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University College London

SYNTHESIS OF TELOMERASE INHIBITORS BASED ON POLYOXAZOLES

A Thesis Presented to the University of London in Partial Fulfilment to the Requirements for the Degree of Doctor of Philosophy

Mona Saadi

August 2007

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London WC1H OAJ
Abstract

This thesis focuses on the synthesis of compounds expected to inhibit telomerase and provide potential for the treatment of cancer. Telomerase is a reverse transcriptase enzyme, which codes for telomeres and define the ends of chromosomes. Abnormal telomerase activity occurs in 85% of cancer cells and consequently has gained considerable interest as a target for cancer therapy. A natural product known as telomestatin can target G-quadruplexes and is shown to be very potent with activity at 5 nM. The aim of this thesis was to synthesise analogues of Telomestatin which contain a polyoxazole macrocycle.

Chapter 1 discusses the enzyme telomerase and describes compounds which are able to inhibit its activity; a literature survey on 2, 4-disubstituted oxazole chemistry is also described.

Chapter 2 describes the attempted synthesis of a dipyridyltrisoxazole and a dipyridyl macrocycle. The synthetic approach involved the Hantzsch method to form oxazoles from amides and bromoketone. The Hantzsch method proved largely ineffective for obtaining the required oxazoles. A second approach involving the Williams-Wipf reaction was carried out to synthesise oxazoles. The procedure involved the cyclisation of L-serine methylester derived compounds with diethylaminosulfur trifluoride, followed by an oxidation reaction with 1,8-diazabicyclo[5.4.0]undec-7-ene and bromotrichloromethane. The Williams-Wipf approach helped to synthesise the half fragment required for the macrocycle.

Chapter 3 involved the synthesis of a tetraoxazolylbipyridyl system. The Williams-Wipf approach was successful in delivering the half fragment of the desired system. However, the palladium cross coupling was ineffective in forming the tetraoxazolylbipyridyl system.

Chapter 4 the aim was to synthesise a symmetrical octaoxazole ring system. The Williams-Wipf approach helped to develop the core structure to telomestatin which consists of five consecutive oxazole rings. A novel hepta oxazolyl ring system was also synthesised; however, owing to insolubility of key intermediates the desired target could not be made.
Acknowledgements

First of all I would like to thank Professor Charles M. Marson for giving me the opportunity to undertake this project which I really enjoyed. I thank him for his advice and enthusiasm throughout the three years. I would also like to thank the Marson group past and present, the technical staff in particular Abil, John, Lisa who were extremely helpful.

Finally, I would like to thank my family, especially my mum and Mehrdad for being there always.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>Ac₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>Anal.</td>
<td>Analytical</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-Cell lymphoma 2</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BnBr</td>
<td>Benzyl bromide</td>
</tr>
<tr>
<td>Boc</td>
<td>Butyloxycarbonyl</td>
</tr>
<tr>
<td>BOP</td>
<td>Benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>Cbz</td>
<td>(benzylcarboxy) carbamate</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin-dependant kinases</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>Cu(OTf)₂</td>
<td>Copper(II) triflate</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DAST</td>
<td>Diethylamino sulfurtrifluoride</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>Deoxo-Fluor™</td>
<td>Bis-(2-methoxyethyl)aminosulphur trifluoride</td>
</tr>
<tr>
<td>dGTP</td>
<td>2′-deoxyguanosine 5′-triphosphate</td>
</tr>
<tr>
<td>DIEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DnaA</td>
<td>DNA replication initiation factor</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>equvi.</td>
<td>Equivalent</td>
</tr>
<tr>
<td>FAB</td>
<td>Fast atom bombardment</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
</tr>
<tr>
<td>G1</td>
<td>Gap 1</td>
</tr>
<tr>
<td>G2</td>
<td>Gap2</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HATU</td>
<td>2-(1H-7-Azabenzotriazol-1-yl)-1, 1, 3, 3-tetramethyl uranium hexafluorophosphate methanaminium</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>HMQC</td>
<td>Heteronuclear multiple-quantum coherence experiment</td>
</tr>
<tr>
<td>HOBT</td>
<td>N-Hydroxy benzotriazole</td>
</tr>
<tr>
<td>hTERT</td>
<td>Human telomerase catalytic sub-unit gene</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IBCF</td>
<td>Isobutyl chloroformate</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Half the maximal inhibitory concentration</td>
</tr>
<tr>
<td>J</td>
<td>Spin-spin coupling constant</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-Insulin Dependant Diabetes Mellitus</td>
</tr>
<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>p-TSA</td>
<td>para-Toluenesulfonic acid</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RPMI 8402</td>
<td>Cell culture made from T-cells</td>
</tr>
<tr>
<td>RTER</td>
<td>Telomerase reverse transcriptase</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>S</td>
<td>Stationary</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
</tr>
<tr>
<td>T</td>
<td>Thymine</td>
</tr>
<tr>
<td>TBDPSCl</td>
<td>Tributylidphenylsilyl chloride</td>
</tr>
<tr>
<td>TBSCI</td>
<td>Tributylsilyl chloride</td>
</tr>
<tr>
<td>TEP1</td>
<td>Telomerase associated protein 1</td>
</tr>
<tr>
<td>TESCI</td>
<td>Triethylsilyl chloride</td>
</tr>
<tr>
<td>TETA</td>
<td>Triethylene tetraamine</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>TIPSOTf</td>
<td>Triisopropylsilyl triflate</td>
</tr>
<tr>
<td>TsCN</td>
<td>Tosyl nitrile</td>
</tr>
<tr>
<td>TMANO</td>
<td>Trimethylidamidine N-oxide</td>
</tr>
<tr>
<td>TMSCl</td>
<td>Trimethylsilyl chloride</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl triflate</td>
</tr>
<tr>
<td>TRAP</td>
<td>Telomerase repeat amplification</td>
</tr>
<tr>
<td>TsCl</td>
<td>Tosyl chloride</td>
</tr>
<tr>
<td>TTP</td>
<td>Thymidine triphosphate</td>
</tr>
</tbody>
</table>
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Chapter 1

1.0 Cancer and Inhibitors of Telomerase

1.1 Objectives

This thesis will focus on the synthesis of compounds expected to inhibit telomerase and hence with potential for the treatment of cancer. Abnormal telomerase activity occurs in 85% of cancer cells and consequently has gained considerable interest as a target for cancer therapy. Chapter one will focus on cancer and its treatment, followed by a detailed account of telomerase and a literature survey of oxazole systems found in nature.

1.2 Cancer

Cancer is a disease which is the cause of most deaths in the UK, one third of the population being affected by it at some time in their lives. Cancer involves unregulated cell growth, these cells can act on normal cells to destroy them.

Cancers form as a result of an accumulation of cancer cells in the form of lumps known as tumors. Tumors that arise between epithelial sheets found in inner and outer surfaces in the body are known as carcinomas. Tumors found in connective tissues are termed sarcomas. Leukaemia and lymphomas are a result of circulating tumors found in bone marrow, and lymph nodes. The disease follows a sequence of events which include healthy cells being converted to cancer cells that can proliferate and invade other nearby tissue. Finally, the cells metastasise, which involves liberation of the cells from the tumor mass and migration to other parts of the body. Uncontrollable division of cells will ultimately lead to death, if no treatment is successful.
Chapter 1

Much time and research has been spent trying to understand cancer and what causes it. It is believed that environmental factors such as an over-exposure to ultra-violet light from the sun can be a cause of cancer, as can be radioactive materials and X-rays. Certain compounds or mixtures termed carcinogens,\(^2\) can also lead to cancer; they include tar found in cigarettes, charred food and additives such as phenylalanine found in fizzy drinks. Biological factors including oncogenes from viruses that attack the healthy host can transmit a cancer-causing gene formed as a result of DNA mutations or chromosomal translocation.

Those affected by cancer show certain symptoms which often may include the following: lumps around a particular area of the body, bleeding, change in body weight, change in bowel movement, feeling fatigue and nauseous. Blood tests and scans are used to confirm the presence of the disease.

Treatments available for cancer include chemotherapy, radiation therapy and surgery. Chemotherapy involves giving drugs to the patient to kill the cancer cells. Dire side effects can develop from taking the medication such as fatigue, nausea and improvement of the immune system since healthy cells are also targeted by the therapy. The success of the therapy depends in part on the stage of development of the cancer.\(^2\) Hence, higher success rates are normally found in the early stages of cancer.

Many different targets for cancer treatment have been and are being investigated, especially inhibition of enzymes including: histone deacetylase inhibitors (HDAC), inhibitors of Bcl-2 and telomerase inhibitors.
1.3 Structure of DNA

DNA molecules are found inside the nucleus of a cell. A process known as transcription occurs in cells where information is transferred from the nucleus via messenger RNA which is the complimentary base sequence of single-stranded DNA. The translation of the nucleotide sequence contained in mRNA provides a code for protein synthesis. The length of DNA contains the instructions for making a single protein or polypeptide known as the gene that can code for a sequence of amino acids that comprise a protein.

The code in DNA consists of four bases, adenine (A), thymine (T), guanine (G) and cytosine (C), and one of the two strands is named as the reference strand, adenine bonding with thymine, and guanine with cytosine. During transcription, bases are always read in the same direction.

Genetic information contained in the DNA molecule (Fig 1) can be transmitted to the next generation by mitosis during cell cycle.

![Figure 1. Structure of DNA](image)

Figure 1. Structure of DNA
1.4 Cell Cycle

![Cell Cycle Diagram]

**Figure 2.** The cell cycle

The cell cycle involves a sequence of events by which a cell grows to develop and divide, forming new daughter cells which then go on to carry out the same cycle. As cells divide they distribute hereditary information to their daughter cells. The process of cell division can take from a few hours to a few months; however, the actual division itself (mitosis) takes a few minutes. The cell cycle is controlled by many proteins which include: cyclins (cyclin D, cyclin E and cyclin A) and cyclin-dependant kinases (CDK4, CDK6 and CDK2). These proteins indicate the appropriate time to grow, divide and halt. The cycle begins with G₀, the resting stage whereby cells are not dividing. At this stage external stimulus induces the process of cell division. The second step of the cycle, G₁ is referred to as the pre-replication stage, during which the cell assembles certain constituents needed for chromosomes to replicate. This step takes from a few hours to a few months to complete. The cycle then reaches the synthesis or S phase, which involves DNA replication and also histone synthesis; the duration of this step can be 10 hours. G₂ is the next step, known as the postmitotic stage and lasts about two hours. Normal metabolism takes place here, allowing the cells to make proteins for cell growth. The cells then go
Chapter 1

through the same sequence of events to form more cells. Cancer treatment focuses on controlling one or more of those stages.

DNA contains all the genetic information of a cell and is characterised by its diversity. Variability in DNA heterocyclic bases is caused by random modifications of the DNA sequence. However, the ability of cell to survive depends on the instructions carried by the DNA, so if the DNA is damaged, then the cell will not synthesise the correct proteins. The problematic time involving DNA is during replication because errors in reading bases can occur leading to mutations. Some of the random modifications can be advantageous; however, others lead to malignant cancers. Cancer is caused by accumulation of alterations in the genome.

1.5 Amino acids

All proteins are made from polymers of various amino acid subunits to form complex structures. There are twenty naturally occurring amino acids and all occur in the laevo configuration except glycine which has no stereocentre.

Each amino acid contains an amino group, a carboxyl group and a different R group. When an amino acid is dissolved in water it can dissociate so that the acidic carboxyl group loses a hydrogen atom and the amino group gains a hydrogen atom due to its basicity, making it a Zwitterion. The different R groups present determine the physical properties of the structure. Alanine, valine, leucine, isoleucine, phenylalanine, tryptophan are all hydrophobic, containing hydrocarbon side chains. Aspartic acid, aspargine, glutamine, tyrosine and cysteine are some of many amino acids which are hydrophilic in nature hence, quite water-soluble. Cysteine contains a highly nucleophilic thiol which can react with another cysteine amino acid to form disulfide linkages that permit for complex bridged protein structures (fig 3).
Chapter 1

Amino acid  dissociated form

\[
\begin{align*}
R &= & \text{H} & \text{CH}_3 & \text{CH}_3 & \text{H}_2 \text{C} \text{CH}_3 & \text{CH}_2 \text{CH}_3 & \text{CH}_2 \text{CH}_3 \\
\text{Glycine} & \quad \text{Alanine} & \quad \text{Valine} & \quad \text{Leucine} & \quad \text{Isoleucine} \\
(Gly, G) & \quad (Ala, A) & \quad (Val, V) & \quad (Leu, L) & \quad (Ile, I) \\
\text{Phenylalanine} & \quad \text{Tyrosine} & \quad \text{Tryptophan} & \quad \text{Methionine} \\
(Phe, F) & \quad (Tyr, Y) & \quad (Trp, W) & \quad (Met, M) \\
\text{Proline} & \quad \text{Serine} & \quad \text{Threonine} & \quad \text{Cysteine} \\
(Pro, P) & \quad (Ser, S) & \quad (Thr, T) & \quad (Cys, C) \\
\text{Lysine} & \quad \text{Arginine} & \quad \text{Histidine} & \quad \text{Aspartine} & \quad \text{Glutamine} \\
(Lys, K) & \quad (Arg, R) & \quad (His, H) & \quad (Asp, D) & \quad (Glu, E)
\end{align*}
\]
Proteins are made from amino acids linked by peptide bonds which are strong covalent bonds. The amino group attacks the acid group to form a bond via a condensation reaction with loss of water. Two linked amino acid units are termed dipeptide; a third amino acid sequence is termed tripeptide. Longer amino acid polymer chains are known as polypeptides; the chain contains an amino and carboxyl terminus.

\[
\begin{align*}
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \\
&\text{H} \text{R} \text{H} \text{O}^- \\
&\text{H}^+ \text{N}^+ \text{C} = \text{O}^- \end{align*}
\]

**Scheme 1.** Peptide formation

Proteins are usually a sequence of 100 to 10,000 amino acids which depending on their inter-and intramolecular bonding, can form different structures. Linear sequences of amino acids form a primary structure of alpha helixes that take the shape of a telephone cord. The structure is held together by hydrogen bonding between N-H and C=O groups. An example of a secondary structure protein is that of keratin which is found in a strand of hair.

Proteins with a longer amino acid chains can form more complex structures. When two or more polypeptide chains lie side by side, hydrogen bonding is crossed linked to form beta-pleated sheet structure. Such protein structures are found in silk. Bonds involved in these protein structures involve electrostatic forces, van der Waals, and hydrophobic interactions.

Proteins can fold together and form a tertiary structure which is highly complex. As well as hydrogen bonding, disulfide linkages can also take place.
between cysteine residues to form a three-dimensional globular shape. Ribonuclease is made up of a tertiary structure which helps it to bind to an RNA molecule and splits RNA apart. Two to three polypeptide chains that form tertiary and secondary proteins can go to form quaternary structures. The three-dimensional shape is held by weak bonds, an example of which is haemoglobin.

1.6 Enzymes

Studies on enzymes have been carried out for more than a hundred years. Findings have proved that enzymes play a key role in biological reactions in the body; the main reason for this is due to specificity. An enzyme reacts with one type of substrate (the reactant) to form the desired product. An enzyme acts as a biological catalyst owing to its ability to lower the activation energy of a reaction without the enzyme being consumed or altered in any way.

![Enzyme activation graph](image)

**Figure 4.** Enzyme activation

Enzymes are generally globular in structure and contain a unique three-dimensional configuration as a result of the specific amino acid sequence. Within every enzyme there is a region known as the active site; the shape of this area is specific to the substrate which it binds to by covalent and hydrogen bonding, among others. The active site is an indentation of the enzyme’s surface
established by the tertiary structure of the protein. In 1894, Emil Fischer\(^6\) first postulated the specific action of an enzyme with a single substrate can be explained using a ‘lock and key’ model. The analogy proposed that the enzyme is a lock and that the substrate is the key. An important development, known as induced fit model, proposes that the enzyme undergoes a conformational change in shape in order to accommodate the substrate. Some active sites require the aid of a prosthetic group such as magnesium or zinc ions.

![Diagram of enzyme-substrate interaction](image)

**Figure 5.** Depiction of Fischer’s Lock-and-key model

Each enzyme has a unique three-dimensional structure which can be altered as a result of many factors. If the pH of the enzyme environment is altered then the bonds holding the enzyme together can change. Temperature is another factor that if increased can break the bonds (hydrogen bonds, hydrophobic interactions etc.) which hold the enzyme in a given conformation.

### 1. 6. 1 Enzyme kinetics

The study of enzyme kinetics was first described by Michaelis and Menten in 1913.\(^7\) They both developed a quantitative theory of enzyme catalysis and kinetics. Their theory postulates first the enzyme (E) binds with the substrate (S) to form an enzyme-substrate complex (ES). The second step
postulated involves the enzyme-substrate breaking down to form a product (P) with regeneration of the free enzyme.

\[ E + S \xrightarrow{K_1} ES \]
\[ ES \xrightarrow{K_2} E + P \]

Michaelis-Menten equation

\[ V_{max} \]
\[ V_0 \]
\[ \frac{V_{max}}{2} \]
\[ K_M \]
\[ [S] \]

Figure 6. Michaelis-Menten kinetics: the effect of substrate on the initial rate

\[ K_1, K_2, K_3 \text{ and } K_4 \text{ are the rate constants of the reaction. The equations describe a reversible binding, although enzyme release and product formation is not always reversible. When the rate of enzyme substrate concentration is equal to the rate of breakdown a steady state is reached where } [ES] \text{ is constant:} \]

\[ K_1([E_{free}] - [ES]) [S] = K_2 [ES] + K_3 [ES] \]

Figure 7
This equation can be rearranged and expressed in terms of a $K_m$ constant known as the Michaelis-Menten constant, which expresses the enzymes relationship to the substrate. The initial rate of a reaction is defined as $V$ and is the measure of < 10% conversion of substrate into product. Once all the enzyme is converted into the enzyme substrate complex a maximum velocity rate is achieved, abbreviated as $V_m$. Michaelis-Menten derived an equation (i) to quantify the rate of reaction for the conversion of a one enzyme-one substrate reaction.

\[ V = \frac{V_m [S]}{K_m + [S]} \] (i)

**The Michaelis-Menten equation**

However, the Michaelis-Menten equation does not readily disclose the $V_m$ when plotted onto a graph. In the Lineweaver-Burk representation\(^8\) used the corresponding double reciprocal plot of the equation is used for the determination of an accurate value of $V_m$. When $1/V$ is plotted against $1/[S]$, a linear plot is obtained (Fig 8).
Figure 8. Lineweaver-Burk plot

Once \(1/V\) is plotted against \(1/[S]\), a linear relationship is obtained. The plot intercepts the y-axis at \(1/V_m\) and extends onto the x-axis at \(1/K_m\).

1.6.2 Enzyme Inhibition

Compounds known to inhibit enzymatic reactions are termed inhibitors. These inhibitors consist broadly of two types: known as reversible and irreversible. Reversible inhibition can be further subdivided into competitive, non-competitive and uncompetitive inhibition. Irreversible inhibition includes suicide and transition-state inhibition.
Chapter 1

1.6.2.1 Reversible inhibition

i) Competitive inhibition involves the substrate and the inhibitor competing for the same active site. Such inhibitors can exhibit a similar conformation to that of the substrate.

ii) Non-competitive inhibition includes inhibitors that are not competing for the active site but are able to bind to another location on the enzyme.

iii) Uncompetitive inhibition arises when an inhibitor is not competing with the enzyme or substrate but is in fact attacking the enzyme substrate complex.

1.6.2.2 Irreversible inhibition

Irreversible inhibitors bind to an enzyme and are not able to dissociate themselves from it. The inhibitor can usually form covalent bonds with the enzyme which makes it very stable. The inhibitor can act in two ways; firstly, it can destroy the active site or it can prevent the substrate from binding to the enzyme. *Suicide inhibition* is one form of irreversible inhibition. The enzyme would carry out its normal biotransformation with the inhibitor believing it to be the substrate but in fact the inhibitor forms a strong covalent bond which can deactivate the enzyme. *Transition state inhibitors* are inhibitors that can mimic the conformation of the active site when a substrate is bound to an enzyme. A well known transition state inhibitor is that of penicillin.
1.7 Telomerase

Telomerase is a reverse transcriptase enzyme and contains RNA as a template for telomeres. Telomeres are stretches of repetitive DNA structures which define the ends of chromosomes. The sequence of DNA is guanine-rich, consisting of (TTAGGG)ₙ bases; however, one strand is longer than the complimentary strand exposing one G base and is known as G-strand overhang. Telomeres can form a higher-order chromatin structure known as a T-loop which can hide the 3'-chromosome end during cellular activities, as shown in figure 9. TRF1 and TRF2 are telomere binding proteins found around the T-loop which prevent telomere lengthening.

![Diagram of T-loop](image)

Figure 9. Telomerase is a specialised reverse transcriptase that synthesises telomeric repeats onto chromosomal ends and thus compensates for progressive telomere shortening caused by the end-replication problem. Research into telomere biology has increased over the last decade, providing more understanding of its role in cancer and its prevention. Telomerase is an essential component of cellular immortalization and tumorigenesis.

It is uncommon to find somatic cells which contain telomerase unless they are germ cells or stem cells; conversely, telomerase is shown to be activated in 85-90% tumour cells. In most human somatic cells, telomeres shorten progressively by 50-200 nucleotides with each cell division. It is believed that
reduction in telomere length can lead to growth arrest. In old age, telomerase activity lessens and telomere shortening increases, which over the course of evolution has become one of the body’s defences to cancer.\textsuperscript{16} Anti-sense oligonucleotides and related compounds exhibit potent inhibition of telomerase in extremely low picomolar concentrations.\textsuperscript{15}

From many viewpoints inhibition of telomerase is seen as an important target for a new anticancer treatment.

As mentioned, telomerase is a reverse transcriptase enzyme which consists of two major components comprising a RNA moiety (RTER) and a catalytic moiety subunit (hTERT) (Fig 10). These sites have been used as targets for telomerase inhibition. Many regulatory proteins associate themselves with the core, including hsp 90, p23 and TEP1.\textsuperscript{17}

\textbf{Figure 10.} Structure of telomerase enzyme complex\textsuperscript{1}

There has been much investigation into the direct targeting of the core telomerase components RTER and hTERT. In 1995 the first successful attempt to inhibit the enzyme directly was reported, results showing that telomeres of the treated cells had their nucleotides shortened. Kondo \textit{et al.}\textsuperscript{18} carried out similar tests using the same vector on glioma cells showed the same results.
In 1997 a gene was discovered for the catalytic subunit of the telomerase enzyme which was later targeted by Zhang et al.; their results showed that telomeres had shortened, and that eventual apoptosis of the cell occurred.

1.7.1 Biochemistry of Telomerase

The first hint of an activity capable of telomere synthesis was found by McClintock who found that specific maize tissues could repair themselves from broken chromosome ends; this was done by telomeres fusing the ends together. Then, 40 years after the discovery of telomere synthesis, Blackburn and co-workers cloned the first telomerase DNA found in *Tetrahymena thermophila.*

Telomerase represents a truly unique enzyme that can synthesise telomeric DNA, one nucleotide at a time, in an apparently template-independent manner. Greider *et al.* found that telomerase can elongate almost any G-rich, single-stranded DNA but the resulting sequence will always contain the newly added bases TTGGGG, as found in *tetrahymena* (fig 11).

**Figure 11** The telomerase elongation reaction.

---

16
Greider proposed that the RNA component served as a template for telomere addition. The reaction mechanism found for telomerase involves the replication of the telomerase RNA template to form a new telomeric DNA.

1.7.2 Telomere Targeting

Other targets of telomerase inhibition involve targeting the telomeres alone, the approach being taken in this project. Telomeres are comprised of chromatin and have specific binding protein sites on them. Telomeres help to protect the chromosomes from recombination and fusion. At every cell division that takes place at least 100 bases of telomeric DNA are lost owing to DNA polymerase being unable to replicate the ends. However, in cancerous cells the telomere length is maintained and stabilized by the enzyme telomerase which catalyses the synthesis of TTAGGG repeats onto the 3’ends of the telomeres.

1.7.3 G- Quadruplexes

Telomeric DNA can fold into quadruplex structures comprising four planar guanine molecules, by intermolecular and intramolecular hydrogen bonding between bases (fig 12). One quadruplex contains typically four G-quartets stacked on top of each other (fig 13). Zahler et al investigated the capacity of the different folded forms of telomeric DNA to serve as primers for the Oxytricha nova telomerase in vitro. It was found that formation of K+ can inhibit telomerase. The mechanism works by a molecule stabilising the 3’ ends of the quadruplex which eventually leads to cessation of the DNA synthesis.
Figure 12

A property of G-rich, single stranded DNA is that it can fold into a four-stranded structure assembled around a core stack of guanines arranged in almost-planar hydrogen-bonded tetrads. Molecules which are able to carry out this mechanism are planar, electron deficient ring systems usually bearing acyclic substituents (fig 14). Molecules that are planar tend to be selective at stabilizing G-quadruplexes because they can fit through the folds easily and interact with the bases. Small molecules that stabilise or promote the formation of quadruplexes also show inhibitory activity.
Only a few small molecules have been identified as quadruplex ligands; they are mostly negatively charged cationic molecules and aromatic electron-rich compounds that require a cavity, such as the groove of the quadruplex. It is likely that the negatively charged compounds could fit into the groove of the quadruplex, flanked by positively charged moieties that can form hydrogen bonds with the quadruplex bases. Examples include the positively charged guanine (G) bases, which are part of the G-quadruplex.

**Figure 13.** Model showing the intramolecular bonding occurring in human quadruplex.

**Figure 14.** Model showing how a planar and electron deficient molecule fits between the G-quadruplex to inhibit the enzyme telomerase.
Chapter 1

Only a few small molecules have been identified as quadruplex ligands; they are subdivided into two categories: groove binders and aromatic chromophores. Examples of aromatic chromophores include porphyrins that contain a cation, anthraquinones, and several tri-and tetra-cyclic ring systems.

Porphyrins with cations attached to them are potent inhibitors, primarily because of the planar chromophore structure which has the ability to fit into the grooves of the quadruplex (fig. 15). Secondly, the cations bind to the negatively charged phosphate back bone of DNA to stabilise the G-quadruplex followed by telomerase inhibition. Such compounds have provided moderate telomerase inhibition with IC$_{50}$ values of 1-25 μM.\textsuperscript{28}

![Figure 15\textsuperscript{28}](image)
1.7.4 Biological studies on telomerase inhibitors

In the TRAP assay, telomerase activity can be measured \textit{in vitro} by a primer extension assay in which telomerase synthesises telomeric repeats onto oligonucleotide primers.\textsuperscript{30} Hence, telomerase synthesises extension products are used in PCR amplification.\textsuperscript{31} In most biological assays for telomerase inhibition a TRAP assay is used.

Many groups have synthesised compounds that inhibit telomerase by targeting G-quadruplex DNA. A series of metalloporphyrins was prepared by Maraval and co-workers;\textsuperscript{32} the porphyrins were metallated with either manganese or nickel. Results showed that the complexes were able to inhibit the telomerase enzyme with IC\textsubscript{50} values in the micromolar range.\textsuperscript{33} Neidle and co-workers\textsuperscript{34} have synthesised telomerase inhibitors that are symmetrical 2,6-disubstituted aminoalkylamido anthraquinones (fig 16) and which possessed IC\textsubscript{50} values $< 10 \ \mu$M.

\begin{figure}[h]
\centering
\includegraphics[scale=0.5]{figure16.png}
\caption{2,6-Disubstituted aminoalkylamido anthraquinone telomerase inhibitors\textsuperscript{33}}
\end{figure}
Sasaki et al found a successful inhibitor of a protein which activates the DNA replication in *E.coli*. Further tests of these compounds have shown that these inhibitors can also suppress telomerase by up to 90%. The structures of these inhibitors are based on the acetoxy-substituted bi-indole template shown in (figure 17). SAR data shows that a carboxylic acid group and a spacer between the bi-indole of exactly not more or less than 14 methylene groups provide successful inhibitors.

![Figure 17](image)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>$R^1$</td>
</tr>
<tr>
<td>1</td>
<td>NH(CH$<em>2$)$</em>{11}$COOH</td>
</tr>
<tr>
<td>2</td>
<td>NH(CH$<em>2$)$</em>{11}$COOCH$_3$</td>
</tr>
<tr>
<td>3</td>
<td>OH</td>
</tr>
<tr>
<td>4</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>5</td>
<td>NH(CH$_2$)$_2$COOH</td>
</tr>
<tr>
<td>6</td>
<td>NH(CH$_2$)$_3$COOH</td>
</tr>
<tr>
<td>7</td>
<td>NH(CH$_2$)$_3$CONHCH$_2$COOH</td>
</tr>
<tr>
<td>8</td>
<td>NH(CH$<em>2$)$</em>{11}$CONHCH$_2$COOH</td>
</tr>
<tr>
<td>9</td>
<td>NH(CH$_2$)$_4$CONH(CH$_2$)$_3$COOH</td>
</tr>
<tr>
<td>10</td>
<td>NH(CH$<em>2$)$</em>{11}$CONH(CH$<em>2$)$</em>{11}$COOH</td>
</tr>
<tr>
<td>11</td>
<td>NH(CH$<em>2$)$</em>{11}$COOH</td>
</tr>
<tr>
<td>12</td>
<td>NH(CH$<em>2$)$</em>{11}$COOCH$_3$</td>
</tr>
<tr>
<td>13</td>
<td>NH(CH$<em>2$)$</em>{11}$COOH</td>
</tr>
</tbody>
</table>

Table 1
Results (Table 1) showed that a terminus carboxyl group provided the best result; a phosphate group (fig 18) was also tested as it possesses the same anionic charge at physiological pH. SAR data (table 2) showed very low IC<sub>50</sub> values of 2.5 μM, making it the most potent telomerase inhibitor in this series.

