SYNTHESISING NUCLEOSIDE ANALOGUES FOR IMAGING PROLIFERATION IN CANCER AND OTHER BIOMEDICAL APPLICATIONS

A Thesis Submitted By

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In partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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Declaration of Originality

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy. It describes the work carried out in the Department of Surgery and Cancer, Imperial College London, under the joint supervision of Professors Anthony G. M. Barrett and Eric O. Aboagye. Unless stated otherwise, the research is my own work and not the product of collaboration.

Andreas Doepner
London, 30th September 2013
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Synthesising nucleoside analogues for imaging proliferation in cancer and other biomedical applications

Andreas M. Doepner

Abstract

Rapidly proliferating cells, such as cancerous cells, show increased reliance on deoxyribonucleic acid (DNA) salvage pathways for producing nucleotides required for DNA synthesis. As such targeting these salvage pathways using radiolabelled nucleosides can provide a means of imaging the extent of proliferation within a tissue using positron emission tomography (PET). This permits the detection of malignant growths. Thiophymidine and 2’-deoxy-2’,2’-difluoro nucleoside analogues are currently being evaluated for theoretically superior properties in comparison to 3’-deoxy-3’-[^18F]-fluorothymidine (FLT), the current standard PET proliferation marker. Investigations towards the design and synthesis of radiolabelled analogues of these nucleosides for evaluation as PET tracers were carried out.

Synthesis and radiosynthesis of the 2’,2’-difluoro nucleoside analogue i was completed using a Stille coupling to introduce the carbon-11 radiolabel. The synthesis of ii, a precursor to a carbon-11 methylated gemcitabine analogue and iii, an intermediate in the synthesis of thiophymidine nucleosides are also reported.

Based on the synthetic route developed for accessing iv an analogue of ii suitable for radiolabelling with fluorine-18, a series of difluoro nucleoside analogues with potential uses in PET, HIV therapy and fluorescent imaging were also synthesised v-viii.
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Finally I would like to thank Cancer Research UK, the Engineering and Physical Sciences Research Council, the Medical Research Council and the Department of Health for funding my research.
Abbreviations

\(^1\text{H}\)
\(^{11}\text{C}\)
\(^{13}\text{C}\)
\(^{13}\text{N}\)
\(^{15}\text{O}\)
\(^{18}\text{F}\)
\(^{19}\text{F}\)
\(^{64}\text{Cu}\)
\(^{68}\text{Ga}\)
\(^{124}\text{I}\)
\(\text{Å}\)
\(\text{Ac}\)
\(\text{AIDs}\)
\(\text{ATP}\)
\(\text{app}\)
\(\text{aq}\)
\(\text{Ar}\)
\(\text{ATP}\)
\(\text{AZT}\)
\(\text{Bq}\)
\(\text{Bn}\)
\(\text{br}\)
\(\text{Bu}\)
\(\text{Bz}\)
\(\text{°C}\)
\(\text{CAMs}\)
\(\text{CDI}\)
\(\text{Cl}\)
\(\text{Ci}\)
\(\text{COSY}\)
\(\text{CT}\)

Hydrogen-1
Carbon-11
Carbon-13
Nitrogen-13
Oxygen-15
Fluorine-18
Fluorine-19
Copper-64
Gallium-68
Iodine-124
Angstrom
Acetyl
Acquired immunodeficiency syndrome
Adenosine triphosphate
Apparent
Aqueous
Unspecified aromatic group
Adenosine triphosphate
Azidothymidine
Becquerel
Benzyl
Broad
Butyl
Benzoyl
Degree celsius
Cell adhesion molecules
1,1'-carbonyldiimidazole
Chemical ionization
Curie
Homonuclear correlation spectroscopy
Computed tomography
D  Distribution coefficient

d  Doublet

d  Deuterated

d  Deuteron

DAST  $N,N$-Diethylaminosulfur trifluoride

DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene

DCE  1,2-Dichloroethane

dCK  Deoxycytidine kinase

DEPT  Distortionless enhancement by polarisation transfer

DIAD  Diisopropyl azodicarboxylate

DIPEA  Diisopropylethylamine

DMAP  4-(Dimethylamino)pyridine

DMDO  Dimethyldioxirane

DMF  $N,N$-Dimethylformamide

DMSO  Dimethylsulfoxide

DNA  Deoxyribonucleic acid

Ds  Dansyl

e-  Electron

EI  Electron ionization

eq  Equivalent

E1cB  Elimination unimolecular conjugate base

Et  Ethyl

ESI  Electrospray ionization

eV  Electronvolt

g  Gram

Glut  Glucose transporter

h  Hour

ID/g  Injected dose per gram tissue

HIF  Hypoxia inducing factor

HIV  Human immunodeficiency virus

HMBC  Heteronuclear multiple-bond correlation spectroscopy

HMDS  Hexamethyldisilizane

HPLC  High performance liquid chromatography

HRMS  High resolution mass spectrometry
HSQC  Heteronuclear single-quantum correlation spectroscopy  
HTS  High-throughput screening  
Hünig’s base  Diisopropylethylamine  
Hz  Hertz  
IC50  Half maximal inhibitory concentration  
^1Pr  Isopropyl  
IR  Infra-red  
J  \(^1\)H-\(^1\)H Coupling constant  
J\(_{C-F}\)  \(^{13}\)C-\(^{19}\)F Coupling constant  
J\(_{C-^{117}Sn}\)  \(^{13}\)C-\(^{117}\)Sn Coupling constant  
J\(_{C-^{119}Sn}\)  \(^{13}\)C-\(^{119}\)Sn Coupling constant  
J\(_{H-F}\)  \(^1\)H-\(^{19}\)F Coupling constant  
J\(_{H-^{117}Sn}\)  \(^1\)H-\(^{117}\)Sn Coupling constant  
J\(_{H-^{119}Sn}\)  \(^1\)H-\(^{119}\)Sn Coupling constant  
k  Kilo  
Kd  Dissociation constant  
LDA  Lithium diisopropylamide  
log  Logarithm  
LTBA  Lithium tri-\(\text{tert}\)-butoxyaluminum hydride  
m  Multiplet  
M  Molar  
Me  Methyl  
mg  Milligram  
MHz  Megahertz  
min  Minute  
mL  Millilitre  
mmol  Millimole  
mol  Mole  
MOM  Methoxymethyl  
mp  Melting point  
MRI  Magnetic resonance imaging  
Ms  Methanesulfonyl  
MS  Mass spectrometry  
MW  Molecular weight
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>n</td>
<td>Nano</td>
</tr>
<tr>
<td>n</td>
<td>Neutron</td>
</tr>
<tr>
<td>NDPK</td>
<td>Nucleoside diphosphate kinase</td>
</tr>
<tr>
<td>NIS</td>
<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>NMPK</td>
<td>Nucleoside monophosphate kinase</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>P</td>
<td>Partition coefficient</td>
</tr>
<tr>
<td>p</td>
<td>Pico</td>
</tr>
<tr>
<td>p</td>
<td>Proton</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>PG</td>
<td>Protecting group</td>
</tr>
<tr>
<td>pKₐ</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>PMP</td>
<td>para-Methoxyphenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>pRB</td>
<td>Retinoblastoma associated protein</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>R</td>
<td>Unspecified alkyl group</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
</tr>
<tr>
<td>SM</td>
<td>Starting material</td>
</tr>
<tr>
<td>Sₙ₂</td>
<td>Biomolecular nucleophilic substitution</td>
</tr>
<tr>
<td>SₙAr</td>
<td>Nucleophilic aromatic substitution</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SUV</td>
<td>Standard uptake value</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>'Bu</td>
<td>tert-Butyl</td>
</tr>
<tr>
<td>TBAB</td>
<td>Tetra-n-butylammonium bromide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetra-(n)-butylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>\textit{tert}-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBDPS</td>
<td>\textit{tert}-Butyldiphenylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TK</td>
<td>Thymidine kinase</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>Ts</td>
<td>\textit{para}-Toluenesulfonyl</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>W</td>
<td>Watt</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>Alpha particle</td>
</tr>
<tr>
<td>(\beta^+)</td>
<td>Positron</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>Gamma radiation</td>
</tr>
<tr>
<td>([\alpha]_D^{25})</td>
<td>Specific rotation</td>
</tr>
<tr>
<td>(\delta_H)</td>
<td>(^1)H chemical shift in ppm downfield from tetramethylsilane</td>
</tr>
<tr>
<td>(\delta_C)</td>
<td>(^{13})C chemical shift in ppm downfield from tetramethylsilane</td>
</tr>
<tr>
<td>(\nu_e)</td>
<td>Neutrino</td>
</tr>
<tr>
<td>(\mu)</td>
<td>Micro</td>
</tr>
</tbody>
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INTRODUCTION

1.0 Background

Cancer, or malignant neoplasm, is a set of mammalian diseases in which groups of affected cells show uncontrolled growth and proliferation causing damage to nearby tissues.\(^1\) In later stages of the disease cells gain the ability to spread to other areas of the body (metastasize), creating multiple tumours which can ultimately lead to death.\(^1\) The disease occurs as the result of genetic mutations which can be caused by a number of factors including: exposure to chemical pollutants, radiation, specific infections, lifestyle choices and hereditary factors, or indeed, a combination of many of these.\(^2\)

Cancer is a leading cause of death in the world, and was responsible for approximately 7.6 million deaths in 2008, contributing to 13% of all deaths worldwide.\(^3\) The incidence of cancer has been shown to increase with age.\(^4\) Therefore, as the result of a growing, aging population the occurrence of cancer is projected to increase dramatically within the next 20 years with an estimated 22.2 million new cases predicted for 2030 in comparison to 12.7 million cases reported in 2008.\(^5\)

Due to the enormous number of people affected by cancer and subsequent mortality rates, it is imperative to continue research into discovering new treatments for this set of diseases and improved methods for its detection.

1.0.1 The Hallmarks of Cancer

In 2000 Hanahan and Weinberg published their seminal paper proposing six specific cellular traits or “Hallmarks” common to cancerous cells that differentiate them from normal healthy cells. These were: self-production of growth signals, indefinite division, insensitivity to anti-growth signals, avoidance of cell death (apoptosis), metastasis and the acquisition of sustained angiogenesis (Figure 1).\(^6\) More recently the authors have published an updated review outlining the emergence of two new hallmarks: reprogramming of cellular energetics and avoiding immune destruction.\(^7\)
Figure 1- The hallmarks of cancer.  

Self-Production of Growth Signals

In general, a cell can only enter a state of proliferation when activated by exogenous growth factors. Growth factors are proteins and hormones which bind to specific trans-membrane receptors. These receptors then relay signals into the cell, and the cell nucleus, inducing growth and cellular division. Cancerous cells have the ability to synthesise their own growth factors as well as expressing the corresponding receptors for binding them. As such, they show a decreased dependence on external growth signals.

Insensitivity to Anti-Growth Signals

In normal cells tumour suppressor genes act in conjunction with anti-proliferative signals (for example transforming growth factor-β (TGF-β)) to regulate cell growth and division within a tissue. However, when these genes mutate causing disruption to the pathways governing cell growth, it can lead to insensitivity to anti-growth signals. For example, in certain human cancers mutation of the tumour suppressor gene coding for the retinoblastoma associated protein (pRB) causes a down-regulation in the production of cell surface receptors for TGF-β. The result is a loss of response to this anti-growth signal, allowing the cell to continue proliferation.
Indefinite Division

It has been demonstrated that normal, healthy cells have a limited potential for replication. Generally a non-proliferative state, known as senescence, is attained after a certain number of cell divisions. Following each division DNA protecting the end of the chromosomes (the telomere) is shortened. When the telomere is eroded to a critical point, fusion of chromosomes can occur. This leads to the formation of dimeric chromosomes and unviable cells, resulting in mass cell death in a state known as crisis. In malignant cells, up-regulation of the DNA polymerase enzyme, telomerase, prevents shortening of the telomere. Found in around 90% of tumour cells, telomerase counteracts the effects of cell division by extending the end of telomeric DNA, granting limitless replicative ability.

Avoidance of Apoptosis

Apoptosis, or programmed cell death, can be induced by detection of both intracellular (for example from DNA damage and hypoxia) and extracellular signals, such as the cytokine tumour necrosis factor (TNF). When these signals are detected, a cascade is initiated which ultimately leads to destruction of the affected cell at the hands of effector caspases. In order for cancerous cells to avoid apoptosis they must either: disable receptors for apoptotic signals or disable their corresponding effectors. A prime example, occurring in approximately 50% of human cancers, is mutation of the gene coding for the protein p53. The p53 protein is a key sensor for detecting abnormalities in DNA. As a result of its inactivation malignant cells are able to ignore apoptotic signals.

Metastasis

The arrangements of cells and maintenance of their quiescent state within tissues is governed by several classes of proteins, particularly cell-cell adhesion molecules (CAMs). These proteins are deregulated as part of the invasion-metastasis cascade. Malignant cells can then detach from the surrounding tissue and travel to other areas of the body, via the bloodstream and lymph, to create secondary tumours. Perhaps the most well studied example involves mutations to the epithelial cell protein E-cadherin. E-cadherin transmits signals to maintain specific cellular assemblies and limit growth by forming cell to cell bridges; alterations to this protein prevent these interactions, leading to metastatic behaviour.
Sustained Angiogenesis

In order for a cell to function, it must be provided with oxygen and other specific nutrients which are delivered via blood vessels. When a tissue is fully developed the ability to encourage growth of new blood vessels (angiogenesis) is carefully controlled by angiogenic inducing and inhibiting signals. In tumours the balance of these signals is altered. For example, by increasing the expression of the initiator vascular endothelial growth factor (VEGF), and decreasing the expression of the inhibitor β-interferon, the growth of new blood vessels to provide additional resources for the tumour is promoted.\textsuperscript{16}

Reprogramming Cellular Energetics and Avoiding Immune Destruction

Reprogramming of cellular energetics is the adjustment of energy metabolism to support increased cell growth and proliferation.\textsuperscript{7} Cancerous cells alter the machinery of a cell so that anaerobic respiration is carried out even in the presence of oxygen, an effect first observed by Warburg.\textsuperscript{17} The mechanisms behind this process are examined in greater detail in Section 1.3.1.1.

It is well known that immunocompromised humans show greater susceptibility to certain opportunistic cancers although these generally arise as a result of viral infection.\textsuperscript{18} However, it has been shown in murine models that tumour cells from immunodeficient mice are generally unsuccessfully transplanted into immunocompetent mice whereas cancerous cells originating from healthy mice can effectively cause further tumours when transplanted.\textsuperscript{19, 20} This evidence suggests that only cancerous cells which have developed the ability to avoid destruction at the hands of the immune system develop into tumours.\textsuperscript{7}

It should be noted that both of these capabilities have yet to be completely validated. They are therefore classified as emerging hallmarks and required further investigation before they can be accepted alongside the other established hallmarks.\textsuperscript{7}

In these reviews Hanahan and Weinberg highlight the progress made in understanding the nature of cancer and the causes behind it. However, there is still much to be learned and it is important to continue research into understanding this set of diseases. In terms of therapeutic uses, these
characteristic differences between healthy and malignant cells provide targets for the development of new chemotherapeutic drugs and methods of detection.

1.0.2 Detecting Cancer

There are various methods available for detecting cancer including: endoscopies, biopsies, molecular diagnostics and imaging techniques. Imaging techniques are not only useful in detecting cancer, but can be used in all aspects of treatment including: screening, evaluation of the success of therapy, planning for future therapies and providing guidance for palliation. These modalities also have the advantage of being non-invasive and, in certain cases, are able to provide real time monitoring of a patient.\(^{21}\) There are a variety of imaging methods, including: magnetic resonance (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET), ultrasound, X-ray computerised tomography (CT) and optical imaging.\(^{22}\)

MRI takes advantage of the inherent magnetic moment of hydrogen atoms within the body which will produce a signal known as free induction decay (FID), when placed in an applied magnetic field. The FID produced by these protons can be detected and analysed to give anatomical information on the area of the body being examined (Figure 2).\(^{23,24}\)

![Figure 2- MRI of the brain, showing a metastatic tumour.](#)

MRI is a powerful technique for providing high resolution (up to 10 μm) images of the body and detecting cancers. However, it is a relatively costly modality and has fairly long scanning times (minutes to hours).\(^{22}\)
Ultrasound is defined as sound with a frequency greater than 20 kHz. However, for medical imaging purposes sound between the frequencies of 2-10 MHz are generally used. Diagnostic ultrasound imaging involves directing a pulse of ultrasound into the body, detecting its reflections/echoes and converting these signals into an image (Figure 3).

![Figure 3- A pancreatic tumour visualised using ultrasound.](image)

Compared to other imaging modalities, ultrasound is cheap and portable and can also offer high resolutions (50 μm) and real time imaging. However, it does require a skilled operator in order to achieve the best images and is unsuitable for imaging certain areas of the body such as the lungs.

X-Ray CT uses similar principles to conventional x-ray imaging i.e. an x-ray will be differentially absorbed and reflected in different tissues of the body. As such the transmitted radiation can be detected and used to form an image. However, CT imaging uses multiple x-ray tubes and detectors which are rotated around the patient. Multiple cross sections are taken to give a three dimensional insight into the body (Figure 4).

![Figure 4- X-ray CT scan of the lungs with a tumour highlighted.](image)

CT scans offer high resolution imaging (50 μm) in a very short time space (50-100 ms) and have excellent specialist usage e.g. for imaging lungs. However, unlike the other
modalities discussed thus far CT scans involve a relatively high dose of ionising radiation and therefore has extra safety considerations.\textsuperscript{28}

Optical imaging techniques such as bioluminescence and fluorescence, in general, have minimal uses in directly imaging patients since light in the ultraviolet-visible wavelength (100-650 nm) is absorbed by chromophores in the body. They can however be used as an aid for analysing pathology of biopsied tissues.\textsuperscript{21} One exception involves the use of molecules labelled with fluorescent tags (fluorochromes) emitting near infrared (NIR) light (650-900 nm) detected using Fluorescence Molecular Tomography (FMT).\textsuperscript{30} In this method, energy from an external light source is used to illuminate tissue causing excitation of the fluorochrome. This subsequently de-excites emitting NIR radiation which is detected (\textit{Figure 5}).\textsuperscript{22}

\textit{Figure 5-} FMT imaging of a murine tumour using a fluorochrome activated by the protease Cathepsin B.\textsuperscript{30}

Although this modality is still under development it is an attractive proposition due to its relative low cost, rapid imaging times (seconds to minutes) and use of targeted reporters.\textsuperscript{22}

SPECT involves the detection of photons emitted from radiopharmaceuticals by a gamma camera which is rotated around a patient allowing construction of three dimensional images.\textsuperscript{31} Radiopharmaceuticals in SPECT generally consist of a gamma emitting radionuclide such as technetium-99 combined with a ligand which targets a disease specific biomarker.\textsuperscript{21} Although SPECT has a particularly low resolution (1 cm) and involves the use of ionising radiation it allows imaging of physiological processes (for example blood flow in the brain) at a lower cost than comparable techniques like PET.\textsuperscript{22, 31}
1.1 Positron Emission Tomography (PET)

1.1.1 The Principles of PET

The majority of the aforementioned techniques (excluding SPECT and FMT) provide anatomical images of the body and are used to identify cancers based on structural changes within a tissue. PET on the other hand, provides a highly sensitive method of cancer detection by imaging physiological processes (e.g. glucose metabolism and cellular proliferation) occurring in the body. This is performed through the use of imaging agents, or molecular probes, which are molecules labelled with positron (an antimatter electron) emitting radionuclides. Frequently used radionuclides include: fluorine-18 \(^{18}\text{F}\), carbon-11 \(^{11}\text{C}\), oxygen-15 \(^{15}\text{O}\) or nitrogen-13 \(^{13}\text{N}\) and less commonly used radionuclides are: copper-64 \(^{64}\text{Cu}\), iodine-124 \(^{124}\text{I}\), and gallium-68 \(^{68}\text{Ga}\).\(^{32}\) For the most part these are produced in a particle accelerator known as a cyclotron, a notable exception being \(^{68}\text{Ga}\) which is produced in a specific generator.\(^{32, 33}\)

Positron emission is a form of radioactive decay occurring in proton-rich nuclei, whereby a proton is converted into a neutron releasing a positron and a neutrino in the process. This results in the formation of a more stable nucleus. Carbon-11 for example, will decay to form elemental boron and in the same way fluorine-18 will produce oxygen-18 (Figure 6).\(^{32}\)

\[\text{Figure 6- Positron emission by decay of carbon-11 to boron, followed by positron-electron annihilation to produce two gamma photons.}\]
Once synthesised, a radiolabelled probe is either injected intravenously or inhaled by a patient and it then localises within specific areas of the body. Positrons emitted by the radionuclide travel a short distance within a tissue (typically 2 mm) before colliding with an electron; this collision causes an annihilation resulting in the release of two 511 keV gamma photons at 180 degrees to each other (Figure 6). These photons are observed by arrays of detectors in PET scanners in a process known as coincident detection: only photons created in the same positron-electron annihilation event arriving at opposite detectors within a few nanoseconds of each other are counted.

