cm⁻¹: 3235 (O–H, br w), 2999 (C–H, w), 1644 (C=N, s). **¹H NMR (400 MHz, CDCl₃) δ/ppm:** 7.85 (d, J = 7.5 Hz, 2H, H1), 7.45 (t, J = 7.5 Hz, 1H, H2), 7.35 (t, J = 7.5 Hz, 2H, H3), 4.50–4.32 (m, 3H, H7&8), 3.97 (d, J = 9.0 Hz, 1H, H6a), 3.66 (d, J = 9.0 Hz, 1H, H6b), 2.92 (br s, 1H, OH). **¹³C NMR (101 MHz, CDCl₃) δ/ppm:** 165.6 (C5), 131.6 (C2), 128.3 (C1&3), 127.1 (C4), 69.3 (C8), 68.0 (C7), 63.9 (C6). **HRMS (ESI)** C₁₀H₁₂NO₂ m/z: [M+H]⁺ 178.0861 (calc. 178.0863).

The physical and spectroscopic data were found to be in agreement with Braga *et al.*²³⁷
**Ethyl O-(tert-butyldimethylsilyl)-L-serinate, 224**

![Ethyl O-(tert-butyldimethylsilyl)-L-serinate](image)

L-serinate ethyl ester hydrochloride (2 g, 11.79 mmol, 1.0 eq) and tert-butyldimethylsilyl chloride (3.55 g, 23.58 mmol, 2.0 eq) were dissolved in dichloromethane (150 mL). Imidazole (2.41 g, 35.38 mmol, 3.0 eq) was added at 0 °C and the reaction was warmed to RT and stirred overnight. This was quenched by the addition of water (100 mL) and extracted with dichloromethane (3 × 100 mL) to give the pure product as a colourless oil (2.2867 g, 9.24 mmol, 78%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 2930 (C-H, w), 1739 (C=O, s), 1095 (C-O, s).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 4.20 (q, $J = 7.0$ Hz, 2H, H7), 3.94 (dd, $J = 9.5$, 4.0 Hz, 1H, H2a), 3.83 (dd, $J = 9.5$, 4.0 Hz, 1H, H2b), 3.51 (t, $J = 4.0$ Hz, 1H, H1), 1.70 (br s, 2H, NH$_2$), 1.30 (t, $J = 7.0$ Hz, 3H, H8), 0.89 (s, 9H, H5), 0.07 (s, 3H, H3a), 0.06 (s, 3H, H3b). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm: 174.1 (C6), 65.4 (C2), 60.9 (C7), 56.5 (C1), 25.7 (C5), 18.2 (C4), 14.2 (C8), −5.5 (C3a), −5.6 (C3b). HRMS (ESI) C$_{11}$H$_{25}$NO$_3$SiNa $m$/z: [M+Na]$^+$ 270.1493 (calc. 270.1501). $[\alpha]_D^{20}$ −6.1 (c = 1.00, CHCl$_3$).

The physical and spectroscopic data were found to be in agreement with Hakimelahi et al.$^{238}$

**Ethyl O-(tert-butyldimethylsilyl)-N-(2-chloroacetyl)-L-serinate, 225**

![Ethyl O-(tert-butyldimethylsilyl)-N-(2-chloroacetyl)-L-serinate](image)

Triethylamine (1.18 mL, 8.49 mmol, 2.1 eq) was added to a solution of 224 (1.00 g, 4.04 mmol, 1.0 eq) in dichloromethane (40 mL). This was cooled to 0 °C and chloroacetyl chloride (0.32 mL, 4.04 mmol, 1.0 eq) was added dropwise. The reaction was warmed to RT and stirred for 90 min. The reaction was then quenched with sat. aq. NH$_4$Cl (20 mL), and stirred for 10 min. This mixture was extracted with dichloromethane (2 × 10 mL). The combined organics were
dried over anh. MgSO₄, filtered and concentrated *in vacuo* to give the crude product. This was purified by column chromatography (20% ethyl acetate in hexane) to yield the pure product as a colourless oil (723.6 mg, 2.23 mmol, 55%); Rₚ 0.20 (20% ethyl acetate in hexane); IR (thin film) v/cm⁻¹: 2932 (C-H, w), 1745 (C=O ester, m), 1679 (C=O amide, s), 1110 (C-O, s), 776 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.38 (d, J = 8.0 Hz, 1H, NH), 4.61 (dt, J = 8.0, 3.0 Hz, 1H, H₃), 4.22 (q, J = 7.0 Hz, 2H, H₅), 4.12 – 4.04 (m, 3H, H₁&₇a), 3.84 (dd, J = 10.0, 3.0 Hz, 1H, H₇b), 1.28 (t, J = 7.0 Hz, 3H, H₆), 0.86 (s, 9H, H₆), 0.04 (s, 3H, H₈a), 0.02 (s, 3H, H₈b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 169.7 (C₄), 165.7 (C₂), 63.1 (C₇), 61.7 (C₅), 54.5 (C₃), 42.5 (C₁), 25.6 (C₁₀), 18.1 (C₆), –5.7 (C₈a), –5.7 (C₈b). HRMS (ESI) C₁₃H₂₆ClNO₄SiNa m/z: [M+Na]⁺ 346.1203 (calc. 346.1212). [α]_D²⁰ +29.2 (c = 1.00, CHCl₃).