![Chemical structures](image)

**Figure 18**

<table>
<thead>
<tr>
<th>No.</th>
<th>Type</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Inhibition (%)</th>
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<tr>
<td>1</td>
<td>14</td>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CN</td>
<td>OC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;-Cl-&lt;i&gt;m&lt;/i&gt;</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CN</td>
<td>OC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;-Cl-&lt;i&gt;m&lt;/i&gt;</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>OH·Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>OC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;-Cl-&lt;i&gt;m&lt;/i&gt;</td>
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<tr>
<td>4</td>
<td>15</td>
<td>OH·Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CN</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
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<td>100</td>
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<tr>
<td>6</td>
<td>15</td>
<td>OH·Et&lt;sub&gt;3&lt;/sub&gt;N</td>
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<tr>
<td>7</td>
<td>15</td>
<td>OH·Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>O-cyclohexyl</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2.** SAR studies of novel telomerase inhibitors based on phosphate derivatives.
Yin et al\textsuperscript{26} has reported a novel telomerase inhibitor which can successfully stabilise the G-quadruplex. These compounds are linear structures made up of triethylene tetramine (TETA) (fig 19). The compound possesses the desired properties for G-quadruplex stabilisation; which are planar electron deficient chromophore, basic side chains, similar to that of anthraquinones.

\begin{center}
\includegraphics[width=0.5\textwidth]{image19}
\end{center}

\textbf{Figure 19}

Biological results showed that TETA was very potent obtaining an IC\textsubscript{50} value of 7.8 \textmu M. The precise mechanism of contact with G quadruplex remains unclear, however, it is possible to assume the compound interacts by inter and intramolecular and hydrogen bonds.

Maraval et al\textsuperscript{32} synthesised a tetracationic porphyrin, known as mesotetrakis(4-N-methylpyridinyl)porphyrin (fig 20) which possesses properties that make it bind well with the G-quadruplex.

\begin{center}
\includegraphics[width=0.5\textwidth]{image20}
\end{center}

\textbf{Figure 20}

M=Mn(III) or Ni(II)
n=5 for Mn
n=4 for Ni
Chapter 1

The metalloporphyrins were prepared from a condensation reaction involving pyrrole and 4-methylpyridinylbenzaldehyde.\textsuperscript{37}

CD and UV thermal melting studies have shown that telomerase inhibition takes place by $\pi$-stacking between the G-quartet, owing to its planar, electron deficient structure. Maraval et al.\textsuperscript{32} showed that manganese metal ions can undergo an oxidation reaction involving KHSO$_3$ into an oxomanganese species; which can oxidise guanine residues in DNA. \textit{In vivo} oxygen in a molecular form is available to oxidise manganese ion in the porphyrin structure.

Optimal inhibition of telomerase was carried out by changing the pyridinyl groups into aminoquinoline groups, 16 (Fig 21). IC$_{50}$ values of around 5-10 µM were obtained for these compounds.

![Figure 21]
Dong-Fang Shi et al. have researched into telomerase inhibitors focusing on G-quadruplexes. The compounds they synthesised were of a porphyrin type structure TMPyP4 (fig 22) similar to that of Maraval et al.; however, different functional groups were attached in order to accumulate as much SAR data.

![Diagram of TMPyP4](image)

**Figure 22** Structure of TMPyP4

From their results they concluded that a cationic porphyrin showed enhanced potency which worked at IC\textsubscript{50} values at 5-25 μM. It is found that using free porphyrins, photocleavage of DNA can arise which is a dangerous consequence for *in vivo* studies. Using Cu(II) can provide an unhindered porphyrin square planar conformation, providing 75% inhibition without causing any photocleavage. Mn (III) and Mg (II) both form octahedral complexes that help prevent the stacking inside the G-quartet, hence, providing low telomerase inhibition values summarised in Table 3.
<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Metal ion</th>
<th>Geometry</th>
<th>% Inhibition (25μM)</th>
</tr>
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<tbody>
<tr>
<td>TMPyP4</td>
<td>H2</td>
<td></td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Zn (II)</td>
<td>Py</td>
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<td></td>
<td>Co (II)</td>
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<td></td>
<td>Fe (III)</td>
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<td>63</td>
</tr>
<tr>
<td></td>
<td>Ni (II)</td>
<td>sq pl-oh</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Mn (III)</td>
<td>oh</td>
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</tr>
<tr>
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<td>Cu (II)</td>
<td>sq pl</td>
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<td></td>
<td>Mg (II)</td>
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<tr>
<td></td>
<td>Pt (II)</td>
<td>sq pl</td>
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<tr>
<td></td>
<td>Pd (II)</td>
<td>sq pl</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 3.

For effective telomerase inhibition, substituents containing a positive charge would be desirable. The reason for this is based on the nature of DNA, which has a backbone of phosphate anions; accordingly, ligands can interact better with DNA if a positive charge is present. SAR results show that with more charged substituents present on the porphyrin structure, the telomerase inhibition can increase to 25 μM Table 4.
<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>5-Ar</th>
<th>10-Ar</th>
<th>15-Ar</th>
<th>20-Ar</th>
<th>inhibition (%)</th>
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<td>TMPyP4</td>
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<td><img src="image2" alt="Structure" /></td>
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<td>31</td>
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</table>

Table 4.

Positively charged N-position substituents are essential for telomerase inhibition. Such alkyl groups used in the SAR studies have included methyl, ethyl and hydroxyethyl groups which have shown some inhibition; however, with increasing chain length the inhibition is decreased. The substituents providing the highest degree of inhibition are that of the N-methylpyridinium series. If the substituent contains a Zwitterion, no net charge is present to interact with the DNA, resulting in a decrease in telomerase inhibition as shown in Table 5.
<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>meso-Ar</th>
<th>inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>TMPyP4</td>
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</tr>
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<td></td>
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<td>0</td>
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<tr>
<td></td>
<td>![Chemical Structure]</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 5
Chapter 1

1.8 Natural products

Many natural products can act as anticancer agents, and many research groups are searching for new compounds from marine sponges for their neoplastic effects. Unsurprisingly, numerous natural products are in clinical trials for the treatment of cancer.

1.8.1 Ascididemin and Meridine

Ascididemin was isolated by Kobayashi *et al.*\textsuperscript{35} in 1988 from the *Okenuratumal didemnum*. Meridine was isolated by Schmitz *et al.*\textsuperscript{36} in 1991 from *Amphicarpa meridiana*; these natural products belong to the pyridoacridine family which are found in marine sponges. The main structural differences (fig. 23) are that Ascididemin has a pyridine ring attached to the acridine skeleton, whereas Meridine contains fused phenolic type ring.

![Ascididemin and Meridine](image)

**Figure 23**

These natural products have been shown to behave as telomerase inhibitors by stabilising the G-quartet. Using the TRAP assay to measure the degree of telomerase inhibition it was found that Ascididemin and Meridine had IC\textsubscript{50} values of 87 \(\mu\)M and 11 \(\mu\)M respectively.
1.8.2 Dictyodendrins 17-20

There are many compounds that can act as telomerase inhibitors.

![Chemical structures](image)

**Figure 24**

Such examples include Korean mistletoe, tea catechins and compounds from the Japanese marine sponge *dictyodendrilla verongiform*. These compounds are thought to act as telomerase inhibitors via the G-quadruplex which causes telomerase shortening.

Dictyodendrins **17-20** showed 100% inhibition of telomerase at a concentration of 50 μg/mL scale. These natural products were the first known
telomerase inhibitors and researchers are synthesising analogues of them to enhance potency.

1.8.3 Telomestatin

A natural product known as telomestatin (21) (Fig 25) can target G-quadruplexes and is shown to be very potent with activity at 5 nM. In 2001 Shin-ya et al.\textsuperscript{38} successfully isolated telomestatin from \textit{steptomyces anulatus} 3533-SV4. Telomestatin can specifically inhibit telomerase without affecting the DNA polymerase or reverse transcriptase found in the enzyme, making it a very selective compound.

![Figure 25. Structure of the natural product telomestatin](image)

Telomestatin was successfully isolated by first cultivating \textit{Steptomyces anulatus}\textsuperscript{39} 3533-SV4 in 2% glycerol, 1.0% polypepton and 0.4% CaCO\textsubscript{3} for three days in a fermentor. The mycelium was then collected after centrifugation. The organism was then extracted into acetone, which was evaporated to dryness and the residue was partitioned between ethyl acetate and water. The organic layer was isolated, dried and purified via column chromatography (methanol and chloroform). Chemical analysis was carried out on the purified compound using FAB-MS which found a molecular formula of C\textsubscript{26}H\textsubscript{14}N\textsubscript{8}O\textsubscript{7}S was (M+H)$^+$, \textit{m/z} 583.0790 (calcd 583.0784). Further spectral data including $^1$H NMR and $^{13}$C
NMR were obtained for the product. The $^1$H NMR spectrum showed 5 aromatic signals $\delta_H$ 8.12, 8.13, 8.24 and 8.34 which were connected to carbon signals $\delta_C$ 137.5-141.2 in the HMQC spectrum. The long range coupling involving $\delta_C$ 130.4-136.7 and $\delta_C$ 156.2-157.3 quaternary carbons established the presence of the linked polyoxazole system. Two methyl groups located on the oxazoles were found at $\delta_H$ 2.55 and 2.65 further confirmed the structure proposed. The thiazoline ring showed two peaks at $\delta_H$ 3.49 and 3.93. A simulated annealing docking approach was carried out on telomestatin to determine the binding interactions with the G-quadruplex of DNA. The docking approach showed that two molecules of telomestatin bind to the G-quartet in the intramolecular orientation shown in Fig. 26.

![Figure 26](image)

Such natural products with telomerase inhibitory activity may be relatively non-cytotoxic and hence as potential of anticancer agents. Minhas et al\(^{10}\) has reported the synthesis of a series of macrocycles (Fig 27) which contain a 24-membered ring similar to that present in telomestatin.
Macrocycles 22-24 were evaluated for G-quadruplex binding and inhibition. UV studies were carried out on the macrocycles with G-quadruplex DNA being the function of temperature. According to the UV results there was no change in the melting transition temperature when the macrocycles were tested. Macrocycle 22 showed no change in the melting transition temperature even with d (TTAGGG)$_4$, indicating that no binding was taking place with G-quadruplex DNA. Macrocycles 23 and 24 exhibited an increase in the melting transition temperature of 73.5 °C, 62.5 °C respectively, showing that some binding was taking place. The macrocycles 23 and 24 were tested for inhibition of human lymphoblastoma RPMI 8402 cells, IC$_{50}$ values obtained being 0.21 and 0.8 μM respectively.
1.9 Oxazoles

1.9.1 Introduction

Oxazoles are numbered around the ring starting from the oxygen ring (figure 28).

![Figure 28](image)

The proton acidities decrease in the order: \(2 > 5 > 4\), owing to the electron-withdrawing effect caused by the oxygen and nitrogen atoms. Oxazoles exhibit characteristic resonances from 7.0 to 8.0 ppm in proton NMR spectra and between 128 and 153 ppm in \(^{13}\)C NMR spectra. These chemical shifts depend somewhat on which substituents are present at the different positions.

1.9.2 Synthesis of 1, 2, 4-trisubstituted oxazoles from nitriles

The synthesis involves the reaction of diazocarbonyl compounds with nitriles under thermal, photochemical, Lewis acid or metal catalysed conditions.\(^1\) The reaction proceeds via a 1,3-dipolar cycloaddition of the carbonylcarbene to the nitrile (path A) or formation and subsequent 1,5-cyclisation of a nitrile ylide (path B).\(^1\)

![Scheme 2](image)
Helquist et al.\textsuperscript{42} investigated metal-catalysed reactions involving rhodium with diazocarbonyl compounds and nitriles. Helquist tested metal salts which included \(\text{Rh}_2(\text{NHAc})_4\), \(\text{Rh}_2(\text{O}_2\text{CC}_3\text{H}_7)_4\) but chose rhodium(II) acetate, \(\text{Rh}_2(\text{OAc})_4\) as it provided the highest yield of the desired product.\textsuperscript{42} The catalyst was reacted with dimethyl diazomalonate with benzonitrile which subsequently gave oxazole 26 (scheme 3).

\begin{equation}
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{N}_2 \\
\text{O} \\
\text{O} \\
\end{array}
\xrightarrow{\text{Rh}_2(\text{OAc})_4, \text{PhCN, reflux}}
\begin{array}{c}
\text{Ph} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{C}_2\text{H}_3 \\
\end{array}
\end{equation}

\textbf{Scheme 3}

Helquist proposed a mechanism for the reaction (scheme 4). The diazo compound 25 reacts with the rhodium catalyst to generate a carbene complex 27 which is subject to nucleophilic attack at the electrophilic carbon centre found on the nitrile. A nitrilium species 29 then undergoes internal attack by an enolate oxygen to give the observed oxazole product 26.\textsuperscript{42}

\begin{equation}
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{N}_2 \\
\text{O} \\
\text{O} \\
\end{array}
\xrightarrow{\text{Rh}_2(\text{OAc})_4}
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{Rh}_\text{Lm} \\
\text{O} \\
\text{O} \\
\end{array}
\xrightarrow{\text{PhCN}}
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{L}_\text{mR}_\text{H} \\
\text{N} \\
\text{Ph} \\
\end{array}
\end{equation}

\textbf{Scheme 4}
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The 5-methoxy group can be removed to leave a 2,4-disubstituted oxazole (Scheme 5), by reduction using LiB(Et)$_3$H (Aldrich Super Hydride®) which acts as the reducing agent. Helquist did this because several naturally occurring compounds bear these 2,4-disubstituted oxazoles, e.g. Mandumycin II, telomestatin (Fig. 29).

Scheme 5

Mandumycin II

Figure 29

Helquist et al.$^{12}$ went on further to improve this method by eliminating the reduction step, by using diazoaldehyde ester 30 with nitriles (scheme 6).

Scheme 6
Chapter 1

The diazoaldehyde ester was readily available by a Vilsmeier-Haack formylation. For the oxazole formation the authors investigated a number of catalysts including Rh$_2$(OAc)$_4$, BF$_3$:Et$_2$O, Cu(OTf)$_2$ and Pd(OAc)$_2$, but only Rh$_2$(OAc)$_4$ was found to be effective. The optimum conditions used excess nitrile as the solvent and a temperature in the range of 65-95 °C (scheme 6) but provided only low yields (18-45 %).

Kozmin et al$^{43}$ used the metal catalysed reaction in their synthesis of the natural product Leucascandrolide A$^{43}$ (Figure 30). The 2,4-disubstituted oxazole side chain was prepared by the reaction of a nitrile with diazomalonate in the presence of Rh$_2$(OAc)$_4$. The reaction was followed by protodesilylation to give the oxazole 33 (scheme 7). Further reactions were carried out to furnish the desired oxazole side chain 34.

![Leucascandrolide A](image)

**Figure 30**
Scheme 7

Hermitage and co-workers\textsuperscript{44} reported the synthesis of the 2,4-disubstituted oxazole 36, from dichloroacetonitrile 35 (scheme 8). The reaction proceeds readily, to give the oxazole in a satisfactory yield of 48%. The reagents are readily available and are environmentally acceptable for an industrial scale synthesis.

Scheme 8
1.9.3 2,4-Disubstituted oxazoles synthesised from amides

Part of the 2,4-linked polyoxazole assembly present in telomestatin is also found in other natural products. A cyclopeptide known as YM-21631\textsuperscript{45} (Fig. 31), isolated from \textit{streptomyces nobilis}, shares similar structural and biological properties to that of telomestatin. The total synthesis of the natural product was accomplished by Pattenden \textit{et al.}\textsuperscript{43} The synthesis was carried out by preparing a tris-oxazole ring system derived from serine.

![Figure 31. Cyclopeptide YM-21631](image)

A procedure discovered by Wipf\textsuperscript{46} and Williams\textsuperscript{47} to synthesise an oxazole in one pot using diethylaminosulfur trifluoride (DAST), 1,8-diazabicyclo[5.4.0]undecene (DBU), bromotrichloromethane (BrCCl\textsubscript{3}) and reacting these reagents with an amido alcohol. Wipf \textit{et al.} also showed that a one-pot reaction to obtain an oxazole from an amido alcohol succeeded using bis (2-methoxyethyl) aminosulfur trifluoride (Deoxo-Fluor\textsuperscript{TM}, DBU and BrCCl\textsubscript{3}. DeoxoFluor\textsuperscript{TM} is found to be an alternative to DAST and preferable owing to its thermal stability. Unlike DAST which requires -78 °C to cyclise the amino alcohol (as above), Deoxo Fluor\textsuperscript{TM} requires only -40 °C (scheme 9). However, DAST provides better yields for serine-derived oxazoles, in comparison to Deoxo-Fluor\textsuperscript{TM}, which gives higher yields on threonine-derived compounds.
Scheme 9. Reagents for oxazole formation

The 2,4-disubstituted oxazoles are linked to form a consecutive trisoxazole system. Scheme 10 below outlines the route taken. Of two oxazoles, one contained a terminal acid group and the other an amine. These two compounds were coupled together using EDCI, HOBT and NMM, giving amide 39 in 56% yield. The benzyl group was then deprotected by hydrogenolysis over palladium hydroxide to give a free hydroxyl group. The alcohol group had been previously protected in order to avoid elimination of water, a major side-reaction.

The Wipf procedure was used in the synthesis of YM-21631 (step iii scheme 10). The overall yield using the DAST, DBU and BrCCl3 method was 57%, which is reasonable for the two-step process.
**Scheme 10**

*Reagents and conditions:* i, EDC, HOBr, NMM, CH$_2$Cl$_2$, 0 °C, 24 h, 56 %; ii, H$_2$, 20% Pd(OH)$_2$/C, MeOH-THF (2:1), rt, 81 %; iii, DAST, CH$_2$Cl$_2$, -78 °C, 1.5 h; iv, BrCCl$_3$, DBU, CH$_2$Cl$_2$, 0 °C, 24 h, 57 %; v, NaOH, THF, H$_2$O, rt, 24 h, 88%.
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Reagents and conditions: i, EDC, HOBr, NMM, CH₂Cl₂, 0 °C to rt, 24 h, 87%; ii, Lawesson’s reagent, THF reflux, 18 h, 50%; iii, 4.0 M HCl solution in dioxane, rt, 24 h, 91%; iv, EDC, HOBr, NMM, CH₂Cl₂, 0 °C to rt, 48 h, 68%; v, NaOH, THF, H₂O, rt, 24 h, 88%, vi, 4.0 M HCl solution in dioxane, rt, 18 h, 65% over two steps.

Scheme 11
Reagents and conditions: i, HATU, NMM, CH₂Cl₂-DMF (2:1), 0 °C to rt, 72 h, 88%; ii DAST, CH₂Cl₂, -78 °C, 2 h, 88%; iii, MnO₂, CH₂Cl₂, rt, 48 h, 27%.

Scheme 12

1. 9. 4 2,4-Disubstituted oxazoles by the Hantzsch synthesis

The Hantzsch synthesis of oxazoles involves reaction of an amide with an α-halo ketone, and is one of the most general and reliable routes to 2,4'-disubstituted oxazoles. The commercial availability of the starting materials and its efficiency proves advantageous for parallel synthesis. Reactions using bromoethyl pyruvate appear to require an amide where the carbonyl is attached to a sp² hybridised carbon (aromatics, alkenes) as shown in scheme 13. Nelson et al²⁰ successfully prepared an aryl oxazole 51 from an aryl amide and ethyl bromopyruvate on 87% yield. Kelly and Lang⁴⁸ also used the Hantzsch approach to prepare 52 as a model for the synthesis of dimethylsulfomycin. The reaction was carried out by heating methacrylamide with 2-(bromoacetyl) pyridine in THF at reflux, giving 52 in 62% yield (scheme 13).
Scheme 13. Hantzch type routes to bis-oxazoles$^{48,49}$

The mechanism of formation of 52 proceeds via the attack of the amide onto the bromoketone shown in scheme 14.
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Armstrong and co-workers\textsuperscript{50} used the Hantzsch synthesis in their approach to Calyculin and related marine natural products (figure 32).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{calyculin.png}
\caption{calyculin\textsuperscript{51}}
\end{figure}

They condensed 1,3-dichloroacetone with sec-butylamide or benzamide which afforded the 2,4-disubstituted oxazoles (scheme 15). Then they converted the 2,4-disubstituted oxazoles into their desired trans-4-alkenyl oxazoles.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {$\text{Cl} \xrightarrow{\text{EtOH, reflux}} \text{Cl}$};
\node at (2,0) {Ph \text{O} \text{CH} \text{NH}_2};
\node at (2.5,1) {Ph \text{O} \text{CH} \text{NH}_2 \text{C} \text{O} \text{Cl} \text{H}};
\node at (3.5,0) {Ph \text{O} \text{CH} \text{NH}_2 \text{C} \text{O} \text{Cl} \text{H}};
\node at (4,1) {67\%};
\end{tikzpicture}
\end{center}

\textbf{Scheme 15}

Faul \textit{et al.}\textsuperscript{52} used a similar method to Armstrong; they reacted ethyl 4-chloroacetoacetone with benzamide in their preparation of insulin-sensitive enhancers for the treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM). They prepared 55 by reacting benzamide 53 with ethyl 4-chloroacetoacetate 54. This was then easily converted into the desired oxazole 57 (scheme 16).
Scheme 16

Tris-oxazole units are found in marine natural products that are secondary metabolites, including the ulapualides, halichondramides, mycalolides and kabiramides.

Figure 33. Halichondramide$^{53}$

Panek et al$^{53}$ attempted the synthesis of the tris-oxazole fragment found in both Halichondramide and Kabiramide C$^{54}$. The synthesis (scheme 17) began with a Hantzsch condensation involving cinnamamide and ethyl bromopyruvate. Sodium hydrogen carbonate was also used as a buffer in the reaction to absorb the HBr liberated. TFAA works as the dehydrogenating agents to convert the oxazoline into the oxazole 60; the Hantzsch method provided an excellent yield of 83%. The terminal ester was then converted quantitatively into the amide using aqueous ammonia. The amide 62 was then condensed with bromo ethylpyruvate to form the bis-oxazole 63 whose ester group was converted into the amide with ammonia, prior to condensation with bromo ethylpyruvate to
form the tris-oxazole intermediate 65, common to both natural products. For the 13 steps, an overall yield of 26% was achieved.

Scheme 17

1. 9. 5 2,4-Disubstituted oxazoles from N-acylaziridines

Eastwood et al.\textsuperscript{55,56} was also interested in the synthesis of Halichondramide. This group investigated the rearrangement of N-acylaziridines carrying substituents which could allow for possible bis- and tris-oxazole fragments. Eastwood et al. developed a regioselective method to synthesise 2,4-disubstituted oxazoles from N-acylaziridines. 2-Positioned N-acylaziridines were chosen to undergo a ring expansion to form the oxazoline. Eastwood found that the R group on the aziridine must be electron-donating in order to form a 2,4-disubstituted oxazole. If the R group is electron-withdrawing, a 2, 5-disubstituted oxazole is obtained (scheme 18).
Scheme 18

The proposed mechanism of formation of oxazoline 71 begins by the iodo anion species attacking the less hindered carbon atom of the aziridine 69, leading to the imidate ion 70. This oxy-anion then attacks the CH₂ position displacing iodide (scheme 19).

Scheme 19

The ring-expansion of the aziridine was conducted in DMF at room temperature (scheme 20) using NaI. A good yield (89%) was obtained; however, some 2,5-substituted product was also formed. Dehydrogenation using NiO₂ then afforded the oxazoline providing a 74%.

Scheme 20

The Williams-Wipf protocol seems to be the most general route by far, although the Hantzsch is the least costly and most favoured on a large scale.
Chapter 1

References

(4) Hopson, L. J.; Wessells, N. K. *Essential Biology.* 158.
Chapter 1


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Chapter 2

2.0 Approaches to the synthesis of Polyoxazoles 75 and 76

2.1 Introduction

One of the aims of this project was to synthesise a capped polyoxazole 75 and a similar macrocyclic polyoxazole system such as 76 (fig 34) which can eventually be evaluated for specific binding to telomeric DNA G–quadruplexes and inhibitors of the human telomerase enzyme. The structure of 76 resembles telomestatin being planar, electron deficient and containing multiple oxazole rings, and especially an internal planar location of eight sp²-hybridised nitrogen atoms.

![Figure 34. Polyoxazole targets](image-url)
2.2 Synthetic Approaches to Dipyridyltrisoxazole 75

2.2.1 Retrosynthetic Analysis of Dipyridyltrisoxazole 75

From a retrosynthetic view point the synthesis of 75 would require the following intermediates shown in scheme 21. It was decided that the central oxazole rings of 75 should be disconnected to form two fragments which include the amide 77 and the bromoketone 78 for the subsequent Hantzsch reaction discussed in chapter one.

![Scheme 21]

The bromoketone 78 can be formed by a bromination reaction using molecular bromine and acetic acid on the ketone 79 (scheme 22).

![Scheme 22]
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A haloform reaction\textsuperscript{1,2} on the ketone 79 using NaOBr would provide the carboxylic acid 80. The ketone 79 could be synthesised by ozonolysis of the alkene 52.\textsuperscript{3}

Scheme 23

Another Hantzsch type reaction involving 2-bromoacetylpyridine (81) and methacrylamide 82 in a sealed tube can synthesise olefinic oxazole 52 (scheme 23).\textsuperscript{3} The bromoketone 81 can be synthesised from 2-acetylpyridine 83 (scheme 24).\textsuperscript{4,5}

Scheme 24
2. 2. 2 Approaches to the synthesis of Dipyridyltrisoxazole 75

2. 2. 3 An approach via the Hantzsch condensation

The synthesis of 75 required the synthesis of the intermediate bromo ketone 81. 2-Acetyl pyridine 83 was reacted with bromine in chloroform under reflux to give pyridyl bromoketone 81 with a high yield (scheme 25). ⁶

![Scheme 25](image)

The attempted synthesis of the olefinic pyridyl oxazole 52 began with a Hantzsch reaction. The reagents used were pyridyl bromoketone 81, very soluble in dichloromethane, and methacyramide, heated in a sealed tube (scheme 26). After 2 days at 100 °C, the mixture was evaporated to leave a brown oil. The tlc of the reaction (Rf 0.5 EtOAc) showed a luminous blue spot which was isolated in 75% yield using column chromatography and NMR had shown it to be the oxazole 52. ³

![Scheme 26](image)

Oxidation of 52 using osmium tetroxide and sodium periodate gave ketone 79 (scheme 27). The yield of this reaction was very low, mainly because of the work-up. It was found that the ketone 79 partitioned between the organic
and aqueous layers; presumably the sodium ion chelates to the nitrogen atom of the pyridine ring and the nitrogen atom of the oxazole. An alternative reaction involving ozonolysis of the alkene 52 to give the ketone 79 was carried out to avoid the problem of chelation, and gave an improved yield of 60%.  

\[
\begin{align*}
52 & \xrightarrow{\text{OsO}_4/\text{NaIO}_4} 79 \\
42\% & \\
\text{or } \text{O}_3, \text{CH}_2\text{Cl}_2 & \times 60\%
\end{align*}
\]

Scheme 27

Preparation of 2-bromo-1-(4-pyridin-2-yl-oxazol-2-yl)-ethanone (78) proved quite difficult. Procedures carried out involved reacting bromine with the corresponding ketone 79 at reflux using chloroform for 24 hours (scheme 28).  
There was no reaction taking place; therefore, alternative conditions using a 100 W light bulb together with heat were tried, but to no avail. Chloroform was then replaced by dioxane in order to increase the temperature of the reaction; however, the increase in energy did not cause any products to form. Acetic acid was also used as the solvent, but with no success.

\[
\begin{align*}
79 & \xrightarrow{\text{Br}_2/\text{CHCl}_3} 78 \\
\end{align*}
\]

Scheme 28

In order to prepare the bromoketone 78 a radical reaction was attempted on the ketone 79 in the presence of benzoyl peroxide and recrystallised N-bromosuccinimide in carbon tetrachloride (scheme 29). The mixture was heated under reflux for more than 48 hours; however, tlc showed no reaction had taken place. A 100 W lamp was then placed near the reaction flask, also in the presence of benzoyl peroxide to initiate the radical process, but again no reaction took place. Benzoyl peroxide was then replaced by the initiating reagent TMSOTf, but nor was that successful.
Scheme 29

Another radical-based route was then attempted, involving bromination of the alkene 52. Once obtained, the allylic bromide 84 would be converted into the ketone via ozonolysis to give 78 (scheme 30).\(^9\) Tlc of the reaction mixture showed a new spot; the \(^1\)H NMR spectrum showed that the alkene peaks had disappeared but that the methyl group remained intact; hence, some attack of bromine on the alkene had taken place.

Scheme 30

In summary, none of the above attempts was successful. It appears that bromination of a methyl ketone attached to an oxazole ring is difficult, and not much is mentioned in the literature, despite the fact that the corresponding acyl pyridines can be readily brominated.\(^10\),\(^11\),\(^12\)

Owing to the inability to obtain 78 directly from the ketone 79 an alternative route was devised, involving intermediates prepared from Baylis-Hillman reactions (scheme 31), which was efficient, high-yielding and required inexpensive reagents. The products also underwent allylic isomerize to give 86,
91\textsuperscript{13} and 88\textsuperscript{14} again in good yields. The next step was to perform a Hantzsch reaction using the corresponding isomerised amides 90, 85,\textsuperscript{15} 89,\textsuperscript{16} 92, with the pyridyl bromoketone 81.

\[ \text{Scheme 31} \]

Compounds 85, 86 and 89 (schemes 32, 33 and 34) were used in the Hantzsch reaction in order to try to obtain the key bromo ketone intermediate 78.

Firstly, the amide 85 was reacted with the bromoketone 81 in a sealed tube for 2 days (scheme 32). It was thought that if the oxazole 93 was obtained then a bromination reaction would take place to form the allylic bromide 94; a subsequent reagent would cleave the phenyl group. However, the tlc of the reaction showed 6 spots which each were isolated but none proved to be the Hantzsch product. The conditions were modified; for example THF was replaced
Chapter 2

by dioxane and microwave irradiation maintained at 150 W, but the reaction did not succeed.