Within the detector, these gamma photons interact with the crystalline material of a scintillator generating a pulse of light, which is amplified by photomultipliers creating an electrical impulse. This is then further processed and corrected for errors such as attenuation and accidental coincidences giving rise to usable data. The detector array forms a ring around the patient, therefore detection of photons by scintillators in the scanner arrays gives information on the concentration and location of the molecular probe within the patient allowing a 3-D image of the body to be generated (Figure 7).

![Figure 7- PET image of a lung tumour using fluorine-18 labelled glucose (\[^{18}\text{F}\]-fluorodeoxyglucose or \[^{18}\text{F}\]-FDG)](image)

The example depicted in Figure 7 shows a PET image of glucose metabolism in the body using the glucose analogue \[^{18}\text{F}\]-FDG. The darker areas represent higher concentrations of the labelled glucose probe and hence areas of higher metabolic rate, corresponding to a carcinoma of the lung. Other darkened areas denote clearance of the probe in the bladder. For further information on the uptake of \[^{18}\text{F}\]-FDG see Section 1.3.1.1.

PET images are commonly analysed semi-quantitatively to gain further information on a malignancy using a parameter known as the standardised uptake value (SUV). In cancer the SUV provides an index of the radioactivity concentration of the tumour compared to the initial injected radioactivity, divided by the body mass. Areas of abnormal molecular probe uptake are
firstly identified, followed by calculation of the SUV in these regions of interest (ROI).\textsuperscript{37} Quantification of the intensity of probe uptake in the ROI yields data which can be analysed to give information on a variety of aspects of the malignancy. For example, SUVs allow differentiation between aggressive and indolent tumours, prediction of prognosis and can give early assessments of a patient’s response to therapy.\textsuperscript{21,37,38}

This highlights the major advantage of PET over other imaging modalities: its ability to provide physiological information on the area of the body being studied. Apart from the aforementioned information gained from SUVs, further beneficial data gained from PET studies include: highlighting biological changes that occur in cancer before anatomical abnormalities can be detected and providing entire body imaging of a primary tumour and its metastases. It is not only in cancer therapy where PET can provide additional insights into diseases; for example $[^{18}\text{F}]$-FDG can also be used as early indicator of Alzheimer’s disease.\textsuperscript{36} Moreover, the scanning time required to generate a PET image is relatively short (minutes), meaning less inconvenience for patients.\textsuperscript{22}

However, PET does have various limitations. For example, in comparison to ultrasound, CT and particularly MRI it offers images with a particularly poor resolution of 1-2 mm, compared to 10-100 μm in MRI. This is a result of the distance travelled by the positron within a tissue prior to annihilation. Furthermore, PET offers little anatomical imaging, to the extent that it becomes difficult to estimate tumour invasion of neighbouring tissues.\textsuperscript{22} One solution to this problem involves the use of joint PET/CT and PET/MRI systems. These hybrid techniques allow the combination of the high resolution anatomical imaging of CT and MRI with the functional information gained from PET (Figure 8).\textsuperscript{39}

\textit{Figure 8}- Combined PET/CT image showing multiple malignant tumours using $[^{18}\text{F}]$-FDG.\textsuperscript{21}
Other problems associated with PET stem from the radionuclides themselves which have short half-lives (Table 1). As a result they have to be synthesised on site by cyclotrons or generators and must be quickly incorporated into their respective molecular probes to prevent significant loss of activity by radioactive decay. This has cost implications and, as will be discussed in following sections, it can present synthetic challenges for probe generation.\textsuperscript{22,36}

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{15}$O</td>
<td>2 min</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>10 min</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>20 min</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>109 min</td>
</tr>
</tbody>
</table>

*Table 1*- Half-lives of commonly use radionuclides.\textsuperscript{36}

1.1.2 Development of PET Molecular Probes

The development of new PET molecular probes for use in clinical settings is a complex and multidisciplinary process, consisting of a number of steps: selection of a suitable target, organic synthesis, radiolabelling, *in vitro* and *in vivo* evaluation and finally kinetic modelling of data.

Development begins with identification of a suitable target (e.g. an enzyme, receptor or transporter) for imaging the desired process. After selection of a target an appropriate compound to bind this target must be discovered. This is normally performed using high throughput screens or by rational design based on crystal structures. The properties of the compound can then be tailored based on *in vitro* and *in vivo* assays in a lead optimisation process.\textsuperscript{40}

With a suitable ligand identified an appropriate radionuclide must be chosen for labelling. The choice of radionuclide is dependent on the biological process being studied: ideally the half-life of the radionuclide and that of the biological process should match. Additionally, the half-life should be sufficient to allow radiolabelling, purification and formulation of the molecular probe.\textsuperscript{41} Techniques for introducing different radiolabels are discussed in further detail in *Section 1.2.*
The formulated molecular probe can then be subjected to *in vitro* testing for plasma stability and affinity studies carried out. Finally, *in vivo* testing for can be carried out to evaluate pharmacokinetic properties of the PET probe. For example, biodistribution studies can be used to determine the percentage of injected dose per gram tissue, (% ID/g) which in turn can be used to calculate the SUV.\textsuperscript{40}

1.1.3 **Desirable Properties of PET Molecular Probes**

As alluded to in the previous section there are a number of desirable properties for PET probes to have. These are defined by both the physical characteristics of the radionuclide and the chemical structure of the probe and include: specificity, selectivity, lipophilicity, affinity, specific activity, metabolic stability, clearance and radiochemical yield.

**Specificity and Selectivity**

A molecular probe should selectively bind to its target to ensure only the desired process is being measured. Additionally, the probe should be designed to reduce the amount of non-specific binding to plasma proteins, cell membranes and other non-target tissues. Excessive non-specific binding gives rise to a high background signal (noise), which reduces the contrast of the enzyme or receptor being targeted. Non-specific binding has been shown to be related to the lipophilicity of a compound.\textsuperscript{41,42}

**Lipophilicity**

Lipophilicity is the affinity of a molecule for lipophilic environments and is expressed as a logarithm of the partition coefficient ‘\(P\)’. The partition coefficient is measured as a ratio between the concentration of a compound in octanol versus water (*Equation 1*).\textsuperscript{43}

\[
P = \frac{[\text{compound}]_{\text{octanol}}}{[\text{compound}]_{\text{water}}}\quad D = \frac{\sum [\text{compound ionised} + \text{ionised}]_{\text{octanol}}}{\sum [\text{compound ionised} + \text{ionised}]_{\text{water}}}
\]

*Equation 1*- Partition coefficient and distribution coefficient.
More accurately this value may be measured using the pH dependent distribution coefficient ‘D’. This value takes into account the degree of ionisation occurring at a specific pH and is the ratio of the concentration of unionised and ionised compound in both phases. In PET, typical logD values are measured at physiological pH. In general a probe needs to be sufficiently lipophilic to pass through cell membranes but not so lipophilic as to show significant non-specific binding to fatty tissues; ideal logD values will be less than three. It should be noted, that non-specific binding cannot be attributed to lipophilicity alone and that a molecules ability to hydrolyse phospholipid bilayers can also play a role.

Clearance

Ideally the probe should be rapidly cleared from plasma to reduce the blood pool background signal at the target tissue. Similarly the probe should also be rapidly cleared from non-specific areas again to improve the signal to noise ratio.

Affinity

Affinity is the ability of a molecular probe to bind to its target. Useful molecular probes generally have affinities in the range of 0.01–1.00 nM and in principle higher affinity should give rise to a higher signal to noise ratio. However, as increased affinity is often associated with increased lipophilicity this is not always the case. Additionally the density of the target must be taken into account, with lower target densities requiring probes with higher affinity.

Specific Activity

Specific activity (SA) is defined as the activity (A) of a radiolabelled probe per unit mass (GBq or mCi per μmol) (*Equation 2*).

\[
SA = \frac{A}{m}
\]

*Equation 2- Specific activity.*
High specific activities are desirable as they allow effective imaging of a target using small amounts of material (typical probes are administered in nano-molar quantities) without perturbing the system being studied by fully saturating the target. Furthermore, using such low amounts of tracer means toxicity is less of an issue. It should be noted that although theoretical specific activity values for radionuclides are very high (e.g. carbon-11 ~ 341,000 GBq/µmol), due to isotopic dilution, unwanted side reactions during labelling and activity loss by decay the specific activity for administered probes tends to be much lower (e.g. carbon-11 ~ 3,000 GBq/µmol).

Metabolism and Labelling Position

Ideally, a PET probe will be stable to metabolism during the timeframe of scanning. However, in many cases metabolism is unavoidable and therefore the position of the molecular probe subjected to radiolabelling must be carefully chosen. The production of radiolabelled metabolites that show binding affinity for the target or accumulate within the target tissue must be avoided. In certain cases labelling of a molecule can be advantageous, increasing its metabolically stability, therefore simplifying kinetic modelling. For an example of such a situation see Section 1.3.2.1.

Radiolabelling and Radiochemical Yield

While more of a synthetic concern, it is nonetheless a desirable property that a molecular probe be rapidly radiolabelled and in good radiochemical yields (RCY, the yield after radiochemical purification expressed as a percentage of the activity originally present). This number is often quoted as the decay-corrected radiochemical yield which takes into account the naturally occurring decay of the isotope. Unless specified RCY shall henceforth refer to decay correct radiochemical yields.

These properties are desirable for all molecular probes, however one can further divide radiopharmaceuticals into three classes defined by their specific target: those based on enzyme–mediated metabolic transformations (often analogues of endogenous compounds), probes based on stoichiometric binding interactions with receptors and those which measure perfusion. The first class of probe will be the focus of much of the work contained in this thesis.
1.2 Introduction to PET Radiochemistry

The production of a PET probe involves numerous steps. Initially, the radionuclide is prepared in a cyclotron by bombarding a target system with a beam of protons or deuterons. The target system is a vessel containing a target material required for the production of a specific radionuclide. The radionuclide is trapped in such a form that it can be directly reacted or further functionalised and then reacted with a ‘cold’ non-radioactive precursor with a compatible reactive functionality.\(^{41}\) The cold compound is generally more chemically complex and is reacted with the radioactive synthon in the final stages of the synthesis (Figure 9).

\[ \begin{align*}
2 \xrightarrow{\text{radiolabelling}} & \quad 3 \xrightarrow{\text{deprotection}} 4 \\
\text{Cold Precursor} & \quad \text{PET Probe}
\end{align*} \]

* = radiolabelled position, LG = reactive functionality, PG = protecting group

**Figure 9** - Simple schematic showing the synthesis of a positron emitting molecular probe.

In some cases, removal of protecting groups (PG) is required after radiolabelling although it is desirable to have as few steps as possible after labelling in order to minimise loss of activity by radioactive decay. Finally, the PET probe must be purified, usually by (high performance liquid chromatography) HPLC, and then formulated for use.\(^{48}\) This entire process should ideally be completed within three half-lives from the end of bombardment (EOB) of the target material.\(^{49}\) As such each step must be carried out in a time efficient manner. Lengthy purification in particular can have drastic effects on yield and specific activity. Purification time by HPLC can be minimised by increasing the solvent flow rate, reducing the column length and increasing the temperature of the column itself. This however is dependent on the degree of separation between the product and by-products.\(^{49}\) An alternative is the use of solid phase extraction (SPE): pre-packed chromatographic cartridges which allow the removal of specific contaminants. Using this method can allow rapid purification but is often limited for use with a specific product.\(^{48}\)

As shown in Table 1 there are many common radionuclides available for synthesising molecular probes. However, the short half-lives of \(^{15}\)O and \(^{13}\)N mean there is relatively little complex
radiochemistry that can be performed with these emitters. On the other hand, $^{14}$C and particularly $^{18}$F have sufficiently long half-lives to allow some flexibility in their synthetic use.

1.2.1 Fluorine-18 Radiochemistry

Fluorine-18 is currently the most widely used radionuclide in PET radiochemistry. Attractive properties of $^{18}$F include its long half-life when compared to $^{11}$C, $^{13}$N and $^{15}$O and the fact that it produces the highest resolution PET images due to its low positron energy and hence short linear positron trajectory in tissues. The emergence of $^{18}$F-FDG as an important tool in the detection and staging of cancer has driven interest in $^{18}$F radiochemistry research as well as the use of PET in clinical and research institutions as a whole.\(^\text{41}\) Radiochemistry with $^{18}$F can be broadly divided into two categories: electrophilic and nucleophilic fluorination reactions.

1.2.1.1 Nucleophilic Fluorination Reactions with $^{18}$F Fluoride

Nucleophilic fluorination reactions make use of $^{18}$F fluoride $^{18}$F\(^-\) generated using the $^{18}$O(p,n)$^{18}$F nuclear reaction. In this case the target material is $^{18}$O enriched H\(_2\)O, which undergoes proton bombardment releasing a neutron and forming a solution of $^{18}$F. Fluoride in this form ($^{18}$FHF) requires additional modification in order to increase its nucleophilicity. The obtained solution is therefore trapped on an ion-exchange resin and eluted with a K\(_2\)CO\(_3\) solution. It is then treated with the phase transfer agent kryptofix-222 (K\(_{222}\)) in order to complex the potassium cation, further increasing the reactivity of fluoride anion. Reactions are generally carried out in polar aprotic solvents again with increasing reactivity in mind.\(^\text{50, 51}\)

An important consideration when carrying out nucleophilic fluorinations is the functionality of the cold precursor \textit{i.e.} the requirement of a suitable leaving group. For simple aliphatic substitutions triflates, tosylates, mesylates, bromides and iodides are all acceptable (Scheme 1).\(^\text{41}\)

\begin{center}
\includegraphics[width=\textwidth]{Scheme_1.png}
\end{center}

\textit{Scheme 1- Synthesis of $^{18}$F-FDG 3 by nucleophilic fluorination.}\(^\text{51}\)
The reaction of protected pyranose 5 with $[^{18}\text{F}]\text{KF.K}_{222}$ is the most utilised radiochemical reaction today and provides a highly relevant demonstration of this type of reaction. The requirement of protecting groups to prevent competing nucleophilic attack by other reactive functionalities is a major issue with these procedures. Deprotection and purification increase the synthesis time, limiting the amount of useful steps that can be carried out beforehand.$^{41,51}$

When attempting nucleophilic fluorinations of aromatic compounds the situation becomes more complex. In order for successful reaction, not only is a suitable leaving group necessitated but in addition, the presence of electron withdrawing groups ortho or para to the leaving group are required for activation of the aromatic ring. Despite these constraining factors these nucleophilic aromatic substitutions ($\text{S}_N\text{Ar}$) have become widely used (Scheme 2).$^{52}$

![Scheme 2- Synthesis of 4-$[^{18}\text{F}]$-fluorobenzaldehyde.$^{52}$](image)

Reacting para-nitrobenzaldehyde with $[^{18}\text{F}]\text{KF.K}_{222}$ is an excellent method for generating $[^{18}\text{F}]$-fluorobenzaldehyde 8, which in itself is a highly versatile intermediate. $[^{18}\text{F}]$-fluorobenzaldehyde and analogues of this molecule can be further reacted in a number of different ways to generate more complex molecular probes. For example reacting $N$-(tert-butyl)hydroxylamine with 8 yields 9 designed for use in in vivo visualisation of free radicals.$^{53}$ This is an example of indirect labelling strategy using $[^{18}\text{F}]$.

Another common indirect fluorination strategy involves reacting $[^{18}\text{F}]$ labelled alkyl azides with alkynes using the copper catalysed Huisgen cycloaddition. This reaction is particularly amenable to PET chemistry as a result of the fast reaction times, high yields and mild conditions (Scheme 3).$^{54}$
Scheme 3- Labelling of an isatin-sulfonamide with $^{[18}F$]-fluoroethylazide by Huisgen cycloaddition.\(^{55}\)

$^{[18}F$]-Fluoroethylazide for example, is synthesised by reacting a precursor tosylate 10 with nucleophilic fluoride. This can be then be reacted with various different alkynes, an appropriate illustration being the synthesis of radiolabelled isatin-sulfonamide 13, which binds caspases 3 and 7 and hence can be used for imaging apoptosis.\(^{55}\) Using these indirect labelling strategies permits access to complex molecular probes that are not viable targets for direct labelling due to the harsh conditions employed in the initial radiolabelling step. However, this also results in an increased number of steps in the radiosynthesis which must also be taken into consideration.

1.2.1.2 Electrophilic Fluorination Reactions with $^{[18}F$] Fluorine

Electrophilic fluorination reactions traditionally make use of $^{[18}F$] fluorine gas ($^{[18}F$ F\(_2\)) available from the $^{18}O(p,n)^{18}F$ and $^{20}Ne(d,α)^{18}F$ nuclear reactions. Proton bombardment of $^{[18}O$ O\(_2\) is the preferred method of $^{[18}F$ F\(_2\) synthesis as a result of its higher efficiency.\(^{56}\) This is an example of a carrier added synthesis whereby a small amount of $^{[19}F$ F\(_2\) (0.2%) is added to the target gas. In the absence of stable fluorine, the radiolabelled gas can adsorb to the walls of the vessel containing the target gas reducing the amount of radioactive material isolated.\(^{57}\) Compared to reactions with $^{[18}F$], the use of $^{[18}F$ F\(_2\) suffers from a number of drawbacks, including: being more difficult to handle and use therefore requiring specialist equipment, lower specific activity as a result of carrier added fluorine and the high probability of producing unwanted fluorinated by-products due to reactivity of gaseous fluorine.\(^{58}\)
Nevertheless, $[^{18}\text{F}]\text{F}_2$ has been applied in the successful radiolabelling of a variety of compounds including $[^{18}\text{F}]-5$-fluorouracil 15. Reacting uracil 14 with $[^{18}\text{F}]\text{F}_2$ in acetic anhydride furnishes 15, which can be used as a prognostic agent in cancer therapy (Scheme 4).\(^{59, 60}\)

![Scheme 4 - Synthesis of $[^{18}\text{F}]-5$-fluorouracil using $[^{18}\text{F}]\text{F}_2$.\(^{59}\)](image)

Another example is the synthesis of $[^{18}\text{F}]-\text{fluoro-L-DOPA}$ 17. Fluoro-destannylation of precursor 16 with $[^{18}\text{F}]\text{F}_2$ is followed by acidic deprotection yielding 17, a molecular probe used in the study of Parkinson’s disease (Scheme 5).\(^{61}\)

![Scheme 5 - Synthesis of $[^{18}\text{F}]-\text{fluoro-L-DOPA}$ using $[^{18}\text{F}]\text{F}_2$.\(^{61}\)](image)

Other less reactive and more selective reagents have emerged in the latest literature. A recent report by Gouverneur et al. for example, describes the synthesis of a highly selective electrophilic fluorinating reagent $[^{18}\text{F}]-\text{Selectfluor bis-triflate} ([^{18}\text{F}]-\text{TEDA})$ 19 (Scheme 6).\(^{58}\)

![Scheme 6 - $[^{18}\text{F}]-\text{TEDA}$ synthesised using high specific activity $[^{18}\text{F}]\text{F}_2$.\(^{58}\)](image)
This protocol takes advantage of Solin’s methodology for the synthesis of high specific activity \([^{18}\text{F}] \text{F}_2\). By reacting methyl iodide with \([^{18}\text{F}] \text{KF}, \text{K}^{222}[^{18}\text{F}]-\text{fluoromethane is produced. The fluoromethane is then mixed with a small amount of carrier \([^{19}\text{F}] \text{F}_2\) and subjected to an electrical discharge, resulting in the exchange of \([^{18}\text{F}]\) and \([^{19}\text{F}]\) in plasma, producing \([^{18}\text{F}] \text{F}_2\) with specific activity up to 50 times greater than that available by traditional proton bombardment.\(^{62}\) 1-Chloromethyl-4-aza-1-azoniabicyclo[2.2.2]octane triflate \(18\) was then fluorinated with the high specific activity fluorine to yield \(19\) and this in turn was reacted with silyl-enol ether \(20\) in a proof of concept reaction (Scheme 7).\(^{58}\)

\[
\begin{align*}
\text{OSiMe}_3 & \quad \text{19, MeCN, 80 °C} & \quad \text{20} & \quad \text{21, RY 50%}
\end{align*}
\]

Scheme 7- Fluorination using \(19\).\(^{58}\)

The reaction proceeded successfully with a radiochemical yield of 50%. Owing to its selectivity and mild reaction conditions in comparison to directly using \([^{18}\text{F}] \text{F}_2\), \([^{18}\text{F}]\) selectfluor bis-triflate \(19\) presents an exciting development for \([^{18}\text{F}]\) fluorinations.\(^{58}\)

Perhaps even more remarkable is the reported synthesis of an electrophilic fluorine source available directly from nucleophilic \([^{18}\text{F}]\) fluoride (Scheme 8).

\[
\begin{align*}
\text{22} & \quad 2 \times \text{OTf} & \quad \text{[}^{18}\text{F}] \text{KF, 18-crown-6} & \quad \text{acetone} & \quad \text{23} & \quad \text{24} & \quad \text{25, RY 33%}
\end{align*}
\]

Scheme 8- Electrophilic fluorination of aryl palladium complexes with \(23\).\(^{63}\)

By controlling the properties of palladium (IV) complexes \(22\) and \(23\) through careful ligand selection Ritter et al. have developed complexes which can react with \([^{18}\text{F}]\) and transform it into an electrophilic source. The cold precursor \(22\) is specifically tailored for capturing nucleophilic
fluoride; once trapped the nature of complex 23 causes a reversal in the reactivity of the fluorine, thus furnishing an electrophilic $[^{18}\text{F}]$ fluorine source. This complex can be used to oxidatively transfer fluorine to palladium (II) aryl complexes, before reductive elimination yields the desired product. The utility of this reaction was demonstrated through the fluorination of several aryl palladium precursors, generating $[^{18}\text{F}]$-fluorodeoxyestrone 25 amongst others. Although the applications of complex 23 are potentially very exciting, it should be noted that both this and complex 24 are chemically complex, requiring glove box manipulations for their synthesis. Furthermore, the use of metals such as palladium requires careful purification to avoid toxicity. While this is suitable for research settings it makes transfer to the clinic difficult.