(R)-N-((tert-Butyldimethylsilyl)oxy)-3-hydroxypropan-2-yl)-2-chloroacetamide, 226

Powdered sodium borohydride (37.4 mg, 2.34 mmol, 1.05 eq) was added to a solution of 225 (305 mg, 2.22 mmol, 1.0 eq) and the reaction was stirred at RT for 2 h. Sat. aq. NH₄Cl solution was added dropwise and the mixture was stirred for 30 min. This was filtered and the filtrate was dried over anh. MgSO₄ and filtered and concentrated *in vacuo*. The crude oil was purified by column chromatography (80% ethyl acetate in hexane) to give the pure product as a colourless oil (27 mg, 0.10 mmol, 43%); Rₚ 0.29 (80% ethyl acetate in hexane); IR (thin film) v/cm⁻¹: 3243 (O-H, br m), 2927 (C-H, w), 1645 (C=O, s), 1053 (C-O, m), 720 (C-Cl, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 7.26 (br s, 1H, NH), 4.09 (d, J = 15.5 Hz, 1H, H₁a), 4.05 (d, J = 15.5 Hz, 1H, H₁b), 3.98 (sp, J = 4.0 Hz, 1H, H₃), 3.87–3.84 (m, 2H, H₄a&₅a), 3.78 (dd, J = 10.5, 4.5 Hz, 1H, H₅b), 3.70 (dd, J = 10.5, 4.5 Hz, 1H, H₄b), 2.85 (br s, 1H, OH), 0.90 (s, 9H, H₈), 0.08 (s, 6H, H₆). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 166.2 (C₂), 63.3 (C₄/₅), 63.2 (C₄/₅), 52.0 (C₃), 42.6 (C₁), 25.7 (C₈), 18.1 (C₇), –5.6 (C₆). HRMS (ESI) C₁₃H₂₆ClNO₄Si m/z: [M+H]⁺ 281.1210 (calc. 281.1214). [α]_D²⁰ +18.7 (c = 1.00, CHCl₃).
Experimental

Ethyl O-(tert-butyldimethylsilyl)-N-(3-ethoxy-3-oxopropanoyl)-L-serinate, 228

Compound 224 (500 mg, 2.02 mmol, 1.0 eq) was dissolved in dichloromethane (20 mL) at 0 °C. Triethylamine (0.61 mL, 4.35 mmol, 2.15 eq) and ethyl malonyl chloride (0.41 mL, 3.23 mmol, 1.6 eq) were added dropwise to the reaction which was subsequently warmed to RT and stirred for 4h. This was quenched with sat. aq. NH₄Cl (20 mL) and stirred for 10 min. This mixture was separated and the aqueous was extracted with dichloromethane (2 × 20 mL). The combined organics were dried over MgSO₄, filtered, and the filtrate was concentrated in vacuo to give a crude oil. This was purified via column chromatography (40% ethyl acetate in hexane) to give the pure product as a yellow oil (482.8 mg, 1.34 mmol, 66%); Rf 0.29 (40% ethyl acetate in hexane); IR (thin film) 𝜈/ cm⁻¹: 3337 (N-H, w), 2932 (C-H, w), 1739 (C=O ester, s), 1679 (C=O amide, m), 1609 (C=O ester, s), 1109 (C=O amide, m), 1109 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ ppm: 7.77 (d, J = 7.5 Hz, 1H, NH), 4.65 (dt, J = 7.5, 3.0 Hz, 1H, H6), 4.21 (qd, J = 7.0, 3.0 Hz, 4H, H2&8), 4.08 (dd, J = 10.0, 3.0 Hz, 1H, H10a), 3.84 (dd, J = 10.0, 3.0 Hz, 1H, H10b), 3.35 (s, 2H, H4), 1.28 (td, J = 7.0, 5.5 Hz, 6H, H1&9), 0.87 (s, 9H, H13), 0.03 (s, 3H, H11a), 0.02 (s, 3H, H11b). ¹³C NMR (101 MHz, CDCl₃) δ/ ppm: 170.0 (C7), 168.7 (C3), 164.9 (C5), 63.3 (C10), 61.6 (C2/8), 61.5 (C2/8), 54.5 (C6), 41.4 (C4), 25.7 (C13), 18.1 (C12), 14.2 (C1/9), 14.1 (C1/9), -5.6 (C11a), -5.7 (C11b). HRMS (ESI) C₁₆H₃₁NO₆SiNa m/z: [M+Na]⁺ 384.1812 (calc. 384.1818). [𝛼]₂⁰ δ +32.1 (c = 1.00, CHCl₃).