Scheme 32

Another attempt to obtain bromoketone 78 was made using amide 86. The amide 86 and bromoketone 81 in THF were irradiated with microwaves for 5 minutes at 150 W (scheme 33). However, tlc showed no reaction had taken place as there was only starting material found. Ozonolysis of the alkene 94 was expected to furnish bromoketone 78.

Scheme 33

A Hantzsch reaction was attempted on the amido alcohol 89 and bromoketone 81 via irradiation with microwaves (scheme 34). The Hantzsch reaction was unsuccessful as the tlc showed many spots, none proving to be the
product 95. If successful, the alcohol 95 would have been converted into the bromide 94 using carbon tetrabromide and triphenylphosphine followed by ozonolysis to give the key intermediate 81.

Scheme 34

The only compound made via a Hantzsch synthesis was oxazole 96, prepared from cinnamamide (58) and the pyridyl bromoketone 81 (scheme 35). The product 96 was formed by placing the two reactants dissolved in THF into a sample tube, then irradiating it in a microwave for 5 minutes. Tlc visualised under UV showed a new bright blue luminous spot which was isolated by column chromatography using neat ethyl acetate.

Scheme 35
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An attempt was made to cleave off the phenyl group by ozonolysis, to oxazole 96 was dissolved in dichloromethane and the temperature was lowered prior to the passage of ozone. After 30 minutes the reaction mixture was quenched with DMS; tlc showed no reaction had taken place since only the starting material 96 was present.

The carboxy-oxazole 80 (scheme 36) was needed in order to prepare the amide 77 required for the Hantzsch reaction. The synthesis of the mono-oxazole acid 80 from the ketone 79 was attempted using sodium hydroxide and bromine. Tlc of the reaction mixture showed baseline product, characteristic of an acid. However, the weight of the crude material once evaporated to dryness was too high and it would only dissolve in water, not in DMSO or chloroform, suggesting contamination of the desired product with inorganic salts. The inorganic salts were difficult to separate from the product, so an alternative route to synthesise 77 was investigated.

Scheme 36
2.2.4 An approach via the Williams-Wipf condensation

The route to 75 began with the coupling of picolinic acid with serine methyl ester hydrochloride to form the amide 98 in 75% yield (scheme 37). The reagents used were 1.1 equivalents of isobutyl chloroformate coupling agent in the presence of triethylamine.

The reaction was carried out at -30 °C and then stirred at ambient temperature for 17 hours. Tlc showed two spots, both of which were isolated; one at Rf 0.7 (ethyl acetate) which was found to be the dehydro amide 99, and the lower spot at Rf 0.3, shown to be the desired amido alcohol 98, which was of a high yield with no major problems.

Scheme 37

The next step required the cyclisation of amido alcohol 98 to give the oxazole 100. The procedure involved stirring the amide with TsCl and triethylamine at room temperature for two days. $^1$H NMR data showed that an elimination reaction took place to give the dehydroamide, signals being observed for a new double bond at δ 5.65. Cyclisation may have failed because of the deactivating (carboxyl) group leading instead to the eliminated product 99 (scheme 37).
Other L-serine derived compounds (scheme 38) were used to investigate oxazole ring formation, but to no avail.

![Chemical structures](image)

**Scheme 38**

The amido alcohol 98 was then successfully converted into the oxazoline 104 using a Williams-Wipf procedure\(^\text{19}\) which required DAST at -78 °C (described in chapter 1). The oxazoline 104 was then converted into the oxazole 100 using DBU and bromotrichloromethane, with no significant side-products (scheme 39).

![Chemical structures](image)

**Scheme 39**

The second fragment required for the final Hantzsch reaction to obtain pentacyle 75 was the amide 77 which was prepared by treatment of the methyl ester 100 with aqueous ammonia (35% yield). Amide 77 precipitated almost immediately and was filtered and washed with water to give material sufficiently clean for an attempted Hantzsch reaction.
Scheme 40

In summary, the key amide intermediate 77 was synthesised but the bromoketone partner 78 could not be prepared, and so since the Hantsch reaction could not be attempted, an alternative route was investigated.
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A revised route to 75 was proposed (scheme 41). Coupling of 2-picolinic acid with the oxazole 105 using isobutyl chloroformate and triethylamine (scheme 41) afforded amide 106; the $^1$H NMR spectrum confirmed product and the HRMS data for the parent ion were within 1 ppm of that expected. The next step involved the cyclisation to the oxazoline using DAST and potassium carbonate to give 107; however, many side-products formed and with little or no oxazoline detected. The results may have indicated that the reaction had been left for too long, so a shorter time stirring for 2 instead of 16 hours at room temperature, but tlc still showed many spots.

The synthesis to 75 required the alcohol 112. The synthesis of 112 began by reacting 2-acetylpyridine with m-CPBA in chloroform to give the pyridyl N-oxide \(^{20}\) 109 in high yield (98%) in scheme 41. The pyridyl N-oxide 109 was then converted to 2-oxo-2-(pyridine-2-yl)ethylacetate 110 using acetic anhydride. The product was very difficult to purify owing to the gelatinous property of component 110 which made column chromatography very difficult and hence furnishing a low yield (32%). Many attempts were made to convert the keto group 110 into the amine 111 using ammonium acetate and sodium cyanoborohydride but that proved unsuccessful as the tlc only showed starting material. The route to 75 was abandoned due to many problems in the synthesis.
Scheme 41
2.3 Synthetic approach to the Dipyridyl macrocycle 76

2.3.1 Retrosynthetic analysis of Dipyridyl macrocycle 76

From a retrosynthetic view point the synthesis of 76 would require the intermediates shown in scheme 42. It was decided that the central oxazole rings in 76 should be disconnected to form two fragments which include the bisamide 115 and the bromo ketone 116 for condensation in a Hantzsch reaction.

Scheme 42
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The bromoketone 116 can be formed by a bromination reaction using molecular bromine in acetic acid on the diketone 117, which can be prepared from the diacid 118 by treatment with methyllithium in anhydrous THF at -78 °C (scheme 42). Hydrolysis of the diester 119 was expected to give the diacid 118. Another Hantzsch type reaction involving pyridine-2,6-dicarboxamide 120 and ethyl bromopyruvate was hoped to give diester 119. The second major fragment required the diamide 115 which can be formed by amination of the ester 119.

2.3.2 Approaches to the synthesis of Dipyridyl macrocycle 76

2.3.3 An approach via the Hantzsch condensation

Initially, the modified Hantzsch reaction was pursued in an attempt to obtain the bisoxazolylypyridine 119, proceeding via pyridine 2,6-dicarboxamide 120 (scheme 44). However, many problems were found with the Hantzsch reaction. First, pyridine 2,6-dicarboxamide (120) was very insoluble in most solvents except DMF, the solubility preventing the reaction. Accordingly, the oxazolyl pyridine 52 (scheme 43) was selected as the target, in order to minimise problems with solubility and to provide a better understanding of how to construct each oxazole ring.

\[
\begin{align*}
\text{Scheme 43} \\
\text{81} & \xrightarrow{\text{H}_2\text{N-COO}} \text{52} \\
\end{align*}
\]

To date, using the Hantzsch reaction to prepare an oxazole has been quite difficult, apparently because ethyl bromopyruvate decomposes upon heating; optimal conditions for the reaction have not been identified, despite many attempts.
Scheme 44

It was hoped that the protocol for the synthesis of monooxazole 52 could be applied to the bisoxazole. Similar conditions were chosen for the reaction carried out with the monooxazole 52 whereby the bromopyridyl ketone 81 and methacrylamide were heated in THF in a sealed tube for 5 days at 75 °C (scheme 45). The tlc results looked promising as a new spot comprised of Rf 0.5 (EtOAc) was found and the starting material had been consumed. An attempt was made to isolate the new spot using column chromatography; however, the material decomposed. The reaction proved to be unsatisfactory because many spots were formed, probably indicating polymerisation.

Scheme 45

Another attempt was made to synthesise the bispyridyl macrocycle 76. Since double-Hantzsch had not worked; a mono-Hantzsch approach was considered mindful of previous success (scheme 46).
The new route required the synthesis of amido-oxazole 130 for reaction with the bromo ketone 131 (scheme 46). The amide 127 was synthesised from L-serine methyl ester hydrochloride 126 and methacrylic acid 125. The amide 127 was then converted into the oxazoline 128 using the Williams and Wipf procedure providing a high yield of 82%. The oxazoline 128 was then dehydrogenated to the oxazole 129 with DBU and bromotrichloromethane in a 85% yield. The ester group was converted into the amide 130 using aqueous ammonia (0.880), in a yield of 75%. The starting bromoketone 131 contained an ester group, which could be converted into an amide in order, to attempt a further Hantzsch reaction with the corresponding bromoketone 134 to form the polyoxazole macrocycle. The amide 130 and the bromoketone 131 were dissolved in THF and stirred for 16 hours under reflux. However, tlc showed no reaction had taken place.

Scheme 46
2. 3. 4 An approach via the Willams-Wipf condensation

In view of the problems encountered with the Hantzsch approach to oxazoles an alternative route to the diamide 115 was sought. A literature procedure using the diester 136 was found by which it was cyclised to form the bisoxazoline 137. The cyclisation occurred using DAST in the presence of potassium carbonate and sodium hydrogen carbonate, a procedure pioneered by Williams and Wipf (scheme 47). The pyridyl diester 136 was prepared in 64% yield from the diacid chloride of pyridine-2,6-dicarboxylic acid and L-serine methyl ester using 4.0 equivalents of triethylamine. The $^1$H NMR spectrum showed a new peak at $\delta$ 4.84, corresponding to the methine signals in 136. The diamide 136 was converted into the oxazoline 137 by using 2.2 equivalents of DAST. $^1$H NMR data showed that the reaction was successful, since the N-H peak was not present and the CH$_2$ peak had shifted to a multiplet at 4.69 ppm. The sequence was then taken further than the literature to give the bisoxazole 138, in a net dehydrogenation reaction using 4.0 equiv of DBU and 2.1 equiv of bromotrichloromethane. This reaction was successful, the CH$_2$ peaks being absent and a new oxazolyl singlet peak at $\delta$ 8.40 ppm (2H) appearing. The ester 138 was then converted into the amide 115 by stirring at room temperature with 0.880 aqueous ammonia (scheme 47) but in only 20% yield due to incompleteness of the reaction. The NMR spectrum of the bisamide 115 showed the absence of a methyl ester signal but the presence of a broad singlet at 7.83 ppm. The diamide is one of the two fragments required for the synthesis of macrocycle 76 (scheme 42).
In summary, only one of the two fragments, the diamide 115, was synthesised as required for a Hantzsch reaction to give macrocycle 76. Formation of a bromo ketone from an oxazolyl methyl ketone seemed very difficult, and the synthesis had to be abandoned.

Future work could include a new route to an eight-membered macrocycle (scheme 48). However, rather than having six oxazole rings the macrocycle could comprise of four oxazoles and two thiazole rings. Thiazoles are more readily formed than are oxazoles during the Hantzsch reaction.
Scheme 48
Chapter 2

References

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(10) Pinori, M. L.; Maria; Modena, Daniela; Mascagni, Paolo. PCT Int. Appl. 2005040156 2005.
Chapter 2


3.0 Approaches to Oxazolylpyridines

3.1 Introduction

One of the aims of this project was to synthesise a planar polyoxazole system such as 144 (fig 35) which could be evaluated for specific binding to telomeric DNA G-quadruplexes and as an inhibitor of the enzyme human telomerase. The structure consists of a bipyridyl ring system to which four oxazole rings are attached. The assembly contains nitrogen atoms in the centre of the ring system that could form intermolecular hydrogen bonds with G-quadruplexes.

![Diagram](image)

Figure 35.
3.2 Retrosynthetic analysis of the tetraoxazolyl bipyridyl 144

It was decided that the bipyridyl ring system should be disconnected first to form two fragments which include the bromopyridine 145 and the pyridylstannane 146, to be joined via a Stille coupling.

Scheme 49

In order to obtain stannane 146, via a Stille coupling, a transmetalation reaction\(^1\) is required which involves an organotin species reacting with a halide in the presence of palladium as the catalyst. The reaction mechanism has been well studied (figure 36).\(^2,3\) The initial step in the catalytic cycle involves the reduction of a palladium (II) species 147 to the active palladium (0) species, 148. The next step, oxidative addition, involves addition of the halide species 149 to the palladium (0) species, oxidising it to form palladium (II) intermediate 150.\(^4\)

Transmetalation occurs from the stannane 160 to the palladium (II) species 161, giving the palladium species 162. Finally, a reductive elimination process takes place, in which the desired product 163 is eliminated and the catalytic cycle is perpetuated palladium (0) through the release of the species 148.
Figure 36.

A Suzuki coupling also follows a similar mechanism to the Stille coupling, except that a boronic acid is used as the ligand in place of a stannane.

The stannane 146 can be synthesised from a stannylation reaction involving the bromopyridine 145 using dichlorobis(triphenylphosphine)palladium (II) and bis (tributyltin) heated to high temperatures (scheme 50).
Scheme 50

The oxazolyl species 145 (scheme 50) can be obtained via a dehydrogenation reaction using DBU and bromotrichloromethane in dichloromethane following the Williams-Wipf procedure.\textsuperscript{7,8}

The oxazoline species 164 (scheme 50) can be formed from by the cyclisation of the di amide 165. The cyclisation process can be carried out by the use of DAST and potassium carbonate in dichloromethane.\textsuperscript{8}

A double acylation of L-serine methyl ester with the pyridine diacid species 166 should afford the diamide 165 (scheme 51).\textsuperscript{9}
Chapter 3

Scheme 51

The bromopyridine 167 has been obtained from chelidamic acid 168 using a procedure described by Takalo et al.\(^{10}\) (scheme 51).

3.3 Approaches to the synthesis of the tetraoxazolyl bipyridyl 144

The route required in the preparation of the bromopyridine 167 from chelidamic acid 168 by the treatment with neat phosphorus pentabromide. The reactive acid bromide went on to form the diethylester 167 after the addition of ethanol (scheme 52). The literature states that a 69% yield is obtained after a recrystallisation; fortunately a yield of 80% was obtained after column chromatography (using neat ethyl acetate).

Scheme 52
The diacid 166 was obtained by hydrolysis reaction with lithium hydroxide in a 1:5 mixture of water and THF (scheme 53). After 3 hours, the mixture was acidified to pH 1. The solid material was difficult to handle due to coagulation but with gentle sonication the diacid 166 was obtained. Acid 166 was extremely insoluble in most solvents, preventing diamide 165 from being obtained. Hence, the diacid chloride 169 was prepared from the diacid 166 using thionyl chloride and two drops of DMF.

Scheme 53

The next step involved the amination of diacid chloride 16 a with L-serine methyl ester hydrochloride. However, the reaction proceeded in low yield. Small amounts of thionyl chloride may have been present that may have reacted with the free alcohol to form the eliminated product 170, since many spots were found on the tlc plate (scheme 54).

Scheme 54
The next step in the synthesis was the obtaining of the bisoxazoline 164 from the bisamidopyridine 165. The reaction involved the reagents DAST and potassium carbonate at -78 °C. This reaction was very high yielding, 93% of the bisoxazoline 164 and with no detectable side-product. The $^1$H NMR spectrum showed the characteristic multiplet at $\delta$ 4.70 for the oxazoline hydrogen atom.$^{11}$

$^{12}$ The next step involved the dehydrogenation of the bisoxazoline 164 using DBU and bromotrichloromethane. This reaction proceeded in satisfactory yield; $^1$H NMR showed the absence of the multiplet found for 164, but the presence of new oxazole peaks confirming that the bisoxazole 145 had been formed (scheme 55).
Chapter 3

The core bromopyridyl fragment 145 was successfully obtained. A similar procedure to that of Pryor et al.\(^9\) was carried out to form the stannane 146 using dichlorobis(triphenylphosphine)palladium (II) and bis (tributyltin). After heating at reflux for two hours, tlc of the reaction indicated many compounds had formed. Each spot was isolated and identified although none of the spots indicated that 146 had been obtained.

![Chemical structure of 145 and 146 with reaction conditions](image)

**Scheme 56**

Since dichlorobis(triphenylphosphine)palladium (II) had not been successful, the catalyst was replaced by palladium tetrakis(triphenylphosphine). However, after heating in toluene at reflux for two hours many side products had formed, and this did not lead to a successful outcome.

![Chemical structure of 145 and 146 with reaction conditions](image)

**Scheme 57**

The failure to obtain stannane 146 meant that no progress could be made beyond bromopyridine 145 and the proposed route had to be abandoned.
3.4 Approaches to the synthesis of bisoxazolylpyridine 175

Another goal was to obtain the bisoxazole 175, in which the bromo group of bisoxazole 145 has been displaced by a secondary amine. The sequence began by reacting the bromide 167 with N¹-ethyl-N², N²-dimethylethane-1,2-diamine in the presence of potassium carbonate and DMSO to form the tertiary amine 171. The reaction was successful, although the yield was quite low; many side-products have formed. The next step involved the hydrolysis of the ester to form the diacid 172. The tlc confirmed the reaction went to completion; however, the work-up proved very difficult. It was found that acidification using 3 M HCl protonated the amine which made it extremely difficult to dissolve in any common solvents. It was decided that the lithium salt of 172 would be used to form the bisamide 173, thereby eliminating the work-up step. However, this reaction was also unsuccessful; tlc showed no product was forming. Owing to lack of time the route to 175 was abandoned.

Scheme 58
References

Chapter 4

4.0 Approaches to the synthesis of cyclooctaoxazole 176

4.1 Introduction

The aim of this section was to synthesise an analogue of telomestatin such as the symmetrical polyoxazole system 176. Such cyclic systems would be evaluated for specific binding to telomeric DNA G-quadruplexes and inhibitors of the human enzyme telomerase. The structure resembles telomestatin being planar, electron deficient and containing multiple oxazole rings, as well as containing an internal planar location of eight sp²-hybridised nitrogen atoms.

Figure 37. An important cyclic polyoxazole target
From a retrosynthetic viewpoint the synthesis of 176 would require the following compounds:

176

\[ \xrightarrow{1. \text{DAST, } K_2CO_3, \text{CH}_2\text{Cl}_2} \xrightarrow{2. \text{DBU, BrCCl}_3} \]

177

\[ \xrightarrow{\text{BOP, DIEA, HOBT, DMF}} \]

178

\[ \xrightarrow{\text{LiOH, THF, } H_2O} \xrightarrow{1. \text{p-TSA, MeOH}} \xrightarrow{2. \text{TFA, THF}} \]

179

180

181

\[ \xrightarrow{\text{DBU, BrCCl}_3} \]
Scheme 59
Chapter 4

Results and discussion

4.2 The first approach: condensation of two trisoxazole units using Boc as the protecting group on nitrogen

The attempted synthesis of 176 followed the reverse of the retrosynthetic strategy shown in scheme 60. The route began with N-protection of serine methyl ester hydrochloride using Boc anhydride and triethylamine in THF giving carbamate 190 as a colourless oil in 91 % yield (scheme 60).\(^1\)

\[
\begin{align*}
\text{HO} & \quad \text{NH}_2 \\
126 & \quad \text{HO} \\
(\text{Boc})_2\text{O} & \quad \text{NH} \\
\text{Et}_3\text{N}, \text{THF} & \quad \text{Boc} \\
91\% & \\
\end{align*}
\]

Scheme 60

The next stage of the synthesis required the protection of the amino alcohol unit as the gem-dimethyl oxazolidine 189, using 2,2 dimethoxypropane and boron trifluoride-diethyletherate in acetone (scheme 61). Although this protection step afforded several products (as shown by tlc), a slow elution on a silica column with petroleum ether: ethyl acetate (10: 0.5) afforded 189 in 82 %.\(^1-3\)

\[
\begin{align*}
\text{HN} & \quad \text{OH} \\
\text{Boc} & \quad \text{O} \\
190 & \quad \text{O} \\
\text{2,2-Dimethoxy-} & \quad \text{O} \\
\text{propane} & \quad \text{N-Boc} \\
\text{Acetone} & \quad 82\% \\
\end{align*}
\]

Scheme 61

The methyl ester 189 was then hydrolysed to the acid 188 using one equivalent of lithium hydroxide in aqueous THF. After an acid work-up, acid 188 was isolated in 65% (scheme 62). The hydrolysis step was optimised by
evaporating the solvent of the reaction mixture to dryness to leave the free carboxylated oxazolidine 191 without any acidification. This procedure improved the yield greatly, affording the crude quantitatively.

Scheme 62

The carboxylate 191 was then acylated with L-serine methyl ester using isobutyl chloroformate as the coupling agent (scheme 63). The reaction was allowed two hours to complete; after work-up, the amide 187 was isolated as a colourless oil. However, obtaining the amide 187 pure was undesirable in terms of yield, since isolation required column chromatography (in neat ethyl acetate) and the amide 187 adhered to silica gel, giving an isolated yield of only 40%, even when 20% methanol was used for elution.

Scheme 63

A trial was carried out to see how an impure sample would react in the subsequent step; 200 mg of crude amide 187 was reacted with one equivalent diethylamino sulfur trifluoride to form the corresponding oxazoline 186 (scheme 64). Results showed that an unpurified sample of amide 187, did not affect the
yield of oxazoline 186. The method was successfully repeated on a 5 g scale. However, it was found that if the reaction was repeated on a scale greater than 5 g the yield was dramatically decreased by up to 20%.

Scheme 64

The oxazoline 186 was successfully converted into the oxazole 185 in quantitative yield using DBU and bromotrichloromethane (scheme 65). Compounds 186 and 185 were each purified by column chromatography without complications.

Scheme 65

Corresponding to the retrosynthesis of compound 176 in scheme 59, the synthetic sequence was successful up to the oxazole step and with no major problems. The common intermediate in the sequence was the oxazole 185 which was to be converted in a convergent manner into the acid 184 and amino alcohol 105 (scheme 66). These compounds would then be coupled together to form the trisoxazole precursor 180.
Several methods of deprotection to give the amino alcohol 105 were tried but proved quite troublesome. Firstly, a modified procedure\(^1\) was used whereby trifluoroacetic acid (TFA) (59 eq) in water (18 eq) was used to react with 185 and then left to stir at room temperature for 14 hours. The mixture was later basified to pH 8 using saturated aqueous sodium hydrogen carbonate. Tlc results showed the completion of reaction (as in scheme 67), as a baseline spot appeared and no protected oxazole 185 was found; however, \(^1\)H NMR spectroscopy of the crude reaction mixture showed that the Boc group was still present. The result seemed to indicate that the reaction had not gone to completion; therefore further modifications were made.

Scheme 67
In a second attempt, a different procedure was followed, using 7.9 equivalents of TFA and 9 equivalents of water. The mixture was heated to 50 °C and after 10 minutes no starting material was found by tlc. The reaction mixture was left for a further 2 hours at 50 °C (scheme 68), after which the solvent was evaporated to dryness and the residue basified to pH 8. However, extraction with ethyl acetate gave a poor yield. It was thought that the product may be very polar and remain in the aqueous aqueous layer, according the aqueous layer, was evaporated by azeotropic removal of water using toluene, giving a high yield of brown oil. However, $^1$H NMR spectroscopy again showed only partial removal of Boc group.

Scheme 68

Using TFA and heating 185 to extreme temperatures was not desirable owing to the sensitive and reactive groups present in the product 105. It was found that TFA transformed the oxazole 185 from an oil into a gelatinous material which proved very difficult to handle as it did not dissolve in common organic solvents or even in water. The next attempt was to deprotect the oxazole 185 using $p$-TSA (1 equivalent) in methanol as it was a milder acid (scheme 69). The tlc showed that a new product had formed, and that no starting material remained. However, the product was isolated and shown not to be 105. Since amino alcohol 105 could not be obtained by any of several methods used, hydrolysis of ester 185 to the acid 184 was not attempted because acid 184 could not be used in the synthesis.
Scheme 69

In conclusion, the first approach to octaoxazole 176 resulted in oxazole 185 (scheme 70). However it proved very difficult to prepare the amino alcohol 105 owing to incompleteness of the reaction and losses during manipulation. The route was then discontinued and an alternative one was investigated.
Scheme 70 The projected first route to the cyclic oxazole 176
4.3 A second approach: condensation of two trisoxazole units using Cbz as the protecting group.

The first approach to macrocycle 176 was unsuccessful owing to problems with deprotection of the Boc group. Hence, an alternative route was required to resolve the problem. In the second approach, the Cbz protecting group was chosen because deprotection by hydrogenation is conducted under neutral conditions, which is highly desirable since acids such as TFA had been found to convert 185 into a gelatinous material that was difficult to manipulate. Deprotection and protection are thus accomplished as in scheme 71.

Scheme 71
Chapter 4

The revised retrosynthesis is shown below:

1. DAST, K$_2$CO$_3$, CH$_2$Cl$_2$
2. DBU, BrCCl$_3$

176

1. LiOH, THF, H$_2$O

193

BOP, DIEA, HOBT, DMF

179

1. p-TSA, MeOH
2. H$_2$, Pd/C, MeOH

194

195

DBU, BrCCl$_3$
Scheme 72
Obtaining acid 202 from 203 involved the stirring of benzyl chloroformate, 2 M NaOH and 1,4-dioxane for 48 hours at room temperature to give 202 in 57% yield (scheme 73). However, the purification of the reaction was quite difficult because quantities of benzyl chloroformate appeared to contaminate the product even after carrying out column chromatography; moreover, benzyl chloroformate is difficult to observe on tlc plates, even with staining. However, a very slow elution with 1% ethyl acetate/ petroleum ether was used; once isolated, acid 202 was a bright white solid with a sharp melting point of 119-120 °C.

Scheme 73

The second step in the synthesis of 176 required the formation of the oxazolidine 201. Initially, boron trifluoride diethyl etherate was used as the catalyst (in the presence of 2,2-dimethoxypropane) to aid the formation of the oxazolidine 201, it had been in the Boc protection (first approach) to obtain 189, but unfortunately the reaction was unsuccessful. An alternative attempt involved reacting 202 with p-TSA, 2,2-dimethoxypropane in acetone at 45 °C for 12 hours (scheme 74). Using p-TSA was successful; the reaction proceeded to completion and gave no side-products. It was also found that if the temperature of the reaction was higher than 45 °C, the gem-dimethyl group was cleaved to give the free alcohol 202. The same result also occurred when the reaction mixture was stirred for longer than 12 hours at 45 °C.

Scheme 74
The new route (scheme 72) to cyclooctaoxazole 176 seemed more efficient since the step involving the hydrolysis of the ester 189 (scheme 75) was avoided.\(^8\)

![Scheme 75](image)

**Scheme 75**

In the revised route, acid 201 was coupled with L-serine methyl ester hydrochloride using isobutyl chloroformate to give amide 200 (scheme 76). The reaction went to completion provided that an excess of L-serine methylester (1.6 eq) was used. However, the yield decreased from 85% to 40% when purified on silica gel. Therefore, in this step crude material 200 was not purified by column chromatography, but was used directly in the next step.\(^1\)

![Scheme 76](image)

**Scheme 76**

The amide 200 was then converted into the oxazoline 199 using DAST under anhydrous conditions at -78 °C in CH\(_2\)Cl\(_2\). The reaction proceeded in 73% yield, and with no major side-products. However, oxazoline 199 was shown to be unstable, and if left for a few days appeared to revert to the amide 200 (scheme
Accordingly, once 199 was formed, it was immediately converted into the oxazole 198 using DBU and bromotrichloromethane to give the desired oxazole in 75% yield.

Deprotection of 198 was carried out using hydrogenation. It was thought that the free amine 105 would be difficult to manipulate owing to its insolubility, as previously found when TFA was used 185; accordingly, the gem-dimethyl group was deprotected first using p-TSA, followed by hydrogenolysis to remove the Cbz group (scheme 78).\textsuperscript{1,9}

The first attempt to deprotect 198 to give 204 was attempted using p-TSA and water. Tlc results indicated that the reaction was complete; however, the product formed (which stained red with anisaldehyde) was found to be some unidentifiable compound (scheme 79). \textsuperscript{1}H NMR spectra showed all the major peaks required; however, the CH\textsubscript{2} and oxazole signals had shifted downfield.
Scheme 79

Water was not used in the next attempt, but instead only one equivalent of $p$-TSA in methanol, when heated under reflux. The alcohol 204 was obtained in quantitative yield, and stained orange on silica impregnated with anisaldehyde (scheme 80). $^1$H NMR and $^{13}$C NMR data confirmed that the product 204 was synthesised as no aryl peaks were found. The reaction must not be left to stir for more than 2.5 hours under reflux as the product undergoes decomposition to give unidentified compound.

Scheme 80
Chapter 4

Hydrogenolysis of the Cbz group in 204 was carried out using 10% Pd/C, suspended in methanol (scheme 81). The method proved successful as 105 was formed in the excellent yield 92%. The amino alcohol 105 did not require purification, since NMR spectroscopy showed the product to be free from any significant impurity. In this way, the deprotection step avoided the use of a strongly acidic medium or base work-up, factors which had proved so troublesome in the first approach to the amino alcohol 105.

Scheme 81

The second fragment required for synthesis of precursor 196 of the trisoxazole was the oxazole carboxylic acid 197. Therefore, a hydrolysis reaction was carried out on the ester 198 using lithium hydroxide and water scheme 82. The reaction was high yielding but required heating at 89 °C for 17 hours, since at lower temperatures the reaction was incomplete.

Scheme 82
The purification of acid 197 was carried out by evaporating the THF and extracting the remaining aqueous mixture with ethyl acetate. The organic layer was then discarded as it contained all the organic impurities. The aqueous layer which contained the carboxylate was acidified to pH 1 to liberate the pure acid 197 without the need for column chromatography.