To summarise, while there are a number of challenges in using $[^{18}\text{F}]$ for radiolabelling including: a relatively small pool of biologically relevant molecules containing a carbon-fluorine bond, limited synthetic flexibility and the unknown effects of replacing functionality with $[^{18}\text{F}]$ (see Section 1.3.2.1). It remains an attractive proposition for radiochemists due to its comparatively long half-life. Moreover, recent developments, particularly in the area of electrophilic $[^{18}\text{F}]$ sources, are helping to further expand the usefulness of $[^{18}\text{F}]$ as a radiolabel.

1.2.2 Carbon-11 Radiochemistry

The most common synthesis of $[^{11}\text{C}]$ is via the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction. In this case nitrogen gas is bombarded by a high-energy beam of protons, resulting in the formation of $[^{11}\text{C}]$ and an $\alpha$-particle (helium nucleus). In the presence of trace amounts of oxygen or hydrogen gas the $[^{11}\text{C}]$ reacts to form either $[^{11}\text{C}]$-carbon dioxide ($[^{11}\text{C}]\text{CO}_2$) or $[^{11}\text{C}]$-methane ($[^{11}\text{C}]\text{CH}_4$), which can then be trapped and used directly or manipulated to form other precursors (Scheme 9).
1.2.2.1 \[^{11}\text{C}]\text{Methyl Iodide}\]

The most frequently used \[^{11}\text{C}]\text{precursor is undoubtedly }[^{11}\text{C}]\text{methyl iodide }([^\text{11}\text{C}]\text{CH}_3\text{I}).[^{11}\text{C}]\text{Methyl iodide can be generated using two methods: the reduction of }[^{11}\text{C}]\text{CO}_2 \text{ by lithium aluminium hydride, followed by reaction with hydroiodic acid; or radical iodination reaction of }[^{11}\text{C}]\text{CH}_4 \text{ with iodine at high temperatures.}[^{66},^{67}][^{11}\text{C}]\text{methyl iodide has found the majority of its use in simple nucleophilic substitution reactions with nitrogen, oxygen and sulfur heteroatoms. In cases where }[^{11}\text{C}]\text{CH}_3\text{I is insufficiently reactive, further conversion using a hot column of silver triflate can generate the more reactive }[^{11}\text{C}]\text{CH}_3\text{OTf species.}[^{65}] \text{ Due to their simple nature (a typical methylation involves trapping }[^{11}\text{C}]\text{CH}_3\text{I}/[^{11}\text{C}]\text{CH}_3\text{OTf in solvent with the cold precursor and heating) and rapid reaction times these reactions have become the most widespread way of synthesising }[^{11}\text{C}]\text{labelled molecular probes.}[^{41}]

There are a number of interesting modifications and additions to basic methyl iodide methodology. Jacobsen and Mishani, for example, have demonstrated an appealing approach for introducing radiolabelled \[^{11}\text{C}]\text{-dimethylamine moieties (common to many drug molecules) into PET probes.}[^{11}\text{C}]\text{CH}_3\text{I can be reacted with methylamine to yield }[^{11}\text{C}]\text{-dimethylamine}[^{27}], \text{which can then be used in the synthesis of numerous analogues (Scheme 10).}[^{68}]
Scheme 10- $^{[11]}$C-dimethylamine as a precursor.$^{68}$

The principal advantage of this method is the reversal in reactivity profile: using $^{[11]}$C CH$_3$I as a radiolabel in a nucleophilic form expands the number of labelling strategies available as well as demonstrating differing functional group tolerance to $^{[11]}$C CH$_3$I.$^{65}$

Hooker et al. have developed a novel approach for the generation of $^{[11]}$C-formaldehyde by the oxidation of $^{[11]}$C CH$_3$I (Scheme 11).$^{69}$

Scheme 11- Synthesis and reaction of $^{[11]}$C-formaldehyde.$^{69}$

Treatment of trimethylamine $N$-oxide (TMAO) 29 with $^{[11]}$C CH$_3$I results in the formation of intermediate 30 which decomposes upon heating resulting in the formation of $^{[11]}$C-formaldehyde 31. The usefulness of this intermediate was demonstrated with the synthesis of 33 from tryptamine 32 using the Pictet-Spengler reaction (Scheme 11). Oxidation of $^{[11]}$C CH$_3$I using TMAO provides a rapid and efficient synthesis of $^{[11]}$C-formaldehyde in comparison to previous methods involving reduction and oxidation of $^{[11]}$C CO$_2$. $^{69}$

$^{[11]}$C CH$_3$I has also found use in palladium-catalysed coupling reactions (Scheme 12).$^{41}$
Due to their tolerance to a wide variety of functional groups, Stille couplings have become the most widely used cross couplings in PET radiochemistry. An important example is in the synthesis of the nucleoside 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-[methyl-11C]-thymine or [11C]-FMAU 35, a potential marker for proliferation in cancer. However, a major disadvantage of these reactions is the use of organo-stannanes. These compounds and the subsequent by-products produced are highly toxic and require careful removal during purification. Suzuki couplings represent a viable solution to this problem. Hostetler et al., for example, reported the successful coupling of aryl boronic esters and acids 36 with [11C] CH₃I. Although these reactions have a more restricted functional group tolerance in comparison to Stille couplings, they report high radiochemical yields and purity and avoid the toxicity issues associated with the use of stannanes.

Also worthy of mention are polymer-supported [11C] methylations. In these reactions, the cold precursor 37 is attached to a solid support and then treated with [11C] CH₃I. After completion of the reaction only the successfully labelled precursor can be washed from the polymer-support. This ensures high purity avoiding the need for HPLC purification reducing loss of activity by decay during chromatography (Scheme 13).
Scheme 13- Polymer-supported methylation and release of labelled product.\textsuperscript{71}

Due to their ease of use, high radiochemical yields and versatility so-called microfluidic ‘loop’ methods have found growing use in the methylation of simple precursors. These methods involve coating the inside surface of a plastic or stainless steel HPLC loop with a solution of the cold precursor and then passing a stream of gaseous $[^{11}\text{C}]\text{CH}_3\text{I}$ through the system. With the loop itself acting as a reaction vessel, the crude mixture can be directly injected onto the HPLC column for purification following reaction. This avoids the loss of material due to transfer from reaction vessels (Scheme 14).

Scheme 14- Schematic of a loop system used for $[^{11}\text{C}]$ methylation reactions e.g. methylation to form $[^{11}\text{C}]$-raclopride \textsuperscript{40}.\textsuperscript{72}

Other advantages of this methodology include its efficiency: using smaller volumes of solvent not requiring any temperature control for trapping or reaction, and rapid reaction rates.\textsuperscript{72} See section 3.2.2 for further discussions of microfluidic reactions.
1.2.2.2 $^{[11]}{\text{C}}$ Carbon Monoxide

The carbonyl group is one of the most widely abundant functionalities found in biologically relevant molecules. The ability to introduce $^{[11]}{\text{C}}$ carbon monoxide ($^{[11]}{\text{C}}$ CO) to molecular probes is therefore highly desirable. $^{[11]}{\text{C}}$ CO is produced by the reduction of $^{[11]}{\text{C}}$ CO$_2$ over molybdenum and zinc catalysts at high temperatures and has found its greatest application in palladium-catalysed carboxylation reactions.$^{41}$

These reactions involve four components: the transition metal catalyst, an aryl or vinyl halide, a nucleophile and $^{[11]}{\text{C}}$ CO. They proceed via a typical catalytic cycle: oxidative addition of the halide species, insertion of $^{[11]}{\text{C}}$ CO to form an acyl-palladium complex, followed by nucleophilic attack and reductive elimination to the product (Scheme 15).$^{73}$

![Scheme 15- General mechanism for carboxylative palladium cross couplings.](image)

Initial investigations of this chemistry suffered from low radiochemical yields as a result of the poor solvent solubility of carbon monoxide, thus limiting the rate of the insertion step.$^{73}$ Recent developments using high pressure micro-autoclaves have overcome these issues, allowing reactions to be performed at pressures in excess of 350 atm enhancing the solubility of $^{[11]}{\text{C}}$ CO.$^{74}$

Such advances have allowed the synthesis of a variety of ketones and amides using Suzuki, Stille and Buchwald-Hartwig carboxylative coupling procedures (Scheme 16).$^{75, 76, 77}$
Free-radical carbonylations of alkyl iodides have also been used to synthesise radiolabelled esters, amides and carboxylic acids from $[^{11}C]$ CO (Scheme 17).78, 79

Although seemingly analogous to palladium catalysed carbonylations, these reactions provide several distinct advantages. For example, without the risk of β-hydride elimination radical carbonylations can make use of alkyl halides and triflates as well as aryl and vinyl halides. Additionally this method is tolerant to functional groups such as carboxylic acids and alcohols, which are normally protected in syntheses using $[^{11}C]$ CO$_2$ and Grignard reagents.79

1.2.2.3 $[^{11}C]$ Carbon Dioxide

In contrast to $[^{11}C]$ CO and $[^{11}C]$ CH$_3$I, there are relatively few examples using $[^{11}C]$ carbon dioxide ($[^{11}C]$ CO$_2$) as the labelling species. A notable exception is in the formation of radiolabelled carboxylic acids and acid chlorides by the reaction of $[^{11}C]$ CO$_2$ with Grignard reagents (Scheme 18).
Scheme 18- Reactions of $[^{11}\text{C}]\, \text{CO}_2$ with Grignard reagents.$^{80,81}$

In both these examples, the $[^{11}\text{C}]\, \text{CO}_2$ is first of all reacted to form the Grignard salt 55 and 58 and then further functionalised as required. The synthesis of $[^{11}\text{C}]$ labelled sodium acetate 58, used for the diagnosis of prostate cancer is an important demonstration of this strategy.$^{80,81}$ However, as previously mentioned the functional group tolerance of these methods can limit their usefulness.

1.2.2.4 $[^{11}\text{C}]$ Hydrogen Cyanide and other Reactions

There are numerous biologically relevant molecules containing the nitrile functionality, making it an ideal target for radiolabelling. $[^{11}\text{C}]$ Hydrogen cyanide ($[^{11}\text{C}]\, \text{HCN}$), produced by the reaction of $[^{11}\text{C}]\, \text{CH}_4$ with $\text{NH}_3$ at high temperatures using a platinum catalyst, provides a suitable reagent for the introduction of this group (Scheme 19).$^{82}$

Scheme 19- Introducing $[^{11}\text{CN}]$ group to aryl halides.$^{83,84}$

$[^{11}\text{C}]\, \text{HCN}$ can be reacted directly with aryl halides 59 using palladium catalysis or it may be converted to $[^{11}\text{C}]\, \text{CuCN}$ and then used to introduce the nitrile group via a Rosenmund-von Braun reaction.$^{83,84}$ With the nitrile functionality in place amides, tetrazoles and carboxylic
acids can be conveniently synthesised allowing the generation of a wide range of radiolabelled molecules. It should be noted that there are a number of reactions such as rhodium catalysed carboxylations using and the use of \[^{11}\text{C}\] labelled phosgene that have been omitted. These will be covered in section 2.0.2 discussing the synthesis of \[^{11}\text{C}\] labelled isocyanates.

These examples serve to highlight the diverse array of reactions available to the radiochemist utilising \[^{11}\text{C}\] and demonstrates the synthetic versatility of this radionuclide in comparison to \[^{18}\text{F}\]. Furthermore, as carbon is an element endogenous to the body it means that a tracer labelled with \[^{11}\text{C}\] it will follow the same biological pathways as the original molecule, providing a ‘true tracer’ of the process being analysed. However, there are limitations associated with \[^{11}\text{C}\] chiefly its relatively short half-life. This places restrictions on the number of steps that can be used and unlike \[^{18}\text{F}\] requires the presence of an on-site cyclotron. Additionally gaseous \[^{11}\text{C}\] CO\(_2\) and \[^{11}\text{C}\] CH\(_4\) are difficult to handle requiring specialist equipment. It should be noted that a short half-life can be beneficial clinically, allowing repeat studies to be carried out with a similar or lower dose than radionuclides with a longer half-life.

1.2.3 Oxygen-15 and Nitrogen-13 Radiochemistry

As is this case with \[^{11}\text{C}\] both \[^{15}\text{O}\] and \[^{13}\text{N}\] are examples of nuclei whose stable isotopes are elements endogenous to the body. They are found in most biologically active compounds and therefore are seemingly ideal candidates for radiolabelling molecular probes. Unfortunately, as previously mentioned, their short half-lives restrict the amount of useful synthetic radiochemistry that can be performed using these radionuclides (Table 1).

Oxygen-15 can be produced in two forms: as \[^{15}\text{O}\] O\(_2\), made in the \(^{14}\text{N}(d,n)^{15}\text{O}\) nuclear reaction by bombarding a nitrogen-oxygen mixture with high energy deuterons, and as \[^{15}\text{O}\] H\(_2\)O made by irradiation of water with protons \(^{16}\text{O}(p,\alpha)^{15}\text{O}\). Both \[^{15}\text{O}\] O\(_2\) and \[^{15}\text{O}\] H\(_2\)O can be used for blood flow studies in the brain and other organs. Nitrogen-13 is generated from H\(_2\)O using the \(^{16}\text{O}(p,\alpha)^{13}\text{N}\) nuclear reaction in the form of \[^{13}\text{N}\] nitrate and nitrite salts. The commonly used form for labelling is as \[^{13}\text{N}\] ammonia. This can be prepared via the reduction of the aforementioned nitrogen salts using an Al/Cu/Zn alloy or can be
made directly by the addition of ethanol to the target to scavenge radical hydroxyl groups thus preventing oxidation. Much like $^{15}\text{O}$ O$_2$ and H$_2$O $^{13}\text{N}$ NH$_3$ can be used in blood flow studies. Other uses include the enzymatic synthesis of amino acids, the reaction of acid chlorides to form amides and the synthesis of $^{13}\text{N}$-cisplatin 62 for measuring uptake of cisplatin in the brain (Scheme 20).$^{88,89}$

\[
\begin{align*}
\text{KCl} & \quad \text{Kl} \quad ^{13}\text{N} \quad \text{NH}_3 \\
\text{AgCl} \quad \text{H}_2\text{O} & \quad \text{cis-PCI}_{2}(^{13}\text{N})\text{NH}_3) \\
\text{RCY 80\%} & \quad 62
\end{align*}
\]

Scheme 20- Synthesis of $^{13}\text{N}$-cisplatin.$^{89}$

While $^{15}\text{O}$ and $^{13}\text{N}$ have demonstrated usefulness for the synthesis of a small number of probes, particularly those suited for perfusion studies. When compared to $^{18}\text{F}$ and $^{11}\text{C}$ their use in complex radiochemical syntheses is at best limited.

Now that the desirable properties of PET radiopharmaceuticals have been established and the synthetic methods for generating them described, some currently used molecular probes will be reviewed. This will be followed by an examination of more recent developments in imaging proliferation in cancer as well as outlining potential targets for the next generation of PET proliferation probes.
1.3 PET Imaging Probes

1.3.1 PET Probes for Imaging Glucose Metabolism

1.3.1.1 $^{[18]}$F-Fluorodeoxyglucose ($^{[18]}$F-FDG)

There are a vast number of radiotracers available for imaging cancers using PET. The current ‘gold standard’ molecular probe is undoubtedly the glucose analogue $^{[18]}$F-FDG, in which the C-2 hydroxyl group of glucose is replaced with an $^{[18]}$F radionuclide (Figure 10). The radiosynthesis of $^{[18]}$F-FDG has been previously covered (Scheme 1 Section 1.2.1.1).

![Figure 10- $^{[18]}$F-FDG 1 and d-Glucose 63.](image)

Cancerous cells show increased consumption of glucose and therefore an increased consumption of $^{[18]}$F-FDG in comparison to normal cells. As mentioned previously (see Section 1.0.1) cancerous cells are able to reprogram the manner in which they carry out glucose metabolism, limiting energy production to glycolysis. This causes the net production of two (adenosine triphosphate) ATP molecules per molecule of glucose compared with thirty six ATP molecules in standard aerobic conditions. As such, additional glucose is required to generate the same amount of energy. Enhanced glucose uptake is caused by an upregulation in expression of the glucose transporting membrane protein Glut 1, mediated by the transcription factors hypoxia inducing factor-1α (HIF-1α) and HIF-2α.

When glucose is taken up by a cell for the purpose of glycolysis it follows a specific pathway to ATP production. However, when $^{[18]}$F-FDG is taken up, it only follows the initial steps of the glycolytic pathway (Figure 11).
When $^{18}\text{F}$-FDG is transported into a tissue it is phosphorylated to 6-phospho-$^{18}\text{F}$-FDG under the control of hexokinase. Once in this state it cannot be further metabolised due to the presence of the C-2 fluorine, which is incompatible with phosphohexose isomerise and glycolysis cannot continue. In addition, Glut transporters cannot deal with phosphorylated forms of glucose and remove 6-phospho-$^{18}\text{F}$-FDG from the cell. $^{18}\text{F}$-FDG is therefore, trapped within the cancerous cell in its mono-phosphorylated state and will accumulate permitting imaging of a tumour. The amount of $^{18}\text{F}$-FDG retained in the lesion is proportional to the rate of glycolysis and so $^{18}\text{F}$-FDG is said to be a marker that images the rate of glycolysis.

1.3.2 PET Probes for Imaging Proliferation: Nucleosides

Another class of compound commonly used for imaging tumours, and the primary focus of this thesis, are radiolabelled nucleosides. These probes differ from $^{18}\text{F}$-FDG in the physiological processes they image and their biodistribution patterns. Although these different classes have overlapping uses their different characteristics ensure they also have specific roles. For example, the natural biodistribution of $^{18}\text{F}$-FDG sees significant accumulation in the brain, heart, inflammatory tissues and bladder with lesser uptake in the liver and kidneys; whereas the nucleoside analogue $^{18}\text{F}$-FLT shows reduced accumulation in the brain and in inflamed tissues.