4-Ethoxy-5-methylene-1,5-dihydro-2H-pyrrol-2-one, 230

Compound 224 (400 mg, 1.62 mmol, 1.0 eq), Bestmann’s Ylide (489 mg, 1.62 mmol, 1.0 eq), benzoic acid (39.5 mg, 0.32 mmol, 0.2 eq) were dissolved in THF (8 mL) and refluxed overnight at 65 °C in the dark. The reaction mixture was concentrated in vacuo and purified via column
Experimental chromatography (diethyl ether) to give the pure product as a yellow oil (139.2 mg, 1.00 mmol, 62%); Rf 0.28 (diethyl ether) IR (thin film) \(\tilde{\nu}/\text{cm}^{-1}: 2980 (\text{C-H, w}), 1686 (\text{C=O, s}), 1652 (\text{C=C, s}), 1593 (\text{C-O, s})\). ¹H NMR (400 MHz, CDCl₃) \(\delta/\text{ppm}: 6.90 (\text{br s, 1H, NH}), 5.09 (\text{s, 1H, H2}), 5.00 (d, \text{ J = 1.0 Hz, 1H, H5a}), 4.74 (t, \text{ J = 1.5 Hz, 1H, H5b}), 4.05 (q, \text{ J = 7.0 Hz, 2H, H6}), 1.42 (t, \text{ J = 7.0 Hz, 3H, H7})\). ¹³C NMR (101 MHz, CDCl₃) \(\delta/\text{ppm}: 171.4 (\text{C1}), 165.2 (\text{C4}), 139.4 (\text{C3}), 93.7 (\text{C2}), 91.8 (\text{C5}), 67.1 (\text{C6}), 14.1 (\text{C7})\). HRMS (ESI) \(\text{C}_7\text{H}_{10}\text{NO}_2 \text{ m/z: } [\text{M+H}]^+ 140.0705 (\text{calc. 140.0705})\).

tert-Butyl 3-(2-ethoxy-2-oxoethylidene)azetidine-1-carboxylate, 236

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\text{Tert}-butyl 3-oxoazetidine-1-carboxylate (2.90 g, 16.93 mmol, 1.0 eq) and ethyl (triphenylphosphoranylidene)acetate (6.49 g, 18.62 mmol, 1.1 eq) were dissolved in dichloromethane (56 mL) and refluxed for 6 h. The reaction was concentrated \textit{in vacuo} and submitted to column chromatography (20% ethyl acetate in hexane) to yield the pure product as colourless crystals (3.8496 g, 15.95 mmol, 94%); Rf 0.29 (20% ethyl acetate in hexane); IR (thin film) \(\tilde{\nu}/\text{cm}^{-1}: 2978 (\text{C-H, w}), 1725 (\text{C=O carbamide, s}), 1704 (\text{C=O ester, s}), 1197 (\text{C-O, s})\). ¹H NMR (400 MHz, CDCl₃) \(\delta/\text{ppm}: 5.75 (\text{s, 1H, H7}), 4.79 (\text{s, 2H, H6}), 4.57 (\text{s, 2H, H4}), 4.15 (q, \text{ J = 7.0 Hz, 2H, H9}), 1.43 (s, \text{ 9H, H1}), 1.26 (t, \text{ J = 7.0 Hz, 3H, H10})\). ¹³C NMR (101 MHz, CDCl₃) \(\delta/\text{ppm}: 165.2 (\text{C8}), 156.2 (\text{C3}), 152.6 (\text{C5}), 113.7 (\text{C7}), 80.0 (\text{C2}), 60.4 (\text{C6\&9}), 57.8 (\text{C4}), 28.3 (\text{C1}), 14.3 (\text{C10})\). HRMS (ESI) \(\text{C}_{12}\text{H}_{16}\text{NO}_4\text{Na} \text{ m/z: } [\text{M+Na}]^+ 264.1215 (\text{calc. 264.1212})\).

The physical and spectroscopic data were found to be in agreement with Collier.²³⁹
2-(tert-Butyl) 8-ethyl 7-phenyl-5-oxa-2,6-diazaspiro[3.4]oct-6-ene-2,8-dicarboxylate, 237

Compound 236 (300 mg, 1.24 mmol, 1.0 eq) and 209 (1.16 g, 7.46 mmol, 6.0 eq) were dissolved in dichloromethane (20 mL). Triethylamine (1.17 mL, 8.43 mmol, 6.8 eq) was added dropwise and the reaction was stirred at RT for 1 h. Water (10 mL) was added and the reaction was washed with brine (10 mL). The combined organics were concentrated in vacuo and purified by column chromatography (30% ethyl acetate in hexane) to yield the product as an orange oil (349.6 mg, 0.97 mmol, 78%); R<sub>f</sub> 0.38 (30% ethyl acetate in hexane) IR (thin film) ν/cm<sup>-1</sup>: 2978 (C-H, w), 1736 (C=O, s), 1698 (C=N, s), 1392 (N-O, s), 1153 (C-O, s).<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ/ ppm: 7.68–7.66 (m, 2H, H14), 7.43–7.38 (m, 3H, H15&13), 4.45 (s, 1H, H7), 4.33 (dd, <i>J</i> = 10.5, 1.0 Hz, 1H, H4/5a), 4.25–4.14 (m, 4H, H9&4/5b), 4.05 (dd, <i>J</i> = 10.0, 1.0 Hz, 1H, H4b), 1.45 (s, 9H, H1), 1.19 (t, <i>J</i> = 7.0 Hz, 3H, H10).<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ/ ppm:167.0 (C8), 155.9 (C3), 154.8 9C11), 130.8 (C15), 128.9 (C13), 128.0 (C12), 126.8 (C14), 83.4 (C5), 80.3 (C2), 64.7 (C4/5), 62.4 9C9), 60.5 (C7), 57.3 (C4/5), 28.3 (C1), 14.0 (C10). HRMS (ESI) C<sub>19</sub>H<sub>25</sub>N<sub>2</sub>O<sub>5</sub> m/z: [M+H]<sup>+</sup> 360.6087 (calc. 360.6085).