4.3.1 Acylation of the trisoxazole precursor 196

The intermediates 197 and 105 required for the synthesis of amide 196 were successfully prepared (scheme 83). Initially, a similar coupling procedure to obtain amide 200 was attempted using the intermediates (197 and 105), in the presence of triethylamine and isobutyl chloroformate at -30 °C. According to the tlc results, a new compound had formed and no starting material remained. The new spot on the tlc (Rf 0.7, EtOAc) was isolated; however, the NMR spectrum showed that the product formed may be an over reacted species, possibly of type E (figure 37).

![Image of compound E](image)

**Figure 37** Side-product E
The method of obtaining the hydroxy amide 196 required careful reconsideration owing to this unexpected result. Accordingly, many trial reactions were carried out, each involving 200 mg of acid 197.

First, the temperature of the reaction was altered. The intermediates 197 and 105 were stirred at 0 °C for 2 hours, then to room temperature with DCC. These conditions afforded two new compounds which were each isolated; the required amide 196 (Rf = 0.3 in EtOAc) in very poor yield (40%), in comparison to the side-product E (70%) (scheme 83). Another coupling reagent, EDCI, was used under similar conditions however, only a poor yield of 15% was obtained.

It seemed that the temperature of the reaction was not the only problem and that a new coupling agent was needed. Again, 200 mg of the acid 197 was used to try out various reagents. A more specialised coupling agent was chosen involving BOP, PyBroP and DIEA; initial results were promising since the isolated yield of 196 was increased from 15% to 40% using BOP (scheme 84).
Scheme 84

However, PyBroP only formed the side product E. Owing to the promising result obtained with BOP, optimisation of the conditions was attempted. The amount of BOP was varied from 1.1 to 1.5 to 2.0 equivalents; by increasing the quantity of coupling agent, a marked decrease in the yield of 196 arose (table 6). Accordingly, the number of equivalents was then kept at 1.2 (77 %, entry 5).

The solvent in the reaction involving BOP was changed from DMF to \( \text{CH}_2\text{Cl}_2 \), but the yield was no better; NMR spectra showed that only E was formed. Variations in temperature of the reaction and the effects are summarised below. Results showed that E was the thermodynamic product and the desired amide 196\(^1\) was the kinetically favoured product. If the temperature of the reaction was increased by just one degree above -62 °C, the yield fell dramatically.
Table 6. Reaction of 105 and 197 under various conditions

Endoh et al\(^1\) had successfully synthesised the amide 196 using the same reagents BOP, DIEA and DMF excluding HOBT. However, the literature did not describe the conditions and so a number of trial reactions had to be carried out.

4.3.2 Protection of the alcohol 105 by silylation

Whilst optimising the coupling reaction, O-silylations were being attempted on the alcohol 105, in the hope that if the alcohol was O-protected then the side product E would be avoided. Various silylating agents were used, including TESCl, TMSCl and TBSCI, in the presence of imidazole as the base (scheme 85). It was found that the solvent used in the protection had an effect on the yield, as summarised in table 7.
Scheme 85

<table>
<thead>
<tr>
<th></th>
<th>TESCI</th>
<th>TBSCI</th>
<th>TMSCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂Cl₂</td>
<td>50 %</td>
<td>46 %</td>
<td>60 %</td>
</tr>
<tr>
<td>DMF</td>
<td>19 %</td>
<td>62 %</td>
<td>35 %</td>
</tr>
</tbody>
</table>

Table 7
Chapter 4

Attempts were made to couple the protected alcohols 205, 206, 207 with the corresponding acid 197 using various coupling agents including; isobutyl chloroformate, DCC, EDCI and BOP. All those attempts were unsuccessful; only the coupled acid intermediates were formed, implying that the amine had failed to attack such intermediates. This may have been due to the silyl groups being quite bulky and causing steric hindrance (scheme 86).

Scheme 86

Reverting to the synthesis involving BOP (scheme 84), it was required that a larger scale acylation reaction was attempted using the amine 105 and the acid 197. In the first trial, 1 g of 197 and 0.45 g of 105 were reacted using 1.1 equivalents of BOP. Unexpectedly, the reaction went well, giving 196 in 65% yield. The amide 196 was then reacted with DAST in CH₂Cl₂ at -78 °C to form the corresponding oxazoline 195 in 72% yield. The amidation reaction was again repeated on a 1 g scale, giving 196 (1.0 g, 83%) an improved yield.
Scheme 87

The key tris-oxazole intermediate 194 was synthesised via the Williams-Wipf \textsuperscript{5} procedure which included the cyclisation of the amido alcohol 196 using DAST to form 195 and then dehydrogentating it with the use of DBU and BrCCl\textsubscript{3} to form 194 with a high yield of 69\% (scheme 87).

Having prepared trisoxazole 194, different possibilities for the synthesis of 176 presented themselves, including that in (scheme 72). The common intermediate 194 of the synthesis would need to be converted into the corresponding acid 193, and also into the amino alcohol 179 (scheme 88).
Scheme 88

Owing to the insolubility of tris-oxazoles 194 and 193, another tris-oxazole derivative, which was more soluble in organic solvents was sought; the benzyl ester group 208, was chosen to replace the methyl ester 194 (figure 38)

Figure 38
The benzyl ester 208 was prepared in 90% yield by reacting the acid 193 with benzyl bromide in the presence of triethylamine. The gem-dimethyl moiety was cleaved by \( p \)-TSA in methanol, but a poor yield of 36% was obtained because side-products had formed. Next, a regioselective hydrogenolysis was tried in order to remove the Cbz group of oxazolidine 209 in the presence of the benzyl ester group (scheme 89). The procedure was carried out using 5% Pd/C but took 9 hours to complete, which is quite slow owing to the use of a non-protic solvent CH\(_2\)Cl\(_2\), although a protic solvent methanol (2:1) was also present. Tlc of the reaction showed a baseline spot, however, \(^1\)H NMR showed no product.

Scheme 89
Chapter 4

Amidation of amine 210 with acid 193 was not attempted due to problems in obtaining the free amine.

Scheme 90

Because scheme 90 had not been successful, the original sequence in scheme 91 was then pursued, in which the trisoxazole 194 needed to be deprotected to give the amino alcohol 179.
Scheme 91

Fortunately, to deprotect the gem-dimethyl group, hydrogenolysis over 10% Pd/C was tried first (instead of p-TSA) (scheme 91); tlc (EtOAc) showed the product to be baseline and the isolated product was found to be the free amino alcohol 179 obtained with no side-products. Accordingly, the synthesis of 179 was carried out in one step from 194 rather than the two shown in scheme 92. This useful result obtained in the deprotection was then applied to the earlier sequence (scheme 81) to give the amino alcohol 105, also in high yield of 83%. 
Scheme 92

The amino alcohol 179 was coupled with the acid 193 using the coupling reagents BOP, DIEA and HOBT (scheme 93). TLC results showed only two spots had formed in the reaction, an improvement compared with using the benzyl ester 211 (scheme 90). The two spots were isolated by column chromatography, and NMR data confirmed that the product obtained with Rf = 0.1 (EtOAc) was in fact the desired amide 215.

Scheme 93
The amide 215 was very insoluble in dichloromethane and hence the formation of the oxazoline 216 proved difficult. The amide 215 dissolved sparingly in dichloromethane upon heating; after cooling to −78 °C the reagents diethylamino sulfur trifluoride and potassium carbonate were added. As soon as DAST was added to the amide 215 in dichloromethane the solid dissolved and immediately the oxazoline 216 precipitated as a white solid isolated in 80% yield. The $^1$H NMR spectrum was obtained at 120 °C in DMSO in which it was sparingly soluble. Oxazoline peaks were found at $\delta_H$ 4.85 (CH$_2$) and 5.28 (CH), HRMS confirmed the accurate mass. However, $^{13}$C NMR data could not be obtained since the probe required for the NMR cannot be heated for long experiments; $^{13}$C NMR spectra at room temperature were unresolved. Unfortunately, the synthesis of macrocycle 176 using this route had to be abandoned owing to the insolubility of oxazoline 216.

4.4 A third approach: synthesis of the penta-oxazole core 218

With the successful isolation of the functionalised trisoxazole fragment 194, many different approaches to macrocycle 176 could be investigated. A third approach would require the synthesis of the penta-oxazole 218 (figure 39) which contains all the unsubstituted oxazole rings in telomestatin and five of these in macrocycle 176. The sequence was investigated concurrently with the second approach.

![Figure 39](image-url)
Chapter 4

The retrosynthesis to form the penta-oxazole 218 is shown in scheme 37:
Scheme 94

The retrosynthesis to form the pentaoxazole is shown in scheme 94 and follows a similar approach to the previous two routes, in which an acid and an amino alcohol would be united to give an amide, here **224**. Then a Williams-Wipf reaction would be carried out to give the pentaoxazole **218**. Hydrolysis of ester **218** would give the acid **222**. Which would then be coupled with the amino alcohol **105**, to give the amide **221** (scheme 94) and finally a Williams-Wipf procedure would furnish macrocycle **176**.
The synthesis began with the amidation of the amino alcohol 105 with the acid 193 (scheme 95). The reaction was carried out similarly to the second approach and involved BOP, DIEA, HOBT and DMF. Pattenden et al.\textsuperscript{10} carried out a similar procedure to synthesise the natural product YM-21613. In the synthesis a monothiazole amino alcohol was reacted with a trisoxazole acid together however, the synthesis used EDCI, NMM, HOBT in CH\textsubscript{2}CH\textsubscript{2} as the reagents (Ch 1, page 41). Tlc results showed that the reaction had progressed; however, some starting material was still present even after stirring at -62 °C for 4 days. It was decided that the amide 224 would be purified via column chromatography since the starting materials in the crude material would interfere with the next step. After column chromatography, the isolated yield of 224 was 40%.

![Chemical structure](image)

**Scheme 95**

The next step involved the synthesis of the oxazoline 223; the pure amide 224 was reacted with DAST and potassium carbonate in CH\textsubscript{2}Cl\textsubscript{2} at -78 °C (scheme 97). As the reaction progressed a solid material formed during the first five minutes. After five minutes the solid foam was filtered and washed with CH\textsubscript{2}Cl\textsubscript{2}. \textsuperscript{1}H NMR and HRMS data showed that oxazoline 223 had been obtained (scheme 96). However, the white foam was highly insoluble even in DMSO at 90 °C.
Eventually, the oxazoline 223 was dissolved in a copious amount of hot CH$_2$Cl$_2$ (30 mL). Once the sparingly soluble oxazoline 223 had been dissolved DBU and BrCCl$_3$ were added; immediately a solid material formed which was quite crystalline (scheme 97). Initially it was thought that the solid material may have been the oxazoline, but the spectral data confirmed that penta-oxazole 218$^{11}$ had been formed. It was even more difficult to obtain a $^1$H NMR spectrum for 218, since it failed to dissolve in DMSO at 90 °C. However, at 110 °C a satisfactory $^1$H NMR spectrum was obtained.
The insolubility of 218 in DMF or DMSO made continuation of the synthesis very difficult (scheme 98). The next step would have involved the dehydrogenation of the oxazolidine to the amino alcohol 105. However, 218 could not be dissolved in CH$_2$Cl$_2$ or methanol which led to the discontinuation of the synthesis. In summary, the synthesis went as far as the pentaoxazole 218.
Scheme 98

Owing to the insolubility of the pentaaxazole 218, a more hydrophobic ester group was chosen; it was decided that a benzyl ester group should replace the methyl ester of 218, ultimately to synthesise ester 233 (scheme 101). Manipulation of the functionality in acid 197 was satisfactorily achieved by O-
benzylation (benzyl bromide in the presence of triethylamine) to give ester 228, followed by cleavage of the hemiaminal carbon atom (p-TSA in methanol) to give the alcohol 229. Subsequent hydrogenolysis afforded the amino alcohol 230 (scheme 99).

Scheme 99

Acylation of the amino alcohol 230 with the trisoxazole carboxylic acid 193 was attempted using BOP, DIEA, HOBT in DMF (scheme 100). The temperature of the reaction was kept at – 62 °C and stirred for 4 days. However, tlc results were quite disappointing, since there were five spots. Each compound was isolated, but \textsuperscript{1}H NMR data showed none was the amide 231. The route was discontinued owing to time constraints and lack of materials.
Scheme 100

The proposed route to macrocycle 176 using the benzyl ester amide 231 had proved very troublesome. It appeared that the acylation of amine 230 with acid 193 formed products other than the amide 231. This failure to react indicated that the revised synthesis (scheme 101) could not be continued. Hence, routes to the macrocycle 176 via penta-oxazole intermediates were not investigated further.
Scheme 101
Chapter 4

References


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EXPERIMENTAL

General: Melting points were determined on a microscope hot-stage Electrotical 9100 apparatus, and are uncorrected. Infra-red (IR) spectra were recorded on a Perkin-Elmer PE-983 spectrophotometer; absorptions are quoted in wavenumbers ($\nu_{\text{max}}$ in cm$^{-1}$). $^1$H NMR spectra were recorded on the Brucker AC300 (300MHz) spectrometer; data reported in parts per million ($\delta$), with tetramethylsilane (TMS) as an internal standard. Coupling constants ($J$) are given in Hertz (Hz). The following abbreviations are used in signal assignments: singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m). $^{13}$C NMR spectra were obtained using a Brucker AMX 300 (75MHz), Brucker AMX 400 (100 MHz), Brucker AMX 500 (125 MHz), spectrometer and are recorded in parts per million ($\delta$) with CHCl$_3$ as an internal standard. Mass spectra were recorded on a VG7070H mass spectrometer with Finigan Incos II data system at University College London. Thin-layer chromatography was performed on Merck 0.2mm aluminium-backed silica gel 60 F$_{254}$ plates and visualised by ultra violet light or an alkaline potassium permanganate spray with subsequent heating. Flash chromatography was performed using Merck 0.040-0.063 mm, 230-400 mesh silica gel. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Evaporation refers to the removal of the solvent under reduced pressure. Glassware, syringes and needles for moisture-sensitive reactions were pre-dried in an oven (130°C). Temperatures below 0 °C were obtained from various mixtures of water, freezing salt and ice, acetone and ice, acetone and dry ice, or liquid nitrogen. Measurements of optical rotation were obtained on AA Series Automatic polarimeter (PolAAR 2000) at 23 °C.
Chapter 5

2-Bromo-1-pyridin-2-yl ethanone (81)\(^1\)

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{Br}
\end{array}
\]

To a solution of 2-acetylpyridine (2.00 g, 16.5 mmol) in benzene (20 mL), was added glacial acetic acid (5 mL, 87 mmol). Bromine (0.84 mL, 16.8 mmol: CAUTION) in benzene (12.5 mL) was added to the reaction mixture over 10 min. A yellow precipitate formed; after stirring for 48 h the mixture was filtered and dissolved in saturated potassium carbonate (20 mL). The orange oil formed was dissolved in diethyl ether (20 mL) and extracted. The organic layer was separated, dried over MgSO\(_4\), filtered and evaporated to give 81 (2.80 g, 89\%) as a brown oil; Rf 0.5 (EtOAc); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta_H\) 8.68 (1H, ddd, \(J=\) 0.88, 1.60, 4.74 Hz, pyridyl), 8.07 (1H, ddd, \(J=\) 0.92, 2.10, 7.8 Hz, pyridyl), 7.88 (1H, ddd, \(J=\) 1.72, 7.6, 9.3 Hz, pyridyl), 7.52 (1H, ddd, \(J=\) 1.25, 4.76, 7.6 Hz, pyridyl), 4.84 (2H, s, CH\(_2\)Br); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta_C\) 192.49 (C=O), 151.46 (s, pyridyl), 149.16 (d, pyridyl), 137.15 (d, pyridyl), 127.78 (d, pyridyl), 122.69 (d, pyridyl), 32.23 (CH\(_2\)Br).

2-(1-Isopropenyl)-4-(2-pyridyl)oxazole (52)\(^1\)

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{N}
\end{array}
\]

2-Bromo-1-pyridin-2-yl ethanone (81) (0.42 g, 2.10 mmol) and methacrylamide (0.35 g, 4.11 mmol) were dissolved in THF (15 mL) in a reinforced tube. The tube was sealed using a polythene cap and heated at 100°C using a paraffin oil bath for 72 h. After being cooled to room temperature, the tube was opened (CAUTION) and the mixture was evaporated. The residue was purified by column chromatography (dichloromethane) to give 52 (0.29 g, 75 \%) as a brown
oil; Rf 0.3 EtOAc; $^1$H NMR (300 MHz, CDCl$_3$) $\delta_{H}$ 8.57 (1H, ddd, $J$= 1.0, 1.7, 4.8 Hz, pyridyl), 8.19 (1H, s, CH, oxazolyl), 7.92 (1H, ddd, $J$= 0.92, 1.9, 8.0 Hz, pyridyl), 7.73 (1H, ddd, $J$= 1.0, 7.6, 9.3 Hz, pyridyl), 7.19 (1H, ddd, $J$= 1.0, 4.8, 7.5 Hz, pyridyl), 6.01 (1H, s, C=CH), 5.41 (1H, s, C=CH), 2.20 (3H, s, CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta_{C}$ 162.93 (s, pyridyl), 150.85 (O=C=N, oxazolyl), 149.38 (d, pyridyl), 136.84 (C=C=O), 136.47 (C=O, oxazolyl), 131.69 (C=C, oxazolyl), 122.68 (d, pyridyl), 120.33 (d, pyridyl), 119.13 (d, pyridyl), 118.47 (C=CH$_2$), 18.98 (CH$_3$).

2-Acetyl-4-(2-pyridyl)oxazole (79)$^1$

Method A: To a solution of 2-(1-methylethenyl)-4-(2-pyridyl) oxazole (52) (0.33 g, 1.77 mmol) in dioxane (75 mL) and water (75 mL) was added 5 drops of an OsO$_4$ solution in water and sodium periodate (1.30 g, 6.1 mmol). The reaction mixture was left to stir at 50°C over 16 h. Dichloromethane (30 mL) was added to the reaction mixture and washed with aqueous sodium metabisulfite (30 mL), water (30 mL) and brine (20 mL), the organic layer was dried over Na$_2$SO$_4$, filtered and evaporated. The residue was purified by column chromatography (dichloromethane) to afford 79 (0.14 g, 42 %) as a white solid; mp 121-123 °C, lit.$^1$ mp 122-123.5 °C ; Rf 0.25 (EtOAc); $^1$H NMR (300 MHz, CDCl$_3$) $\delta_{H}$ 8.61 (1H, ddd, $J$= 0.9, 1.76, 4.8 Hz, pyridyl), 8.42 (1H, s, oxazolyl), 7.97 (1H, ddd, $J$= 0.98, 2.0, 7.9 Hz, pyridyl), 7.79 (1H, ddd, $J$= 1.78, 7.6, 9.4 Hz, pyridyl), 7.29 (1H, ddd, $J$= 1.2, 4.8, 7.5 Hz, pyridyl), 2.74 (3H, s, CH$_3$); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta_{C}$ 185.65 (CH$_3$C=O), 157.43 (s, oxazolyl), 149.58 (s, pyridyl), 142.67 (d, pyridyl), 139.66 (d, oxazolyl), 137.09 (s, oxazolyl), 127.67 (d, pyridyl), 123.52 (d, pyridyl), 120.51 (d, pyridyl), 26.7 (CH$_3$).
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Method B: To a solution of 2-(1-methylethenyl)-4-(2-pyridyl) oxazole (52) (2.18 g, 11.7 mmol) in dichloromethane (150 mL). A bubbler was placed inside the solution and ozone gas was added to the solution at -78 °C for 1 h. Then the reaction was purged with oxygen for 10 min. Triphenylphosphine (9.20 g, 35.1 mmol) was added to the reaction until the reaction mixture turned yellow. The reaction was left to stir for 16 h at ambient temperature. The residue was purified by column chromatography (dichloromethane) to afford 79 (1.32 g, 60%) as a white solid.

\[ \text{2-(Hydroxy-phenyl-methyl)acrylamide (85)} \]

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
& \quad \text{=CH}_2 \\
& \quad \text{NH}_2
\end{align*}
\]

To a solution of benzaldehyde (4.79 mL, 49.0 mmol) in methanol (4 mL) was added acrylamide (3.34 g, 47.0 mmol) and 3-hydroxyquinolidine (2.98 g, 23.5 mmol). The mixture was stirred at 20 °C for 72 h. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give 85 (2.18 g, 40%) as a white solid, mp 98-100 °C, lit.\(^2\) mp 97-99 °C; Rf 0.2 (1:5 EtOAc: petroleum ether); \(^1\)H NMR (300 MHz, CD\(_3\)OD-d\(_4\)) \(\delta\)H 7.29 (5H, m, aryl), 6.25 (1H, s, C=CH\(_2\)), 5.66 (1H, s, C=CH), 5.63 (1H, s, CHO\(_2\)), \(^1\)H NMR (75 MHz, CD\(_3\)OD-d\(_4\)) \(\delta\)C 172.42 (NH\(_2\)-C=O), 147.66 (C=CH\(_2\)), 143.08 (s, aryl), 129.50 (d, aryl), 128.81 (d, aryl), 127.61 (d, aryl), 121.06 (C=CH\(_2\)) 74.16 (CHO\(_2\)).
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2-Bromomethyl-3-phenylacrylamide (86)

![Chemical Structure]

2-(Hydroxy-phenylmethyl)-acrylamide (85) (0.50 g, 2.8 mmol) was dissolved in hydrobromic acid (3.08 mL, 57.0 mmol). The mixture was stirred at 20 °C over 16 h. The mixture evaporated and the residue was purified by column chromatography (ethyl acetate) to give 86 (0.56 g, 83%) as a brown oil; IR (thin film) (ν_max) 3050, 2983, 1650, 1468, 1356, 630 cm⁻¹; Rf 0.4 (1:3 EtOAc: petroleum ether); ¹H NMR (300 MHz, CDCl₃) δ_H 7.77 (1H, C=CH), 7.46 (5H, m, aryl), 4.43 (2H, s, CH₂Br); ¹³C NMR (75 MHz, CDCl₃) δ_C 167.96 (HN-C=O), 136.38 (C=CH), 134.57 (s, aryl), 132.55 (HC=CCH₂Br), 129.04 (d, aryl), 128.83 (d, aryl), 128.34 (d, aryl), 28.83 (CH₂Br); LRMS m/z (EI) 241 (M⁺H, 11%), 239 (M⁺H, 12%), 160 (100%), 143 (23%), 143 (7%), 115 (97%), 91 (13%), 80 (9%); HRMS calc'd for C₁₀H₁₀BrNO 238.9946. Found 238.9949.

2-Methyl(hydroxyphenylmethyl)-acrylate 87³

![Chemical Structure]

To a solution of benzaldehyde (4.79 mL, 47.0 mmol) in chloroform (3 mL) was added 3-hydroxyquinaldinine (0.29 g, 2.3 mmol). The mixture was stirred at 20 °C for 5 min. Methyl acrylate (6.39 mL, 71.0 mmol) was then added and the mixture was stirred at 20 °C over 72 h. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give 87 (8.58 g, 95 %) as a colourless oil; Rf 0.32 (1:5 EtOAc: petroleum ether); ¹H NMR (300 MHz, CDCl₃) δ_H 7.28 (5H, m, aryl), 6.32 (1H, s, C=CH), 5.84 (1H, s, C=CH), 5.54 (1H, s, OH), 3.69 (3H, s, OCH₃); ¹³C NMR
Chapter 5

(75 MHz, CDCl₃) δc 166.75 (C=O), 142.04 (s, aryl), 141.34 (s, C=CH₂), 128.42 (d, aryl), 127.81 (d, aryl), 126.62 (d, aryl), 126.62 (d, C=CH₂), 73.14 (CHOH), 51.94 (OCH₃).

3-Hydroxymethyl-3-phenylacrylate (88)¹

To a stirred solution of methyl 2-(hydroxyphenylmethyl)acrylate 87 (1.0 g, 5.2 mmol) and acetic anhydride (0.60 mL, 6.2 mmol) in dichloromethane (20 mL) was added trimethylsilyl trifluoromethanesulphonate (0.1 mL) at 20 °C. After 2 h, the mixture was evaporated and methanol (20 mL), potassium carbonate (2.16 g) were added. The mixture was stirred for 1h at 20 °C. Then mixture was evaporated, diluted with water (50 mL) and extracted with diethyl ether (50 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give 88 (0.77 g, 77 %) as a colourless oil; Rf 0.5 (1:2 EtOAc: petroleum ether); ¹H NMR (CDCl₃, 300 MHz) δH 7.83 (1H, s, C=CH), 7.42 (5H, m, aryl), 4.40 (2H, s, CH₂OH), 3.87 (3H, s, OCH₃); ¹³C NMR (75 MHz, CDCl₃) δc 168.46 (C=O), 142.68 (C=CH), 134.52 (s, aryl), 130.92 (C=CH), 129.61 (d, aryl), 129.27 (d, aryl), 128.61 (d, aryl), 57.90 (CH₂OH), 52.21 (OCH₃).

2-Hydroxymethyl-3-phenyl-acrylamide (89)
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To 3-hydroxy methyl-3-phenyl-acrylate 88 (0.2 g, 1.0 mmol) was added 0.88 aqueous ammonia (3 mL). The mixture was stirred at 50 °C for 16 h. The mixture was evaporated, the residue was purified by column chromatography (ethyl acetate) to give 89 (0.14 g, 76 %) as colourless oil. IR (thin layer) (ν_max) 3400, 2923, 1633, 1593, 1402, 989 cm⁻¹; Rf baseline (EtOAc); ¹H NMR (CDCl₃, 300 MHz) δ_H 7.48 (1H, s, C=CH), 7.33 (5H, m, aryl), 7.28 (2H, m, NH₂), 4.28 (2H, s, CH₂OH); ¹³C NMR (CDCl₃, 75 MHz) δ_C 170.30 (H₂N-C=O), 136.30 (C=CH), 135.93 (s, aryl), 135.76 (C=CH), 129.32 (d, aryl), 129.22 (d, aryl), 128.29 (d, aryl), 57.20 (CH₂OH); LRMS m/z (EI) 177 (M⁺, 14%), 160 (23%), 148 (21%), 131 (100%), 115 (51%), 103 (42%), 91 (11%): HRMS calcd for C₁₀H₁₁N₂O₂ 177.0784. Found 177.0787.

Acetic acid 2-carbamoyl-3-phenyl-allyl ester (90)

To a stirred solution of 2-(hydroxyphenylmethyl)-acrylamide (85) (0.50 g, 2.8 mmol) and acetic anhydride (0.33 mL, 3.38 mmol) in dichloromethane (1 mL) was added trimethylsilyl trifluoromethanesulfonate (0.1 mL) at 20 °C. After 24 h, the mixture was evaporated to leave a yellow residue which was purified by column chromatography (1:2.5 ethyl acetate:petroleum ether) to give 90 (0.55 g, 89%) as a yellow oil: IR (thin film) (ν_max) 3384, 1664, 1595, 1450, 1031 cm⁻¹; Rf 0.1 (EtOAc); ¹H NMR (CD₃OD, 300 MHz) δ_H 7.66 (1H, s, C=CH), 7.40 (5H, m, aromatic-H), 4.90 (2H, s, CH₂OAc), 2.10 (3H, s, OCH₃); ¹³C NMR (75 MHz, CD₃OD) δ_C 172.66 (H-N-C=O), 172.58 (O-C=O), 142.15 (C=CH), 141.49 (C=CH), 135.98 (s, aryl), 131.74 (d, aryl), 130.31 (d, aryl), 129.77 (d, aryl), 60.80 (CH₂OAc), 20.83 (OCH₃); LRMS m/z (EI) 219 (M⁺, 24%), 159 (100%), 148 (14%), 131 (89%), 115 (96%), 103 (53%), 91 (18%): HRMS calcd for C₁₂H₁₃NO₃ 219.0895. Found 219.0891.
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**Methyl 2-acetoxyethyl-3-phenyl-3-phenylaclrate (91)**

![Chemical Structure](image)

To a solution of methyl (2-hydroxyphenylmethyl)-acrylate (87) (0.50 g, 2.8 mmol) in dichloromethane (20 mL) was added acetic anhydride (0.33 mL, 3.4 mmol) and trimethylsilyl trifluoromethanesulfonate (0.056 mL). The mixture was stirred for 2 h at 20 °C. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give 91 (1.10 g, 96 %) as a colourless oil; Rf 0.2 (1:5 EtOAc: petroleum ether); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ H 7.97 (1H, s, C=CH), 7.36 (5H, m, aryl), 4.94 (2H, s, CH$_2$OAc), 3.82 (3H, s, OCH$_3$), 2.07 (3H, s, OC=OCH$_3$, acetate); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 170.74 (C=O, acetate), 167.30 (C=O, methyl ester), 145.50 (C=CH), 134.19 (s, aryl), 129.60 (C=CHCH$_2$), 129.45 (d, aryl), 128.73 (d, aryl), 128.20 (d, aryl), 59.35 (CH$_2$OC=OCH$_3$), 52.30 (OCH$_3$), 20.95 (OC=OCH$_3$, acetate).

**Acetic acid 2-carbamoyl-1-phenyl-allyl ester (92)**

![Chemical Structure](image)

To a stirred solution of 2-(hydroxyphenylmethyl)-acrylamide 88 (0.20 g, 1.18 mmol) in pyridine (2 mL) was added acetic anhydride (0.14 mL, 1.35 mmol) at 20 °C. After 24 h the mixture was evaporated and the residue was purified by column chromatography (1:2 ethyl acetate: petroleum ether) to give 92 (0.15 g, 58 %) as a yellow oil; IR (thin film) ($v_{max}$) 3384, 1664, 1595, 1450, 1031 cm$^{-1}$; Rf 0.2 (1:3 EtOAc: petroleum ether); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ H 7.35 (5H, m, aryl), 6.84 (1H, s, CHOAc), 6.04 (1H, s, C=CH), 5.86 (2H, bs, NH$_2$), 5.63 (1H, s, C=CHH), 2.09 (3H, s, OCH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ C 169.57 (HN-C=O), 167.94 (O-C=O), 142.69 (C=CH$_2$), 137.45 (s, aryl), 128.66 (d, aryl), 128.47 (d, aryl), 127.08 (aryl), 122.47 (C=CH$_2$), 73.77 (CHOAc), 21.11 (OCH$_3$); LRMS m/z (EI) 219 (M$^+$, 2%), 202 (3%). 176 (100%), 159 (53%), 143 (2%), 132
(23%), 115 (52%), 105 (84%), 91 (13%): HRMS calc for C_{12}H_{13}NO_2 219.0895. Found 219.0893.