1.3.2.1 3'-Deoxy-3'-$^{18}\text{F}$-fluorothymidine ($^{18}\text{F}$-FLT)

As was previously mentioned, cancerous cells show rapid proliferation and hence exhibit increased DNA synthesis in comparison to ordinary cells. It can be easily envisaged that
imaging proliferation would allow for the detection of cancers; 3’-deoxy-3’-\([^{18}\text{F}]\)fluorothymidine ([\(^{18}\text{F}\)-FLT]) 65 is a PET molecular probe highly suited to this purpose.\(^{94}\) [\(^{18}\text{F}\)]-FLT is an analogue of the deoxynucleoside thymidine, 64 in which the hydroxyl group in the 3’ position has been replaced by an [\(^{18}\text{F}\)] radionuclide (Figure 12).

![Figure 12- Deoxynucleoside 64 and \([^{18}\text{F}]\)-FLT 65.](image)

The synthesis of [\(^{18}\text{F}\)]-FLT proceeds via a simple nucleophilic substitution reaction on protected nucleoside precursor 66 using [\(^{18}\text{F}\)] KF.K\(_{222}\), followed by hydrolysis to the product (Scheme 21).\(^{95}\)

![Scheme 21- Synthesis of \([^{18}\text{F}]\)-FLT.](image)

There are two routes a cell can make use of to generate the nucleotides required for DNA synthesis, namely the DNA salvage and de novo pathways. In general, tissues utilise the de novo pathway. However, rapidly proliferating tissues show a strong reliance on the salvage pathway.\(^{96}\)

The salvage pathway involves the synthesis of nucleotides using nucleosides and bases formed during DNA degradation. Thymidine and [\(^{18}\text{F}\)]-FLT are therefore actively transported into cells by carrier protein. Once in the cell they are phosphorylated by thymidine kinase 1 (TK1), an enzyme expressed during the DNA synthesis phase of the cell cycle (Figure 13).\(^{96}\)
Thymidine is subsequently subjected to further phosphorylation by nucleoside monophosphate and nucleoside diphosphate kinases (NMPK and NDPK) before being incorporated into DNA by DNA polymerase.\textsuperscript{96} \[^{18}\text{F}\]-FLT also undergoes further phosphorylation by NMPKs and NPDKs but cannot be incorporated into DNA due to the incompatibility of the 3’ fluorine with DNA polymerase.\textsuperscript{97} As such the detection of \[^{18}\text{F}\]-FLT accumulation within rapidly proliferating cells provides a measure of TK1 activity. Since TK1 activity has been shown to correlate with the extent of cellular replication, \[^{18}\text{F}\]-FLT serves to image proliferation. \[^{18}\text{F}\]-FLT has also been shown in various studies to be a sensitive probe for assessing tumour response to treatment.\textsuperscript{98, 99}

However, there are also a number of issues with \[^{18}\text{F}\]-FLT. The main concern is that its uptake and retention by tumours is in general significantly lower than that of thymidine.\textsuperscript{98} The likely cause of the problem of \[^{18}\text{F}\]-FLT uptake will be evaluated by comparison with carbon-11 radiolabelled thymidine.

1.3.2.2 \[^{11}\text{C}\]-Thymidine

The most widely studied molecular probes for cellular proliferation are \[^{11}\text{C}\] labelled thymidine compounds. \[^{11}\text{C}\]-thymidine nucleosides can be synthesised by labelling in two positions on the pyrimidine ring: either the 5-methyl position or the 2-carbonyl position. 5-\[^{11}\text{C}\]-methyl thymidine \textit{70} is synthesised by the double deprotonation of bromide precursor \textit{68}, followed by reaction of intermediate di-anion \textit{69} with \[^{11}\text{C}\] CH\textsubscript{3}I and then deprotection (\textit{Scheme 2}).\textsuperscript{100} Synthesis of 2-\[^{11}\text{C}\]-thymidine \textit{74} is a slightly more involved. Firstly \[^{11}\text{C}\] phosgene is generated by reaction of \[^{11}\text{C}\] CH\textsubscript{4} with chlorine at high temperature to produce \[^{11}\text{C}\] CCl\textsubscript{4}, which can then be oxidised over iron to form phosgene. This is further reacted with liquid ammonia to generate \[^{11}\text{C}\] urea \textit{72}, which can be heated with diethyl β-methylmalate in the presence of sulfuric acid and sulfur trioxide to yield the radiolabeled thymine \textit{73}. Finally the
base is enzymatically condensed with 2’-deoxyribose-1-phosphate using thymidine phosphorylase (Scheme 22).\textsuperscript{101}

\begin{center}
\textbf{Scheme 22- Synthesis of 5-[methyl-\textsuperscript{11C}]thymidine 70 and 2-[\textsuperscript{11C}]thymidine 74.}\textsuperscript{100, 101}
\end{center}

Unfortunately, these probes have several disadvantages which have led to [\textsuperscript{18F}-FLT being considered a more suitable candidate for cancer imaging.\textsuperscript{98} Principally is the fact that [\textsuperscript{11C}]thymidine compounds are subject to rapid \textit{in vivo} degradation by thymidine phosphorylase. This results in the production of numerous metabolites, creating images of a lower quality than [\textsuperscript{18F}-FLT and making kinetic studies more complex.\textsuperscript{98, 102}

Nevertheless, thymidine probes show a variety of properties that should be taken into account when considering the design of novel cancer imaging probes. For example, these compounds are better substrates for many nucleoside carrier proteins and for TK1, in comparison to [\textsuperscript{18F}-FLT.\textsuperscript{97} Additionally, and in contrast to [\textsuperscript{18F}-FLT, [\textsuperscript{11C}]thymidine compounds are suitable substrates for DNA polymerase. Upon entering the salvage pathway (Figure 13) they are completely phosphorylated and incorporated into DNA.\textsuperscript{97} Studies in A549 tumour cells have shown that since [\textsuperscript{18F}-FLT is not incorporated into DNA it is subject to a degree of dephosphorylation by a putative nucleotidase (dNT). As a result the phosphorylated [\textsuperscript{18F}-FLT can be transported out of the cell following dephosphorylation by this nucleotidase, which could explain its poor uptake.\textsuperscript{103} Since thymidine shows improved uptake and retention within proliferating cells when compared to [\textsuperscript{18F}-FLT, it suggests that if one could design a [\textsuperscript{11C}]-thymidine molecule stable to thymidine phosphorylase, it could overcome the limitations of both FLT and thymidine compounds.\textsuperscript{103} This would provide a metabolically robust compound that allows sensitive proliferation imaging.
Unfortunately, the stability of $^{[18F]}$-FLT to breakdown by thymidine phosphorylase has been attributed to the replacement of the 3’ hydroxyl with $^{[18F]}$. As was previously mentioned, this $^{[18F]}$ radiolabel contributes to the uptake issues of FLT making it a poorer substrate for TK1 and unreactive with DNA polymerase. It is clear that a different approach to enhancing the stability of nucleoside molecular probes is required.

1.3.3 Recent Developments in Imaging of Proliferation

1.3.3.1 Sulfur Based Pyrimidine Nucleosides

A recent study by Toyohara et al. reported the synthesis of a sulfur based thymidine nucleoside proliferation marker 5-[Methyl-$^{11}$C]thiothymidine using a Stille coupling with $^{[11]}$C CH$_3$I (Scheme 23).

![Scheme 23- Synthesis of 5-[Methyl-$^{11}$C]thiothymidine 76.](image)

Although it should be noted that the results given are for in vivo studies in mice and not clinical data, this probe displays a number of exciting properties. For example, it exhibits relative metabolic stability in plasma, indicating that it will not suffer from the poor image quality seen in $^{[11]}$C-thymidine molecules. It is also efficiently incorporated into DNA via the salvage pathway due to the presence of the 3’ hydroxyl group. As a result, 5-[methyl-$^{11}$C]-thiothymidine shows improved uptake in rapidly proliferating tissues in comparison to [methyl-$^{3}$H]-FLT; these results were mirrored in tumour models with 76 again showing higher accumulation.

Based on the initial data from in vivo mice studies for 5-[methyl-$^{11}$C]-thiothymidine, $^{[11]}$C thiothymidine nucleosides present an exciting prospect for the imaging of proliferation in cancers using PET. They appear to show superior properties to the current PET proliferation markers $^{[18F]}$-FLT and $^{[11]}$C-thymidine and hence warrant further investigation.
Radu et al have reported the synthesis of a deoxycytidine based nucleoside analogue: 1-(2'-deoxy-2'-$^{18}$F]-fluorarabinofuranosyl) cytosine also known as $^{18}$F-FAC 78 (Figure 14).  

\[ \text{Deoxycytidine 77 and } ^{18}\text{F}-\text{FAC 78.} \]

Synthesis of $^{18}$F-FAC begins with introduction of the $^{18}$F fluoride radiolabel to furanose precursor 79. This is subsequently transformed into bromide 81 using HBr in acetic acid, before being subjected to the Vorbrüggen glycosylation with bis-trimethylsilylated cytosine 82 and finally deprotected to the desired product 78 (Scheme 24).

\[ \text{Scheme 24- Synthesis of } ^{18}\text{F}-\text{FAC.} \]

In a similar fashion to deoxthymidine and $^{18}$F-FLT, $^{18}$F-FAC is taken up via a nucleoside salvage pathway in this case the deoxycytidine pathway. There are however, a number of differences in the pathways and the uptake of $^{18}$F-FAC itself. The initial phosphorylation for example, is performed by deoxycytidine Kinase (dCK) in place of TK1 (Figure 15). Another
key difference is evidence showing that $[^{18}\text{F}]$-FAC is a substrate for DNA polymerase and therefore is incorporated into DNA.\textsuperscript{96, 106}

\begin{center}
\includegraphics[width=\textwidth]{DNA_salvage_pathway.png}
\end{center}

*Figure 15*- The DNA salvage pathway showing routes taken by deoxycytidine (Cyd) and $[^{18}\text{F}]$-FAC.\textsuperscript{96}

Thus, $[^{18}\text{F}]$-FAC uptake occurs in cells showing increased expression of dCK including: myeloid and lymphatic cancers, a number of solid tumours, as well as lymphocytes and activated T-cells. $[^{18}\text{F}]$-FAC therefore serves to image dCK activity in these cells, allowing imaging of immune activation. Usefully $[^{18}\text{F}]$-FAC shows a different biodistribution pattern to $[^{18}\text{F}]$-FLT and $[^{18}\text{F}]$-FDG with significant quantities of the probe found in the thymus, spleen, intestines, bone marrow and liver.\textsuperscript{106, 108} Another interesting potential use of $[^{18}\text{F}]$-FAC is in predicting the response to specific classes of nucleoside based chemotherapeutic agents. Cytarabine, fludarabine, gemcitabine and decitabine are all cytotoxic pro-drugs activated by dCK that suffer from poor response in tumour cells displaying reduced dCK activity.\textsuperscript{109} Measuring $[^{18}\text{F}]$-FAC uptake would allow evaluation of the likelihood of a positive response to these drugs.\textsuperscript{108}

Although $[^{18}\text{F}]$-FAC was the primary focus of Radu’s report, mention was also made of another deoxycytidine nucleoside analogue, 2‘-deoxy-2’,2’-difluorocytidine (dFdc or gemcitabine)\textsuperscript{84}, which showed superior selectivity and retention in proliferating T-cells in comparison to $[^{18}\text{F}]$-FAC. This could be attributed to the additional hydrogen bonding in the dCK active site between the ribo-fluorine and a tyrosine residue.\textsuperscript{110} dFdc was not investigated for use as a PET imaging probe due to envisaged synthetic difficulties in generating a precursor to dFdc suitable for labelling with $[^{18}\text{F}]$ fluoride (*Figure 16*).\textsuperscript{106}
While not strictly used for imaging proliferation, the useful properties exhibited by $^{18}$F-FAC and the improvements shown by dFdc warrant further investigation of this molecule through the use of different radiolabelling strategies.

Furthermore, the principle of replacing an arabino-fluorine with a germinal difluoro moiety could be applied to other nucleosides with the aim of altering their selectivity for thymidine kinase and deoxycytidine kinase enzymes (Figure 17).

For example, $^{11}$C and $^{18}$F-FMAU have both been shown to be stable in vivo and are incorporated into DNA giving them advantageous properties in comparison to $^{18}$F-FLT and $^{11}$C-Thymidine (Figure 17). Unfortunately, FMAU is known to be a better substrate for the cell cycle independent TK2 in comparison to TK1. This suggests that FMAU is perhaps not an ideal candidate for imaging the proliferation of tumours, as the uptake of TK2 selective nucleosides has been previously demonstrated not to correlate with the proliferation of tumours. However, FMAU has also been shown to be a substrate for dCK. Bearing in mind the aforementioned favourable binding interactions for difluoro nucleoside analogues with dCK, it would be of interest to synthesise a di-fluoro analogue of this molecule and compare its selectivity for TK1, TK2 and dCK and utility as PET proliferation probe with that of $^{11}$C/$^{18}$F-FMAU.
1.4 Aims and Objectives

For the past ten years the Aboagye group has researched PET based imaging of proliferation in cancerous cells with a focus on $[^{18}\text{F}]-\text{FLT}$. As a result of the recent developments in sulfur based pyrimidine and 2’-deoxy-2’,2’-difluoro nucleoside analogues, chemistry discovery efforts will now be focussed on the study of these molecules.

Sulfur Based Pyrimidine Nucleosides

The aim of this project was the development of novel methodology to facilitate synthesis of a number of $[^{11}\text{C}]$ labelled thiothymidine nucleoside analogues. This was to be followed by an evaluation of their use as PET probes for imaging proliferation (Figure 18).

![Figure 18- 2-$[^{11}\text{C}]$-thiothymidine analogue with variable ‘R’ group.](image)

The synthesis of these compounds was based on the generation of a novel precursor $\beta$-lactam nucleoside 86. This would be heated to undergo an elimination reaction to yield an intermediate azetone 87, which would then be reacted with a variety of $[^{11}\text{C}]$-isocyanates to allow the late stage introduction of the radiolabel (Scheme 25).

![Scheme 25- A simplified illustration of the proposed synthesis of the 2-$[^{11}\text{C}]$-thiothymidine analogues.](image)
The theoretical basis for azetones, their potential reactivity and the methods for producing $[^{11}\text{C}]$-isocyanates will be discussed in full in Sections 2.0.1 and 2.0.2.

2’-Deoxy-2’,2’-difluoro Nucleosides

The aim of this project was to synthesise and radiolabel an analogue of dFdc with an $[^{11}\text{C}]$ radiolabel in the 5-position of the cytosine ring 89* using a Stille coupling strategy (Scheme 26).\(^6^4\)

![Scheme 26- Proposed synthesis of analogue 89*.](image)

It was hoped that using stannane precursor 88 would permit easy access to 89*, circumventing the envisioned synthetic difficulties of producing an $[^{18}\text{F}]$-dFdc analogue while maintaining the desirable properties of selectivity and retention. Furthermore, a similar synthetic strategy could be applied to the synthesis of a 2’,2’-difluoro analogue of $[^{11}\text{C}]$-FMAU 90* (Figure 19).

![Figure 19- Di-fluoro $[^{11}\text{C}]$-FMAU analogue $[^{11}\text{C}]$-dFMAU 90*.](image)
RESULTS AND DISCUSSION

2. Sulfur Based Pyrimidine Nucleosides

2.0 Overview

The aims of this project, as previously discussed (Section 1.4), were twofold: development of novel methodology to allow radiolabelling of precursor β-lactam nucleoside 86 and synthesis of precursor 86 itself (see Section 2.2.1 for a proposed route).

2.0.1 Azetones

Outlined in Scheme 27 the generation of an azetone intermediate and subsequent reaction with an \(^{[11}\text{C}]\) isocyanate would allow the late stage induction of a radiolabel as part of novel methodology for the synthesis of pyrimidine bases. Azetones, as a result of the anti-aromatic nature of their structure and its resonance forms (in violation of Hückel’s \(4n + 2\) rule), are highly reactive and unstable compounds (Scheme 27).

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

Scheme 27- Resonance forms of a generic unfunctionalised azetone.

During the late 1960s and early 1970s, Arbuzov and co-workers reported the synthesis of a number of stable azetones through the reaction of benzoyl isocyanates with a variety of alkynes (Scheme 28). Unfortunately, these results could not be reproduced by other researchers. Pericás et al., reported the synthesis of 2-phenyl-4,5-di-t-butoxy-1,3-oxazin-6-one 95 from similar starting materials to those used by Arbuzov and unambiguously assigned the structure using X-ray crystallography. They suggested that perhaps the products isolated by Arbuzov were not azetones but rather the corresponding 1,3-oxazin-6-ones derivatives (Scheme 28).
Scheme 28- Azetone synthesis reported by Arbuzov and 1,3-oxazin-6-one synthesis by Pericás.\textsuperscript{117, 120}

In fact, the only other claim to isolation of an azetone was made by Wentrup et al. who reported the formation of an N-adamantylazetone 98 in equilibrium with its corresponding imidoyletene 97. Using vacuum pyrolysis (FVP) of 1-adamantyl-4-methyl-5-phenyl-2,3-dihydropyrrole-2,3-dione 96, products were monitored by mass spectrometry and I.R. within an argon matrix at 18 \(^0\)K (Scheme 29).\textsuperscript{121}

Scheme 29- Generation of an azetone by FVP.\textsuperscript{121}

Benzo-fused forms of azetones (benzazetones) on the other hand, are known to be stable and have been synthesised and fully characterised.\textsuperscript{114} Initially reported to be formed by the reaction of \textit{N}-alkyl anthranil salts with triethylamine, benzazetones have been occasionally described in the literature including a more recent radiochemical synthesis by Långström et al (Scheme 30).\textsuperscript{114, 122, 123}
Unfortunately, these stable azetone derivatives are not suited for the desired methodology and the isolation of un-fused azetones in ambient conditions seems unlikely. However, despite discounting the possibility of forming stable azetones, Pericás et al. suggested the probability of an intermediate azetone 103 in their synthesis of 95. These claims were substantiated through the use of computational methods (Scheme 31).120

Moreover, there have been sporadic reports in the literature of reactions purporting to proceed via an intermediate azetone 103 and imidoylketene 104.120

120 Crucial diagrams have not been included due to formatting constraints, but they are referenced in the text. Further details can be found in the original source.
Scheme 32- Synthesis of 1,3-oxazin-6-one derivative 108.

In this case, the proposed pathway involved an E1cB style β-elimination of acetate from 105 to generate the azetone 106. This underwent a retro-[4-exo-dig] ring opening to generate the imidoylketene 107, which reacted in a [6-exo-dig] ring closure to form 108.\textsuperscript{127} The authors subsequently published follow up computational studies on this process. Results complemented those of earlier work suggesting that proceeding \textit{via} an azetone is the most energetically favourable pathway, despite being un-favoured by Baldwin’s rules, to accessing the 1,3-oxazin-6-one analogues.\textsuperscript{128}

From these observations one can conclude that there appears to be limited physical evidence for the existence of azetones such as 103 and 106. However, indirect evidence from computational studies and from reactions purporting to proceed \textit{via} these intermediates, suggests that developing methodology with the aim of utilising an azetone intermediate is an endeavour worthy of investigation.

In more detail, our proposed methodology involved the use of a β-lactam nucleoside precursor functionalised with a sulfoxide 109, which could be heated to undergo \textit{syn}-elimination forming the intermediate azetone 87 and benzene sulfenic acid (Scheme 33).\textsuperscript{129}
Scheme 3- Proposed mechanistic route to nucleosides 113.

Electrocyclic ring opening of the unstable anti-aromatic lactam would result in the formation of intermediate imidoylketene 111. This would subsequently undergo, by analogy with related transformations, stepwise addition with a variety of [¹¹C]-isocyanates leading to the isolation of nucleosides 113.¹³⁰,¹³¹

2.0.2 Carbon-11 Labelled Isocyanates

Having outlined the proposed methodology for generating novel 2-[¹¹C]-thiothymidine nucleosides and discussed the theoretical basis behind this proposition, it would be prudent to briefly review the methods available for generating the required ¹¹C radiolabelled isocyanate precursors.