2-(tert-Butyl) 8-ethyl 7-benzyl-2,5,6,7-tetraazaspiro[3.4]oct-5-ene-2,8-dicarboxylate, 238

Compound 236 (386 mg, 1.60 mmol, 1.0 eq) was dissolved in benzyl azide (2 mL, 16.0, 10.0 eq) and heated in a sealed tube at 100 °C for 48 h. This was purified via column chromatography
(30% ethyl acetate in pet. ether) to give the pure compound as a yellow oil (204 mg, 0.54 mmol, 34%); \(R_f\) 0.49 (30% ethyl acetate in pet. ether) IR (thin film) \(\tilde{\nu}\) /cm\(^{-1}\): 2988 (C-H, w), 1726 (C=O, s), 1631 (C=C, m), 1115 (C-O, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\)/ppm: 7.30–7.17 (m, 5H, H\(_{13,14,15}\)), 5.68 (br s, 2H, H\(_{4a&5a}\)), 4.89 (br s, 2H, H\(_{4b&5b}\)), 4.41 (q, \(J = 7.0\) Hz, 2H, H\(_9\)), 2.65 (br s, 3H, H\(_{7&11}\)), 1.42–1.38 (m, 12H, H\(_{1&10}\)). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\)/ppm: 154.3 (C8), 138.1 (C3), 128.9 (C12), 128.2 (C14), 127.6 (C13), 126.8 (C15), 89.5 (C7), 79.8 (C2), 59.0 (C9), 54.8 (C6), 51.1 (d, \(J = 12.0\) Hz, C4/5), 50.3 (d, \(J = 20.0\) Hz, C4/5), 48.4 (C11), 28.4 (C1), 14.6 (C10). HRMS (ESI) C\(_{19}\)H\(_{26}\)N\(_4\)O\(_4\)Na m/z: [M+Na]\(^+\) 397.1840 (calc. 397.1846).

(2-Hydroxy-5-oxo-5,6-dihydro-2H-pyran-2-yl)methyl acetate, 242

To a suspension of sodium borohydride (225 mg, 5.95 mmol, 0.5 eq) in ethanol (26 mL) was added at 0 °C a solution of (5-acetylfuran-2-yl)methyl acetate (2 g, 11.89 mmol, 1.0 eq) in ethanol (34 mL) and the reaction was stirred for 10 min at 0 °C. Acetic acid was added dropwise until the sodium borohydride had been quenched. The resulting mixture was concentrated in vacuo and dissolved in water (80 mL). To this a solution of bromine (1.9 g, 11.89 mmol, 1.0 eq) in methanol (6 mL) was added dropwise and the reaction was stirred for 2 h. Sat. aq. NH\(_4\)Cl was added dropwise until the mixture had reach pH 5. Brine was added and the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organics were dried over anh. MgSO\(_4\), filtered, and concentrated in vacuo to give the pure product as an orange oil (1.3491 g, 7.25 mmol, 61%); IR (thin film) \(\tilde{\nu}\) /cm\(^{-1}\): 3375 (O-H, br s), 1740 (C=O ester, s), 1699 (C=O ketone, s), 1044 (C-O, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\)/ppm: 6.90 (d, \(J = 10.5\) Hz, 1H, H4), 6.18 (d, \(J = 10.5\) Hz, 1H, H3), 4.61 (d, \(J = 17.0\) Hz, 1H, H1a), 4.46 (d, \(J = 12.0\) Hz, 1H, H6a), 4.19 (d, \(J = 17.0\) Hz, 1H, H1b), 4.08 (d, \(J = 12.0\) Hz, 1H, H6b), 3.37 (br s, 1H, OH), 2.16 (s, 3H, H8). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\)/ppm: 194.0 (C2), 171.1 (C7), 144.5 (C4), 128.2 (C3), 92.2 (C5), 67.6 (C6), 66.5 (C1), 20.8 (C8). HRMS (ESI) C\(_{10}\)H\(_{12}\)O\(_3\)Na m/z: [M+Na]\(^+\) 209.0416 (calc 209.0420).