2-(2-styrvl-oxazol-4-yl pyridine) (96)

![Chemical Structure](image)

To a solution of 2-bromo-1-pyridin-2-yl-ethanone 81 (0.20 g, 0.99 mmol) in methanol (15 mL) was added cinnamamide (0.58 g, 3.99 mmol). The mixture was placed in the microwave (900 Watts) for 20 sec. The mixture was evaporated and the residue was dissolved in dichloromethane (30 mL), purified by column chromatography (1:4 ethyl acetate: petroleum ether) to give 96 (0.08 g, 35 %) as a colourless oil. Rf 0.2 (1:4 EtOAc: pet spt); IR (thin film) (\(\nu_{\text{max}}\)) 3813, 1427, 1303, 1120, 1056, 693 cm\(^{-1}\);\(^{1}\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\)H 8.59 (1H, ddd, \(J=\) 0.9, 1.68, 4.7 Hz, pyridyl), 8.24 (1H, s, oxazolyl), 7.91 (1H, ddd, \(J=\) 0.98, 2.0, 7.9 Hz, pyridyl), 7.77 (1H, ddd, \(J=\) 1.74, 7.3, 9.8 Hz, pyridyl) 7.54 (2H, m, aryl), 7.56 (1H, d, \(J=\)16.38 Hz, C=CH), 7.36 (3H, m, aryl), 7.22 (1H, ddd, \(J=\) 1.2, 4.8, 7.5 Hz, pyridyl), 6.97 (1H, d, \(J=\)16.38 Hz C=CH);\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\)C 161.85 (s, pyridyl), 150.65 (s, oxazolyl), 149.52 (d, pyridyl), 142.25 (d, oxazolyl), 136.94 (d, oxazolyl), 136.84 (d, pyridyl), 136.43 (s, aryl), 135.40 (d, C=CH), 129.34 (d, aryl) 128.93 (d, aryl), 127.84 (d, aryl), 127.27 (d, pyridyl), 122.85 (d, C=CH), 120.23 (d, pyridyl); m/z +(Cl) 249 (M+H, 2%), 214 (1%), 166 (4%), 148 (100%), 136 (2%): HRMS calc for C\(_{16}\)H\(_{12}\)N\(_2\)O [M+H] 249.1028. Found 249.1019.
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3-Hydroxy-2-[(pyridine 2 carbonyl) amino] propionic acid methyl ester 98

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

To a solution of propionic acid (3.0 g, 24.0 mmol) in anhydrous THF (80 mL) was added triethylamine (6.14 mL, 48.0 mmol). Isobutyl chloroformate (3.32 g, 24.0 mmol) was added dropwise to the mixture at -30 °C. The reaction mixture was stirred over 2 h at -30 °C. Then L-serine methyl ester hydrochloride (7.46 g, 48.0 mmol) was added and the mixture was stirred at ambient temperature over 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (20 mL) and water (20 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography (1:1 ethyl acetate: petroleum spirit) to give 98 (3.93 g, 72 %) as a colourless oil; Rf 0.25 (EtOAc); \(^1\)H NMR (300 MHz, CDCl₃) \(\delta_H\) 8.83 (1H, bd, NH, \(J = 6\) Hz), 8.59 (1H, ddd, \(J = 0.9\), 1.67, 4.76 Hz, pyridyl), 8.17 (1H, , ddd, \(J = 1.0\), 2.11, 7.8 Hz, pyridyl), 7.86 (1H, ddd, \(J = 1.72\), 7.7, 9.4 Hz, pyridyl), 7.46 (1H, ddd, \(J = 1.24\), 4.7, 7.6 Hz, pyridyl), 4.87 (1H, m, CHCH₂OH), 4.03 (2H, m CHCH₂OH), 3.82 (3H, s, OCH₃); \(^{13}\)C NMR (75 MHz, CDCl₃) \(\delta_C\) 170.72 (H₂CO-C=O), 164.70 (HN-C=O), 149.10 (s, pyridyl), 148.31 (d, pyridyl), 137.49 (d, pyridyl), 126.60 (d, pyridyl), 122.47 (d, pyridyl), 63.55 (CH₂OH), 54.95 (CHCH₂OH), 52.84 (O-CH₃).
Methyl-2-(picolinamido)acrylate (99)

![Chemical Structure Image]  

To a solution of 3-Hydroxy-2-[pyridine 2 carbonyl] amino] propionic acid methyl ester 98 (3.00 g, 13.30 mmol) in dichloromethane (30 mL) was added triethylamine (1.80 mL, 14.5 mmol) and p-toluenesulfonyl chloride (2.50 g, 13.30 mmol). The mixture was stirred at 20 °C for 2 h. The mixture was evaporated and the residue was purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give 99 (2.20 g, 82 %) as an orange oil; IR (thin film) $v_{\text{max}}$ (cm$^{-1}$) 3438, 1628, 1525 1126; Rf 0.5 (EtOAc); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H 8.61 (1H, ddd, $J$ = 0.9, 1.48, 4.77 Hz, pyridyl), 8.19 (1H, ddd, $J$ = 0.9, 2.09, 7.78 Hz, pyridyl), 7.86 (1H, ddd, $J$ = 1.67, 7.6, 9.3 Hz, pyridyl), 7.48 (1H, ddd, $J$ = 1.2, 4.78, 7.6 Hz, pyridyl), 6.81 (1H, s, C=CH), 6.01 (1H, s C=CH$_2$), 3.85 (3H, s, OCH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$C 164.37 (H$_3$CO-C=O), 163.01 (HN-C=O), 149.47 (s, pyridyl), 148.35 (d, pyridyl), 137.52 (d, pyridyl), 131.19 (C=CH$_2$), 126.58 (d, pyridyl), 122.22 (d, pyridyl), 109.18 (C=CH$_2$), 52.95 (O-CH$_3$); LRMS m/z (EI) 207 (M+H, 100%), 191 (7%), 177 (3%), 147 (4%), 96 (11%); HRMS calcd for C$_{10}$H$_{16}$N$_2$O$_3$ 207.0770. Found 207.0801.
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2,6-Pyridinedicarboxylic acid monobenzyl ester (101)\textsuperscript{7,8}

\begin{center}
\begin{tikzpicture}
\path (0,0) -- (0,0.5) -- (0.5,0.5) -- (0.5,1.0) -- (1.0,1.0) -- (1.0,0) -- (0,0);
\path (0,0) -- (0.5,0) -- (0.5,0.5) -- (1.0,0.5) -- (1.0,0);
\path (0.5,0) -- (0.5,0.5);
\path (0.5,0.5) -- (1.0,0.5);
\end{tikzpicture}
\end{center}

A mixture of pyridine-2,6-dicarboxylic acid (2.00 g, 1.2 mmol), water (4.8 mL), benzyl alcohol (13.8 mL, 130 mmol) and concentrated sulfuric acid (0.66 mL) was heated at reflux for 2 h, and then allowed to stir at 25 °C for 16 h. The mixture was neutralised with saturated aqueous sodium hydrogen carbonate (170 mL) and extracted with chloroform (150 mL) to remove the dibenzyl ester (20%). The aqueous layer was acidified to pH 2.0, at which point the monoester crystallized as white needles. Additional monoester was obtained by extracting the resulting mother liquor with chloroform to give 101 as white needles (1.9 g, 61%). mp 130-132 °C, lit.\textsuperscript{8} mp 132-133 °C ; Rf 0.2 (1:4 EtOAc: petroleum ether);
\textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz) \textdelta\textsubscript{H} 8.21 (3H, m, pyridyl), 7.37 (5H, m, aryl), 5.40 (2H, m, OCH\textsubscript{2}), 4.12 (1H, bs, OH); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 75 MHz) \textdelta\textsubscript{C} 165.52 (HO-C=O), 164.01 (CH\textsubscript{2}O-C=O), 148.05 (s, pyridyl), 147.52 (s, aryl), 139.15 (s, pyridyl), 128.46 (d, pyridyl), 128.27 (d, pyridyl), 128.15 (d, pyridyl), 127.95 (d, aryl), 127.87 (d, aryl), 127.43 (d, aryl), 66.81 (OCH\textsubscript{2}).
(S)-6-(2-Hydroxy-1-methoxycarbonyl-ethyl carbamoyl)-pyridine-2-carboxylic acid benzyl ester (102)

Isobutyl chloroformate (0.28 mL, 2.14 mmol) was added to a solution of pyridine 2,6-dicarboxylic acid monobenzyl ester 101 (0.55 g, 2.14 mmol) and triethylamine (0.6 ml, 4.28 mmol) in dichloromethane (40 mL) at -30 °C. After 1 h, L-serine methyl ester hydrochloride (0.67 g, 4.28 mmol) was added in portion over 10 min. The mixture was left to stir and warm up from -20 °C to ambient temperature over 16 h. Then dichloromethane (30 mL) was added and the mixture was extracted with water (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated to leave a residue which was purified via column chromatography (1:1 ethyl acetate: petroleum ether) to give 102 (0.40 g, 52 %) as white plates, mp 88-90 °C; IR (KBr) νₘₐₓ 3525, 3269, 1741, 1687, 1539, 1045 cm⁻¹; Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (CDCl₃, 300 MHz) δ_H 9.04 (1H, d, J= 3Hz, NH), 8.29 (1H, d, J= 6Hz, pyridyl), 8.22 (1H, d, J= 6Hz, pyridyl), 8.17 (1H, t, J= 7.8 Hz, pyridyl), 7.45 (5H, m, aryl), 5.41 (2H, s, OCH₂), 4.84 (1H, m, CH), 4.01 (2H, m, CH₂OH), 3.78 (3H, s, OCH₃);¹³C NMR (CDCl₃, 75 MHz) 170.51 (HN-C=O), 164.36 (CH₂O-C=O), 163.84 (CH₂O-C=O), 149.72 (s, pyridyl), 146.52 (s, aryl), 138.63 (s, pyridyl), 135.23 (d, pyridyl), 128.74 (d, pyridyl), 128.57 (d, pyridyl), 128.31 (d, aryl), 127.57 (d, aryl), 125.74 (d, aryl), 67.77 (OCH₂), 63.17 (CH₂-OH), 55.23 (HOCH₂-CH), 52.73 (OCH₃). LRMS m/z +(FAB) 381 (M+Na, 100%), 359 (92%), 338 (42%), 321 (6%), 269 (3%), 235 (2%): HRMS calcd for C₁₈H₂₈N₂O₆ [M+Na] 381.1063. Found 381.1069. Calculated for C₁₈H₁₈N₂O₆ C, 60.33, H, 5.06, N 7.82%. Found C, 60.09, H, 5.17, N, 7.45%.
3-Hydroxy-2-[(pyridine 2 carbonyl) amino] propionic acid methyl ester 98 (1.0 g, 4.5 mmol) placed under an argon atmosphere and anhydrous dichloromethane (20 mL) was then added. The temperature of the reaction was reduced to -78 °C; DAST (0.63 mL, 4.8 mmol) was then added dropwise (forming a yellow solution). The mixture was left to stir at -78 °C for 2 h. Potassium carbonate (1.20 g, 9.01 mmol) was then added to the mixture, which was left to stir to ambient temperature for 16 h. The mixture was then poured into a solution of sodium carbonate (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (3:1 hexanes: ethyl acetate) to give 104 (0.66 g, 72 %) as a colourless oil; [α]D 23 = -12 ° (c= 1.0, CHCl₃); IR (thin film) νmax (cm⁻¹) 3411, 2916, 1737, 1639, 1045, 966; Rf 0.35 (EtOAc); ¹H NMR (300 MHz, CDCl₃) δH 8.67 (1H, ddd, J= 0.9, 1.7, 4.78 Hz, pyridyl), 8.06 (1H, ddd, J= 1.0, 2.0, 7.8 Hz, pyridyl), 7.76 (1H, ddd, J= 1.72, 7.7, 9.4 Hz, pyridyl), 7.40 (1H, ddd, J= 1.2, 4.8, 7.6 Hz, pyridyl), 4.98 (1H, CH₃, oxazoliny1), 4.68 (1H, m, CH₂, oxazoliny1), 3.84 (3H, s, OCH₃); ¹³C NMR (75 MHz, CDCl₃) δC 171.18 (H₂CO-C=O), 165.34 (s, O-C=N), 149.80 (s, pyridyl), 145.96 (d, pyridyl), 136.73 (d, pyridyl), 126.05 (d, pyridyl), 124.41 (d, pyridyl), 70.17 (t, CH₂ oxazoliny1), 68.70 (d, CHCH₂, oxazoliny1), 52.78 (O-CH₃); LRMS m/z (EI) 207 (M+H, 4%), 193 (2%), 147 (100%), 119 (24%), 106 (8%), 92 (68%), 78 (47%): HRMS calcd for C₁₀H₁₀N₂O₃ 206.06914. Found 206.06916.
2-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester (100)

![Chemical structure of 2-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester](attachment:chemical_structure_image.png)

To a solution of 2-pyridin-2-yl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester 104 (0.15 g, 0.727 mmol) in dichloromethane (10 mL) was added DBU (0.29 mL, 1.45 mmol). The mixture was left to stir for 5 min at -10 °C. To the mixture was added bromotrichloromethane (0.10 mL, 0.75 mmol). The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. Saturated aqueous ammonium chloride (20 mL) was added and the organic layer was isolated, dried over MgSO₄ and evaporated. The residue was purified by column chromatography (ethyl acetate) to give 100 (0.125 g, 85%) as a beige solid, mp 124.5-126.5 °C; IR (KBr) νmax 3415, 2925, 1711, 1633, 1047, 999 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δH 8.74 (1H, ddd, J= 0.9, 1.7, 4.8 Hz, pyridyl), 8.38 (1H, s, oxazole), 8.28 (1H, ddd, J= 1.0, 2.0, 7.8 Hz, pyridyl), 7.86 (1H, ddd, J= 1.7, 7.8, 9.3 Hz, pyridyl), 7.44 (1H, ddd, J= 1.1, 4.78, 7.6 Hz, pyridyl), 3.96 (3H, s, OCH₃); ¹³C NMR (300 MHz, CDCl₃) δC 164.66 (CH₃O-C=O), 161.50 (s, pyridyl), 150.01 (C=N, oxazolyl), 145.12 (d, pyridyl), 144.91 (C=CH, oxazolyl), 137.26 (C=CH, oxazolyl), 134.60 (d, pyridyl), 125.53 (d, pyridyl), 122.89 (d, pyridyl), 52.38 (OCH₃): HRMS calcd for C₁₀H₈N₂O₃: 204.05349. Found 204.05367: Anal. Calcd for C₁₀H₈N₂O₃: C, 58.82; H, 3.95; N, 13.72. Found: C, 58.64; H, 3.92; N, 13.50.
2-Pyridin-2-yl-oxazole-4-carboxylic acid amide (77)

2-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester 100 (0.10 g, 0.48 mmol) was dissolved in 0.880 aqueous ammonia (4 mL) and dioxane (1 mL). The mixture was left to stir for 30 h at ambient temperature. The precipitate was filtered and washed with water to leave 77 (0.07 g, 79%) as a beige crystalline solid, mp 189 °C; IR (KBr) (ν \text{max}) 3398, 2925, 1638, 1061, 968 cm\(^{-1}\); Rf baseline (EtOAc); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) \text{H} 8.73 (1H, s, oxazolyl), 8.73 (1H, ddd, \(J\) = 1.0, 1.7, 4.8 Hz, pyridyl), 8.13 (1H, ddd, \(J\) = 1.0, 2.0, 7.8 Hz, pyridyl), 8.02 (1H, ddd, \(J\) = 1.72, 7.9, 9.3 Hz, pyridyl), 7.78 (1H, bs, NH), 7.61 (1H, bs, NH), 7.57 (1H, ddd, \(J\) = 1.1, 4.8, 7.5 Hz, pyridyl); \(^1^3\)C NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) 161.59 (H\(_2\)N-C=O), 160.34 (s, pyridyl), 149.97 (C=N, oxazolyl), 144.83 (d, pyridyl), 143.01 (d, C=CH, oxazolyl), 137.60 (C=CH, oxazolyl), 134.68 (d, pyridyl), 125.58 (d, pyridyl), 122.28 (d, pyridyl). Anal. Calcd for C\(_9\)H\(_7\)N\(_3\)O\(_2\): C, 57.43; H, 3.88; N, 21.48. Found: C, 56.99; H, 3.80; N, 21.36.
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**Methyl-2-(2-hydroxy-1-(picolinamido) ethyl)oxazoles-4-carboxylate (106)**

![Chemical Structure](image)

To a solution of picolinic acid (0.20 g, 1.62 mmol) in DMF (20 mL) was added BOP (0.80 g, 1.78 mmol), DIEA (0.68 mL, 3.72 mmol) and HOBT (0.24 g, 1.78 mmol). The mixture was stirred for 1.5 h at 20 °C, then the temperature was lowered to -62 °C. A solution of 2-(1-amino-2-hydroxyl-ethyl)-oxazole-4-carboxylic acid methyl ester 105 (0.29 g, 1.55 mmol) in DMF (10 mL) was added to the mixture dropwise. The solution was stirred for 24 h at -62 °C, then ethyl acetate (50 mL) was added. The organic layer was washed with water (3x 50 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 106 (0.18 g, 40%) as a brown oil; [α]D²³ = -27.3° (c=0.76, CHCl₃); IR (thin layer) (νmax) 3436, 1662, 1265, 1095 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δH 8.66 (1H, m, pyridyl), 8.52 (1H, s, oxazolyl), 8.09 (1H, m, pyridyl), 7.96 (1H, m, pyridyl), 7.58 (1H, m, pyridyl), 5.38 (1H, m CHCH₂), 4.08 (2H, m, CH₂OH), 3.86 (3H, s, OCH₃); ¹³C NMR (100 MHz, CD₃OD) δC 166.56 (HNC=O), 164.70 (OC=O), 162.93 (s, pyridyl), 150.37 (s, oxazolyl), 149.88 (d, pyridyl), 146.46 (d, oxazolyl), 138.86 (d, pyridyl), 134.19 (d, oxazolyl), 128.14 (d, pyridyl), 123.33 (d, pyridyl), 63.38 (CH₂OH), 52.57 (CHCH₂OH), 51.38 (OCH₃); LRMS m/z + (FAB) 219 (M+Na, 100%), 221 (9%), 199 (31%), 177 (96%): HRMS calcd for C₁₃H₁₃N₂O₅ [M+Na] 314.0752. Found 314.0758.

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2-Acetylpyridine 1-oxide (109)

To a solution of 2-acetylpyridine (6.05 g, 49.9 mmol) in chloroform (50 mL) was added \textit{m}-CPBA (11.0 g, 64.9 mmol) and stirred at 20 °C for 0.5 h then heated at reflux for a further 0.5 h. The mixture was evaporated and the residue was dissolved in ether (50 mL) and water (50 mL). The organic layer was washed with water (2x50 mL), the water washings was evaporated to leave 109 (6.7 g, 98%) as a brown residue. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta_{H} 8.53\) (1H, dd, \(J=2.2, 7.8\) Hz, pyridyl), 7.40 (1H, m, pyridyl), 7.17 (2H, m, pyridyl), 2.52 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta_{C} 194.86\) (C=O), 146.60 (s, pyridyl), 140.42 (d, pyridyl), 128.04 (d, pyridyl), 126.48 (d, pyridyl), 125.42 (d, pyridyl), 30.39 (CH\textsubscript{3}).

\textit{Acetoxy}methyl 2-pyridyl ketone (110)

A solution of 2-acetylpyridine 1-oxide 109 (2.44 g, 18.0 mmol) in acetic anhydride (35 mL) was heated at 140 °C for 1.5 h. The mixture was evaporated and the residue was purified by column chromatography (4:1, 40-60 °C petroleum ether: ethyl acetate). The mixture was evaporated to leave 110 (1.02 g, 32%) as a yellow oil. Rf 0.2 (CHCl\textsubscript{3}); \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta_{H} 8.96\) (1H, ddd, \(J= 0.9, 1.73, 4.81\) Hz, pyridyl), 8.03 (1H, ddd, \(J= 1.0, 2.2, 7.8\) Hz, pyridyl), 7.83 (1H, ddd, \(J= 1.7, 7.68, 9.3\) Hz, pyridyl), 7.51 (1H, ddd, \(J= 1.0, 4.8, 7.7\) Hz, pyridyl), 5.62 (2H, s, OCH\textsubscript{2}), 2.21 (3H, s, OCH\textsubscript{3}); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta_{C} 175.98\) (C=O, ketone), 171.71 (C=O, acetate), 170.97 (C=O, ester), 146.49 (s, pyridyl), 137.44 (d, pyridyl), 123.28 (d, pyridyl), 123.07 (d, pyridyl), 121.12 (d, pyridyl) 65.85 (CH\textsubscript{2}), 21.73 (CH\textsubscript{3}).

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2,6-Dipicolinic acid (2.00 g, 12 mmol) and thionyl chloride (11.7 mL, 130 mmol) was heated at reflux for 2 h. The excess thionyl chloride was evaporated, and the residue dissolved in dry benzene (13.3 mL). After the solution had been stirred at 0 °C for 15 min, ethanol (4.0 mL) was added dropwise. The mixture was then heated at reflux for 2 h, cooled to 25 °C, and aqueous 20% sodium carbonate (20 mL) was added, the mixture was then left to stir for 10 min. The organic layer was collected and the aqueous layer extracted with diethyl ether (2 x 10 mL). The combined organic layers were dried over MgSO₄, and evaporated to give 122 (2.37 g, 89%) as a pale yellow oil: Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (300 MHz, CDCl₃) δH 8.27 (2H, d, J=7.8 Hz, 3-H, 5-H), 7.99 (1H, t, J=8.1 Hz, 4-H), 4.47 (4H, q, J=6.3, 9 Hz, 2 x CH₂), 1.43 (6H, m, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃) δc 164.49 (C=O), 148.49 (s, pyridyl), 138.26 (d, pyridyl), 128.76 (d, pyridyl), 62.20 (OCH₂), 15.17 (CH₃).

Pyridine-2,6-dicarboxamide (120)¹⁰

To pyridine-2,6-dicarboxylic acid diethyl ester 122 (2.00 g, 8.96 mmol) was added concentrated aqueous ammonia (0.880, 7.0 mL) and the resulting white suspension was stirred 16 h. The white solid formed was collected, washed with water to give 120 (0.75 g, 51%) as white platelets; mp 298 °C (dec), lit mp¹⁰ 317 °C; Rf baseline (EtOAc); ¹H NMR (DMSO-d₆, 300 MHz) δH 8.86 (2H, bs, N-H), 8.14 (3H, m, pyridyl), 7.70 (2H, bs, N-H); ¹³C NMR (DMSO-d₆, 75 MHz) δc 165.27 (C=O), 148.98 (s, pyridyl), 139.08 (d, pyridyl), 124.07 (d, pyridyl).
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**1,1-(Pyridine-2,6-diyl)bis(2-bromoethanone) (123)**

![Chemical Structure](image)

To a solution of the 1,1-(pyridine-2,6-diyl)bisethanone (236) (1.25 g, 7.66 mmol) in chloroform (50 mL) was added bromine (0.87 mL, 16.9 mmol) dropwise. The mixture was heated at reflux for 16 h. Evaporation to afford 123 (2.4 g, 98%) as a brown solid, mp 159-161°C, lit. mp 160-162 °C; Rf 0.2 (1:2 EtOAc: petroleum ether); $^1$H NMR (300 MHz, CDCl$_3$) δ$_H$ 8.33 (2H, d, J=7.8 Hz, pyridyl), 8.21 (1H, t, J=9 Hz, pyridyl), 4.61 (4H, s, 2CH$_2$); $^{13}$C NMR (75 MHz, CDCl$_3$) δ$_C$ 191.36 (BrCH$_2$-C=O), 154.21 (s, pyridyl), 138.41 (d, pyridyl) 126.67 (d, pyridyl), 39.10 (CH$_3$Br).

**3-Hydroxy-2-(2-methacyrrolaminio)-propionic acid methyl ester (127)**

![Chemical Structure](image)

To a solution of methacrylic acid (0.36 g, 3.79 mmol) in anhydrous THF (20 mL) under an argon atmosphere was added triethylamine (0.99 mL, 7.58 mmol). The ice bath temperature was lowered to -30°C, then isobutyl chloroformate (0.49 mL, 3.79 mol) was added. The mixture was left to stir at -30°C for 2 h. Then L-serine methyl ester hydrochloride (0.59 g, 3.79 mmol) was added in one portion. The thick slurry was stirred at -30°C then allowed to warm from -30°C to ambient temperature for 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (30 mL) and water (30 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated. The residue was purified by flash chromatography (5:1 petroleum ether: ethyl acetate) to give 127 (0.32 g, 45%) as a colourless oil; [α]$_D^{23}$ = -9.0° (c 1.0 in CHCl$_3$); IR (thin film) $\nu_{max}$ 3364, 2852, 1733, 1661, 1520, 1079, 935 cm$^{-1}$; Rf 0.2 (1:5 EtOAc: petroleum ether); $^1$H NMR (300 MHz, CDCl$_3$) δ$_H$ 6.81 (1H, bs, NH$_2$), 5.80 (1H, s, C=CH), 5.41 (1H, s, C=CH), 4.72 (1H, m, CH$_2$CH), 3.95 (2H, m, CH$_2$OH), 3.80 (3H, s, OCH$_3$),
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1.98 (3H, s, CH₃); ¹³C NMR (75 MHz, CDCl₃) δₑ 171.08 (HN-C=O), 168.65 (CH₂O-C=O), 139.04 (C=C-C=O), 121.08 (C=C-C=O), 63.37 (CH₂OH), 54.90 (HC-CH₂OH), 52.87 (O-CH₃), 18.48 (CH₃); LRMS m/z (EI): 188 (M+H, 100%), 170 (74%), 157 (7%), 141 (2%), 128 (13%), 97 (11%): HRMS calcd for C₆H₁₃NO₃ 187.08445, found 187.08527.

2-Isoprenyl-4, 5-dihydro-oxazole-4-carboxylic acid methyl ester (128)

To a solution of amide 127 (0.26 g, 1.37 mmol) under an argon atmosphere was added anhydrous dichloromethane (10 mL). The temperature of the reaction was lowered to -78 °C. Then DAST (0.29 mL, 2.20 mmol) was added dropwise (forming a light yellow solution). The mixture was stirred at -78 °C for 6 h. Potassium carbonate (0.35 g, 2.6 mmol) was then added and the stirred mixture was allowed to warm from -78 °C to ambient temperature over 16 h. The mixture was then poured into aqueous sodium carbonate (20 mL). The organic layer was isolated, and dried over MgSO₄, filtered and evaporated. The residue which was purified by column chromatography (3:1, petroleum ether: ethyl acetate) to give 128 (0.16 g, 82%) as a colourless oil; [α]D⁺²² = 6.8° (c=1.0, CHCl₃); IR (thin film) νmax 3416, 2963, 1743, 1608, 1441, 1066, 989 cm⁻¹; Rf 0.2 (1:3 EtOAc: petroleum ether); ¹H NMR (300 MHz, CDCl₃) δ H 5.80 (1H, s, C=CH), 5.39 (1H, s, C=CH), 4.83 (1H, m, CH₂CH), 4.47 (2H, m, CH₂, oxazoliny), 3.77 (3H, s, OCH₃), 2.01 (3H, s, CH₃); ¹³C NMR (75 MHz, CDCl₃) δc 171.58 (C=O), 167.08 (C=N, oxazoliny) 132.28 (C=CH₂), 123.12 (C=CH₂), 69.30 (CHCH₂, oxazoliny), 68.68 (CHCH₂, oxazoliny), 52.64 (OCH₃), 19.28 (CH₃); LRMS m/z (CI): 169 (M⁺, 94%), 145 (100%), 131 (6%), 117 (23%), 101 (33%), 96 (11%): HRMS calcd for C₆H₁₃NO₃ [M+H] 170.08171, found 170.08172.
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2-Isoprenyl-oxazole-4-carboxylic acid methyl ester (129)

To a solution of oxazoline 128 (0.13 g, 0.77 mmol) in dichloromethane (10 mL) was added DBU (0.22 mL, 1.5 mmol). The temperature was then lowered to -10 °C then bromotrichloromethane (0.08 mL, 0.81 mmol) was added. The mixture was allowed to warm up with stirring from -10 °C to ambient temperature over 16 h. The mixture was evaporated to leave a brown residue which was purified by column chromatography (ethyl acetate) to leave 129 (0.10 g, 85%) as a yellow oil; IR (thin film) ν max 3414, 2933, 1726, 1572, 1438, 1004, 933 cm⁻¹; Rf 0.2 (1:2 EtOAc: petroleum ether); H NMR (300 MHz, CDCl₃) δH 8.23 (1H, s, oxazolyl), 6.03 (1H, s, C=CH), 5.41 (1H, s, C=CH₂), 3.87 (3H, s, OCH₃), 2.19 (3H, s, CH₂); C NMR (75 MHz, CDCl₃) δC 163.30 (CH₃O-C=O), 161.34 (C=N, oxazolyl), 143.71 (C=CH, oxazolyl), 134.09 (C=CH, oxazolyl), 131.13 (C=CH₂), 120.08 (C=CH₃), 52.21 (OCH₃), 19.00 (CH₃); LRMS m/z (EI): 169 (M+H, 94%), 170 (32%), 157 (5%), 136 (36%), 110 (100%), 86 (48%); HRMS calcd for C₈H₉NO₃ 169.0738, found 169.0740.