The most widely investigated method for generating [¹¹C]-isocyanates involve reactions with radiolabelled phosgene. As previously mentioned [¹¹C] phosgene is generated either by the reaction of [¹¹C] CH₄ with chlorine at elevated temperatures, followed by oxidation over iron granules in a stream of oxygen; or by reacting [¹¹C] CO with platinum tetrachloride.³ Phosgene is then typically reacted directly with an amine, sometimes in the presence of base, to generate an isocyanate (Scheme 34).¹³²,¹³³
Scheme 34- Formation of phenyl-[\textsuperscript{11}C]-isocyanate 115 and urea by-product 116.\textsuperscript{132}

Note: RCY given for urea formed by administering excess aniline.

However, these methods tend to suffer from side reactions leading to the production of symmetrical [\textsuperscript{11}C]-ureas as a result of administration of excess amine. Particularly in the case of PET where microscale quantities are used and especially when using gaseous amines, this can prove to be problematic.\textsuperscript{133}

Brown and co-workers have developed a number of methods for overcoming the problems associated with the formation of ureas as a side product. One such example involves the reaction of phosgene with sulfinylamines (Scheme 35).\textsuperscript{134}

Scheme 35- Synthesis of [\textsuperscript{11}C]-isocyanates 118 using sulfinylamines.\textsuperscript{134}

Sulfinylamines are well suited to this purpose being sufficiently reactive to form an isocyanate with phosgene but without sufficient nucleophilicity to further react with the isocyanate and form ureas. This permits further functionalization to unsymmetrical ureas as desired.\textsuperscript{134} A further alternative developed by Brown et al. involves making use of the \textit{N,N-}bis(trimethylsilyl)methylamine 121 (Scheme 36).\textsuperscript{135}

Scheme 36- Synthesis of methyl-[\textsuperscript{11}C]-isocyanate 122 using silyl-amines.\textsuperscript{135}
Unfortunately, these methods generally suffer from low radiochemical yields and the production of phosgene itself is capricious. More attractive methods make use of $[^{11}\text{C}]\text{ CO}$ or $[^{11}\text{C}]\text{ CO}_2$ which are much more readily available.

An effective method for producing radiolabelled isocyanates and ureas without the use of $[^{11}\text{C}]$ phosgene has been demonstrated by Långström et al. This method utilises the rhodium catalysed carbonylation of azides to form isocyanates (Scheme 37).

![Scheme 37- Proposed pathway for $[^{11}\text{C}]$-isocyanate formation by carbonylation.]

The initial decomposition of azide 123 results in the production of an intermediate nitrene, which undergoes reaction with $[^{11}\text{C}]$ CO and the rhodium catalyst to yield a further intermediate in the form of isocyanate 126 or an isocyanate co-ordinated rhodium complex 125. The isocyanates can then undergo further conversion to ureas 128 and carbamates 127.

The ideal situation would involve the use of $[^{11}\text{C}]$ CO$_2$ as it is available directly from the cyclotron without the need for any further manipulation. A promising example was reported by Van Tilburg et al. whereby phenyl-$[^{11}\text{C}]$-isocyanate was formed by the reaction of phenyl-triphenylphosphinimine with carbon dioxide (Scheme 38).
Scheme 38- Reaction of $[^{11}\text{C}]$ CO$_2$ with phenyl-triphenylphosphinimine to form isocyanate 115.$^{138}$

Trapping of $[^{11}\text{C}]$ CO$_2$ in a solution of the triphenylphosphinimine 129 in THF with subsequent heating allowed the formation of the desired product 115, which was then further reacted to form an unsymmetrical $[^{11}\text{C}]$ labelled urea 130. This was the only example used to illustrate the usefulness of this protocol. The potential for use of a variety of other phosphinimines was suggested, although the structural diversity of available phosphinimines may still prove be a limiting factor.$^{138}$

Inspired by the work of Hooker et al. Wilson and co-workers have recently published a method for the synthesis of a wide variety $[^{11}\text{C}]$-isocyanates from $[^{11}\text{C}]$ CO$_2$ (Scheme 39).$^{69,139}$

Scheme 39- Synthesis of $[^{11}\text{C}]$-isocyanates 135 by fixation of $[^{11}\text{C}]$ CO$_2$. $^{139}$

This protocol involves the fixation of $[^{11}\text{C}]$ CO$_2$ using 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) 131 in a solution of acetonitrile at ambient temperatures and pressures. Displacement of BEMP by an amine results in the formation of a carbamate salt 133, which then upon addition of POCl$_3$ is dehydrated to form the isocyanate 135.
The authors demonstrated the scope of this reaction by successfully synthesising a variety of isocyanates and reacting these to form both $[^{11}\text{C}]$ carbamates and ureas. As a result of the wide scope, mild conditions and the use of readily available $[^{11}\text{C}]\text{CO}_2$ this appears to be a most promising method. However, it should be noted this protocol does require careful stoichiometric control of reagent addition to avoiding the formation of symmetrical ureas, a problem inherent in many of these discussed methods.$^{139}$
2.1 Methodology Studies

2.1.1 Synthesis of Sulfoxide Precursors

To assess the validity of the devised strategy for synthesising thymidine analogues from the corresponding β-lactam nucleosides and isocyanates, preliminary investigations were performed using more chemically simple systems. It was envisaged that a suitable sulfoxide precursor could be accessed by the thiation and oxidation of an unsubstituted nitrogen-protected β-lactam (Scheme 40).

Scheme 40- Sulfoxide precursors for trial methodology studies.

Consultation of the literature prior to attempting this synthesis revealed conflicting views regarding the viability of enolates formed from unsubstituted β-lactams. Urbach and Kano, reported successful enolisation and reaction of N-silyl and N-para-methoxyphenyl (PMP) protected unsubstituted lactams. However, Wilson and Miller suggested that these systems were unsuitable for this purpose as a result of enolate instability, self-condensation and reaction via the alkoxide anion. To test these competing theories, synthesis and enolisation of both N-tert-butyldimethylsilyl (TBS) and N-PMP protected lactams 141 and 146 were performed (Scheme 41).

Scheme 41- Synthesis of N-silyl protected β-lactam 143.

Protection of 2-azetidinone 140 with TBSCI proceeded smoothly giving 141 in good yields. In corroboration with the report of Williams et al. initial attempts at thiation of 141 using standard
conditions (addition of the lactam to LDA, followed by stirring at −78 °C and then treatment with a solution of diphenyl disulfide) yielded only starting material. Fortunately, it was discovered that by modifying the addition of reactants so that both the lactam 141 and diphenyl disulfide were added simultaneously to a solution of LDA, sulfide 142 could be isolated in satisfying yields. Perhaps concurrent addition of the lactam and electrophile permitted immediate reaction of diphenyl disulfide with the enolate, which was highly unstable over more prolonged periods.

With multiple grams of sulfide 142 now in hand a variety of conditions were screened for oxidation to the sulfoxide (Table 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Time</th>
<th>Sulfoxide Yield (%)</th>
<th>Sulfone Yield (%)</th>
<th>Starting Material (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NBS, Phosphate Buffer, H₂O₂, MeCN, 35°C</td>
<td>48 h</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>NaIO₄: SiO₂ CH₂Cl₂, 140 °C Microwave</td>
<td>2.5 min</td>
<td>18</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Ac₂O, H₂O₂, SiO₂, CH₂Cl₂</td>
<td>36 h</td>
<td>79</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Table 2* - Conditions evaluated for oxidation of 142.

Utilising hydrogen peroxide in acetonitrile with a phosphate buffer and catalytic N-bromosuccinimide resulted mainly in decomposition with some starting material recoverable (Table 2, entry 1). Microwave mediated oxidation using sodium metaperiodate supported on silica gel was more successful (Table 2, entry 2). However, full conversion was not observed, with over oxidation to the sulfone and decomposition also evident. Making use of milder conditions by reacting 142 with hydrogen peroxide and catalytic acetic anhydride at ambient temperature resulted in full conversion after 36 hours, with no over oxidation or decomposition observed (Table 2, entry 3).

Having developed a suitable synthetic route for accessing diastereomeric sulfoxide precursors for the methodology studies, N-PMP protected lactam-sulfoxide 148 was also synthesised (Scheme 42).
Following the protocol of Easton et al., reaction of 3-bromopropionyl chloride 144 with para-anisidine gave amide 145 in excellent yields. In an improvement over the literature procedure it was discovered that β-lactam 146 could be cyclised and isolated in a 72% yield by recrystallization from DMF, compared to 58% reported by flash silica column chromatography. Problems were encountered during initial attempts at thiation of 146 due to insolubility of this lactam at −78 °C in THF. Thankfully, the enolate of 146 proved to be sufficiently soluble at low temperature. Therefore, by increasing the temperature of the lactam-diphenyl disulfide solution, followed by slow addition to a −78 °C solution of LDA in THF, solubility was maintained, allowing for successful reaction. Subsequent oxidation furnished exclusively sulfoxide 148.

Through completion of the synthesis of sulfoxides 143 and 148 it was demonstrated that enolisation and subsequent reaction of lactams 141 and 146 is possible given suitable modifications of addition methods. Moreover, attempts could now be made at syn-elimination of the sulfoxides and trapping of any resulting imidoylketenes.

2.1.2 Trial Reactions of Sulfoxide Precursors

For the purpose of these studies having two differently protected lactams was beneficial: the N-silyl protected lactam could be easily deprotected and subjected to further functionalization if the
methodology proved successful, whereas the N-PMP protected lactam would provide more robust protection should the TBS group prove labile to the heating needed for elimination of the sulfoxide to occur.\textsuperscript{129, 148}

Sulfoxide 143 was subjected to various different conditions in an attempt to induce elimination of the sulfoxide (Table 3).

\centering
\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Entry & Conditions* & Yield (\%) \\
\hline
1 & Toluene-d\textsuperscript{8}, 130 °C, 0.1 M, 4 h & 0 \\
 & 5eq. Tosyl isocyanate, Toluene, 130 °C, 0.1 M, 4 h & 0 \\
2 & THF, 130 °C, 0.1 M, 24 h & 0 \\
 & 5eq. Tosyl isocyanate, Furan, THF, 130 °C, 0.1 M, 24 h & 0 \\
\hline
\end{tabular}
\caption{Table 3- Attempted elimination of sulfoxide 143.}
\end{table}

Initial attempts were promising: a test reaction performed in deuterated toluene showed consumption of starting material leading to decomposition (Table 3, entry 1). It was hoped that this decomposition was proceeding via the azetone and therefore the imidoylketene, thus a number of attempts were made at trapping this intermediate. Trapping reactions carried out using tosyl isocyanate in either toluene or THF were unsuccessful leading only to decomposition (Table 3, entries 2 and 3). It was thought that benzenesulfinic acid produced as a by-product of the syn-elimination could be causing the observed decomposition. A further attempt was
therefore made using furan as a trap for the acid (Table 3, entry 4); however this again was unsuccessful. Another possible reason behind these observations could be the poor thermal stability of the sulfoxide itself, particularly due to the potential lability of the TBS group.

Indeed, these concerns proved to be justified as demonstrated in the attempted elimination of sulfoxide 148 (Table 4).

![Diagram of sulfoxide 148](image)

**Table 4- Attempted elimination of sulfoxide 148.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene-d₈, 130 °C, 0.1 M, sealed tube, 24 h</td>
<td>Starting material</td>
</tr>
<tr>
<td>2</td>
<td>Toluene-d₈, 130 °C, 0.1 M, microwave, 24 h</td>
<td>Starting material</td>
</tr>
<tr>
<td>3</td>
<td>Toluene-d₈, 160 °C, 0.1 M, sealed tube, 24 h</td>
<td>Starting material</td>
</tr>
<tr>
<td>4</td>
<td>Toluene-d₈, 160 °C, 0.1 M, microwave, 24 h</td>
<td>Starting material</td>
</tr>
</tbody>
</table>

As in the case for sulfoxide 143 a test reaction was firstly carried out to observe consumption of 148. Despite prolonged heating at high temperatures only starting material was recovered (Table 4, entry 1). Using microwave radiation and increasing the reaction temperature again gave no conversion (Table 4, entries 2, 3 and 4). Clearly syn-elimination of the sulfoxide was not occurring in these systems. Consequently, this also suggested that the observed decomposition of lactam 143 was due to instability of the TBS group to high temperatures rather than elimination and formation of the azetone.
2.1.3 Synthesis and Trial Reactions of Selenoxide Precursors

As a result of the unsuccessful sulfoxide fragmentations a more reactive system was proposed: the syn-elimination of selenoxides to their corresponding alkenes is known to occur spontaneously at room temperature.\(^{149}\) It was therefore hoped that the increased reactivity of the selenoxides would allow the desired elimination reactions to occur where they had been unsuccessful in the more stable sulfoxide systems (Scheme 43).

![Scheme 43- Proposed synthesis and elimination of N-PMP protected selenoxide.](image)

It was envisaged that selenylation of 146 could be carried out using a procedure similar to that described for thiation of 146. Oxidation of selenide 149 would be performed with DMDO at low temperatures to reduce the number of by-products in the mixture before warming the selenoxide 150 to room temperature to undergo elimination.

Problems were encountered when attempting selenylation of 146: using a slight excess of LDA and phenylselenyl bromide resulted in the recovery of a large portion of starting material (60%), as well as a mixture of the desired mono-selenide 149 and the bis-selenide 151. Presumably the pK\(_a\) of alpha carbonyl proton in 149 was lower than those in 146 resulting in a second deprotonation and reaction with another equivalent of the selenyl bromide to form 151. It was therefore decided to attempt full conversion to 151 by using additional equivalents of phenylselenyl bromide and LDA. This resulted in starting material consumption and the formation of a mixture of the mono and bis-selenylated products in more acceptable yields (Scheme 44).
Scheme 44- Synthesis of selenide 149.

The bis-selenide was then successfully mono-de-phenylselenylated using the enolate of acetophenone resulting in exclusive generation of 149 (Scheme 44).

With the desired selenide now available, oxidation to the selenoxide was performed using DMDO. As previously mentioned, DMDO was seen as an ideal reagent for this step due to the production of relatively unreactive acetone as a by-product which would make attempted reaction with the isocyanate more likely (Scheme 45).\textsuperscript{150}

\begin{center}
\begin{tikzpicture}
\node at (-3,0) {146};
\node at (-3,-1) {149};
\node at (0,0) {$\text{LDA, PhSeBr}$};
\node at (0,1) {THF, -78 °C};
\node at (-3,-1.5) {11%};
\node at (0,-1.5) {48%};
\end{tikzpicture}
\end{center}

\begin{center}
\begin{tikzpicture}
\node at (-3,0) {151};
\node at (-3,-1) {149};
\node at (0,0) {$\text{LDA, Acetophenone}$};
\node at (0,1) {THF, -78 °C};
\node at (-3,-1.5) {65%};
\end{tikzpicture}
\end{center}

\textit{Scheme 44- Synthesis of selenide 149.}

\begin{center}
\begin{tikzpicture}
\node at (-3,0) {149};
\node at (-3,-1) {150};
\node at (0,0) {$\text{DMDO, Acetone,}$};
\node at (0,1) {Toluene, 115 °C};
\node at (-3,-1.5) {98%};
\node at (0,-1.5) {33%};
\node at (3,0) {149};
\node at (3,-1) {152};
\end{tikzpicture}
\end{center}

\textit{Scheme 45- Oxidation to and attempted elimination of selenoxide 150.}

As in previous examples, attempted eliminations were first carried out in the absence of an isocyanate to ascertain starting material consumption. Unexpectedly oxidation of 149 resulted in almost quantitative formation of the stable selenoxide 150. This was considered somewhat peculiar as stable selenoxides are generally only isolatable in systems lacking β-protons.\textsuperscript{151, 152} Nevertheless, isolation of 150 was deemed advantageous as the elimination reaction could be carried out as a separate step using solvents entirely unreactive to the intermediate ketene. When refluxed in toluene the selenoxide was fully converted, undergoing disproportionation to furnish
both keto β-lactam 152 and selenide 149 as products. This disproportionation implied that the reaction was occurring \textit{via} a bimolecular process. In order to avoid this and favour elimination, the experiment was repeated at a low concentration (0.005 M) and at higher temperature by slow addition (10 hours) of 150 to refluxing xylenes. Unfortunately the same result was obtained.

By nature β-lactams are strained systems.\textsuperscript{153} It is therefore possible that elimination was not observed as a result of this strained nature preventing the selenoxide from effecting deprotonation in the β-position (Scheme 46).

$$\text{Scheme 46- Transition state required for elimination.}$$

### 2.1.4 Synthesis of Alternative Azetone Precursors

With both the sulfoxide and selenoxide systems failing to react \textit{via} the anticipated pathways it was clear that an alternative approach to generating the azetone was needed. Pyrolysis of carboxylate salts is a common method for inducing decarboxylation. It was therefore proposed that heating potassium salt 153 in the presence of 18-crown-6 would result in decarboxylation, followed by elimination, giving rise to the desired azetone intermediate (Scheme 47).

$$\text{Scheme 47- Formation of the azetone 154 by decarboxylative elimination from 153.}$$

β-lactam 155 has been reported in the literature and was suggested as suitable starting point for the synthesis of a carboxylate salt analogous to 153 (Scheme 48).\textsuperscript{154}
Starting from 155, base mediated epimerisation leading to the thermodynamically favoured trans-diastereomer could be followed by cleavage of the benzyl group of 156 to give the corresponding alcohol. Consequently, functionalization as a tosylate 157 and hydrolysis of the ester would furnish potassium salt 158.

The synthesis began with the Staudinger cycloaddition of imine 160 (formed by condensation of ethyl glyoxylate 159 with para-anisidine) with benzyloxyacetyl chloride to give the cis-β-lactam 155 (Scheme 49). \(^{154,155}\)

Epimerisation alpha to the ethyl ester proved to be difficult and a variety of conditions were evaluated (Table 5).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\text{Na}_2\text{CO}_3, \text{MeCN}:\text{H}_2\text{O}, 0.03 \text{ M})</td>
<td>Starting material</td>
</tr>
<tr>
<td>2</td>
<td>(\text{NHMe}_2, \text{benzene}, 0.1 \text{ M})</td>
<td>Starting material</td>
</tr>
<tr>
<td>3</td>
<td>(\text{DBU, CH}_2\text{Cl}_2, 0.1 \text{ M})</td>
<td>Starting material</td>
</tr>
</tbody>
</table>

*Table 5- Attempted epimerisation of lactam 155.*
The initially used conditions were based on the work of Alcaide et al. who reported the instability of similarly functionalised β-lactams to harsher conditions.\textsuperscript{156} Unfortunately in these cases only starting material was recovered (\textit{Table 5}, entries 1 and 2). Using a stronger base also resulted in no observable conversion (\textit{Table 5}, entry 3).\textsuperscript{157} It was thought that by synthesising the corresponding aldehyde of 155 the system could be made more susceptible to the epimerisation conditions by decreasing the pK\textsubscript{a} of the proton alpha to the carbonyl functionality.