The physical and spectroscopic data were found to be in agreement with Snider et al.\(^{206}\)
Experimental

(2-Acetoxy-5-oxo-5,6-dihydro-2H-pyran-2-yl)methyl acetate, 243

![Structure of (2-Acetoxy-5-oxo-5,6-dihydro-2H-pyran-2-yl)methyl acetate]

To a solution of 242 (1.34 g, 7.20 mmol, 1.0 eq) in dichloromethane (85 mL) was added acetic anhydride (14.4 mL, 152.3 mmol 20.0 eq), 4-dimethylaminopyridine (0.09 mL, 0.72 mmol, 0.1 eq), and pyridine (7.2 mL, 89.4 mmol, 12.5 eq) at 0 °C. The reaction was stirred for 30 min at 0 °C. This was then washed with 10% aq. CuSO₄ (50 mL), water (50 mL), sat. aq. NaHCO₃ (50 mL), and brine (50 mL). The organic layer was dried over anh. MgSO₄, filtered and concentrated in vacuo to give the pure product as an amorphous blood-red solid (1.3357 g, 5.85 mmol, 81%); IR (thin film) $\tilde{\nu}$/cm⁻¹: 1736 (C=O ester, s), 1699 (C=O ketone, m), 1211 (C-O, s).

$^1$H NMR (400 MHz, CDCl₃) δ/ppm: 7.22 (d, $J = 10.5$ Hz, 1H, H7), 6.21 (d, $J = 10.5$ Hz, 1H, H6), 4.59 (d, $J = 17.0$ Hz, 1H, H4a), 4.54 (d, $J = 11.5$ Hz, 1H, H8a), 4.42 (d, $J = 11.5$ Hz, 1H, H8b), 4.29 (d, $J = 17.0$ Hz, 1H, H4b), 2.09 (s, 3H, H1/10), 2.07 (s, 3H, H1/10). $^{13}$C NMR (101 MHz, CDCl₃) δ/ppm: 193.2 (C5), 170.1 (C2/9), 169.3 (C2/9), 143.3 (C7), 128.1 (C6), 97.3 (C3), 68.0 (C4), 65.0 (C8), 21.2 (C1/10), 20.7 (C1/10). HRMS (ESI) C₁₀H₁₂O₆ m/z: [M+Na]⁺ 251.0519 (calc. 251.0526).

The physical and spectroscopic data were found to be in agreement with Snider et al.²⁰⁶

10,12-Dioxo-3,11-dioxatricyclo[5.3.1.1²⁶]dodeca-4,8-diene-4,7-diyldiis(methylene) diacetate, 244

![Structure of 10,12-Dioxo-3,11-dioxatricyclo[5.3.1.1²⁶]dodeca-4,8-diene-4,7-diyldiis(methylene) diacetate]

The title compound was isolated as a by-product from the reaction to synthesise 245. Dark orange oil (30.1 mg, 0.09 mmol, 5%); Rₚ 0.12 (40% ethyl acetate in hexane); IR (thin film) $\tilde{\nu}$/cm⁻¹: 2977 (C-H, w), 1742 (C=O ester, s), 1698 (C=O ketone, s), 1223 (C-O, s). $^1$H NMR (400 MHz, CDCl₃) δ/ppm: 6.83 (d, $J = 10.5$ Hz, 1H, H7), 6.30 (d, $J = 10.5$ Hz, 1H, H8), 4.91 (d, $J = 9.0$ Hz,
Experimental

1H, H4), 4.80–4.76 (m, 2H, H2&10), 4.44–4.25 (m, 4H, H11&14), 3.27 (dd, J = 7.5, 2.5 Hz, 1H, H5), 2.21 (s, 3H, H16), 2.09 (s, 3H, H13). $^{13}$C NMR (101 MHz, CDCl3) δ/ppm: 198.3 (C1), 189.0 (C9), 170.4 (C15), 170.3 (C12), 150.8 (C3), 147.9 (C7), 129.6 (C8), 101.0 (C2), 81.5 (C4), 81.4 (C6), 75.9 (C10), 66.7 (C14), 61.7 (C11), 48.5 (C5), 20.8 (C13), 20.6 (C16). HRMS (ESI) C16H16O6Na m/z: [M+Na]+ 359.0743 (calc. 359.0743).

The physical and spectroscopic data were found to be in agreement with Lee et al.240

L-(+)-Prolinol, 254

To a suspension of lithium aluminium hydride (2.47 g, 65.14 mmol, 15 eq) in THF (65 mL) was added portion-wise L-proline (5 g, 43.43 mmol, 1.0 eq) at 0 °C. The reaction was refluxed under nitrogen for 2h. This was cooled to RT and quenched with 20% aq. KOH (6.5 mL) the mixture was filtered and the residue was refluxed in THF for 45 min. This was filtered and combined to the previous filtrate and dried over MgSO4. This was filtered and concentrated in vacuo to give the crude product. This was purified by vacuum distillation (77–78 °C, 5 mbar) to give the pure product as a colourless oil (3.95 g, 39.09 mmol, 90%); IR (thin film) $\tilde{\nu}$/cm$^{-1}$: 3294 (O-H, br s), 2869 (C-H, s), 1457 (C-H, m), 1047 (C-O, s). $^{1}$H NMR (400 MHz, (CD$_3$)$_2$SO) δ/ppm: 4.40 (br s, 1H, OH), 3.23 (d, J = 6.0 Hz, 2H, H5), 2.99 (qn, J = 6.0 Hz, 1H, H4), 2.80–2.75 (m, 1H, H1a), 2.72–2.66 (m, 1H, H1b), 1.71–1.54 (m, 3H, H3a&2), 1.35–1.27 (m, 1H, H3b). $^{13}$C NMR (101 MHz, (CD$_3$)$_2$SO) δ/ppm: 64.7 (C5), 59.7 (C4), 46.0 (C1), 27.9 (C3), 25.2 (C2). HRMS (ESI) C9H14ClO2 m/z: [M+H]$^+$ 102.0913 (calc. 102.0913).