2-Isoprenyl-oxazole-4-carboxylic acid amide (130)

The ester 129 (0.09 g, 0.53 mmol) was dissolved in 0.880 aqueous ammonia (2 mL) and dioxane (1 mL). The mixture was left to stir for 30 h at room
temperature. The reaction mixture was then evaporated and the residue was extracted with ethyl acetate (15 mL) and water (15 mL). The organic layer was dried over MgSO₄, filtered to give 130 (0.06 g, 75%) as a beige foam; IR (thin film) ν_max 3383, 2922, 1464, 1377 cm⁻¹; Rf baseline (EtOAc); ¹H NMR (300 MHz, CDCl₃) δ_H 8.16 (1H, s, oxazolyl), 6.90 (1H, bs, NH), 6.53 (1H, bs, NH), 5.96 (1H, s, C=CH), 5.35 (1H, s, C=CH), 2.14 (3H, s, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ_C 163.22 (NH₂C=O), 162.36 (C=N, oxazolyl), 141.30 (C=CH), 136.54 (C=CH, oxazolyl), 131.04 (C=CH, oxazolyl), 119.66 (C=CH₂), 18.82 (CH₃); LRMS m/z (EI): 152 (M⁺, 100%), 136 (22%), 124 (7%), 108 (9%), 85 (83%), 69 (48%); HRMS calcd for C₇H₅N₂O₂ 152.0530, found 152.0534.

6-(2-Bromoacetyl)-pyridine-2-carboxylic acid ethyl ester (131)

![Chemical Structure](image)

To a suspension of sodium methoxide (0.39 g, 7.30 mmol) in benzene (8.5 mL), ethyl acetate (1.25 mL) was added pyridine-2,6-dicarboxylic acid diethyl ester 122 (0.50 g, 2.23 mmol). After 10 min the reaction was cooled and then 10 M hydrochloric acid (2.23 mL) was added dropwise. Then the suspension was heated at reflux for 16 h, then the organic layer was separated and discarded. The aqueous layer was neutralised using solid anhydrous sodium carbonate. Ether (20 mL) was added and then the organic layer was dried over MgSO₄, filtered and evaporated. The residue was then dissolved in chloroform (20 mL) and bromine (0.07 mL) was added dropwise. The reaction was stirred at reflux for 3 h. The mixture was then evaporated down and then purified by column chromatography (dichloromethane) to afford 131 (0.24 g, 64%) as a brown oil; IR ν_max 2930, 1708, 1456, 1377, 761 cm⁻¹; Rf 0.2 (DCM); ¹H NMR (CDCl₃, 300 MHz) δ_H 8.22 (2H, d, J=6 Hz, pyridyl), 8.18 (1H, t, J=8.8 Hz, pyridyl), 4.78 (2H, m, CH₂O), 4.47 (2H, s, CH₂Br), 1.23 (3H, m, CH₃); ¹³C NMR (CDCl₃, 75 MHz) δ_C 191.36 (BrCH₂OC=O), 163.35 (CH₂CH₂O-C=O), 150.34 (s, pyridyl), 149.49 (s, pyridyl), 147.80 (d, pyridyl), 129.28 (d, pyridyl), 128.39 (d, pyridyl), 127.40 (d, pyridyl), 63.16 (CH₂O), 39.49 (CH₂Br), 14.29 (CH₃CH₂); LRMS m/z
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(EI): 272 (M^{81+}, 60%), 270 (M^{79+}, 62%), 242 (54%), 226 (100%), 178 (61%): HRMS calcd for C_{10}H_{10}BrNO_3 270.9844, found 270.9857.

**Pyridine-2,6-dicarboxylic acid** (235)

![Pyridine-2,6-dicarboxylic acid](image)

To a stirred solution of pyridine-2,6-dicarboxylic acid (1.00 g, 5.86 mmol) and thionyl chloride (2.14 mL, 30 mmol) at 25 °C was added two drops of DMF. The mixture was then heated at reflux for 2 h. The mixture was cooled to 25 °C and excess thionyl chloride was evaporated to afford 235 (2.77 g, 77%) as an oil that was used without further purification.

**3-** Hydroxy-2-[[6-(2-hydroxy-1-methoxycarbonyl-ether carbamoyl)-pyridine-2-carboxyl]-amino]-propionic acid methyl ester (136)

![Hydroxy-2-[[6-(2-hydroxy-1-methoxycarbonyl-ether carbamoyl)-pyridine-2-carboxyl]-amino]-propionic acid methyl ester](image)

To a solution of L-serine methyl ester hydrochloride (2.04 g, 13.9 mmol) in chloroform (20 mL) was added triethylamine (4.02 mL, 29.3 mmol) at ambient temperature. To the mixture was added pyridine-2,6-dicarboxylic acid dichloride (235) (1.21 g, 5.96 mmol) in chloroform (10 mL). The resulting mixture was stirred for 24 h at room temperature. The white solid was filtered off and the filtrate was evaporated and the residue was purified by column chromatography (20:1 ethyl acetate: methanol) to give 136 (1.32 g, 64 %) as white flakes, mp 165.0-168.0°C, lit.\textsuperscript{12} mp 165.0-167.0 °C; Rf 0.2 (1:1 EtOAc: petroleum ether); \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ\textsubscript{H} 8.85 (2H, d, NH, J=7.9 Hz), 8.13 (2H, d, J = 7.8 Hz, pyridyl), 7.82 (1H, t, J = 7.8 Hz, pyridyl), 4.84 (2H, m, CHCH\textsubscript{2}OH), 4.22 (4H, m CH\textsubscript{2}OH), 3.83 (6H, s, OCH\textsubscript{3}); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ\textsubscript{C} 171.21 (HN-C=O),
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167.92 (O-C=O), 148.17 (s, pyridyl), 138.86 (d, pyridyl), 125.32 (d, pyridyl), 62.90 (CH$_3$OH), 55.34 (HC-CH$_2$OH), 53.01 (O-CH$_3$).

**Pyridine-2,6-bisoxazozaline methyl ester 137**

![Chemical Structure](image)

3-Hydroxy-2-[[6-(2-hydroxy-1-methoxycarbonyl-ether carbamoyl)-pyridine-2-carbonyl]-amino]-propionic acid methyl ester (136) (0.50 g, 1.26 mmol) was placed under argon atmosphere and anhydrous dichloromethane (10 mL) was added. The temperature of the mixture was lowered to -78 °C; DAST (0.37 mL, 2.77 mmol) was then added dropwise over 5 min. The mixture was left to stir at 78 °C for 2 h. Potassium carbonate (0.195 g, 1.41 mmol) was then added to the mixture which was left to stir from -78 °C to ambient temperature. The mixture was then poured into saturated aqueous sodium hydrogen carbonate (20 mL). The organic layer was dried with MgSO$_4$, filtered and evaporated to give 137 (0.40 g, 90 %) as a yellow oil; Rf 0.2 (EtOAc); $^{1}$H NMR (300 MHz, CDCl$_3$) δ$_H$ 8.17 (2H, d, J = 7.7 Hz, pyridine), 7.89 (1H, t, J = 7.8 Hz, pyridine), 5.01 (2H, m, CH oxazoliny1), 4.69 (4H, m CH$_2$ oxazoliny1), 3.80 (6H, s, OCH$_3$); $^{13}$C NMR (300 MHz, CDCl$_3$) δ$_C$ 171.06 (H$_3$CO-C=O), 164.85 (s, O-C=N, oxazoliny1), 146.17 (s, pyridyl), 137.58 (d, pyridyl), 126.74 (d, pyridyl), 70.43 (CH$_2$, oxazoliny1), 68.67 (s, OCH-CH$_2$, oxazoliny1), 52.85 (O-CH$_3$).
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**Pyridine-2,6-bisoxazole methyl ester 138**

![Chemical structure of pyridine-2,6-bisoxazole methyl ester 138]

To a solution of pyridine-2,6-bisoxazolazine methyl ester 137 (0.20 g, 6.0 mmol) in dichloromethane (8 mL) was added DBU (0.35 g, 2.44 mmol). After stirring for 10 min bromotrichloromethane (0.13 mL, 1.22 mmol) was added at -10°C to the mixture and stirred over 20 h. The mixture was evaporated to leave a residue that was purified by column chromatography (ethyl acetate) to give 138 (0.15 g, 80 %) as a white solid; mp 158.0-160.0 °C; IR (KBr) ν_{max} 3429, 1747, 1574, 1325, 1155, 1107 cm\(^{-1}\); Rf 0.2 (EtOAc); \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ\(_H\) 8.40 (2H, s, oxazolyl), 8.29 (2H, d, \(J=7.9\) Hz pyridyl), 8.02 (1H, t, \(J=7.8\) Hz, pyridyl), 3.97 (6H, s, OCH\(_3\)); \(^13\)C NMR (75 MHz, CDCl\(_3\)) δ\(_C\) 161.32 (H\(_3\)CO-C=O), 160.26 (s, pyridyl), 145.51 (C=N, oxazolyl), 145.31 (C=CH, oxazolyl), 138.42 (d, pyridyl), 134.73 (C=CH, oxazolyl), 124.44 (d, pyridyl), 52.41 (O-CH\(_3\)); Calculated for C\(_{15}\)H\(_{11}\)N\(_3\)O\(_6\) C, 54.72, H, 3.37, N 12.76% Found C, 54.37, H, 3.34, N, 12.54%.

**2,6-Pyridin-2-yl-oxazole-4-carboxylic acid amide 115**

![Chemical structure of 2,6-Pyridin-2-yl-oxazole-4-carboxylic acid amide 115]

2,6-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester (138) (0.20 g, 0.60 mmol) was dissolved in 0.880 aqueous ammonia (4 mL) and dioxane (1 mL). The reaction was stirred for 30 h at room temperature. The precipitate was filtered and washed with water to give 115 (0.08 g, 88 %) as a white solid, mp 268 °C (dec); IR (KBr) ν_{max} 3506, 2324, 1638, 1271, 1066 cm\(^{-1}\); Rf baseline (EtOAc); \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) δ\(_H\) 8.81 (2H, s, oxazolyl), 8.27 (3H, m,
pyridyl), 7.83 (2H, bs, NH), 7.63 (2H, bs, NH), 7.57 (1H, m, pyridyl); $^{13}$C NMR (125 MHz, DMSO-d$_6$) δc 161.54 (H$_2$N-C=O), 158.92 (s, O-C=N, oxazolyl), 145.25 (s, pyridyl), 143.49 (s, C=CH, oxazolyl), 139.38 (d, C=CH oxazolyl), 137.78 (d, pyridyl), 123.85 (d, pyridyl); LRMS m/z (FAB): 322 (M+Na, 34%), 300 (57%), 273 (13%), 245 (24%), 176 (100%); HRMS calcd for C$_{13}$H$_9$N$_5$O$_4$ [M+Na] 322.0552, found 322.0533.

4-Bromopyridine-2,6-dicarboxylic acid diethyl ester (167)$^{13}$

[Image of the compound structure]

Chelidamic acid monohydrate (168) (0.20 g, 1.1 mmol) and phosphorus pentabromide (3.05 g, 7.1 mmol) were heated to 90 °C whereupon it formed a melt. The mixture was then stirred at 120 °C at reflux for 16 h. After the mixture was cooled chloroform (20 mL) was added and the mixture was filtered. The filtrate was cooled to 0 °C and then ethanol (16 mL) was added dropwise. The mixture was heated at reflux for 3 h. The mixture was evaporated and the residue was purified by column chromatography (1: 1 ethyl acetate: 40-60 °C petroleum ether), to give 167 (0.26 g, 80%) as yellow semi-solid; Rf 0.5 (EtOAc)

$^1$H NMR (300 MHz, CDCl$_3$): δH 8.42 (2H, s, pyridyl), 4.48 (4H, q, J= 6.0, 15Hz, CH$_2$CH$_3$), 1.45 (6H, t, J= 6.0 Hz, CH$_2$CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$) δc 163.55 (C=O), 149.53 (s, pyridyl), 136.50 (s, pyridyl, C-Br), 131.07 (d, pyridyl), 62.74 (OCH$_2$CH$_3$), 14.20 (OCH$_2$CH$_3$).
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4-Bromopyridine-2, 6-dicarboxylic acid (166)\textsuperscript{13}

\[
\begin{array}{c}
\text{N} \\
\text{Br}
\end{array}
\]

To a solution of the diester 167 (0.19 g, 0.63 mmol) in THF (5 mL) was added a solution of lithium hydroxide monohydrate (0.06 g, 1.5 mmol) in water (0.83 mL). The mixture was stirred for 17 h at 38 °C. Then the solution was acidified to pH 1 with 3M HCl. The mixture was evaporated and the residue was dissolved in ethyl acetate and filtered. The filtrate was evaporated to give 166 (0.12 g, 78%) as a beige solid, mp 206 °C (dec), lit.\textsuperscript{13} mp 205-207 °C (dec); Rf baseline (EtOAc); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz) \( \delta \text{H} \) 8.35 (2H, s, pyridyl); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 300 MHz) \( \delta \text{C} \) 165.15 (C=O), 152.39 (s, pyridyl), 136.86 (s, pyridyl, C-Br), 128.29 (d, pyridyl).

4-Bromo-pyridine-2,6-dicarboxylic dichloride (169)\textsuperscript{13}

\[
\begin{array}{c}
\text{Cl} \\
\text{Br}
\end{array}
\]

Thionyl chloride (1.39 mL, 19.0 mmol), dimethylformide (3 drops) were added to 166 (0.40 g, 1.62 mmol) and stirred at reflux for 2 h. Thionyl chloride was evaporated off using the rotary evaporator. To leave 169 as an orange oil (0.46 g, quantitative yield).
S-2-[(4-Bromo-6-(2-hydroxy-1-methoxycarbonyl-ethylcarbonyl)-pyridine-2-carbonyl]-amino}3-hydrox-propionic acid methylester (165)

To a stirred suspension of L-serine methyl ester (0.63 g, 4.06 mmol) in chloroform (50 mL) was added triethylamine (10.19 mL, 79.5 mmol). Then diacid chloride 169 (0.46 g, 1.62 mmol) in chloroform (5 mL) was added to the mixture dropwise at 0 °C. The reaction was stirred at 20 °C for 17 h. The mixture was evaporated then diluted with ethyl acetate (30 mL) and washed with water (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (1:1, ethyl acetate: 40-60 °C petroleum ether) to give 165 (0.22 g, 30%) as a white foam; $[\alpha_\lambda]^{25}_D = -23.1^\circ$ (c=1.0, CH₂Cl₂); IR (thin film) $\nu_{max}$ 3350, 2265, 1724, 1687, 1536, 1229, 1109 cm⁻¹; Rf 0.2 (EtOAc); $^1$H NMR (DMSO-d₆, 300 MHz) δ₉ 9.30 (2H, m, NH), 8.33 (major rotamer), 8.23 (min rotamer), (2H, s, 3,5-pyridyl), 5.18 (2H, t, J=6 Hz, CH₂OH), 4.60 (2H, m, CHCH₂OH), 3.89 (4H, m, CH₂OH), 3.66 (6H, s, OCH₃); $^{13}$C NMR (DMSO-d₆, 75 MHz); δ₉ 170.39 (O-C=O), 162.20 (N-C=O), 150.13 (s, pyridyl), 135.22 (s, pyridyl, C-Br), 127.76 (d, pyridyl), 60.46 (CH₂OH), 55.39 (CHCH₂OH), 52.02 (OCH₃). LRMS m/z (El) 448 (M+81, 2%), 446 (M+79, 4%), 419 (2%), 382 (13%), 282 (3%), 147 (72%) 106 (15%) 92 (56%) 78 (100%); HRMS calcd for C₁₅H₁₅BrN₃O₈ [M]⁺ 447.02772. Found 447.02616.
4-Bromopyridine-2, 6-bisoxazoline methyl ester 164

![Chemical structure](image)

To a stirred solution of amide 165 (0.20 g, 0.45 mmol) in anhydrous dichloromethane (20 mL) at -78 °C was added DAST (0.13 g, 0.98 mmol) dropwise. The mixture was stirred at -78 °C for 4 h. Potassium carbonate (1.85 g, 13.65 mmol) was then added to the mixture, which was left to stir from -78 °C to ambient temperature over 17 h. The mixture was then poured into saturated aqueous sodium hydrogen carbonate (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give 164 (0.20 g, 89%) as a yellow oil; [α]D²³

= - 16.2° (c=1.0, CH₂Cl₂); IR (thin film) (νmax) 3236, 1733, 1627, 1560, 1386, 1218, 748 cm⁻¹; Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (CDCl₃, 300 MHz) δ 8.43 (2H, s, 3,5-pyridyl), 4.82 (2H, m, CHCH₂), 4.70 (4H, m, CHCH₃), 3.81 (6H, s, OCH₃); ¹³C NMR (CDCl₃ 75 MHz) δ 170.79 (O-C=O), 163.95 (s, pyridyl), 147.36 (s, C=N oxazolinyl), 134.06 (s, pyridyl, C-Br), 129.85 (d, pyridyl), 70.64 (N-CHCH₂, oxazolinyl), 68.64 (CH₂CH, oxazolinyl), 52.89 (OCH₃). LRMS m/z +(CI) 411 (M⁺+H, 65%), 409 (M⁺⁻H, 67%), 365 (37%), 351 (8%), 331 (100%), 306 (4%) 272 (12%) 115 (3%): HRMS calcld for C_{15}H_{14}BrN₃O₆ [M]⁺ 412.0144. Found 412.0139.
To a stirred solution of oxazoline 164 (0.20 g, 0.48 mmol) in dichloromethane (15 mL) was added DBU (0.30 mL, 2.04 mmol). After 10 min bromotrichloromethane (0.16 mL, 1.1 mmol) was added dropwise at -10 °C. The mixture was stirred for 17 h at 20 °C. The mixture was evaporated and purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give 145 (0.10 g, 51 %) as a beige foam. IR (thin film) (v\text{max}) 3236, 1733, 1627, 1560, 1386, 1218, 748 cm\textsuperscript{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ\textsubscript{H} 8.66 (1H, s, oxazolyl), 8.44 (2H, s, pyridyl), 3.98 (6H, s, OCH\textsubscript{3}); \textsuperscript{13}C NMR (CDCl\textsubscript{3} 75 MHz) δ\textsubscript{C} 161.11 (O-C=O), 158.36 (s, pyridyl), 146.47 (s, C=N, oxazolyl), 145.60 (d, oxazolyl, N-C=CH), 134.91 (s, pyridyl, C-Br), 127.46 (s, oxazolyl, N-C=CH), 127.46 (d, pyridyl), 52.51 (OCH\textsubscript{3}); LRMS m/z (EI) 409 (M\textsuperscript{81+}, 1%), 407 (M\textsuperscript{79+}, 2%), 363 (2%), 206 (4%), 147 (100%), 119 (22%) 92 (65%) 78 (41%) 65 (6%); HRMS calcd for C\textsubscript{15}H\textsubscript{10}BrN\textsubscript{3}O\textsubscript{6} [M] \textsuperscript{+} 406.9753. Found 406.9749.
Diethyl 4-((2-(dimethyl amino) ethyl)(ethyl) amino) pyridine-2,6-dicarboxylate 171

To a suspension of 4-bromopyridine-2,6-dicarboxylate 167 (0.20 g, 0.66 mmol) in DMF (6 mL) was added anhydrous potassium carbonate (0.10 g, 0.78 mmol) and N1-ethyl-N2, N2-dimethylethane-1,2-diamine (0.8 mL). The yellowish-brown mixture was heated at 60 °C for 48 h. The resultant brown suspension was cooled to room temperature and poured onto water (100 mL) and the mixture was extracted with ethyl acetate (100 mL). The organic layer was dried over MgSO4, filtered and evaporated. The brown residue was purified via column chromatography (5% methanol: ethyl acetate) to give 171 (0.08 g 40%) as a yellow oil. IR (thin film) (νmax) 3404, 1645, 1450, 1124, 1022 cm⁻¹; Rf 0.2 (EtOAc); 1H NMR (CDCl3, 300 MHz): δH 7.56 (2H, s, pyridyl), 4.41 (4H, m, CH3CH2), 3.48 (4H, m, 2xNCH2), 2.51 (2H, t, CH2N(CH3)2, J = 6 Hz), 2.32 (6H, s, N(CH3)2), 1.43 (6H, t, CH2CH3 J = 6 Hz), 1.20 (3H, t, J = 6 Hz, NCH2CH3), 13C NMR (CDCl3, 75 MHz) δc 165.86 (C=O), 153.96 (s, pyridyl), 149.13 (s, pyridyl), 109.91 (d, pyridyl), 62.14 (2xCH2CH3), 56.00 (CH3CH2N(CH3)2), 47.98 (CH2CH2N(CH3)2), 45.71 (N(CH3)2), 45.08 (NCH2CH3), 14.20 (NCH2CH3), 11.96 (2xCH2CH3); LRMS m/z +(Cl) 338 (M+H, 6%), 310 (1%), 270 (30%), 197 (16%), 114 (15%), 74 (100%); HRMS calcd for C17H27N3O4 [M+H] 338.2074. Found 338.2084.
mixture was stirred for 1 h at ambient temperature. The mixture was evaporated and the residue was dissolved in dichloromethane (50 mL) and washed with aqueous solution of sodium hydrogen carbonate (50 mL). The organic layer was dried over MgSO₄, filtered, and evaporated. The residue was purified by column chromatography (9:1, petroleum ether:ethyl acetate) to give 189 (5.52 g, 82 %) as a colourless oil; Rf 0.3 (1:1 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 75 °C, 400 MHz) δ 4.40 (1H, m, CHCH₂), 4.15 (1H, dd, J= 8, 12 Hz, CHH, oxazolidinyl), 3.93 (1H, dd, J= 4, 8 Hz, CHH oxazolidinyl), 3.01 (3H, s, OCH₃), 1.55 (3H, s, CH₃), 1.45 (3H, s, CH₃), 1.34 (9H, s, CH₃, Boc); ¹³C NMR (DMSO-d₆, 75 °C, 100 MHz) δ 170.54 (H₃C-C=O), 150.40 ([CH₃]₃O-C=O), 93.50 (NC(Me)_₂O), 79.13 ([CH₃]₃C), 65.14 (OCH₂CH, oxazolidinyl), 58.36 (OCH₂CH, oxazolidinyl), 51.24 (OCH₃), 27.41 ([CH₃]₃C), 24.68 (CH₃), 23.95 (CH₃).

2,2-Dimethyloxazolidine-3,4-dicarboxylic acid 3-tert-butyl ester (188)¹⁴-¹⁶

![Chemical Structure](image)

To a stirred solution of ester 189 (0.50 g, 2.04 mmol) in THF: water (20:10 mL) was added lithium hydroxide (8.5 mg, 0.20 mmol) at 20 °C. The mixture was heated at 50 °C for 14 h. The mixture was then evaporated and neutralised to pH 7. Extraction with ethyl acetate (15 mL) gave an organic layer which was dried over MgSO₄, filtered and evaporated to give 188 (0.31 g, 65%) as a white foam. Rf baseline (EtOAc); ¹H NMR (DMSO-d₆, 75 °C, 400 MHz) δ 4.27 (1H, m, CHCH₂, oxazolidinyl), 4.11 (1H, m, CHH, oxazolidinyl), 3.93 (1H, dd, J=4, 8 Hz, CHH, oxazolidinyl), 1.55 (3H, s, CH₃), 1.45 (3H, s, CH₃), 1.40 (9H, s, CH₃, Boc); ¹³C NMR (DMSO-d₆, 23 °C, 75 MHz) δ 174.65 (HO-C=O), 151.63 ([CH₃]₃O-C=O), 92.94 (NC(Me)₂O), 78.42 ([CH₃]₃C), 67.16 (OCH₂CH, oxazolidinyl), 61.01 (OCH₂CH, oxazolidinyl), 28.09 (C[CH₃]₃), 25.31 (CH₃), 24.48 (CH₃).
2,2-Dimethyl-oxazolidine3,4-dicarboxylic acid 3-tert-butyl ester 191

To a stirred solution of ester 189 (0.50 g, 2.04 mmol) in THF: water (20:10 mL) was added lithium hydroxide (8.5 mg, 0.20 mmol) at 20 °C. The mixture was heated to 50 °C for 14 h. The mixture was evaporated and ethyl acetate (15 mL) was added. The organic layer (containing organic impurities) was discarded. The aqueous layer was evaporated afford 191 (0.55 g, 100%) as a white solid, mp 222 °C (dec).

4-(2-Hydroxy-1-methoxycarbonyl-ethylcarbamyl)2,2-dimethoxyoxazolidine-3-carboxylic acid-tert-butyl ester (187)\textsuperscript{14,15}

To a stirred solution of lithium carboxylate 191 (0.50 g, 2.04 mmol) in anhydrous THF (20 mL) was added triethylamine (0.55 mL, 4.32 mmol). Then isobutyl chloroformate (0.28 mL, 2.16 mmol) was added to the mixture at -30 °C and stirred for 3 h. Then L-serine methyl ester hydrochloride (0.67 g, 4.32 mmol) was added in one portion. The thick slurry was stirred at ambient temperature for 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (20 mL) and water (20 mL). The organic layer was dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated to give 187 (0.70 g, 100%) as a colourless oil; Rf 0.2 (EtOAc); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 90 °C, 400 MHz) $\delta$H 7.80 (1H, bs, NH), 4.62 (1H, m, CHCH\textsubscript{2}, oxazolidinyl), 4.39 (2H, m, CH\textsubscript{2}OH), 4.08 (1H, m, CHCH\textsubscript{2}OH), 3.85 (1H, m, CHH, oxazolidinyl), 3.78 (1H, m, CHH, oxazolidinyl), 3.65 (3H, s, OCH\textsubscript{3}) 1.55 (3H, s, CH\textsubscript{3}), 1.45 (3H, s, CH\textsubscript{3}), 1.38 (9H, s, [CH\textsubscript{3}]\textsubscript{3} Boc); \textsuperscript{13}C NMR
(DMSO-d$_6$, 75 °C, 100 MHz) $\delta$$_C$ 170.69 (HN-C=O), 170.22 (H$_3$CO-C=O), 151.20 ([CH$_3$]$_3$O-C=O), 93.86 (NC(Me)$_2$O), 79.42 ([CH$_3$]$_3$C), 66.29 (OCH$_2$CH, oxazolidinyl), 61.30 (CH$_2$OH), 59.47 (OCH$_2$CH, oxazolidinyl), 54.58 (CHCH$_2$OH), 51.62 (OCH$_3$), 27.92 ([CH$_3$]$_3$C), 25.32 (CH$_3$), 24.37 (CH$_3$): HRMS calcd for C$_{13}$H$_{26}$N$_2$O$_7$ [M] 346.1740. Found 346.1720.

4-(Methoxycarbonyl) oxazoline-2,2 dimethoxy-oxazolidine-3-carboxylic acid-tert-butyl ester (186)$^{15}$

To a stirred solution of amide 187 (0.34 g, 0.98 mmol) in anhydrous dichloromethane (20 mL) was added DAST (0.38 mL, 2.87 mmol) dropwise at -78 °C. The mixture was stirred at -78 °C for 4 h. Potassium carbonate (0.47 g, 3.42 mmol) was then added to the mixture, which was stirred from -78 °C to ambient temperature over 17 h. The mixture was then poured into a solution of sodium hydrogen carbonate (30 mL). The organic layer was dried over MgSO$_4$, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give 186 (0.25 g, 77 %) as a yellow oil; IR (thin film) ($v$_{max}$) 3429, 2979, 1743, 1699, 1392, 1249, 1170 cm$^{-1}$; Rf 0.3 (EtOAc); $^1$H NMR (DMSO-d$_6$, 75 °C, 400 MHz) $\delta$$_H$ 4.74 (1H, m, CHCH$_2$, oxazolinyl), 4.57 (1H, m, CHCH$_2$, oxazolidinyl), 4.41 (1H, m, CHCH$_2$, oxazolinyl), 4.13 (1H, dd, $J$= 6.5, 8.9 Hz, CHH, oxazolidinyl), 3.91 (1H, dd, $J$= 2.9, 8.9 Hz, CHH, oxazolidinyl), 3.68 (3H, s, OCH$_3$) 1.54 (3H, s, CH$_3$), 1.46 (3H, s, CH$_3$), 1.39 (9H, s, CH$_3$, Boc); $^{13}$C NMR (DMSO-d$_6$, 75 °C, 100 MHz) $\delta$$_C$ 171.05 (O-C=O), 167.99 ([CH$_3$]$_3$O-C=O), 155.42 (s, oxazolinyl), 93.86 (NC(Me)$_2$O), 79.29 ([CH$_3$]$_3$C), 69.75 (CH$_2$CH, oxazolinyl), 67.72 (OCH$_2$CH, oxazolidinyl), 66.23 (CH$_2$CH, oxazolinyl), 54.10 (OCH$_2$CH, oxazolidinyl), 52.05 (OCH$_3$), 27.73 ([CH$_3$]$_3$C), 24.84 (CH$_3$), 23.89 (CH$_3$): HRMS calcd for C$_{13}$H$_{24}$N$_2$O$_6$ [M+Na] 351.1532. Found 351.1528.
4-(Methoxycarbonyl) oxazolyl-2,2 dimethoxy-oxazolidine-3-carboxylic acid-tert-butyl ester (185)$^{14,15}$

![Chemical Structure](image)

To a stirred solution of oxazoline 186 (0.20 g, 0.61 mmol) in anhydrous dichloromethane (10 mL) was added DBU (0.18 g, 1.22 mmol) and stirred at ambient temperature for 10 min. Then bromotrichloromethane (0.07 mL, 0.67 mmol) was added to the mixture at -10 °C dropwise. The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. The mixture was evaporated and the residue was purified by column chromatography (1:3 ethyl acetate: petroleum spirit) to give 185 (0.18 g, 89%) as a white solid mp 125-127 °C, lit.$^{15}$ mp 124 °C; Rf 0.35 (EtOAc); $^1$H NMR (DMSO-d$_6$, 75 °C, 400 MHz) $\delta$H 8.66 (1H, s, oxazolyl), 5.08 (1H, m, CHCH$_2$, oxazolidinyl), 4.26 (1H, dd, $J$= 6.60, 9.2 Hz, CHH, oxazolidinyl), 4.02 (1H, dd, CH$_2$, $J$= 3.1, 9.2 Hz, CHH, oxazolidinyl), 3.81 (3H, s, OCH$_3$) 1.63 (3H, s, CH$_3$), 1.52 (3H, s, CH$_3$), 1.32 (9H, s, CH$_3$, Boc); $^{13}$C NMR (DMSO-d$_6$, 75 °C, 100 MHz) $\delta$C 163.29 (H$_3$CO-C=O), 160.49 ([CH$_3$]$_2$O-C=O), 150.43 (s, O=C=N oxazolidinyl), 144.50 (s, CCHO, oxazolyl), 132.23 (d, CH, oxazolyl), 93.71 (NC(Me)$_2$O), 79.42 ([CH$_3$]$_3$C), 66.30 (OCH$_2$CH, oxazolidinyl), 54.08 (OCH$_2$CH, oxazolidinyl), 51.05 (OCH$_3$), 27.38 ([CH$_3$]$_3$C), 25.10 (CH$_3$), 23.79 (CH$_3$).
L-Serine (10.0 g, 95 mmol) was dissolved in 2 M NaOH: dioxane (80:40 mL). The temperature of the reaction was lowered to 0 °C and then benzyl chloroformate (15 mL, 104 mmol) was added dropwise. The mixture was stirred to ambient temperature for 48 h. The mixture was then acidified to pH 4 using 3M HCl and extracted with ethyl acetate (150 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (1% ethyl acetate: petroleum ether) to give 202 (13.0 g, 57 %) as a white solid, mp 119-120 °C, lit.¹⁷ mp 120-122 °C; Rf 0.2 (1:2 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 300 MHz): δH 7.35 (5H, m, aryl), 5.03 (2H, s, CH₂Ph), 4.07 (1H, m, CHCH₂OH), 3.73 (2H, m, CH₂OH); ¹³C NMR (DMSO-d₆, 75 MHz) δc 172.06 (HO-C=O), 155.97 (PhCH₂O-C=O), 136.90 (s, aryl), 128.28 (d, aryl), 127.74 (d, aryl), 127.66 (d, aryl), 65.39 (CH₂Ph), 61.26 (CHCH₂OH), 56.58 (CHCH₂OH).