Aldehyde 164 was easily accessed in three steps using the procedure of Alcaide et al. (\textit{Scheme 50}).\textsuperscript{158}

\begin{center}
\begin{tikzpicture}
\node (161) at (0,0) {161};
\node (162) at (2,0) {162};
\node (163) at (4,0) {163};
\node (164) at (6,0) {164};
\node (Scheme 50) at (3,-1) {\textit{Scheme 50- Synthesis of aldehyde 164}.\textsuperscript{158}};
\path[->] (161) edge node[auto] {Formic Acid, MeOH} (162);
\path[->] (162) edge node[auto] {69\%} (163);
\path[->] (163) edge node[auto] {1.0 M HCl, CHCl\textsubscript{3}} (164);
\end{tikzpicture}
\end{center}

Condensation of \textit{para}-anisidine with glyoxal 161 gave di-imine 162 in good yields.\textsuperscript{159} Staudinger cycloaddition of 162 with benzyloxyacetyl chloride furnished β-lactam 163, which could then be hydrolysed directly to the desired aldehyde. With aldehyde 164 in hand further attempts at epimerisation could be made. Unfortunately these efforts also resulted in the recovery of starting material (\textit{Table 6}).\textsuperscript{156}

\begin{center}
\begin{tikzpicture}
\node (164) at (0,0) {164};
\node at (1.5,0) {epimerisation};
\end{tikzpicture}
\end{center}

\textit{Table 6- Attempted epimerisation of aldehyde 164.}
\begin{center}
\begin{tabular}{|c|c|c|}
\hline
\textbf{Entry} & \textbf{Conditions} & \textbf{Result} \\
\hline
1 & Na\textsubscript{2}CO\textsubscript{3}, MeCN:H\textsubscript{2}O, RT, 0.03 M & starting material \\
2 & NHMe\textsubscript{2}, benzene, RT, 0.10 M & starting material \\
\hline
\end{tabular}
\end{center}
With attempted epimerisation of the β-lactams 155 and 164 proving to be unsuccessful a new strategy was envisaged whereby the desired trans-geometry could be accessed by simple S\textsubscript{N}2 reaction (Scheme 51).

\[
\begin{align*}
&\text{165} \\
\xrightarrow{TIO, CO_2Et, PMP} \\
\text{Nal, Acetone} \\
\xrightarrow{} \\
&\text{166}
\end{align*}
\]

\textit{Scheme 51- Suggested synthesis of trans-β-lactam 166.}

Based on similar literature examples it was thought that displacing the triflate in 165 with sodium iodide would give exclusively the trans-lactam 166.\textsuperscript{160} Synthesis of 165 was relatively simple using lactam 155 as a starting point (Scheme 52).

\[
\begin{align*}
&\text{155} \\
\xrightarrow{H_2, Pd/C, THF, RT} \\
&\text{165} \\
\xrightarrow{NH_2HCO_2, Pd/C, MeOH, reflux, 0.30 M} \\
&\text{155} \\
\xrightarrow{168, 169, 15\%} \\
\xrightarrow{15\%} \\
\xrightarrow{14\%} \\
\end{align*}
\]

\textit{Scheme 52- Synthesis of triflate 165.}

A number of conditions were screened in order to find a suitable procedure for deprotection of benzyl ether 155 (Table 7).
### Table 7- Deprotection of benzyl ether 155.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂, Pd(OH)₂/C, MeOH, 0.12 M</td>
<td>Starting material</td>
</tr>
<tr>
<td>2</td>
<td>NH₄HCO₂, Pd/C, MeOH, reflux, 0.30 M</td>
<td>167 36%, 168 15%, 169 14%</td>
</tr>
<tr>
<td>3</td>
<td>H₂, Pd/C, THF, 0.03 M</td>
<td>167 97%</td>
</tr>
</tbody>
</table>

Initial attempts at hydrogenation using Pearlman’s Catalyst proved to be unsuccessful due to solubility issues of 155 in methanol (Table 7, entry 1). Increasing the temperature to reflux and using ammonium formate overcame the solubility problems but also lead to the formation of two side products (Table 7, entry 2). The presence of these could be attributed to heating of the mixture coupled with the action of ammonia released during the reaction: 168 is a product of trans-esterification with methanol and 169 is the epimerised form of the desired product. In order to prevent the formation of by-products, milder reaction conditions and a different solvent were used. Carrying out the hydrogenation in THF with palladium on carbon permitted deprotection at room temperature and resulted in excellent yields of 167 (Table 7, entry 3).

Functionalization of 167 as its triflate ester occurred quantitatively and was followed by displacement of the triflate using sodium iodide. Interestingly a 1:1 mixture of diastereomers 166 and 170 was recovered after reaction. Since a large excess of sodium iodide was used this outcome can be rationalised by the displacement of one iodide by another until an equilibrium mixture is reached (Scheme 53).

![Scheme 53- Synthesis of potassium salt 171.](image-url)

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Fortunately, these diastereomers could be easily separated. The final step in this synthesis was the hydrolysis of ethyl ester 166 to the corresponding potassium salt, which was accomplished in fair yields using potassium trimethylsilanolate (Scheme 53).\textsuperscript{163}

With desired salt in hand the fragmentation reactions could now be attempted (Table 8).

![Scheme](attachment:scheme.png)

**Table 8- Attempted fragmentation of 171 and trapping of intermediate 172.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Reaction Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH, 60-160 °C, 300 W, 0.1 M</td>
<td>30 min at each temperature</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2</td>
<td>Antracene 5eq., 18-crown-6, ( o )-xylenes, 125 °C, 0.5 M</td>
<td>16 h</td>
<td>Decomposition</td>
</tr>
<tr>
<td>3</td>
<td>175 10 eq., 125 °C</td>
<td>16 h</td>
<td>176 and 177, 55%</td>
</tr>
<tr>
<td>4</td>
<td>Adamantyl isocyanate 5eq., 18-crown-6, ( o )-xylenes, 125 °C, 0.5 M</td>
<td>2 h</td>
<td>180 6%</td>
</tr>
</tbody>
</table>

As was the case with the sulfoxide and the selenoxide preliminary investigations were performed in the absence of other reactants in order to assess starting material consumption. Heating 171 in methanol using microwave irradiation at a variety of temperatures showed that temperatures in excess of 120 °C were required for starting material consumption (Table 8, entry 1). It was hoped that using methanol would result in trapping of the resultant intermediate ketene providing evidence of the azetone, however this proved not to be the case and decomposition was instead observed.

It was therefore decided to attempt trapping with a variety of different reagents. Efforts to capture the proposed imidoylketene intermediate in a Diels-Alder process with anthracene resulted only in decomposition of the starting material (Table 8, entry 2). A similar attempt
using diene 175 again resulted in decomposition of 171 with the isolation of the Diels-Alder product between two molecules of 175 (Scheme 54).

\[
\begin{align*}
\text{173} & \xrightarrow{\text{LIH-MDS, Et$_2$O, reflux}} \text{174} & \xrightarrow{\text{MOMCl, DIPEA, CH$_2$Cl$_2$}} \text{175} & \xrightarrow{\text{171, neat, 125 °C}} \text{176} + \text{177} \\
& & & \text{58% combined}
\end{align*}
\]

Scheme 54- Synthesis of diene 175 and reaction to form regioisomers 176 and 177.

Diene 175 was synthesised in two steps by the opening and isomerisation of commercially available 2-methyl-2-vinylloxirane 173, followed by protection of the resultant alcohol with chloromethyl methyl ether (MOMCl). As a consequence of the highly volatile nature of both 174 and 175 yields for this synthesis were lower than may be expected, however sufficient material was synthesised in order to attempt the trapping reactions.

A final attempt at trapping was made using adamantyl isocyanate, which was chosen as the distinctive $^1$H-NMR adamantyl peaks would allow easy identification of incorporation into products (Table 8, entry 4 and Scheme 55).

\[
\begin{align*}
\text{178} & \xrightarrow{\text{decomposition via other routes}} \text{171} & \xrightarrow{\text{18-crown-6, o-xylene, 125 °C}} \text{180} & \xrightarrow{\text{Ad--NCO}} \text{179} \\
& & & \text{5%}
\end{align*}
\]

Scheme 55- Synthesis of urea 180 and proposed mechanistic route.
In this case, alongside unidentifiable decomposition, small quantities of urea 180 were isolated contaminated with another by-product. While by no means providing clear proof that this is the case, one possible explanation for the isolation of 180 is via an intermediate azetone (Scheme 55). Decarboxylation and elimination of 171 followed by ring opening of the azetone to form imidoylketene 172, could be followed by hydrolysis to para-anisidine, which could then react with adamantly isocyanate to furnish urea 180. While every effort was made to ensure the reaction was performed under anhydrous conditions it is possible that some moisture may have been retained by the hygroscopic salt allowing for hydrolysis of the imine. It can therefore be suggested that if the reaction is proceeding via an azetone intermediate the majority of the imidoylketene is decomposing via other pathways with small amounts being hydrolysed explaining the observations.

However, in the reports of Pericás and Alajarin, reactions of imidoylketenes formed from azetones were hypothesised to proceed via an intramolecular process (see Section 2.0.1 Schemes 31 and 32). It has also been demonstrated that imidoylketenes generated from more stable sources are liable to undergo dimerisations (Scheme 56).

![Scheme 56- Dimerisation of imidoylketene 182 reported by Zhou et al.](image)

This suggests that if the reaction was proceeding through an azetone, one could expect to see some additional evidence of intramolecular reaction or dimerisation. It is therefore also conceivable that decomposition to para-anisidine may be occurring by another unknown pathway.
2.2 Towards the Synthesis of Thiothymidine Nucleoside 85

2.2.1 Overview and Proposed Synthesis

In parallel to the methodology studies, efforts were also focused on the development of a synthetic route for accessing the aforementioned novel β-lactam nucleoside precursor 193 and hence the thio-thymidine analogues themselves. A route was proposed which would complement and make use of the methodology studies (Scheme 57).

![Scheme 57- Proposed synthesis of thiothymidine analogues 85.](image)

The synthesis would begin with Mitsunobu reaction of literature known alcohol 185 with methanesulfonic acid simultaneously introducing a leaving group and the desired stereochemistry. This would be followed by displacement of the mesylate in 186 with triphenylmethyl mercaptan, removal of the silyl protecting group and Appel reaction to furnish iodide 188. Coupling of 188 and 189 and subsequent trans-esterification would yield Barton ester 191. This could then be subjected to a radical decarboxylation, generating 192 which is in a suitable form to be subjected to the methodology that would be developed concurrently.
2.2.2 Synthesis of β-Lactam Coupling Partner 189

β-lactam 189 was obtained using a two-step procedure described by Baggaley et al (Scheme 58). 166

Scheme 58- Synthesis of lactam 189. 166

The acetylation of glycine ethyl ester hydrochloride with 3-bromopropionyl chloride gave amide 195 in high yields. Cyclisation to lactam 189 was carried out in the presence of potassium hydroxide. Yields, while being a good match for the literature, were relatively low as the result of the competing elimination process producing 196. 166

2.2.3 Synthesis and Inversion of Alcohol 185

Alcohol 185 was easily accessible in four steps starting from D-glucose (Scheme 59).

Scheme 59- Synthesis alcohol 185.

The synthesis began with the protection of glucose 63 as its benzylidene acetal 197 using benzaldehyde dimethyl acetal. 167 Oxidative cleavage of 197 using sodium metaperiodate and an
excess of sodium bicarbonate was directly followed by reduction of the intermediate with sodium borohydride to give benzylidene-erythritol 198 in excellent yields. Finally the primary alcohol of 198 was protected as its silyl ether using TBSCI and standard conditions to furnish 185.

It was originally envisaged that alcohol 185 could be displaced using methane sulfonic acid under Mitsonobu conditions to yield the inverted mesylate 186 as described by Anderson et al (Scheme 57). Unfortunately these conditions were sufficiently acidic to result in silyl-deprotection of 185. Milder conditions using benzoic acid, followed by hydrolysis and functionalization of the resultant alcohol to from 186 were therefore proposed (Scheme 60).

Scheme 60- Proposed route to mesylate 186.

Despite prolonged heating at reflux, the Mitsonobu reaction would not proceed to completion giving poor yields of benzoate ester 199. Possible reasons for the lack of full conversion can be attributed to hindrance associated with the cyclic system and the bulk of the silyl protecting group which could also hinder reaction at the alcohol.

These results were unsatisfactory and it was clear a different approach was required for accessing the inverted alcohol 200. A two-step oxidation and selective reduction protocol proved to be more successful (Scheme 61).

Scheme 61- Oxidation-reduction protocol for synthesis of 200.
Swern oxidation of alcohol 185 to ketone 201 was followed by reduction using L-selectride. This process was high yielding and the reduction was highly diastereoselective furnishing almost exclusively axial alcohol 200.\textsuperscript{173, 174} The conformation was unambiguously assigned based on \textsuperscript{1}H-NMR coupling constants.

2.2.4 \textbf{Introducing the Sulfide Functionality: Synthesis of Thioether 203}

With the inverted alcohol now accessible, the next step was to introduce the sulfur moiety to form thioether 187. As was previously outlined (Scheme 5 Section 2.2.1) the lithium salt of triphenylmethyl mercaptan was to be used to form 187. However, due to the difficulty in handling this thiolate, the substitution reactions were first of all trialled using a simpler system with thiophenol as the nucleophile (Table 9)

\begin{center}
\textbf{Table 9- Introducing the sulfide functionality to form 203.}
\end{center}
<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting Material</th>
<th>Reaction Conditions</th>
<th>Temperature</th>
<th>203:204 Ratio</th>
<th>Yield of 203 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mesylate</td>
<td>Thiophenol, NaOH, DMF, Toluene (0.06 M)</td>
<td>Room temp</td>
<td>0:0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Triflate</td>
<td>Thiophenol, NEt₃, DMSO (0.15 M)</td>
<td>Room temp</td>
<td>50:50</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Triflate</td>
<td>Thiophenol, NaH, DMSO-THF</td>
<td>Room temp</td>
<td>14:86</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Triflate</td>
<td>Thiophenol, NEt₃, CH₂Cl₂ (0.10 M)</td>
<td>0°C</td>
<td>64:36</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Triflate</td>
<td>Thiophenol, NEt₃, CH₂Cl₂ (0.50 M)</td>
<td>−10°C</td>
<td>70:30</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>Triflate</td>
<td>Thiophenol, NEt₃, CH₂Cl₂ (0.50 M)</td>
<td>−30°C</td>
<td>80:20</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>Triflate</td>
<td>Thiophenol, NEt₃, CH₂Cl₂ (0.50 M)</td>
<td>−55°C</td>
<td>80:20</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>Triflate</td>
<td>Thiophenol, Na₂CO₃, CH₂Cl₂ (0.10 M)</td>
<td>Room temp</td>
<td>60:40</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>Triflate</td>
<td>Thiophenol, Na₂CO₃, MeCN (0.50 M)</td>
<td>0°C</td>
<td>64:36</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>Triflate</td>
<td>Thiophenol, Na₂CO₃, MeCN (0.50 M)</td>
<td>−10°C</td>
<td>68:32</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>Triflate</td>
<td>Thiophenol, THF 18-crown-6, tBuOK (0.50 M)</td>
<td>−30°C</td>
<td>65:35</td>
<td>25</td>
</tr>
<tr>
<td>12</td>
<td>Triflate</td>
<td>Thiophenol, THF 18-crown-6, tBuOK (0.50 M)</td>
<td>−55°C</td>
<td>72:28</td>
<td>48</td>
</tr>
</tbody>
</table>

This transformation proved to be particularly challenging as a result of the competing elimination reaction. Both the triflate 202 and the mesylate 186 were easily accessible from alcohol 200 using standard conditions. Unfortunately the mesylate proved to be insufficiently reactive, with attempted displacement yielding only starting material (Table 9, entry 1). Attempting the displacement using 202 was met with greater success: a preliminary reaction in DMSO and
triethylamine yielded both the desired 203 and the elimination product 204 in a 50:50 ratio, based on analysis of the crude 1H-NMR (Table 9, entry 2). This provided a solid basis for further optimisation.

One idea was to pre-form the thiolate using sodium hydride to prevent the base from causing elimination (Table 9, entry 3). However, the sulfur anion was found to be sufficiently reactive to cause elimination itself. Following on from this, different conditions of concentration and temperature were evaluated using triethylamine and sodium carbonate as the bases (Table 9, entries 4–10). From these studies it was generally found that lowering the temperature increased the ratio of product to elimination product, with the best conditions utilising a higher concentration and triethylamine as the base (Table 9, entry 6). A final set of conditions using potassium tert-butoxide and THF at high concentrations were attempted. At −55 °C these conditions provided the highest yields (Table 9, entry 12).

Having completed the synthesis of thioether 203, it was deemed prudent to validate the downstream reactions using this compound as a test system (Scheme 62).

\[ \text{Scheme 62- Deprotection and functionalization of thioether 203, followed by attempted alkylation of lactam 189 with 206.} \]

Silyl deprotection of thioether 203 proceeded smoothly using tetra-n-butylammonium fluoride (TBAF) and was followed by iodination of alcohol 205 in good yields using the Appel reaction. Attempted alkylation of the enolate of β-lactam 189 with iodide 206, gave no conversion. Since the focus of this project was the synthesis of 85, with a different thioether in place, further attempts at this alkylation were not made.
Having established conditions to allow the generation of thioethers from triflate 202, attention could now be focussed on the synthesis of 187 containing the triphenylmethane-sulfide moiety. Chadwick et al. reported the synthesis and isolation of lithium triphenylmethanethiolate co-ordinated with 15-crown-5, by deprotection of the corresponding mercaptan with n-BuLi under strictly anhydrous conditions. It was hoped this product could be used directly in similar conditions to the formation of 203 (Scheme 63).

Scheme 63- Attempted synthesis of lithium triphenylmethanethiolate 209.

Unfortunately despite repeated attempts using glovebox techniques and Schlenk equipment to ensure the driest conditions isolation of the product was unsuccessful. As a result, efforts to directly react the mercaptan 208 with 202 were made (Table 10).

Table 10- Attempted reaction of 202 with triphenylmethanethiol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triphenylmercaptan, THF 18-crown-6, BuOK, (0.5 M)</td>
<td>−55 °C</td>
<td>202 and 204</td>
</tr>
<tr>
<td>2</td>
<td>Triphenylmercaptan, Toluene, 15-crown-5, n-BuLi, (0.5 M)</td>
<td>−55 °C</td>
<td>204</td>
</tr>
</tbody>
</table>
Unfortunately, using the conditions established for the reaction of 202 with thiophenol yielded only starting material and the elimination product (Table 10, entry 1). A further attempt made using Chadwick’s conditions also produced similar results (Table 10, entry 2). It is likely that the bulky mercaptan is hindering reaction at the triflate preventing any substitution. Reviewing the literature showed similar types of reaction using conditions at reflux.\textsuperscript{181,182} However these are unsuited for this situation as such conditions are likely to favour elimination as well as decomposition of the unstable triflate 202. An alternative method for generating sulfide \textsuperscript{187} was therefore sought.

2.2.6 \textbf{Alternative Routes to Thioether 187}

It was hoped that reacting 202 with thioacetic acid or a derivative salt, followed by hydrolysis of the resulting thioester 210 would provide the free thiol which could then be protected with trityl chloride to give the desired sulfide (Scheme 64 and Table 11).

\begin{center}
\includegraphics[width=\textwidth]{Scheme_64}
\end{center}

\textit{Scheme 64- Alternative route to thioether 187.}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KSAc, DMF, 0.15 M</td>
<td>204</td>
</tr>
<tr>
<td>2</td>
<td>KSAc, DMF, \textdegree{30} C, 0.15 M</td>
<td>204</td>
</tr>
<tr>
<td>3</td>
<td>KSAc, DMF, \textdegree{55} C, 0.5 M</td>
<td>204</td>
</tr>
<tr>
<td>4</td>
<td>KSAc, THF, 18-crown-6 \textdegree{55} C, 0.5 M</td>
<td>204</td>
</tr>
<tr>
<td>5</td>
<td>AcSH, Cs\textsubscript{2}CO\textsubscript{3}, DMF, \textdegree{55} C, 0.5 M</td>
<td>204</td>
</tr>
</tbody>
</table>

\textit{Table 11- Attempted reaction of 202 with thioacetate salts.}

Based on literature precedence, directly reacting 202 with potassium thioacetate was attempted at room temperature.\textsuperscript{183} This lead only to elimination (Table 11, entry 1). Neither decreasing the
reaction temperature, nor making use of previously established conditions had any effect on the outcome of the reaction (Table 11, entries 2-4). A final attempt using thioacetic acid and a caesium base also resulted in elimination (Table 11, entry 5). These results suggest that the thioacetate anion was insufficiently nucleophilic to favour the desired substitution reaction over the competing elimination.

Potassium ethyl xanthate was proposed as more nucleophilic sulfur source and could be reacted in a similar fashion to the thioacetate salts (Scheme 64 and Table 12).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EtOCS₂K, DMF, 0.04 m</td>
<td>204</td>
</tr>
<tr>
<td>2</td>
<td>EtOCS₂K, DMF, 0 °C, 0.2 m</td>
<td>204</td>
</tr>
<tr>
<td>3</td>
<td>EtOCS₂K, DMF, −20 °C, 0.04 m</td>
<td>204</td>
</tr>
<tr>
<td>4</td>
<td>EtOCS₂K, THF, 18-crown-6 −10 °C, 0.2 m</td>
<td>204</td>
</tr>
<tr>
<td>5</td>
<td>EtOCS₂K, DMF, −40 °C, 0.2 m</td>
<td>204</td>
</tr>
</tbody>
</table>

Table 12- Attempted reaction of 202 with xanthate salts.

Initial attempts at room temperature based on literature procedures again resulted in elimination (Table 12, entry 1). It was quickly apparent that decreasing the reaction temperature and altering concentration had little effect on the outcome of the reaction, with elimination occurring in all cases (Table 12, entries 2-5).