The physical and spectroscopic data was found to be in agreement with Widianti et al.210
Experimental

1,3-Diiodoacetone, 256

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Sodium iodide (260.0 mg, 0.87 mmol, 2.2 eq) was dissolved in acetone (5 mL) and 1,3-dichloroacetone (100 mg, 0.79 mmol, 1.0 eq) was added. The reaction was stirred at RT for 1h before being filtered and concentrated to give the pure product as a pure yellow solid (197 mg, 0.64 mmol, 80%); IR (thin film) \( \tilde{\nu}/\text{cm}^{-1} \): 1710 (C=O, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 4.11 (s, 4H, H1). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 195.6 (C2), 1.3 (C1).

The physical and spectroscopic data was found to be in agreement with Voronkov et al.\(^{110}\)

\textit{tert}-Butyl((1,3-dichloropropan-2-yl)oxy)dimethylsilane, 262

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\text{Cl} \quad \text{Cl}
\]

1,3-Dichloroisopropanol (2 mL, 11.48 mmol, 1.0 eq) and imidazole (1.17 g, 17.22 mmol, 1.5 eq) were dissolved in dichloromethane (35 mL). The mixture was cooled to 0 °C, \textit{tert}-butyldimethylsilyl chloride (2.59 g, 17.22 mmol, 1.5 eq) was added, and the reaction was stirred overnight at RT. This was quenched by addition of sat. aq. NH\(_4\)Cl (30 mL). The layers were separated and the aqueous was extracted with diethyl ether (3 \( \times \) 10 mL). The combined organics were washed with brine (30 mL), dried over anh. MgSO\(_4\), filtered, and the excess solvent was removed in vacuo to give the crude product. This was purified by column chromatography (10% diethyl ether in pet. ether) to yield the pure product as a colourless oil (2.25 g, 9.29 mmol, 81%); \( R_f \) 0.76 (10% diethyl ether in pet. ether); IR (thin film) \( \tilde{\nu}/\text{cm}^{-1} \): 2931 (C-H, w), 1107 (C-O, m), 775 (C-Cl, s). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 4.03 (qn, \( J = 5.5 \) Hz, 1H, H2), 3.62 (dd, \( J = 11.0, 5.5 \) Hz, 2H, H1a), 3.56 (dd, \( J = 11.0, 5.5 \) Hz, 2H, H1b), 0.91 (s, 9H, H5), 0.12 (s, 9H, H3). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \( \delta/\text{ppm} \): 72.2 (C2), 45.9 (C1), 25.7 (C5), 18.1 (C4), –4.7 (C3). HRMS (ESI) \( \text{C}_9\text{H}_{21}\text{Cl}_2\text{SiO} \) \( m/z \): [M+H]\(^+\) 243.0730 (calc. 243.0739).

The physical and spectroscopic data was found to be in agreement with Axenrod et al.\(^ {241} \)
(S)-(+) 4-Methylenehexahydro-1H,3H-pyrrolo[2,1-c][1,4]oxazepane, 266

To a suspension of NaH (53% suspension in mineral oil, 1.86 g, 40.97 mmol, 2.0 eq) in THF (100 mL) at 0 °C under nitrogen L-prolinol (2 mL, 20.48 mmol, 1.0) was added dropwise. This was warmed to RT and stirred for 1 h. 3-Chloro-2-(chloromethyl)prop-1-ene (2.37 mL, 20.48 mmol, 1.0 eq) was added dropwise and reaction was stirred for 2 h at 60 °C. Afterwards it was cooled to RT and the excess solvent was removed in vacuo. This was diluted with ethyl acetate (50 mL) and washed sat. aq. NH₄Cl (30 mL). The aqueous was extracted with ethyl acetate (20 mL). The combined organics were dried over MgSO₄, filtered and concentrated in vacuo to give the crude product. This was purified by column chromatography (10% methanol in dichloromethane) to give a colourless oil (1.1813 g, 7.71 mmol, 38%); Rf 0.35 (10% methanol in dichloromethane); IR (thin film) v/cm⁻¹: 2935 (C-H, m), 1643 (C=C, w), 1102 (C-O, s). ¹H NMR (400 MHz, CDCl₃) δ/ppm: 4.89 (d, J = 14.0 Hz, 2H, H1), 4.34 (d, J = 14.0 Hz, 1H, H3a), 4.25 (d, J = 14.0 Hz, 1H, H3b), 3.90 (dd, J = 12.0, 2.5 Hz, 1H, H8a), 3.63 (d, J = 15.0 Hz, 1H, H9a), 3.27–3.21 (m, 2H, H8b&9b), 3.08–3.03 (m, 1H, H4a), 2.61–2.54 (m, 1H, H7), 2.43–2.37 (m, 1H, H4b), 1.92–1.68 (m, 3H, H6a&5), 1.40–1.31 (m, 1H, H6b). ¹³C NMR (101 MHz, CDCl₃) δ/ppm: 146.9 (C2), 111.8 (C1), 76.5 (C8), 74.9 (C3), 67.8 (C7), 58.5 (C9), 56.2 (C4), 27.2 (C6), 22.6 (C5). HRMS (ESI) C₉H₁₆NO m/z: [M+H]⁺ 154.1221 (calc. 154.1226). [α]ᵢ₀° +110.0 (c = 1.00, CHCl₃).
References