2, 2-Dimethyl-oxazolidine-3, 4-dicarboxylic acid 3-benzyl ester (201)¹⁴,¹⁸

To a stirred solution of acid 202 (5.25 g, 22 mmol) in acetone (100 mL) was added 2, 2-dimethoxypropane (24 mL, 270 mmol). p-Toluenesulfonic acid (0.58 g, 3.0 mmol) in acetone (30 mL) was added to the mixture dropwise. The mixture was stirred for 12 h at 45 °C, then evaporated and basified to pH 8 using saturated sodium hydrogen carbonate. After extraction with ethyl acetate (60 mL), the aqueous layer was acidified to pH 1 using 3M HCl. Ethyl acetate (150 mL) was added and the organic layer was dried over MgSO₄, filtered and
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evaporated to give 201 (6.13 g, 100%) as a white solid, mp 110 °C. Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 90°C, 400 MHz): δₜ 7.35 (5H, m, aryl), 5.06 (2H, m, CH₂Ph), 4.44 (1H, m, CHCH₂, oxazolidinyl), 4.19 (1H, dd, J= 7.2, 9.1 Hz, CHH oxazolidinyl), 4.05 (1H, dd, J= 2.5, 9.1 Hz, CHH oxazolidinyl), 1.58 (3H, s, CH₃), 1.48 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 90 °C, 400 MHz) δc 172.03 (OH-C=O), 152.03 (PhCH₂O-C=O), 136.57 (s, aryl), 128.28 (d, aryl), 127.64 (d, aryl), 126.94 (d, aryl), 94.22 (NC(Me)₂O), 66.24 (OCH₂Ph, oxazolidinyl), 65.74 (OCH₂CH, oxazolidinyl), 58.25 (OCH₂CH, oxazolidinyl), 24.88 (CH₃), 23.62 (CH₃).

4-(2-Hydroxy-1-methoxycarbonyl-ethyl carbamoyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid benzyl ester (200)¹⁴

![Chemical structure of 200]

To a stirred solution of acid 201 (3.07 g, 10.0 mmol) in anhydrous dichloromethane (30 mL) triethylamine (2.79 mL, 22 mmol) was added. The mixture was left to stir for 10 min and isobutyl chloroformate (1.59 mL, 12 mmol) was added dropwise at -30 °C. The mixture was stirred for 2.5 h at -30 °C and L-serine methyl ester (2.54 g, 16.0 mmol) was added. The mixture was stirred for 17 h at ambient temperature. Water (30 mL) was added to the reaction the organic layer was dried over MgSO₄, filtered and evaporated to give 200 (3.55 g, 85%) as a colourless oil; Rf 0.2 (EtOAc); ¹H NMR (DMSO-d₆, 90 °C, 400 MHz) δₜ 7.91 (1H, d, J= 8 Hz, N-H), 7.33 (5H, m, aryl), 5.06 (2H, s, CH₂Ph), 4.75 (1H, t, J= 5.4 Hz, CHCH₂, oxazolidinyl), 4.52 (1H, m, CHCH₂OH), 4.39 (2H, m, CHCH₂OH), 4.16 (1H, dd, J= 7.1, 8.9 Hz, CHH oxazolidinyl), 3.93 (1H, dd, J= 3.2, 9.0 Hz, CHH oxazolidinyl), 3.72 (3H, m, OCH₃), 1.64 (3H, s, CH₃), 1.58 (3H, s, CH₃); ¹³C NMR (DMSO-d₆ 90 °C, 400 MHz): δc 171.24 (HN-C=O), 170.50 (H₃CO-C=O), 152.27 (PhCH₂O-C=O), 137.16 (s, aryl), 128.63 (d, aryl), 128.01 (d, aryl), 127.65 (d, aryl), 94.90 (NC(Me)₂O), 67.30 (CH₂Ph), 66.55 (OCH₂CH, oxazolidinyl), 61.83 (OCHCH₂,
oxazolidiny1), 59.83 (CH₂OH), 55.31 (CHCH₂OH), 52.24 (OCH₃), 25.61 (CH₃), 24.91 (CH₃).

4-(2-Hydroxy-1-methoxycarbonylethyl carbamoyl)-2,2-dimethyloxazolidine-oxazoline methyl ester 199

To a stirred solution of amide 200 (4.27 g, 11 mmol) in anhydrous dichloromethane (30 mL) was added DAST (1.78 mL, 13 mmol) dropwise at -78°C. The mixture was left to stir at -78°C for 2 h. Potassium carbonate (2.80 g, 21.0 mmol) was then added to the mixture, which was stirred at ambient temperature for 16 h. The mixture was then poured into a solution of sodium carbonate (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (3:1 hexanes: ethyl acetate) to give 199 (2.97 g, 73%) as a colourless oil; [α]D = -11° (c=1.0, CH₂Cl₂); IR (thin film) (νmax) 2985, 1712, 1411, 1350, 1211, 1095, 841 cm⁻¹; Rf 0.2 (1:3EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 90°C, 400 MHz): δH 7.36 (5H, m, aryl), 5.14 (1H, m, CHCH₂, oxazolinyl), 5.10 (1H, m, CHCH₂, oxazolidinyl), 4.90 (1H, d, JHH=12.7 Hz, CHHPh), 4.83 (1H, d, JHH=12.7 Hz, CHHPh), 4.40 (2H, m, CHCH₂, oxazolinyl), 4.21 (1H, dd, J= 2.0, 8.7 Hz, CHH, oxazolidinyl), 4.03 (1H, dd, J= 2.0, 8.7 Hz, CHH, oxazolidinyl), 3.71 (3H, s, OCH₃), 1.63 (3H, s, CH₃), 1.52 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 90°C, 100 MHz) δc 171.20 (H₃CO-C=O), 168.18 (PhCH₂O-C=O), 151.89 (s, C=N), 137.01 (s, aryl), 128.60 (d, aryl), 128.02 (d, aryl), 127.68 (d, aryl), 94.82 (NC(Me)₂O), 70.37 (OCH₂CH, oxazolinyl), 68.23 (OCH₂CH, oxazolinyl), 66.94 (OCH₂Ph), 66.49 (OCH₂CH, oxazolidinyl), 54.67 (OCH₂CH, oxazolidinyl), 52.28 (OCH₃), 25.60 (CH₃), 24.56 (CH₃); LRMS m/z (El) 338 (M⁺, 8%), 347 (38%), 303 (64%), 213 (2%), 156 (4%), 123 (1%): HRMS calcd for C₁₈H₂₈N₂O₆ [M⁺] = 362.1478. Found 362.1466.
4-(2-Hydroxy-1-methoxycarbonylethylene carbamoyl)-2, 2-dimethyl oxazolidine-oxazole methyl ester (198)\textsuperscript{14}

![Chemical Structure](image)

To a stirred solution of oxazoline 199 (4.13 g, 11 mmol) in anhydrous dichloromethane (30 mL) was added DBU (3.4 mL, 22.0 mmol). Then bromotrichloromethane (1.24 mL, 12.5 mmol) was added to the mixture at -10 °C dropwise. The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. The mixture was evaporated and the residue was purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give 198 (3.08 g, 75%) as a colourless oil; Rf 0.2 (1:1 EtOAc: petroleum ether); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 90°C, 400 MHz) δ\textsubscript{H} 8.60 (1H, s, oxazolyl), 7.28 (5H, m, aryl), 5.21 (1H, m, CHCH\textsubscript{2}, oxazolinyI), 5.10 (1H, d, J\textsubscript{H}\textsubscript{A}=12.7, CHPh), 4.99 (1H, d, J\textsubscript{H}\textsubscript{B}=12.8, CHPh), 4.30 (1H, dd, J= 6.5, 9.3 Hz, CHH, oxazolidinyl), 4.07 (1H, dd, J= 2.6, 9.3 Hz, CHH oxazolidinyl), 3.81 (3H, s, OCH\textsubscript{3}), 1.64 (3H, s, CH\textsubscript{3}), 1.53 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 90°C, 100 MHz) δ\textsubscript{C} 162.98 (H\textsubscript{3}CO-C=O), 160.61 (PhCH\textsubscript{2}O-C=O), 151.67 (s, C=N, oxazolyl), 144.85 (d, C=CH, oxazolyl), 135.93 (s, C=CH, oxazolyl), 132.31 (s, aryl), 127.87 (d, aryl), 127.40 (d, aryl), 126.96 (d, aryl), 94.31 (NC(Me)\textsubscript{2}O), 66.70 (OCH\textsubscript{2}Ph), 66.00 (OCH\textsubscript{2}CH, oxazolidinyl), 54.12 (OCH\textsubscript{2}CH, oxazolidinyl), 51.24 (OCH\textsubscript{3}), 25.19 (CH\textsubscript{3}), 23.68 (CH\textsubscript{3}); HRMS calcd for C\textsubscript{18}H\textsubscript{20}N\textsubscript{2}O\textsubscript{6} [M+Na] 383.1219. Found 383.1212.
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2-(1-Benzylxycarbonylamino-2-hydroxy-ethyl) oxazole-4-carboxylic acid methyl ester (204)

To a stirred solution of oxazolidine 198 (0.82 g, 2.24 mmol) in methanol (30 mL) was added p-toluenesulfonic acid (0.43 g, 2.24 mmol). The reaction was heated at reflux for 2.5 h. The mixture was then evaporated and purified by column chromatography (1:4 ethyl acetate: 40-60 °C petroleum ether) to give 204 (0.73 g, quantitative) as a white solid, mp 101-102 °C; [α]D 23 = -14 ° (c=1.0, CH2Cl2); IR (thin film) (νmax) 3402, 1720, 1585, 1259, 1111, 1028, 912 cm⁻¹; Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 90 °C, 400 MHz) δH 8.43 (1H, s, oxazolyl), 7.24 (1H, m, NH), 7.12 (5H, m, aryl), 4.89 (2H, m, CH₂Ph), 4.61 (1H, m, CHCH₂OH), 3.60 (2H, m, CH₂OH), 3.57 (1H, m, OCH₃); ¹³C NMR (DMSO-d₆, 90 °C, 100 MHz) δC 163.93 (CH₃OC=O), 161.44 (CH₂OC=O), 156.09 (s, oxazolyl), 145.44 (s, aryl), 137.26 (d, oxazolyl), 132.88 (s, oxazolyl), 128.61 (d, aryl), 128.06 (d, aryl), 127.90 (d, aryl), 66.18 (CH₂OH), 62.30 (CH₃OC=O), 52.31 (CHCH₂OH), 51.87 (OCH₃); LRMS m/z (EI) 320 (M⁺, 26%), 290 (13%), 245 (6%), 213 (3%), 199 (22%), 91 (100%): HRMS calcd for C₁₅H₁₆N₂O₆ [M] 320.1008. Found 320.1009. Anal. Calcd for C₁₅H₁₆N₂O₆: C, 56.25; H, 5.04; N, 8.75. Found: C, 55.85; H, 5.18; N, 8.47.
4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2,2-dimethyloxazolidine-
tris oxazolyl carboxylic acid (105)\(^{14}\)

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

To an evacuated solution of carbamate 204 (2.23 g, 6.96 mmol) in methanol (50 mL) was added 10% palladium-on-carbon (0.223 g, 0.69 mmol). The mixture was evacuated and hydrogen was admitted. The mixture was stirred for 17 h, then evacuated and finally air admitted. The mixture was filtered through a pad of celite and the filtrate was evaporated to give 105 (1.2 g, 92 %) as a brown oil; Rf baseline (EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\)\(_H\) 8.25 (1H, s, oxazolyl), 4.32 (1H, m, CHCH\(_2\)), 4.02 (2H, m, CH\(_2\)OH), 3.91 (1H, s, OCH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\)\(_C\) 162.54 (H\(_3\)CO-C=O), 161.52 (s, C=N, oxazolyl), 144.40 (d, C=CH, oxazolyl), 133.04 (s, C=CH, oxazolyl), 63.87 (CH\(_2\)OH), 52.29 (CH\(_2\)OH), 51.42 (OCH\(_3\)): HRMS calcd for C\(_7\)H\(_{10}\)N\(_2\)O\(_4\) [M+Na] 209.0538. Found 209.0531.

4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2,2-dimethyloxazolidine-
oxazole carboxylic acid (197)\(^{14}\)

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

To a stirred solution of ester 198 (0.60 g, 1.66 mmol) in THF: water (8:1 mL) was added lithium hydroxide (0.08 g, 1.99 mmol) in water (2 mL). The mixture was stirred for 17 h at reflux. The mixture was evaporated and the residue was acidified to pH 1. Ethyl acetate (20 mL) was added to the mixture was extracted with water (20 mL). The organic layer was dried over MgSO\(_4\), filtered and evaporated to give 197 (0.46 g, 81 %) as white platelets, mp 130-133 °C; Rf 0.2 (EtOAc); (DMSO, 90 °C, 400 MHz) \(^1\)H NMR \(\delta\)\(_H\) 8.50 (1H, s, oxazolyl), 7.21
(5H, m, aryl), 5.20 (1H, m, CHCH₂, oxazolidinyl), 5.11 (1H, d, J₉₁₁ = 12.4 Hz, CH₂Ph), 5.01 (1H, d, J₉₁₂ = 12.8 Hz, CH₂Ph), 4.28 (1H, dd, J = 6.5, 9.3 Hz, CHH, oxazolidinyl), 4.16 (1H, dd, J = 2.6, 9.3 Hz, CHH oxazolidinyl), 1.65 (1H, s, CH₃), 1.47 (1H, s, CH₃), 1³C NMR (DMSO, 90°C, 100 MHz) δ: 163.42 (HO-C=O), 162.03 (PhCH₂O-C=O), 151.91 (s, C=N, oxazolyl), 145.10 (d, C=CH, oxazolyl), 136.70 (s, C=CH, oxazolyl), 134.12 (s, aryl), 128.57 (d, aryl), 128.07 (d, aryl), 127.63 (s, aryl), 95.00 (NC(Me)₂O), 67.46 (OCH₂Ph), 66.68 (OCH₂CH, oxazolidinyl), 54.89 (OCH₃), 25.92 (CH₃), 24.42 (CH₃): HRMS calcd for C₁₇H₁₈N₂O₈ [M+H] 347.1243. Found 347.1238.

**Chlorotris(dimethylamino)phosphonium hexafluorophosphate(V) (241)**

\[
\begin{align*}
\text{Cl} & \quad \text{N-P'N} \\
\text{N} & \quad \text{PF}_6
\end{align*}
\]

Potassium hexafluorophosphonate (9.25 g, 50 mmol) was dispersed in acetonitrile (250 mL). Oxalyl chloride (4.3 mL, 50 mmol) and DMF (2.5 mL, 30 mmol) were added under inert atmosphere. To the mixture was added HMPA (8.75 mL, 50 mmol) at 0 °C dropwise. The mixture was left to stir for 4 h at 20 °C. The mixture was filtered and the filtrate was evaporated to give 241 (10.9 g, 64%) as a white solid, mp 109 °C.

**(1H-benzo[d][1,2,3]triazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate (242)**

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{N} & \quad \text{P'N} \\
\text{N} & \quad \text{PF}_6
\end{align*}
\]

To a suspension of phosphate 241 (10.9 g, 31.8 mmol) in acetone (150 mL) HOBT (4.30 g, 31.8 mmol) and triethylamine (4.07 mL, 31.8 mmol) was added. The mixture was stirred for 1 h and then filtered. The filtrate was evaporated to give 242 (11 g, 78%) as a white solid mp 137-138 °C.
2-(1-Amino-2-hydroxy-ethyl)-oxazole-4-carboxylic acid methyl ester (Hydroxy-1-methoxycarbonyl ethyl carbomyl)-2, 2-dimethoxazolidine-oxazole (196)\textsuperscript{14}

To a solution of acid 197 (1.0 g, 2.87 mmol) in DMF (250 mL) was added BOP (1.5 g, 3.4 mmol), DIEA (1.2 mL, 6.60 mmol) and HOBT (0.43 g, 3.51 mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of amine 105 (0.45 g, 2.41 mmol) in DMF (20 mL), pre-cooled at -62 °C, was added to the mixture, dropwise via a cannula. The mixture was left to stir for 72 h at -62 °C; then ethyl acetate (150 mL) was added and this mixture was extracted with water (3x 150 mL). The aqueous layer was extracted with ethyl acetate (3x 150 mL). The organic layer was dried over MgSO\textsubscript{4}, filtered and evaporated to give 196 (0.10 g, 77%) as a colourless oil used in the next step without purification. Rf 0.2 (1:2 EtOAc: petroleum ether); (DMSO-d\textsubscript{6}, 80 °C, 400 MHz) \textsuperscript{1}H NMR δ\textsubscript{H} 8.67 (1H, s, oxazolyl), 8.47 (1H, s, oxazolyl), 8.09 (1H, d, J=8 Hz, NH), 7.25 (5H, m, aryl), 5.22 (2H, m, CH\textsubscript{2}OH), 5.20 (1H, m, CHCH\textsubscript{2}OH), 5.03 (2H, m, CH\textsubscript{2}O-C=O), 4.31 (1H, dd, J=6.8, 9.6 Hz, OCH\textsubscript{2}H oxazolidinyl), 4.12 (1H, dd, J=6.4, 9.6 Hz, OCH\textsubscript{2}H oxazolidinyl), 4.10 (1H, t, J=5.2 Hz, OH), 3.82 (3H, s, OCH\textsubscript{3}), 1.67 (3H, s, CH\textsubscript{3}), 1.55 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 90 °C, 100 MHz); δ\textsubscript{C} 163.00 (CH\textsubscript{2}O-C=O), 162.69 (C=O, Cbz), 160.99 (HN-C=O), 159.65 (s), 145.12 (s), 145.09 (s), 142.11 (s), 136.22 (d), 135.63 (d), 132.50 (s), 128.191 (d), 127.69 (d), 127.24 (d), 94.64 (N(C(Me\textsubscript{3}))O), 67.03 (CH\textsubscript{2}OCO, Cbz), 66.35 (OCH\textsubscript{2}CH, oxazolidine), 61.72 (OCH\textsubscript{2}CH, oxazolidine), 54.50 (OCH\textsubscript{3}), 51.50 (CH\textsubscript{2}OH), 49.50 (NCHCH\textsubscript{2}OH), 25.57 (CH\textsubscript{3}), 24.01 (CH\textsubscript{3}); HRMS calcd for C\textsubscript{30}H\textsubscript{28}N\textsubscript{6}O\textsubscript{11} [M+H] 515.1778. Found 515.1801.
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**Methyl 2-(1-amino-2-(tert-butyldimethylsilyloxy) ethyl) oxazole-4-carboxylate (205)**

![Chemical Structure]

Imidazole (0.18 g, 2.68 mmol), TBSCI (2.5 mL, 1.35 mmol) and DMAP (0.70 mg, 0.054 mmol) were added sequentially to a suspension of amine 105 (0.1 g, 0.54 mmol) in dichloromethane (5 mL) at ambient temperature. Then the mixture was washed with water (2 mL), the organic layer was dried with MgSO₄, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give 205 (0.074 g, 46%) as a yellow oil; [α]²³ = -23 °(c=0.8, CHCl₃); IR (thin film) (νmax) 3299, 2953, 1745, 1664, 1585, 1112, 1002 cm⁻¹; Rf 0.2 (EtOAc); ¹H NMR (CDCl₃, 300 MHz): δH 8.20 (1H, s, oxazolyl), 6.65 (1H, d, J= 6 Hz, NH₂), 5.36 (1H, m, H₂NCH), 4.02 (2H, m, OCH₂), 3.95 (1H, s, OCH₃), 0.78 (15H, s, CH₃); ¹³C NMR (CDCl₃, 75 MHz) δC 162.81 (C=O), 160.59 (s, oxazolyl), 144.27 (s, oxazolyl), 133.39 (d, oxazolyl), 63.98 (CH₂OH), 52.27 (NCH₂CH₂), 48.27 (OCH₃), 25.61 (CH₃), 18.08 (Si-CH₃); LRMS m/z +(Cl) 329 (100%), 301 (M+H, 45%), 267 (2%), 197 (74%), 165 (4%), 133 (6%), 97 (44%), 85 (41%): HRMS calcd for C₁₃H₂₄N₂O₄Si [M+H]⁺ 301.1578. Found 301.1572.
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**Methyl 2-(1-amino-2-(triethylsilyloxy) ethyl) oxazole-4-carboxylate (206)**

![Chemical Structure]

To a suspension of amine 105 (0.10 g, 0.54 mmol) in dichloromethane (5 mL) was added imidazole (0.18 g, 2.68 mmol), TESCl (2.3 mL, 1.35 mmol) and DMAP (0.7 mg, 0.054 mmol). Then the mixture was washed with water (2 mL) and the organic layer was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give 206 (0.08 g, 50%) as a yellow oil; [α_D]²⁺ = -26° (c= 0.95, CHCl₃); IR (thin film) (ν_max) 3293, 2924, 1736, 1664 cm⁻¹; Rf 0.2 (EtOAc); H¹ NMR (CDCl₃, 300 MHz): δ_H 8.19 (1H, s, oxazolyl), 6.73 (1H, d, J=9 Hz, NH₂), 5.37 (1H, m, CH), 4.13 (2H, m, OCH₂), 3.95 (1H, s, OCH₃) 0.91 (6H, m, SiCH₂), 0.53 (9H, t, J=15 Hz, SiCH₂CH₃); ¹³C NMR (CDCl₃, 75 MHz) δ_C 162.83 (C=O), 160.67 (s), 144.30 (s), 133.38 (d), 63.62 (CH₂OH), 52.27 (CHCH₂OH), 48.23 (OCH₃), 6.49 (CH₃), 4.11 (Si-CH₂); LRMS m/z + (Cl) 329 (62%), 301 (M+H, 24%), 281 (11%), 197 (100%), 115 (32%), 85 (92%); HRMS calcd for C₁₃H₂₄N₂O₄Si [M+H]⁺ 301.1578. Found 301.1568.
To a stirred solution of amine 105 (0.10 g, 0.53 mmol) in DMF (2 mL) was added triethylamine (0.14 mL, 1.07 mmol) and DMAP (0.013 g, 0.11 mmol). The mixture was stirred at 0 °C and trimethylsilyl chloride (0.07 mL, 0.54 mmol) was added dropwise. After stirring for 17 h at ambient temperature, ethyl acetate (10 mL) was added and the mixture was washed with water (3x10 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 207 (0.08 g, 60%) as a yellow oil; [α]D²¹ = -25 °(c=0.91, CHCl₃); IR (thin film) (νmax) 3024, 2916, 1735, 1668, 1448, 1326, 1060 cm⁻¹; Rf 0.3 (EtOAc);¹H NMR (CDCl₃, 300 MHz): δH 8.19 (1H, s, oxazolyl), 4.01 (1H, m, CHCH₂Si), 3.89 (2H, s, OCH₂Si), 3.89 (1H, s, OCH₃), 0.055 (9H, s, SiCH₃);¹³C NMR (CDCl₃, 75 MHz) δC 166.37 (C=O), 161.65 (s), 144.19 (s), 133.19 (d), 65.36 (CH₂OSi), 64.68 (CHCH₂), 52.20 (OCH₃), -0.66 (CH₃); LRMS m/z +(Cl) 259 (M+H, 9%), 243 (3%), 197 (8%), 187 (100%), 169 (28%), 155 (94%), 128 (91%), 96 (46%); HRMS calcd for C₁₀H₁₈N₂O₄Si [M+H]⁺ 259.1114. Found 259.1116.
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4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2,2-dimethyl oxazolidine oxazole-oxazolinyl-oxazolyl methyl ester 195

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

To a stirred solution of amide 196 (0.61 g, 1.19 mmol) in anhydrous dichloromethane (20 mL) at -78°C was added DAST (0.19 mL, 1.42 mmol) dropwise. The mixture was stirred for 2.5 h and then potassium carbonate (0.25 g, 1.77 mmol) was added. After 17 h water (30 mL) was added to the reaction and the mixture was extracted with dichloromethane (30 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 195 (0.42 g, 72 %) as an orange oil; [α_D]²³ = -11° (c=1.0, CH₂Cl₂); IR (thin film) (ν_max) 3136, 1714, 1643, 1406, 1348, 1095, 752 cm⁻¹; RF 0.3 (1:1 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 90 °C, 400 MHz): δ_H 8.68 (1H, s, oxazolyl), 8.48 (1H, s, oxazolyl), 7.28 (5H, m, aryl), 5.56 (1H, m, CHCH₂, oxazolinyl), 5.21 (1H, m, CHCH₂, oxazolidinyl), 5.10 (1H, d, J_HA =12.6 Hz, CHHPh), 4.99 (1H, d, J_HB =12.6 Hz, CHHPh), 4.73 (2H, m, CHCH₂, oxazolinyl), 4.31 (1H, m, CHH, oxazolidinyl), 4.09 (1H, m, oxazolidinyl), 3.82 (3H, s, OCH₃), 1.68 (3H, s, CH₃), 1.57 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 90 °C, 100 MHz) δ_c 163.12 (PhO-C=O), 162.84 (H₂CO-C=O), 160.45 (s), 158.66 (s), 151.08 (s), 145.31 (d), 145.27 (d), 142.41 (s), 135.83 (s), 132.17 (s), 129.23 (d), 127.76 (d), 127.24 (d), 126.82 (d), 94.19 (NC(Me₃)₂O), 69.82 (t, CH₂CH, oxazolinyl), 66.62 (CH₂OCO), 65.88 (OCH₂CH₂), 65.88 (d, CH₂CH, oxazolinyl), 62.68 (OCH₂CH₂, oxazolidinyl), 51.42 (OCH₃), 25.10 (CH₃), 23.64 (CH₃); LRMS m/z (El) 496 (M⁺, 11%), 437 (9%), 354 (6%), 275 (25%), 215 (36%), 153 (43%), 121 (100%); HRMS calcd for C₂₅H₂₄N₄O₈ [M]⁺ 496.1594. Found 496.1583.
4-(2-Hydroxy-1-methoxy carbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-tris oxazolyl methyl ester (194)\textsuperscript{14}

To a stirred solution of oxazoline 195 (0.38 g, 0.77 mmol) in anhydrous dichloromethane (10 mL) was added DBU (0.23 mL, 1.53 mmol). After 10 min bromotrichloromethane (0.08 mL, 0.84 mmol) was added dropwise at -10 °C. The mixture was stirred for 17 h at 20 °C. The mixture was evaporated and purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give 194 (0.26 g, 69 %) as a white solid, mp 206-208 °C, lit.\textsuperscript{14} mp 208 °C; Rf 0.5 (EtOAc); H\textsuperscript{1} NMR (DMSO-d\textsubscript{6}, 90°C, 400 MHz): δ\textsubscript{H} 8.91 (1H, s, oxazolyl), 8.83 (1H, s, oxazolyl), 8.76 (1H, s, oxazolyl), 7.25 (5H, m, aryl), 5.28 (1H, m, CHCH\textsubscript{2}, oxazolidinyl), 5.12 (1H, d, J\textsubscript{HA} =12.6 Hz, CHHPh), 5.02 (1H, d, J\textsubscript{HB} =12.9 Hz, CHHPh), 4.33 (1H, dd, J=6.5, 9.2 Hz, CHH, oxazolidinyl), 4.15 (1H, dd, J=2.6, 9.3 Hz, CHH, oxazolidinyl), 3.86 (1H, s, OCH\textsubscript{3}), 1.69 (3H, s, CH\textsubscript{3}), 1.56 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 90°C, 100 MHz) δ\textsubscript{C} 163.55 (H\textsubscript{3}CO-C=O), 160.32 (PhCH\textsubscript{2}C=O), 155.17 (s), 154.51 (s), 151.06 (s), 144.74 (d), 140.42 (d), 140.22 (d), 135.84 (s), 133.09 (s), 129.65 (s), 128.74 (s), 127.70 (d), 127.21 (d), 126.84 (d), 94.22 (NC(Me\textsubscript{2})O), 66.61 (CH\textsubscript{2}Ph, Cbz), 65.88 (OCH\textsubscript{2}CH\textsubscript{2}oxazolidine), 52.13 (OCH\textsubscript{3}), 51.23 (OCH\textsubscript{2}CH\textsubscript{2} oxazolidine), 25.09 (CH\textsubscript{3}), 23.61 (CH\textsubscript{3}); HRMS calcd for C\textsubscript{24}H\textsubscript{22}N\textsubscript{4}O\textsubscript{8} [M+H] 495.1515. Found 495.1506.
4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-
tris oxazolyl carboxylic acid (193)\textsuperscript{14}