Since substitution reactions on 202 were clearly unviable, a different route to 187 was required. It was thought that thionation of ketone 201, followed by reduction of the resulting thioketone 211 to form the thiol and then trityl protection as previously described would yield the desired thioether (Scheme 65).

Scheme 65- Alternative route to 187 via thioketone 211.
A number of attempts were made at the formation of the thioketone 211 making use of both Lawessons’ reagent and phosphorous pentasulfide under a variety of reaction conditions (Table 13).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Reaction Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P₄S₁₀, HMDO, Toluene,</td>
<td>1 h</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>Reflux, 1.0 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lawessons’ reagent,</td>
<td>1 h</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>Toluene, Reflux, 1.0 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>P₄S₁₀, Pyridine, Reflux,</td>
<td>1 h</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>0.1 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>P₄S₁₀, Pyridine, CH₂Cl₂,</td>
<td>1 h</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>Reflux, 0.1 M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 13- Attempted thionation of ketone 201.

Despite a variety of attempts, decomposition was observed in all cases (Table 13, entries 1-4). This can be attributable to the presence of enolizable protons alpha to the ketone which allows for the formation of dimeric sulphides leading to degradation of the compounds.

Having made numerous unsuccessful attempts at synthesising thioether 187, it was apparent that the route being used was not viable. Thioether 187 either needed to be developed from an alternative starting point or perhaps another route for generating the nucleoside altogether was required. Due to time constraints further investigations of this project were not possible.
2.3 Conclusion and Further Work

The goal of developing methodology to allow the synthesis of thymidine nucleobase analogues by reaction of isocyanates with imidoylketene intermediates, generated from β-lactams, was unsuccessful. Nevertheless efficient syntheses were developed for accessing novel β-lactam structures functionalised as sulfoxides, selenoxides and potassium salts 143, 148, 150 and 171 (Figure 20).

![Figure 20- Novel β-lactam structures synthesised.](image)

Of particular note was isolation of the stable selenoxide 150 due to the rarity of such molecules in the literature. Additionally, while no desired trapping of isocyanates was observed, potassium salt 171 may have fragmented to an azetone as evidenced by the isolation of urea 180.

The synthesis of β-lactam nucleoside precursor 193 and hence nucleoside analogues 85 was not completed. Problems were encountered in attempted installation of the sulfide moiety in thioether 187 as a result of a competing elimination process. The route concluded following a ten step synthesis of sulfide 206 (Figure 21).

![Figure 21- Sulfide 206.](image)

Looking forward, an alternative method of generating the desired azetone could be through the use of a nucleoside precursor such as 213 (Scheme 66).
Scheme 66- Alternative method for accessing nucleoside analogues.

This method is particularly attractive as 1,3-oxazine-2,4-(6H)-dione 215 is a literature known compound and thus the usefulness of the proposed methodology could be easily assessed. 215 is readily synthesised from the reaction of maleic anhydride with trimethylsilyl azide (Scheme 67).\(^{189, 190}\)

\[
\text{O} = \text{C} \quad \text{O} \\
\text{(CH)}_{3}\text{SiN}_{3} \quad \text{Benzene, } 50 \degree \text{C} \\
\text{214} \quad \text{215} \quad 52\%
\]

Scheme 67- Synthesis of 215.\(^{189}\)

Furthermore the corresponding nucleoside of 215 has been synthesised via Vorbrüggen reaction with modified ribose sugar 216 by Chwang and Bobek (Scheme 68).\(^{191, 192}\)

\[
\text{Bz} \quad \text{OBz} \\
\text{216} \quad \text{217} \quad 90\%
\]

Scheme 68- Synthesis of nucleoside 217 and alternative glycosyl donor thiofuranose 218.\(^{191}\)

One could therefore envisage that replacement of ribose 216 with benzyl 3,5-di-O benzyl-2-deoxy-1,4-dithio-D-erythropentofuranoside 218 would allow access to a thio-nucleoside analogous to 213 (Scheme 68). 218 is available in seven steps from 2-deoxy-D-ribose and its use as a glycosyl donor in Vorbrüggen reactions is well known.\(^{193}\)
Finally, there are reported examples using similar compounds to those proposed for the formation and reaction of imidoylketenes (Scheme 69).\textsuperscript{165}

\begin{center}
\begin{tikzpicture}
  \node[draw, circle, minimum size=1cm, label=above:\textbf{219}] (A) at (0,0) {\includegraphics[width=0.15\textwidth]{image1}};
  \node[draw, circle, minimum size=1cm, label=above:\textbf{220}] (B) at (3,0) {\includegraphics[width=0.05\textwidth]{image2}};
  \node[draw, circle, minimum size=1cm] (C) at (3.5,0) {\includegraphics[width=0.05\textwidth]{image3}};
  \node[anchor=north] at (2.75,0.75) {Further reaction};
  \node[anchor=south] at (2.75,0.25) {Heat, \textit{-} CO\textsubscript{2}};

  \path[->] (A) edge node[above] {\textit{Heat, \textit{-} CO\textsubscript{2}}} (B);
  \path[->] (B) edge node[above] {} (C);
\end{tikzpicture}
\end{center}

\textit{Scheme 69-} Reported methodology for generating imidoylketenes from \textbf{219}.\textsuperscript{165}
3. Synthesis and Radiolabelling of 2\textsuperscript{'-}Deoxy-2',2\textsuperscript{''}-difluoro Nucleoside Analogues

3.0 Overview

Outlined in the project aims and objectives (*Section 1.4*) the synthesis and radiolabelling of two 2',2''-difluoro nucleoside analogues $90^*$ and $89^*$ was planned. It was believed these could be obtained from their corresponding protected nucleosides $221\beta$ and $222\beta$ by iodination, stannylation and finally Stille coupling with $[^{11}\text{C}]\text{CH}_3\text{I}$ as described by Samuelsson *et al* (Scheme 70).\textsuperscript{64}

![Scheme 70](image)

*Scheme 70*- Planned synthesis of $90^*$ and $89^*$, both compounds were expected to be accessible \textit{via} similar methods.

Chou *et al.* have reported the synthesis of both $221\beta$ and $222\beta$ from simple starting materials and their work was used as a basis for this project.\textsuperscript{194}
3.1 Cold Synthesis of dFMAU

3.1.1 Synthesis of Protected Glyceraldehyde 225

Chou’s methods made use of 2,3-\textit{O}-isopropylidene-\textit{D}-glyceraldehyde 225 as a starting material, which can be readily synthesised from \textit{D}-mannitol (Scheme 71).

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {226};
  \node (b) at (2,0) {227};
  \node (c) at (4,0) {225};
  \draw[->] (a) -- node [midway, above] {\text{ZnCl}_2, \text{acetone}} (b);
  \draw[->] (b) -- node [midway, above] {74\%} (c);
  \draw[->] (b) -- node [midway, above] {\text{SiO}_2, \text{NaIO}_4, \text{CH}_2\text{Cl}_2, 0 \degree \text{C}} (c);
\end{tikzpicture}
\end{center}

\textit{Scheme 71-} Synthesis of protected glyceraldehyde 225.

\textit{D}-Mannitol 226 was easily protected as its diacetonide 227 using acetone and zinc chloride.\textsuperscript{195} Cleavage of the diacetonide to unstable aldehyde 225 was carried out using silica supported sodium metaperiodate as described by Shing \textit{et al}. It was reported that simple filtration of the reaction mixture yielded products of suitable purity for further reaction; however, this was not found to be the case.\textsuperscript{196} Pure aldehyde was hence obtained by distillation of the crude residue as reported by Schmid and Bryant.\textsuperscript{197}

3.1.2 Synthesis of Ribonolactone 228

Ribonolactone 228 was synthesised in four steps from aldehyde 225 starting with a Reformatskii reaction to introduce the key difluoro-moiety (Scheme 72).
The Reformatskii reaction of ethyl bromodifluoroacetate with \( \text{225} \) presented some difficulties.\(^{198}\) An initial attempt made using zinc dust activated by acetic acid lead to poor conversion.\(^{199}\) This was rectified by altering the method of activation: stirring with 0.2 equivalents of iodine served to fully activate the zinc and lead to satisfying yields of diastereomeric \( \text{229} \).\(^{200}\) A 2.3:1.0 ratio of trans and cis diastereomers were recovered, which is in agreement with the polar Felkin-Anh model.

Benzoylation of alcohol \( \text{229} \) was followed by deprotection of the acetonide \( \text{230} \) under acidic conditions. Spontaneous 5-exo-trig cyclisation of the resultant intermediate to \( \text{231} \), under aezeotropic distillation is favoured by Baldwin’s rules. Lactone \( \text{231} \) was further benzoylated to give an epimeric mixture of \( \text{228} \), which was recrystallized to furnish exclusively the ribo anomer in similar yields to the literature.\(^{194}\)

3.1.3 Synthesis of Protected Nucleoside \( \text{221}\beta \) 

With sufficient quantities of \( \text{228} \) available, the synthesis was continued with introduction of the uracil nucleobase (Scheme 73).
Ribonolactone 228 was selectively reduced using lithium tri-tert-butoxyaluminum hydride to yield lactol 232. This was subsequently mesylated to furnish 233 in excellent yields over the two steps. Silylated uracil 234 was synthesised by refluxing uracil 14 with hexamethyldisilizane (HMDS) in the presence of ammonium sulphate. 234 was directly reacted with mesylates 233 under the Vorbrüggen protocol to give the blocked anomic nucleoside. In an improvement from the literature procedure, recrystallization from ethyl acetate then dichloromethane was found to give exclusively the desired β-anomer.194

3.1.4 Completion of the Synthesis

The synthesis of 90 was completed by functionalization of the protected nucleoside 221β as a stannane followed by Stille coupling with methyl iodide (Scheme 74).
Reflexing 221β with iodine monochloride in CH₂Cl₂ resulted in starting material recovery. Altering iodinating conditions to ceric ammonium nitrate and iodine gave 235 in good yields. Removal of the benzoyl protecting groups in 235 using methanolic ammonia was followed by coupling of the iodide 223 with hexamethylditin to furnish stannane 224. Preliminary attempts at the Stille coupling of 224 with methyl iodide were unsuccessful resulting in starting material recovery. It was observed that the reported colour change (from purple to deep yellow) corresponding to the in situ formation of the bis(tris(2-tolyl)phosphine)palladium (Pd[P(o-tolyl)₃]₂) catalyst was not occurring during the course of the reaction. This suggested that there were issues with the quality of the commercially available tris(dibenzylideneacetone)dipalladium (Pd₂(dba)₃) catalyst. The catalyst was therefore synthesised from palladium dichloride (PdCl₂) and dibenzylideneacetone according to the procedures of Ukai et al and recrystallized as its chloroform adduct to ensure high purity (Scheme 75).

Fortunately, this catalyst proved to be sufficiently pure for successful reaction, generating the desired product 90.
3.2 Radiochemical Synthesis of $[^{11}\text{C}]-\text{dFMAU}$ 90*

3.2.1 Production of Carbon-11 Methyl Iodide

Following the successful synthesis of cold dFMAU preparations were made for the synthesis of the radiolabelled nucleoside. Due to the discontinuation of carbon-11 production at the Hammersmith Campus, all radiochemistry was performed with our collaborators at the Wolfson Brain Imaging Centre (WBIC), University of Cambridge headed by Dr Franklin Aigbirhio and under the supervision of Dr Patrick Riss.

$[^{11}\text{C}]\text{CH}_3\text{I}$ used in the radiochemical synthesis was produced using the so-called ‘dry method’ by radical iodination of $[^{11}\text{C}]\text{CH}_4$ at high temperatures using the GE Medical Systems PET trace MeI Microlab instrumentation. In more detail: $[^{11}\text{C}]\text{CO}_2$ from the cyclotron target is firstly adsorbed on molecular sieves in oven A (Figure 22).

![Figure 22- Schematic of $[^{11}\text{C}]\text{CH}_3\text{I}$ synthesis module. VM = valve, OM = oven.](image-url)
Hydrogen is then released into the system and oven A is sealed and heated, releasing the $[^{11}\text{C}]\text{CO}_2$ which is reduced by hydrogen and the nickel catalyst to form $[^{11}\text{C}]\text{CH}_4$. The $[^{11}\text{C}]\text{CH}_4$ is released under a flow of helium into the methyl iodide conversion components of the system containing ovens B, C and D. Iodine is heated to 700 °C and vaporised on oven B and is reacted with $[^{11}\text{C}]\text{CH}_4$ to form $[^{11}\text{C}]\text{CH}_3\text{I}$ on oven C. $[^{11}\text{C}]\text{CH}_3\text{I}$ is subsequently trapped on the adsorbent Porapak polymers in oven D. Recirculation of $[^{11}\text{C}]\text{CH}_4$ through the system is continued until conversion to $[^{11}\text{C}]\text{CH}_3\text{I}$ is complete. Finally, any unreacted $[^{11}\text{C}]\text{CH}_4$ is released as waste before the trapped $[^{11}\text{C}]\text{CH}_3\text{I}$ is released and trapped in a solution of DMF heating oven D in a stream of helium.\textsuperscript{204, 205}

3.2.2 Synthesis of $[^{11}\text{C}]$-dFMAU $90^*$

Radiolabelling of 224 using $[^{11}\text{C}]\text{CH}_3\text{I}$ was successfully carried out according to the procedures of Samuelsson et al. to give the novel radiolabelled nucleoside $90^*$ (Scheme 76).\textsuperscript{64}

$$
\text{Scheme 76- Radiolabelling of 224 with }[^{11}\text{C}]\text{CH}_3\text{I}. \textsuperscript{64}
$$

When the synthesis was performed manually, decay-corrected radiochemical yields of 16% ($n=2$) and radiochemical purity of >97% (after HPLC) were achieved after reacting for five minutes. In order to minimise contact with radioactivity and perform a more streamlined and efficient production, the synthesis was adapted for automation using the GE Tracerlab FXc\textsuperscript{®} synthesis module. This resulted in similar radiochemical purity but unfortunately gave lower radiochemical yields of 5% ($n=3$). This was thought to be a result of blocked filters and tubes during transfer of the reaction mixture from the reaction vessel to the HPLC caused by palladium deposits. Confirmation of the product identity was performed by spiking a crude analytical sample of the radiolabelled material with authentic cold sample of 90 (Figure 23).
Further efforts to optimise the synthesis of 90* involved investigations into developing a method suited for use with a microfluidic reactor. Microfluidic syntheses involve the controlled injection of reaction components through a network of small channels, typically with 10-500 µm dimensions, which form a microfluidic reactor. Microfluidic systems offer numerous advantages which are particularly useful to the PET radiochemist, including: rate acceleration by increased mixing of components and enhanced heat transport as a result of the increased surface area (particularly important when considering the short half-lives of PET radionuclides), the ability to easily perform reactions using small amounts of reagent (in µM-pM range) as is typical in PET syntheses, ease of automation which allows direct integration with HPLC purification for example and the small space requirements which allow easier shielding of the user from radioactivity.

For these reasons, it was hoped that using the Advion NanoTek® microfluidic system would allow for an automated synthesis of the radiolabelled product while also decreasing the reaction time. A variety of conditions were investigated (Table 14).
<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (°C)</th>
<th>Flow Rate (1:1)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>5</td>
<td>170</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>7</td>
<td>190</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>60 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>9</td>
<td>140</td>
<td>30 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>10</td>
<td>140</td>
<td>25 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>11</td>
<td>140</td>
<td>20 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
<tr>
<td>12</td>
<td>140</td>
<td>15 µL min⁻¹</td>
<td>No Conversion</td>
</tr>
</tbody>
</table>

*Table 14*- Conditions evaluated for the microfluidic radiochemical synthesis of 90*.  

Note: Total reactor volume = 30 µL.  
Flow rate corresponds to injection of ¹¹CH₃I in DMF from one pump and the remaining reaction components in DMF from another in a 1:1 mixture.

Initial conditions were based upon those used in the standard radiolabelling reaction to form 90*, the difference being the residence time in the reactor (i.e. reaction time) was 15 seconds in comparison to five minutes when performed in a vial (*Table 14*, entry 1). In this case, no conversion of the starting material was observed. It was thought the problem could be due to temperature differences between the reactor and the reaction mixture itself. The temperature was therefore increased incrementally to a maximum of 200 °C to no avail (*Table 14*, entries 2-8). A second line of thought centred on the residency time within the reactor itself, decreasing the flow rate leading to an eventual residency time of one minute also did not result in conversion of 224 (*Table 14*, entries 9-12). Aside from the reaction time, the failure to isolate any product could be attributed to the lower concentration of reactants caused by the larger surface area of the microfluidic system. Indeed, in Samuelssons’ report, it was noted that lowering the reaction concentration gave reduced yields.⁶⁴ Perhaps it is a combination of both the lower reaction concentration and reduced reaction time which lead to these results.
Despite being unable to isolate any product from the microfluidic synthesis and the relatively low radiochemical yields attained from the Tracerlab system, biological testing of radiolabelled 90* for TK1 selectivity was nevertheless carried out. It should be noted that compounds prepared in radiochemical yields of less than 5% (decay-corrected) are still suitable for biological studies, as such the yield of 90* was less problematic than may be expected.\footnote{208}

3.3 Biological Assay of [\textsuperscript{11}C]-dFMAU 90*

The assay used to test the activity of 90* for TK1 was developed by Roberta Sala our collaborator at the Hammersmith Campus and is adapted from the work of Toyohara et al.\footnote{209} Briefly, TK1 expressing cells (TK1+) were grown until they achieved 60-70% confluency, whereupon they were lysed and suspended in a homogenised buffer suited for reaction. These cells were then incubated with radiolabelled 90* and additional reaction buffer, allowing the extent of phosphorylation to be assessed (Table 15).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions*</th>
<th>Incubation Time</th>
<th>Phosphorylation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer, 90*, 37 °C</td>
<td>40 min</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Buffer, TK1+ cell lysate, 90*, 0 °C</td>
<td>40 min</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Buffer, TK1- cell lysate, 90*, 37 °C</td>
<td>40 min</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Buffer, TK1+ cell lysate, 90*, 37 °C</td>
<td>20 min</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Buffer, TK1+ cell lysate, 90*, 37 °C</td>
<td>40 min</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>6</td>
<td>Buffer, TK1+ cell lysate, 90*, CIP, 37 °C</td>
<td>40 min</td>
<td>0</td>
</tr>
</tbody>
</table>

*Each experiment was repeated three times and carried out using 20 µL of cell lysate, 180 µL of buffer (50 mM Tris-HCl pH 7.5, 2.5 mM ATP, 2.5 mM MgCl\(_2\)) and 100 µL of activity (50 MBq in 5 mL). Experiments performed under the guidance of Dr Patrick Riss at the WBIC University of Cambridge.
A number of control experiments were carried out in order to validate the results of the assay. Negative controls performed with cell lysates not expressing TK1 (TK1-) and with TK1+ cell lysates but at 0 °C, as expected, resulted in zero phosphorylation of 90* (Table 15, entries 2 and 3). A blank experiment containing no cell lysate gave an identical result (Table 15, entry 1). Unfortunately, incubation of TK1+ cell lysate with 90* for 20 minutes and 40 minutes resulted in minimal phosphorylation of the nucleoside (Table 15, entries 4 and 5). These results suggest that 90* is not a substrate for TK1. A final experiment was performed using standard conditions with the addition of the alkaline phosphatase (CIP). Had a new peak been observed in any previous experiments, this would have allowed confirmation that it was a product of phosphorylation.

Since 90* shows no TK1 activity, assays to assess its dCK activity need to be performed. Following discussions with our collaborators several other factors were suggested that could account for these results: 90* was formulated in 6% EtOH:H2O and low phosphorylation rates with non-radioactive samples in EtOH have been observed in the past. Additionally there might be traces of palladium or tin left in the formulation, which could have poisoned the kinase, leading to low conversion.
3.4 Towards the Synthesis of 5-Methyl-Gemcitabine 89

3.4.1 Modification of the Synthetic Route

With the cold and radiosynthesis of 90 completed, focus was turned to the synthesis and radiosynthesis of methylated gemcitabine. As previously mentioned, it was thought that the strategy for generation of this compound would be similar to that used in accessing 90. However, in this case a slight modification of the route was required to account for the N4-amine in 89 (Scheme 77).