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241. Axenrod, T., Watnick, C., Yazdekhasti, H. & Dave, P. R. Synthesis of 1,3,3-
2,2′-(4,5-Dichloro-1,2-phenylene)bis(ethan-1-aminium) dichloride, 29
2,2′-(4,5-Dichloro-1,2-phenylene)diacetonitrile, 30
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![Spectra](image_url)
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**Spectra**

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**NMR Spectra**

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2-Phenyl-3-oxa-1-azaspiro[4.5]deca-1,7-diene, 151
3-Oxa-1-azaspiro[4.5]deca-1,7-dien-2-amine, 152
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[Image of NMR spectrum]
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\[
\begin{align*}
\text{HN} & \quad 2 \\
\text{O} & \quad 3 \\
\text{N} & \quad 4 \\
\text{O} & \quad 5 \\
\text{C} & \quad 6 \\
\text{C} & \quad 7
\end{align*}
\]
**tert-Butyl 3-(2-ethoxy-2-oxoethylidene)azetidine-1-carboxylate, 236**

Spectra

Chemical data parameters:
- **name:** tert-Butyl 3-(2-ethoxy-2-oxoethylidene)azetidine-1-carboxylate
- **CAS:** 20157-75-8
- **formula:** C13H22NO4
- **mass:** 236.364
- **IR:** 2920, 1643, 1462, 1262, 1168, 732, 711, 641 cm⁻¹
- **nmr:**
  - 1H NMR (400 MHz, CDCl₃) δ 1.25 (t, J = 7.1 Hz, 3H), 1.36 (s, 9H), 1.45 (s, 3H), 2.35 (s, 3H), 2.45 (s, 3H), 3.84 (q, J = 7.1 Hz, 2H), 7.30 (d, J = 7.1 Hz, 1H)
- **mass spec:** 236.364

2D NMR experiments:
- **1H-1H COSY**
- **1H-13C HSQC**
- **1H-13C HMBC**
- **13C-13C HSQC**

2D NMR spectra showing the assignments and coupling constants for the various protons and carbons.
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**4-(4-Methoxyphenyl)-1-azaspiro[4.5]deca-3,7-dien-2-one, 176**

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<td><strong>Space group</strong></td>
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<td>c = 11.5405(6) Å</td>
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### 1-Azaspiro[4.5]deca-3,7-dien-2-one, 179

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### 3-Phenyl-1,8-dioxa-2,6-diazaspiro[4.4]non-2-en-7-one, 212

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<td>(c = 12.0561(7)) Å (\gamma = 81.581(4))°</td>
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3-Oxa-1-azaspiro[4.5]deca-1,7-dien-2-amine, 152

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Volume  812.593 Å³
Z  4
Density (calculated)  1.244 gcm⁻³
Absorption coefficient  0.678 mm⁻¹
F(000)  328
Crystal size  0.250 × 0.060 × 0.020 mm³
Theta range for data colletion  4.29–66.73°
Index ranges  
  –12 ≤ h ≤ 12,  –9 ≤ k ≤ 9,  –12 ≤ l ≤ 12
Reflections collected  9075
Independent reflections  1443
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Absorption correction  Multi-scan
Max. and min. transmission  0.987 and 0.849
Refinement method  Full-matrix least-squares on F²
Data/restraints/parameters  1443/0/108
Goodness-of-fit F²  1.094
Final R indices [I > 2σ(I)]  R₁ = 0.0465, wR₂ = 0.1122
R indices (all data)  R₁ = 0.0583, wR₂ = 0.1185
Largest diff. peak and hole  0.189 and –0.184 eÅ⁻³
Appendix C

Computational analysis

C.1. Reference Set

The following are the small molecule drugs from the top 50 best-selling drugs of 2013:

- **Seretide** (Fluticasone + Salmeterol)
- **Abilify**
- **Crestor**
- **Cymbalta**
- **Spiriva**
- **Gleevec**
- **Lyrica**
- **Januvia**
- **Atripla** (Efavirenz + Tenofovir + Emtricitabine)
- **Diovan**
Computational analysis