To a stirred solution of the ester 194 (0.27 g, 0.55 mmol) in THF (10 mL) was
added lithium hydroxide (0.027 mL, 0.66 mmol) in water (1.5 mL). The mixture
was heated at reflux for 16 h. Then the mixture was evaporated and acidified to
pH 1 using 3M HCl. Ethyl acetate (20 mL) was added and the organic layer was
dried over MgSO\textsubscript{4}, filtered and evaporated to give 193 (0.26 g, quantitative) as
white powder, mp 219 °C (dec). Rf baseline (EtOAc); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 90
°C, 400 MHz) \delta\textsubscript{H} 8.88 (1H, s, oxazolyl), 8.71 (1H, s, oxazolyl), 8.68 (1H, s,
oxazolyl), 7.25 (5H, m, aryl), 5.28 (1H, m, CH\textsubscript{2}, oxazolidinyl), 5.09 (1H, d,
\textit{J}_{\text{HH}} =16.4 \text{ Hz}, \text{CHHPh}), 5.02 (1H, d, \textit{J}_{\text{HH}} =12.6 \text{ Hz}, \text{CHHPh}), 4.34 (1H, dd, \textit{J} =6.5, 9.3 \text{ Hz}, \text{CHH},
oxazolidinyl), 4.15 (1H, dd, \textit{J}=2.6, 9.3 Hz, \text{CHH},
oxazolidinyl), 1.69 (3H, s, CH\textsubscript{3}), 1.56 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 90°C,
100 MHz) \delta\textsubscript{C} 168.90 (HO-\text{C}=O), 163.45 (PhCH\textsubscript{2}OC=O), 160.96 (s), 155.03 (s),
151.04 (s), 144.15 (d), 144.14 (d), 140.30 (d), 139.94 (s), 139.92 (s), 135.76 (s),
129.72 (s), 128.73 (d), 127.62 (d), 126.76 (d), 94.14 (NC(Me\textsubscript{2})O), 66.53
(OCH\textsubscript{2}Ph, Cbz), 65.80 (OCH2CH\textsubscript{3}, oxazolidinyl), 54.02 (OCH2CH\textsubscript{3}, oxazolidinyl),
25.06 (CH\textsubscript{3}), 25.02 (CH\textsubscript{3}): HRMS calcd for C\textsubscript{23}H\textsubscript{20}N\textsubscript{4}O\textsubscript{8} [M+H] 479.1202. Found
479.1224.
4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2, 2-dimethyloxazolidine- tris oxazolyl benzyl ester (208)

To a stirred solution of acid 193 (0.20 g, 0.42 mmol) in DMF (15 mL) was added triethylamine (1.3 mL, 10 mmol) and stirred at 20 °C for 10 min. The temperature was lowered to 0 °C and benzyl bromide (0.9 mL, 7.6 mmol) was added dropwise to the mixture and stirred to ambient temperature for 17 h. Then ethyl acetate (20 mL) was added and extracted with water (3x 20 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 208 (0.21 g, 90 %) as white crystals, m.p 160-163 °C. [αD]^{23} = -79.5 ° (c=1.0, CH₂Cl₂); IR (KBr) (ν_{max}) 3136, 1710, 1643, 1406, 1095, 698 cm⁻¹; Rf 0.2 (1:1 EtOAc: petroleum ether); ¹H NMR (DMSO-d₆, 90°C, 400 MHz): δ_H 8.92 (1H, s, oxazolyl), 8.88 (1H, s, oxazolyl), 8.67 (1H, s, oxazolyl), 7.42 (5H, m, aryl), 7.23 (5H, m, aryl), 5.37 (2H, s, CH₂OBn), 5.28 (1H, m, CHCH₂, oxazolidinyl), 5.12 (1H, d, J_HA = 12.8 Hz, CHHPh), 5.00 (1H, d, J_HW = 12.5 Hz, CHHPh), 4.33 (1H, dd, J = 6.5, 9.3 Hz, CHH, oxazolidinyl), 4.15 (1H, dd, J = 2.6, 9.3 Hz, CHH, oxazolidinyl), 1.69 (3H, s, CH₃), 1.56 (3H, s, CH₃); ¹³C NMR (DMSO-d₆, 90°C, 100 MHz) δ_C 163.97 (PhCH₂-C=O), 160.21 (Bn-O-C=O), 155.60 (s), 155.01 (s), 145.46 (s), 140.87 (d), 140.83 (d), 140.70 (s), 140.66, 136.26, 135.78, 133.56 (s), 130.10 (s), 129.22 (d, aryl), 128.37 (d, aryl), 128.13, 127.97 (d, aryl), 127.63 (d, aryl), 127.26 (d, aryl), 94.65 (NC(Me₂)O), 67.03 (O=COCH₃Ph), 66.30 (CH₂OBn), 66.16 (OCH₂CH₂oxazolidinyl), 66.09 (OCH₂CH, oxazolidinyl), 25.53 (CH₃), 24.03 (CH₃): Anal. Calcd for C₃₀H₂₆N₄O₈: C, 63.15; H, 4.59; N, 9.82. Found: C, 62.71; H, 4.69; N, 9.60.
4-(2-Hydroxy-1-methoxycarbonyl ethyl carbomyl)-tris oxazolyl benzyl ester (209)

To a solution of ester 208 (0.10 g, 0.17 mmol) in dichloromethane/methanol (25/5 mL) was added p-toluenesulfonic acid (0.03 g, 0.17 mmol) and stirred at reflux for 3 h. The mixture was evaporated and purified by column chromatography (1:3 ethyl acetate: petroleum spirit) to give 209 (0.09 g, 36%) as a white foam; $[\alpha_D]^{23} = -65.3$ (c= 0.82, CH$_2$Cl$_2$); IR (thin film) ($\nu_{max}$) 3296, 1724, 1699, 1544, 1153, 1109, 729 cm$^{-1}$; Rf 0.2 (EtOAc); $^1$H NMR (DMSO-d$_6$, 90°C, 400 MHz): $\delta$H 8.90 (1H, s, oxazolyl), 8.88 (1H, s, oxazolyl), 8.82 (1H, s, oxazolyl), 7.37 (10 H, m, aryl), 5.37 (2H, s, CH$_2$OBn), 5.07 (2H, m, CH$_2$OPh), 4.88 (1H, m, CHCH$_2$OH), 3.83 (2H, m, CHCH$_2$OH); $^{13}$C NMR (DMSO-d$_6$, 90°C, 100 MHz) $\delta$C 163.75 (PhCH$_2$-C=O), 159.79 (BnO-C=O), 155.36 (s), 154.61 (s), 150.70 (s), 145.08 (d), 145.03 (d), 140.25 (d), 140.22 (s), 136.44 (s), 135.38 (s), 133.13 (s), 129.64 (s), 127.99 (d, aryl), 127.96 (d), 127.79 (d), 127.78 (d), 127.68 (d), 127.66 (d), 66.08 (O=COCH$_2$Ph), 65.77 (CH$_2$Ph), 61.47 (CHCH$_2$OH), 51.95 (CHCH$_2$OH): LRMS m/z (EI) 530 (M$^+$, 74%), 337 (81%), 320 (19%), 301 (11%), 219 (18%), 105 (100%): HRMS calcld for C$_{30}$H$_{26}$N$_4$O$_8$ [M] 530.1437. Found 530.1466.
Amino alcohol-tris oxazolyl-methyl ester 179

A solution of oxazolidine 194 (0.06 g, 0.12 mmol) in methanol (10 mL) and dichloromethane (20 mL) was evacuated was then 10% palladium-on-carbon (6 mg, 0.001 mmol) was added. The mixture was evacuated and hydrogen was admitted. The mixture was stirred for 17 h and then evacuated. The mixture was then filtered through a pad of celite and the filtrate was evaporated to give 179 (0.030 g, 74%) as an orange oil, \([\alpha_D]^{23}_{D}^o = -14.2^o\ (c=1.0, \text{CH}_2\text{Cl}_2)\); IR (thin film) \((\nu_{\text{max}})\) 3425, 1651, 1469, 1124, 617 cm\(^{-1}\); RF baseline (EtOAc); \(^1\)H NMR (DMSO-d6, 500 MHz) \(\delta\)H 9.05 (1H, s, oxazolyl), 9.03 (1H, s, oxazolyl), 8.91 (1H, s, oxazolyl), 4.02 (1H, m, CHCH_3OH), 3.83 (3H, s, OCH_3) 3.66 (2H, m, CH_2OH); \(^1^3\)C NMR (DMSO-d6, 125 MHz) \(\delta\)C 167.76 (C=O), 160.87 (s, oxazolyl), 155.98 (s, oxazolyl), 154.97 (s, oxazolyl), 145.66 (d, oxazolyl), 140.83 (d, oxazolyl), 140.70 (d, oxazolyl), 133.28 (s, oxazolyl), 129.90 (s, oxazolyl), 128.62 (s, oxazolyl), 64.68 (CH_2OH), 51.98 (CHCH_2OH), 51.95 (OCH_3); LRMS m/z + (Cl) 321 (M+H, 6%), 307 (12%), 289 (12%), 273 (7%), 244 (3%), 154 (100%); HRMS calcd for \(\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_6\) [M+H] 321.0835. Found 321.0827.
2-(1-Amino-2-hydroxy-ethyl)-trisoxazole-4-carboxylic acid methyl ester
(2-hydroxy-1-methoxycarbonyl ethyl carbomyl)-2,2-dimethyloxazolidine-
trisoxazole (215)

To a solution of the acid 193 (0.19 g, 0.40 mmol) in DMF (80 mL) was added
BOP (0.16 g, 0.44 mmol), DIEA (0.17 mL, 0.2 mmol) and HOBT (0.059 g, 0.44
mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of amine
179 (0.13 g, 0.04 mmol) in DMF (10 mL) pre-cooled at -62 °C, was added to the
mixture dropwise via a cannula. The mixture was left to stir for 96 h at -62 °C,
then ethyl acetate (50 mL) was added and this mixture was washed with water
(3x 50 mL). The organic layer was dried over MgSO₄, filtered and evaporated.
The residue was purified via column chromatography (ethyl acetate) to give 215
(0.12 g, 38%) as a white solid, mp 212 °C (dec); IR (νmax) (KBr) 3456, 1724,
1658, 1350, 1099 cm⁻¹; Rf 0.1 (EtOAc); (DMSO-d₆, 80 °C, 400 MHz) ¹H NMR
δH 8.91 (1H, s, oxazolyl), 8.87 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.77 (1H,
s, oxazolyl), 8.72 (1H, s, oxazolyl), 8.67 (1H, s, oxazolyl), 8.51 (1H, d, J= 8 Hz,
N-H), 7.24 (5H, m, aryl), 5.34 (1H, m, NCH₂CH₂OH), 5.03 (2H, m, CH₂Ph),
5.00 (2H, m, NCH₂CH₂OH), 4.33 (1H, m, oxazolidinyl), 4.15 (1H, m, oxazolidinyl), 3.86 (3H, s, OCH₃), 1.68 (3H, s, CH₃), 1.56 (3H, s, CH₃); ¹³C
NMR (DMSO-d₆, 80 °C, 400 MHz); δc 163.56 (O-C=O), 161.67 (C=O), 160.36
(N-C=O) 155.34 (s), 155.18 (s), 154.25 (s), 151.11 (s), 150.45 (s), 149.72 (s),
144.79 (d), 142.70 (d), 140.77 (d), 140.46 (d), 140.24 (d), 140.07 (d), 135.87 (s),
133.12 (s), 129.87 (s), 129.69 (s), 128.83 (s), 128.69 (d), 127.75 (d), 127.26 (d),
126.87 (d), 94.23 (NC(Me₂)₂O), 66.63 (CH₂OCO, Cbz), 65.89 (OCH₂CH₂,
oxazolidine), 58.70 (OCH₂CH₂, oxazolidine), 54.10(OCH₃), 51.27(CH₂OH),
49.57(NCH₂CH₂O), 25.14 (CH₃), 23.66 (CH₃); LRMS m/z +(FAB) 783 (M+H,
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9%), 675 (5%), 481 (11%), 381 (2%), 338 (100%), 281 (4%), 207 (3%): HRMS calcd for C_{36}H_{30}N_{6}O_{13} [M+H] 783.2010. Found 783.1996.

4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamyl)-2, 2-dimethyloxazolidine-hepta-oaxazoly benzyl ester 216

To a stirred solution of amide 215 (0.11 g, 0.017 mmol) in anhydrous dichloromethane (10 mL) at -78 °C was added DAST (0.024 mL, 0.018 mmol) dropwise. The mixture was left to stir for 5 min and the solid was filtered and dried to give 216 (0.08 g, 80%) as a yellow solid, mp 289 °C (dec); IR (ν_{max}) (KBr) 3103, 1720, 1678, 1103, 985 cm^{-1}; H NMR (DMSO-d_{6}, 120 °C, 400 MHz) δ_{H} 8.86 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.83 (1H, s, oxazolyl), 8.77 (1H, s, oxazolyl), 8.73 (1H, s, oxazolyl), 8.69 (1H, s, oxazolyl), 7.24 (5H, m, aryl), 5.67 (1H, m, NCH, oxazolidinyl), 5.28 (1H, m, CH_{2}CH, oxazolinyl), 5.12 (1H, d, J=12.7 Hz, OCHHPh), 5.01 (1H, d, J 12.7 Hz, OCHHPh), 4.83 (2H, m, CH_{2}, oxazolinyl), 4.33 (1H, dd, J=6.5, 9.3 Hz, OCHH oxazolinyl), 4.13 (1H, dd, J=2.7, 9.3 Hz, OCHH oxazolinyl), 3.86 (3H, s, OCH_{3}), 1.72 (3H, s, CH_{3}), 1.57 (3H, s, CH_{3}); LRMS m/z +(FAB) 787 (M+Na, 27%), 705 (4%), 638 (3%), 484 (23%), 435 (7%), 329 (100%), 301 (15%); HRMS calcd for C_{36}H_{30}N_{6}O_{13} [M+Na] 787.1724. Found 787.1728.
2-(1-Amino-2-hydroxy-ethyl)-oxazole-4-carboxylic acid methyl ester
(hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2, 2-dimethyloxazolidine-
trisoxazole (224)

To a solution of the acid 193 (0.22 g, 0.46 mmol) in DMF (80 mL) was added BOP (0.22g, 0.50 mmol), DIEA (0.19 mL, 1.05 mmol) and HOBT (0.07 g, 0.52 mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of the amine 105 (0.07 g, 0.38 mmol) in DMF (10 mL), pre-cooled at -62 °C was added to the reaction, dropwise via a cannula. The mixture was left to stir for 96 h at -62 °C, then ethyl acetate (50 mL) was added and this mixture was extracted with water (50 mL x3). The organic layer was dried over MgSO4, filtered and evaporated to leave a residue which was purified via column chromatography (ethyl acetate) to give 224 (0.10 g, 40 %) as a colourless oil, [α]D = -60.6 (c=0.82, CH2Cl2); IR (νmax) (thin film) 3446, 1720, 1653, 1598, 1409, 1350, 1097 cm⁻¹; Rf 0.1 (EtOAc); 1H NMR (DMSO-d6, 90 °C, 400 MHz); δH 8.91 (1H, s, oxazolyl H), 8.77 (1H, s, oxazolyl H), 8.70 (1H, s, oxazolyl H), 8.67 (1H, s, oxazolyl H), 8.36 (1H, d, J= 7.6 Hz, N-H), 7.25 (5H, m, aryl), 5.25 (2H, m, CH2OH), 5.20 (1H, m, NCH2CH2OH), 5.13 (1H, d, JHHA=12.7 Hz, CHHPh), 5.05 (1H, d, JHB =18.2 Hz, CHHPh), 4.34 (1H, dd, J =6.8, 9.6 Hz, OCHH oxazolidinyl), 4.15 (1H, dd, J=6.4, 9.6 Hz, OCHH oxazolidinyl), 3.93 (1H, t, J =5.2Hz, O-H), 3.82 (3H, s, OCH3), 1.69 (3H, s, CH3), 1.54 (3H, s, CH3); 13C NMR (DMSO, 90 °C, 100 MHz); δc 163.99 (O-C=O), 162.96 (C=O), 160.99 (s), 159.56 (s), 155.60 (s),
154.39 (s), 145.20 (d), 142.33 (d), 140.86 (d), 140.62 (d), 136.57 (s), 136.26 (s), 132.49 (s), 130.10 (s), 129.20 (d), 128.14 (d), 127.64 (d), 127.25 (d), 94.63 (NC(Me₂)O), 67.05 (CH₂OCO, Cbz), 66.33 (OCH₂CH, oxazolidine), 54.45 (OCH₂CH, oxazolidine), 51.46 (OCH₃), 49.57 (CH₂OH), 42.63 (NCH₂CH₂O), 25.55 (CH₃), 24.05 (CH₃); LRMS m/z +(FAB) 671 (M+Na, 8%), 646 (7%), 592 (2%), 326 (15%), 281 (16%), 199 (19%), 176 (100%), 147 (48%); HRMS calcld for C₃₀H₂₈N₆O₁₁ [M+Na] 671.1713. Found 671.1699.

4-(2-Hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2,2-dimethyl oxazolidine-tris oxazole-oxazolinyl-oxazolyl methyl ester (223)

To a solution of amide 224 (0.11 g, 0.017 mmol) in anhydrous dichloromethane (10 mL) at -78 °C was added DAST (0.024 mL, 0.018 mmol) dropwise. The mixture was left to stir for 2.5 h and then potassium carbonate (0.03 g, 0.025 mmol) was added. After 17 h, water (10 mL) was added to the mixture and the resulting solution extracted with dichloromethane (10 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 223 (0.09 g, 85%) as a colourless oil, mp 236 °C (dec); IR(νmax) (KBr) 3119, 1720, 1664, 1579, 1409, 1105, 985 cm⁻¹; Rf 0.2 (EtOAc); ¹H NMR (DMSO-d₆, 80 °C 400 MHz) δH 8.90 (1H, s, oxazolyl), 8.77 (1H, s, oxazolyl), 8.75 (1H, s, oxazolyl), 8.73 (1H, s, oxazolyl), 7.25 (5H, m, aryl), 5.60 (1H, m, NCH, oxazolidinyl), 5.27 (1H, m, CH₂CH, oxazolinyl), 5.12 (1H, d, J =12.8 Hz, OCH₂Ph), 4.80 (1H, d, J 12.8 Hz, OCH₂Ph), 4.74 (2H, m, CH₂, oxazolinyl), 4.33 (1H, dd, J =6.5, 9.3 Hz, OCH₂H oxazolinyl), 4.14 (1H, dd, J=2.7, 9.3 Hz, OCH₂H oxazolinyl), 3.82 (3H, s, OCH₃), 1.68 (3H, s, CH₃), 1.57 (3H, s, CH₃); ¹³C NMR (DMSO, 80 °C, 100 MHz) δc 163.92 (O-C=O), 163.22 (C=O), 160.82 (s), 159.02 (s), 155.60 (s),
155.17 (s), 151.49 (s), 145.55 (d), 144.95 (d), 142.70 (d), 140.68 (d), 140.43 (d), 136.23 (s), 132.64 (s), 130.60 (s), 130.16 (s), 129.28 (d), 128.08 (d), 127.58 (d), 127.24 (d), 94.66 (NC(Me)<sub>2</sub>O), 70.35 (t, CH<sub>2</sub>CH, oxazolinyl) 67.02 (CH<sub>2</sub>OCO), 66.32 (OCH<sub>2</sub>CH), 63.21 (t, CH<sub>2</sub>CH, oxazolinyl), 54.41 (OCH<sub>2</sub>CH, oxazolidinyl), 51.46 (OCH<sub>3</sub>), 25.44 (CH<sub>3</sub>), 23.99 (CH<sub>3</sub>); LRMS m/z +(FAB) 653 (M+Na, 8%), 629 (1%), 413 (4%), 360 (56%), 326 (14%), 199 (28%), 176 (100%): HRMS calcd for C<sub>30</sub>H<sub>26</sub>N<sub>6</sub>O<sub>10</sub> [M+Na] 653.1608. Found 653.1620.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-penta-oxazolyl methyl ester (218)

![Chemical structure of 218](image)

To a solution of oxazoline 223 (0.06 g, 0.095 mmol) in anhydrous dichloromethane (30 mL) was added DBU (0.6 mL, 3.9 mmol). After 10 min bromotrichloromethane (0.2 mL, 1.0 mmol) was added dropwise at -10 °C. The mixture was left to stir for 30 min at 20 °C. The precipitate was filtered and washed with dichloromethane to give 218 as a yellow solid (0.04 g, 73%), mp 269 °C (dec); IR (v<sub>max</sub>) (KBr) 3113, 2341, 1717, 1649, 1512, 1406, 1095, 976 cm<sup>-1</sup>; Rf 0.2 (EtOAc); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 313 K, 400 MHz): δ<sub>H</sub> 8.99 (1H, s, oxazolyl), 8.97 (1H, s, oxazolyl), 8.90 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.78 (1H, s, oxazolyl), 7.24 (5H, m, aryl), 5.66 (1H, m, NCH oxazolidine), 5.30 (1H, dd, J=2.7, 6.5 Hz, NCHCH<sub>2</sub>O), 5.14 (1H, d, J=12.7 Hz, CH<sub>2</sub>Ph), 5.02 (1H, d, J=12.7 Hz, CH<sub>2</sub>Ph), 4.34 (1H, dd, J=6.5, 9.3 Hz, OCHH oxazolidine), 4.16 (1H, dd, J=2.7, 9.3 Hz, OCHH oxazolidine), 3.87 (3H, s, OCH<sub>3</sub>), 1.70 (3H, s, CH<sub>3</sub>), 1.57 (3H, s, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 90 °C, 400 MHz) δ<sub>C</sub> 163.35 (O-C=O), 160.09 (C=O), 155.09 (C=N), 154.95 (C=N), 154.87 (C=N), 154.31 (C=N), 150.96 (s), 144.30 (d), 140.12 (d), 140.05 (d), 140.00 (d), 135.68 (s), 133.09 (s), 129.67 (s), 129.62 (s), 129.53 (s), 128.72 (d), 127.46 (d), 126.97 (d),
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126.66 (d), 94.13 (NC(Me₂)O), 66.43 (CH₂OCO), 65.79 (NHCH₂O), 53.96 (NCH CH₂O), 50.89 (OCH₃), 24.92 (CH₃), 23.57 (CH₃). LRMS m/z +(FAB) 651 (M+Na, 18%), 629 (1%), 553 (4%), 510 (3%), 360 (24%), 199 (23%), 176 (100%), 153 (72%): HRMS calcd for C₃₀H₂₄N₆O₁₀ [M+Na] 651.1452. Found 651.1462.

**Benzyl 2-(3-(benzoxycarbonyl)-2,2-dimethoxyoxazolidin-4-yl)oxazoles-4-carboxylate (228)**

![Chemical Structure](image)

To a solution of acid 197 (0.33 g, 0.95 mmol) in DMF (15 mL) was added triethylamine (2.6 mL, 20 mmol); the mixture was stirred at 20 °C for 10 min. The temperature was lowered to 0 °C and benzyl bromide (1.81 mL, 15 mmol) was added dropwise. The mixture was allowed to warm up with stirring from 0 °C to ambient temperature over 17 h. Then ethyl acetate (20 mL) was added and extracted with water (3x 20 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give 228 (0.37 g, 90%) as a colourless oil, [α]D\(^{23}\) = -12 (c=1.0, CHCl₃); IR (thin film) (νₚ₃₅) 3418, 1720, 1585, 1454, 1226, 1068 cm\(^{-1}\); Rf 0.4 (EtOAc); \(^1\)H NMR (DMSO, 90°C, 400 MHz): δH 8.65 (1H, s, oxazolyl), 7.39 (5H, m, aryl), 7.19 (5H, m, aryl), 5.33 (2H, s, CH₂OBn), 5.21 (1H, m, CH oxazolidine), 5.10 (1H, d, J\(_{HA}\) =12.6 Hz, CHHPH), 4.98 (1H, d, J\(_{HB}\) =12.6 Hz, CHHPH), 4.30 (1H, dd, J =6.4, 9.2 Hz, OCHH oxazolidine), 4.09 (1H, dd, J =6.4, 9.2 Hz, CHH oxazolidine), 1.65 (3H, s, CH₃), 1.54 (3H, s, CH₃); \(^{13}\)C NMR (DMSO-d₆, 90 °C, 100 MHz) δC 163.33 (C=O), 160.28 (C=O), 151.46 (s), 145.37 (s), 136.24 (d), 135.89 (s), 132.62 (s), 128.35 (d), 128.13 (d), 128.02 (d), 127.88 (d), 127.64 (d), 127.22 (d), 94.61 (NC(Me₂)O), 67.00 (CH₂OBn), 66.26 (CH₂OCO, Cbz), 65.85 (OCH₂CH, oxazolidine), 54.44 (OCH₂CH, oxazolidine), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%), 25.47 (CH₃), 23.99 (CH₃); LRMS m/z (El) 436 (M⁺, 14%), 421 (11%), 392 (1%)
377 (87%), 239 (24%), 152 (15%), 91 (100%): HRMS calcd for C_{24}H_{24}N_{2}O_{6} [M]^+ 436.1634. Found 436.1642.

**Benzyl2-(1-(benzyl oxycarbonylamino)-2-hydroxyethyl)oxazole-4-carboxylate (229)**

![Structural formula of benzyl2-(1-(benzyl oxycarbonylamino)-2-hydroxyethyl)oxazole-4-carboxylate (229)](image)

To a solution of oxazolidine 228 (0.32 g, 0.73 mmol) in methanol (30 mL) was added p-toluenesulfonic acid (0.14 g, 0.73 mmol). The mixture was heated at reflux for 2.5 h. The mixture was then cooled to 20 °C, evaporated and the residue was purified via column chromatography (1:4 ethyl acetate: petroleum ether) to give 229 (0.15 g, 52%); as a colourless oil, [α]_D^{23} = -23.3 (c=1.0, CHCl_3); IR (thin film) (ν_max) 3290, 1724, 1699, 1544, 1261, 1068 cm⁻¹; RF 0.2 (EtOAc); ¹H NMR (DMSO-d₆, 90 °C, 400 MHz) δ_H 8.69 (1H, s, oxazolyl), 7.35 (10H, m, aryl), 5.32 (2H, s, CH₂OBn), 5.05 (2H, m, CH₂O of Cbz), 4.82 (1H, m, CHCH₂OH), 3.77 (2H, m, CH₂OH); ¹³C NMR (DMSO-d₆, 90 °C, 100 MHz) δ_C 163.20 (C=O), 160.04 (s), 155.25 (C=O), 144.93 (s), 136.42 (d), 135.45 (s), 132.04 (s), 127.96 (d), 127.77 (d), 127.63 (d), 127.52 (d), 127.23 (d), 127.06 (d), 65.44 (CH₂OBn), 65.34 (CH₂OCO, Cbz), 61.48 (OCH₂CH, oxazolidine), 51.48 (OCH₂CH); LRMS m/z + (Cl) 397 (M+H, 6%), 307 (18%), 289 (12%), 273 (7%), 244 (3%), 154 (100%); HRMS calcd for C_{21}H_{20}N_{2}O_{6} [M+H]^+ 397.1399. Found 397.1390.
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**Benzyl 2-(1-amino-2-hydroxyethyl)oxazoles-4-carboxylate (230)**

A solution of carbamate 229 (0.11 g, 0.28 mmol) in methanol (20 mL) was evacuated and then 5% palladium-on-carbon (0.10 g, 0.69 mmol) was added. The mixture was evacuated and hydrogen was admitted into the reaction. The mixture was left to stir for 1.5 h and then evacuated. The resulting mixture was filtered through a pad of celite and the filtrate was concentrated to give 230 (0.06 g, 89%) as a colourless oil, [α$_D$]$^{23} = -18.6$ (c=0.87, CHCl$_3$); IR (ν$_{max}$) (thin film) 3570, 2916, 1633, 1361, 1080 cm$^{-1}$; Rf baseline (EtOAc); $^1$H NMR (CD$_3$OD, 400 MHz) δ$_H$ 8.28 (1H, s, oxazolyl), 7.27 (5H, m, aryl), 5.09 (2H, s, OCH$_2$), 4.92 (1H, t, $J$=3Hz, OH), 4.03 (1H, m, NH$_2$CH), 3.91 (2H, m, CH$_2$OH); $^{13}$C NMR (CD$_3$OD, 100 MHz) δ$_C$ 165.00 (C=O), 158.19 (s), 145.23 (s), 138.00 (d), 136.12 (s), 129.42 (d), 128.99 (d), 128.81 (d), 67.99 (CH$_2$OBn), 63.63 (CH$_2$OH), 53.17 (NH$_2$CHCH$_2$); LRMS m/z +(Cl) 263 (M+H, 6%), 228 (7%), 199 (34%), 181 (3%), 91 (100%): HRMS calcd for C$_{13}$H$_{14}$N$_2$O$_4$ [M+H]$^+$ 263.1031, found 263.1035.
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