Lipitor

Celebrex

Zetia

Alimta

Micardis

Palvix

Velcade

Atacand

Xarelto

Gilenya

Viagra

Janumet (Sitagliptin + Metformin)

Invega
C.2. Principle Moment of Inertia

Using MOE 2018.0602 the following parameters were used in conformer searching:

**Table C.1** Conformational search settings.

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<tr>
<td>Solvation</td>
<td>Born</td>
</tr>
<tr>
<td>Method</td>
<td>LowModeMD</td>
</tr>
<tr>
<td>Rejection Limit</td>
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</tr>
<tr>
<td>RMS Gradient</td>
<td>0.005</td>
</tr>
<tr>
<td>Iteration Limit</td>
<td>10000</td>
</tr>
<tr>
<td>MM Iteration Limit</td>
<td>500</td>
</tr>
<tr>
<td>RMSD Limit</td>
<td>0.15</td>
</tr>
<tr>
<td>Energy Window</td>
<td>3</td>
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<tr>
<td>Conformation Limit</td>
<td>200</td>
</tr>
</tbody>
</table>
C.3. Principle Component Analysis

Using MOE 2018.0602 the following structural and physico-chemical parameters were used in PCA:

**Table C.2** Structural and physico-chemical parameters used in PCA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>2D or 3D</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA_H</td>
<td>Total hydrophobic surface area</td>
<td>3D</td>
</tr>
<tr>
<td>ASA_P</td>
<td>Total polar surface area</td>
<td>3D</td>
</tr>
<tr>
<td>a_acc</td>
<td>Number of hydrogen bond acceptor atoms</td>
<td>2D</td>
</tr>
<tr>
<td>a_arom</td>
<td>Number of aromatic rings</td>
<td>2D</td>
</tr>
<tr>
<td>a_don</td>
<td>Number of hydrogen bond donor atoms</td>
<td>2D</td>
</tr>
<tr>
<td>a_nN</td>
<td>Number of nitrogen atoms</td>
<td>2D</td>
</tr>
<tr>
<td>a_nO</td>
<td>Number of oxygen atoms</td>
<td>2D</td>
</tr>
<tr>
<td>b_rotN</td>
<td>Number of rotatable bonds</td>
<td>2D</td>
</tr>
<tr>
<td>chiral</td>
<td>Number of chiral centres</td>
<td>2D</td>
</tr>
<tr>
<td>KierFlex</td>
<td>Molecular flexibility</td>
<td>2D</td>
</tr>
<tr>
<td>logS</td>
<td>Log solubility in water</td>
<td>2D</td>
</tr>
<tr>
<td>mr</td>
<td>Molar refractivity</td>
<td>2D</td>
</tr>
<tr>
<td>rings</td>
<td>Number of rings</td>
<td>2D</td>
</tr>
<tr>
<td>SlogP</td>
<td>Log octanol/water partition coefficient</td>
<td>2D</td>
</tr>
<tr>
<td>TPSA</td>
<td>Topological polar surface area (Å²)</td>
<td>2D</td>
</tr>
<tr>
<td>vol</td>
<td>Van der Waals volume</td>
<td>3D</td>
</tr>
<tr>
<td>weight</td>
<td>Molecular weight</td>
<td>2D</td>
</tr>
</tbody>
</table>

The following settings were used in the principle component analysis:

**Table C.3** Principle component settings.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Weight field</td>
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</tr>
<tr>
<td>Prefix</td>
<td>PCA</td>
</tr>
<tr>
<td>Component limit</td>
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</tr>
<tr>
<td>Minimum variance (%)</td>
<td>95</td>
</tr>
<tr>
<td>Condition limit</td>
<td>1e+006</td>
</tr>
</tbody>
</table>
C.4. LLAMA Parameters

The following reactions were used in the LLAMA program to elaborate the spirocyclic scaffolds

**Table C.4** Reactions used in LLAMA elaboration.

- Reductive amination
- Buchwald-Hartwig amination
- Sulfonamide formation
- Urea formation
- Alcohol alkylation
- Carbamate formation
- Secondary amide alkylation
- Esterification
- Secondary amide arylation
- Amide formation
- Alcohol arylation
Appendix D

Publication

Novel non-ATP competitive small molecules targeting the CK2 α/β interface

Paul Brear, Andrew North, Jessica Iegre, Kathy Hadje Georgiou, Alexandra Lubin, Laura Carro, William Green, Hannah F. Sore, Marko Hyvönen, David R. Spring

https://doi.org/10.1016/j.bmc.2018.05.011

This paper contains the research the Spring Group has done into elaborating NMR154 and NMR154L to find novel non-ATP competitive fragments which inhibit the interface site CK2.

I was given responsibility to write a publication bringing together all the group’s work in this field. This first involved analysing the 144 molecules which had been synthesised to bind at the interface. These were then sorted into groups based on the vectors of NMR154 which had been elaborated, or if they were not NMR154 based. With the data in hand I wrote the entire manuscript, compiled the supplementary information, and synthesised a couple of compounds to record missing data.