Amines are known to be poisons for a number of catalysts including platinum and palladium.\(^{210}\) Consequently, it was envisaged that protection of the N4-amine of 238 would be required to prevent catalyst poisoning as well as decrease the chance of other side-reactions with methyl iodide and during the palladium catalysed steps. Indeed, Reddington *et al.* have reported the necessity of such protection in 5-iodo-2'-deoxycytidine for successful Heck reaction with N-allyl-trifluoroacetamide.\(^{211}\)

3.4.2 Synthesis of Gemcitabine 84

Making use of mesylate intermediate 233 as a starting point, the synthesis of benzoyl-protected gemcitabine were completed according to methods of Chou (Scheme 78).\(^{194}\)
Cytosine was silylated to form 82 by refluxing in HMDS in the presence of ammonium sulfate. This was then reacted with mesylate 233 (synthesis described in Scheme 71 Section 3.1.3) using the Vorbrüggen reaction to give the blocked anomeric nucleoside 222. The yield of 222 was slightly lower in comparison to the given literature values. This was thought to be partly due to changing solvents from 1,1,2-trichloroethane to 1,2-dichloroethane which meant the reaction was performed at a lower temperature and took longer to complete. Should the reaction be repeated 1,1,2-trichloroethane will be used. Isolation of the desired β-anomer 222β could be easily performed by slurrying the anomeric mixture in warm methanol followed by filtration to recover the insoluble beta-product. Basic deprotection of the blocked nucleoside led to almost quantitative yields of cold gemcitabine.

3.4.3 Attempted Synthesis of 89

Iodination of the 5-position of cytosine ring proved to be somewhat challenging, requiring a variety of conditions to be screened before a suitable method was discovered (Table 16).
Protocols making use of iodine and iodic acid were found to be amongst the most commonly used methods for iodinating cytidine and deoxycytidine in the literature. Unfortunately using these conditions with 84 in a mixture of tetrachloromethane, acetic acid and water resulted in poor yields of 238 as well as the formation of an unidentified by-product (Table 16, entry 1). This side-product proved difficult to separate from 238 using flash silica column chromatography and contributed to the low yields. Using microwave irradiation and N-iodosuccinimde resulted in incomplete reaction and poor yields of 238 (Table 16, entry 2). Cheng et al reported high yields for the iodination of cytidine, using iodine and iodic acid, with acetyl protection of the alcohol functionalities in place. Disappointingly attempts at replicating this procedure with 222β gave low yields of 243 with a number of side products also observed. A final set of conditions whereby a lower concentration was used and purification was performed by recrystallization, led to good yields of 238 (Table 16, entry 4). It is probable that recrystallization of the crude mixture from water was the main factor behind the observed increase in yield as this negated the need for difficult chromatographic separation of 238 and the by-products.
With iodide 238 now easily accessible, acetylation of the N4-amine was carried out to ensure the nucleoside was suitably functionalised for use with palladium chemistry (Scheme 79).

\[
\begin{align*}
\text{238} & \xrightarrow{\text{Ac}_2\text{O, DMAP, pyridine}} \text{244} \\
\end{align*}
\]

Scheme 79- Acetylation of 238.

Efforts to selectively protect the amine with a single equivalent of acetic anhydride, in DMF, resulted in incomplete reaction and low yields. Increasing the equivalents of acetic anhydride to a slight excess resulted in a mixture of protected products. As a result it was decided to attempt global protection of 238. Fortunately this strategy proved to be more successful furnishing 244 in adequate yields.

The penultimate step of the synthesis was stannylation of 244 to generate a precursor suitable for radiolabelling (Scheme 80).

\[
\begin{align*}
\text{244} & \xrightarrow{\text{Sn}_2\text{Me}_6, \text{Pd}_2\text{PPh}_3\text{Me}_3, \text{THF, reflux}} \text{245} \\
\end{align*}
\]

Scheme 80- Protodestannylation of 244 to 245.

Attempted stannylation of 244 with hexamethylditin lead mainly to decomposition with 245 the product of protodestannylation identifiable by $^1$H NMR. Unfortunately 245 could not be isolated cleanly, although high resolution mass spectrometry allowed confirmation of its identity. The replacement of hexamethylditin with the more sterically encumbered and less reactive bis(tributyltin) was suggested as a means of avoiding this problem. However, with only small quantities of precursor 222β available and other projects showing promising developments, as well as providing possible alternative solutions to the problems of radiolabelling deoxycytidine
analogues, it was decided to switch focus to these assignments with the possibility of revisiting the stannylation reaction in the future.

3.5 Conclusion and Further Work

The synthesis and radiolabelling of the novel nucleoside analogue 90* was successfully completed in 14 steps, making use of a Stille coupling to introduce the radiolabel (Figure 24).

![Figure 24- Novel radiolabelled nucleoside 90*](image)

Despite proving not to be a substrate for TK1, 90* could still prove to be useful for PET applications should it show activity for dCK. It is hoped assays for dCK will be performed in the future in order to assess this probe's utility. It must also be noted that carrying out the radiosynthesis of 90* in a clinical PET lab meant only intermittent visits to Cambridge were possible. As a result of the sporadic nature of this work, as well as the departure of the authors’ primary laboratory supervisor, the specific activity of 90* remains uncalculated. Should the probe prove useful in other assays and carbon-11 production facilities remain unavailable at Hammersmith Hospital, further collaborations will need to be established in order to calculate this value.

Attempted synthesis of 5-methyl-gemcitabine concluded with an unexpected protodestannylation reaction yielding 245 during efforts to form stannane 244 (Figure 25).

![Figure 25- 245 product of protodestannylation](image)
Potential solutions to a number of the challenges experienced in the syntheses of these nucleosides were identified in work which is discussed in the following chapter.
4. 2’-Deoxy-2’,2’-difluoro Nucleoside Analogues for Radiolabelling with Fluorine-18 and other Biomedical Applications

4.0 Overview

4.0.1 Alternative Radiolabelling Strategies for 2’,2’-difluoro Nucleosides

One of the main problems encountered during radiosynthesis of 90* was not specifically a synthetic challenge but rather the lack of carbon-11 production facilities locally. The ability to synthesise 2’-deoxy-2’,2’-difluoro nucleosides analogues suitable for radiolabelling with fluorine-18 was desirable for two reasons: firstly, it would allow use of the fluorine-18 services available at Hammersmith Hospital and secondly, it would also provide a radiolabelled nucleoside with a substantially longer half-life.

With this in mind, a deoxyuridine-nucleoside analogue hydroxymethylated in the 5-position was proposed as a suitable precursor for fluorination (Scheme 81).

Scheme 81- Proposed route to a [18F]-dFMAU analogue 248.

Hydroxymethylation of uracil bases using a KOH (aq) solution and para-formaldehyde was first reported with full characterisation by Cline et al. in the 1950s. Since then there have been a number of reports describing the use of this reaction and its modifications with varying degrees of success. Recent examples include the hydroxymethylation of uracil, uridine and deoxyuridine analogues by Gavriliu, Chung, Hudson and Dai. If a nucleoside such as 247 could be accessed then functionalization of the alcohol as a tosylate followed by displacement with [18F]-KF would provide a viable strategy to accessing a [18F] labelled analogue of dFMAU 90.
The synthesis of 247 would also present the opportunity for the synthesis of a variety of other nucleoside analogues with chemically interesting structures and potentially useful biological properties (Figure 26).

![Figure 26- 2'-Deoxy-2',2'-difluoro nucleoside analogue targets.](image)

Nucleoside reverse transcriptase inhibitors have revolutionised the treatment of HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome) since their introduction in the late 1980s. Azidothymidine (AZT) 254 in this respect is a pioneer for this class of drug molecule and remains clinically relevant today (Figure 27).

![Figure 27- AZT.](image)

The success of AZT can be attributed to its high selectivity for HIV reverse transcriptase in comparison to DNA polymerase as well as the presence of the azido group which permits facile
crossing of phospholipid bilayers as well as the blood brain barrier. An AZT analogue such as 249 could perhaps mimic some of the interesting properties of this drug. Starting from 247, azide 249 should be relatively simple to synthesise using the methods of Seio et al. (Scheme 82).

Scheme 82- Proposed synthesis of AZT analogue 249.

\[^{18}\text{F}\]-FOT 255 is a nucleoside analogue previously described in our group with potential use in the imaging of proliferation in cancer. During the synthesis a Huisgen cycloaddition was used to introduce the triazole functionality (Figure 28).

Figure 28- \[^{18}\text{F}\]-FOT.

Assuming the synthesis of the protected azide 256 (Scheme 82) was successful, the opportunity to carry out a Huisgen cycloaddition to generate the corresponding nucleoside triazole analogue 252 would become available (Scheme 83).
The cold synthesis and radiosynthesis of 252 would be a logical step in assessing the utility of nucleosides bearing the triazole functionality as PET proliferation markers for cancer.

Covalently linked bivalent nucleosides have also shown potential for use in several areas, including: combined allosteric and direct inhibition of HIV reverse transcriptase, the study of DNA repair mechanisms and as inhibitors of ribonucleotide reductase (an important target for chemotherapeutic agents).\textsuperscript{227, 228, 229} Using 247 and a simple linker would provide a means of accessing these types of structure, with the addition of the difluoro-moiety, in the form of dinucleoside 250 (Scheme 84).

Optical imaging techniques such as fluorescent imaging (see Section 1.0.2 for a greater discussion of optical imaging techniques) have applications for \textit{in vitro} cellular imaging and in fluorescence based assays.\textsuperscript{230} Labelling 247 with dansyl chloride, for example, could provide another means of evaluating the usefulness of 2'-deoxy-2',2'-difluoro nucleoside analogues for imaging tumours (Scheme 85).
Finally, the modified nucleoside analogue 5-(β-D-glucopyranosyloxymethyl)-2’-deoxyuridine 258 is expressed by a number of Kinetoplastida protozoa including the parasites Trypanosoma Brucei and Leishmania which cause the diseases sleeping sickness and Leishmaniasis.\textsuperscript{231} It has been demonstrated that 258 is essential for the survival of the Leishmania parasite, although its function has yet to be unambiguously determined.\textsuperscript{231} Synthesis of 258 was carried out by de Kort et al. with the aims of evaluating the biological and physical effects of incorporating this nucleoside into DNA in order to further understand its function.\textsuperscript{232} Generation of a similar analogue could be used to complement these studies (Scheme 86).

Scheme 85- Proposed syntheses of dansylated nucleoside 251.

\begin{center}
\textbf{Scheme 86- Proposed synthesis of glycosylated nucleoside 253.}\textsuperscript{232}
\end{center}
4.1 Synthesis of Hydroxymethylated Nucleoside 270β

4.1.1 Trial Reactions with Uracil

In order to verify the suitability of the hydroxymethylation procedure, trial reactions were carried out with uracil (Table 17).

![Chemical structure](image)

**Table 17-** Trial reactions with uracil.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(CH₂O)n, 0.5 N KOH (aq) 60 °C, 72 h</td>
<td>66%</td>
</tr>
<tr>
<td>2</td>
<td>(CH₂O)n, 0.5 N KOH (aq) 200 W, 90 °C, 2 h</td>
<td>58%</td>
</tr>
<tr>
<td>3</td>
<td>(CH₂O)n, NEt₃, H₂O 60 °C, 16 h</td>
<td>73%</td>
</tr>
</tbody>
</table>

Stirring uracil with para-formaldehyde in 0.5 N KOH (aq) according to the protocol described by Cline gave the product in 66 % yield (Table 17, entry 1).²¹⁷ Abdel-Raham et al. reported an improvement of this procedure using microwave heating and higher temperatures to dramatically accelerate the reaction; these conditions allowed the generation of 259 in two hours, albeit in reduced yields (Table 17, entry 2).²³³ The final conditions investigated used triethylamine in water in place of 0.5 N KOH (aq) and gave the best yields of 259 after stirring for 16 hours (Table 17, entry 3).²²⁰ It was decided to adopt these conditions for future endeavours as they not only gave best yield, but also had the simplest purification avoiding the use of acidic resins as in the previous examples (Table 17, entries 1 and 2).²¹⁷, ²³³
4.1.2 Attempted Hydroxymethylation of Nucleosides

Having established the most suitable method for hydroxymethylation of uracil, the reaction could now be used for the synthesis of a nucleoside analogous to 247 (Scheme 87).

![Reaction scheme](image)

Scheme 87- Hydroxymethylation of 260

The reaction was firstly attempted on the free nucleoside 260. It was found that acetonitrile was required in addition to water in order to maintain full solubility of the reactants. Yields for this step were poor and completion required heating for 72 hours. Nevertheless, 261 could be isolated and it was hoped that further attempts would prove to be more successful (Scheme 88).

![Reaction scheme](image)

Scheme 88- Hydroxymethylation of 262

260 was firstly protected as its methoxymethyl ether 262. Protection was necessary to allow differentiation between the primary hydroxyl groups following hydroxymethylation, to permit further functionalization. A MOM protection was chosen as it would be stable to the reaction conditions while also ensuring the molecule was sufficiently polar to maintain solubility. The protected nucleoside 262 was further reacted to furnish 263 along with small amounts of an inseparable side product, a result corroborated by the reports of Chung et al. Unfortunately yields for this reaction were also poor. Attempts made using the other conditions outlined in Table 17 gave similarly low yields.
With small quantities of the hydroxymethylated nucleoside 263 available it was deemed prudent to utilise this material to trial the synthesis of one of the desired analogues. In this case introduction of the dansyl group, considered to be the simplest transformation, was performed (Scheme 89).

![Scheme 89- Attempted synthesis of dansylated analogue 251.](image)

Dansylation of 263 furnished 264 in low yields. This was attributed to the presence of impurities from the previous step. Nonetheless, sufficient material was recovered to allow attempts to be made at deprotection on a small scale (Table 18).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MgCl₂, n-PrSH, CH₂Cl₂, 0.5 M</td>
<td>Starting Material</td>
</tr>
<tr>
<td>2</td>
<td>ZnCl₂, n-PrSH, CH₂Cl₂, 0.5 M</td>
<td>Starting Material</td>
</tr>
<tr>
<td>3</td>
<td>ZnBr₂, n-PrSH, CH₂Cl₂, 0.5 M</td>
<td>Starting Material</td>
</tr>
<tr>
<td>4</td>
<td>6.0 N HCl, THF, 0.5 M</td>
<td>Epimerisation</td>
</tr>
</tbody>
</table>

Table 18- Attempted deprotection of nucleoside 264.

Unfortunately despite trying numerous conditions removal of the methoxymethyl ether groups of 264 proved to be unsuccessful. Initial efforts using milder conditions with a variety of Lewis acids and 1-propanethiol yielded only starting material (Table 18, entries 1, 2 and 3). Making use of harsher conditions resulted in epimerisation of the nucleoside (Table 18, entry 4).
As a result of the poor yields for the hydroxymethylation step and difficulties in removing the methoxymethyl ether protecting groups, it was clear a different approach to generating large quantities of an intermediate analogous to 247 needed for synthesis of the desired analogues was required.

4.1.3 Alternative Routes to Hydroxymethylated Nucleosides

Dai et al. also reported difficulties when attempting hydroxymethylation on a similar protected nucleoside system. As a solution they performed a palladium catalysed carbonylation on a 5-iodo-nucleoside precursor, followed by Luche reduction to the corresponding primary alcohol. Inspired by these results attempts were made to apply this protocol to the generation of a nucleoside similar to 247 (Scheme 90).

![Scheme 90- Attempted carbonylation of 265](image)

The synthesis started with protection of iodide 223 (reported in section 3.1.4) using 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane (TIPDSCI) proceeding in a good yield. Unfortunately attempts at carbonylation led to incomplete reaction as well as the production of a number of side-products which prevented clean isolation of the desired material. Dai et al. reported the carbonylation using 50 psi of carbon monoxide. These pressures could not be attained using the available equipment and was cited as a probable cause for the incomplete reaction.

Reviewing the literature revealed other alternative strategies for accessing hydroxymethylated nucleosides akin to 247 including photochemical mono-bromination of thymidine nucleosides followed by hydrolysis. However, since the original reactions with uracil (Table 17) were high yielding and did not require the use of specialist equipment it was decided to revisit these and use a different approach to incorporating this moiety.
Brulíková and Uchida described the synthesis of nucleotide analogues modified in the 5-position by pre-functionalization of the nucleobases used in the Vorbrüggen reaction.\textsuperscript{241, 242} Encouraged by these reports attempts were made at directly using 5-hydroxymethyluracil \textit{259} with the mesylate precursor \textit{233} (Scheme 91).

\begin{align*}
\text{Scheme 91- Attempted Vorbrüggen reaction of 266 and 233.}
\end{align*}

Silylation of this \textit{259} and subsequent coupling with mesylates \textit{233} (see Section 3.1.3 for synthesis) was unsuccessful. Zhang \textit{et al.} also reported problems with this transformation on similar nucleosides, citing solubility issues of \textit{266} during the glycosylation. Silyl protection of \textit{259} prior to coupling was purported to alleviate these difficulties (Scheme 92).\textsuperscript{243}

\begin{align*}
\text{Scheme 92- Vorbrüggen glycosylation of 268 and 233.}\textsuperscript{243}
\end{align*}

Protection of \textit{259} with \textit{tert}-butyldiphenylsilyl chloride (TBDPSCl) and subsequent silylation with HMDS proceeded smoothly to furnish \textit{268}. Coupling of \textit{268} with mesylates \textit{233} gave the desired nucleoside \textit{269} as a mixture of anomers. Separation of the anomers proved to be difficult. Only partial separation was possible by column chromatography and re-crystallisation was not possible. Based on the crystallisation of similar nucleosides in the literature, it was
hoped that removing the bulky non-polar TBDPS group would render the nucleoside more susceptible to re-crystallisation (Scheme 93).²⁴⁴

![Scheme 93 - Deprotection of 269 and separation of the anomers.]

Unexpectedly deprotection of 269 using excess of TBAF lead to epimerisation of the nucleoside giving rise to a one-to-one anomerico-mixture.²⁴⁵ This could be rationalised by the presence of water in the TBAF solution, which would lead to the formation of HF and this could in turn cause the observed epimerisation. Indeed, Hogrefe et al. have reported the presence of significant quantities of water even in newly purchased bottles of TBAF.²⁴⁶ Pleasingly, recrystallization of the anomic mixture from chloroform allowed complete separation of the anomers. The identity of the β-anomer was ascertained by 2D-NOESY experiments (see Supporting Data). Further confirmation of this assignment was obtained by deprotection of 270β to 261 and comparison of the NMR data with that of an authentic sample of 261 formed from literature known compounds. (Scheme 87, Section 4.1.2).

Using this route, multi-grams quantities of 270β could be made rapidly and efficiently, thus permitting continued efforts into synthesis of the desired analogues (Figure 24).
4.2 Synthesis of 2’-Deoxy-2’,2’-difluoro Nucleoside Analogues

4.2.1 Synthesis of Fluorinated Nucleoside 271

The fluorinated nucleoside analogue 271 was the primary target in the hope that a rapid turnaround of the ‘cold’ compound would give sufficient time for radiochemistry to be performed. As outlined previously (Scheme 79 Section 4.0.1) functionalization of 270β with a suitable leaving group was to be followed by displacement with fluoride (Table 19).

![Chemical structure of 270β and 271]

Table 19- Synthesis of protected fluorinated nucleoside 271.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TfO, Pyridine, THF, −78 °C to 0 °C</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2</td>
<td>TsCl, Pyridine, THF</td>
<td>Decomposition</td>
</tr>
<tr>
<td>3</td>
<td>DAST, THF, −15 °C</td>
<td>271, 35%</td>
</tr>
</tbody>
</table>

Unfortunately efforts at forming the tosylate and triflate of 270β resulted in decomposition (Table 19, entries 1 and 2). It was thought this could be due to reaction through the unprotected uracil amide of 270β, a process which was also evident in the protection of 260 with MOMCl (Scheme 86 Section 4.1.2). Furthermore, reaction at this position is known in the literature an example being in the synthesis of [18F]-FOT. The use of fluorinating reagents such as diethylaminosulfur trifluoride (DAST) was suggested as alternative method for producing the desired nucleoside. DAST was the reagent of choice as the synthesis of [18F]-DAST has been reported in the literature meaning this protocol would be translatable to a radiochemical synthesis. Fluorination of 270β using DAST under standard conditions was successful (Table 19, entry 3).
The final step of the synthesis was deprotection of the benzoyl groups in \textbf{271} using ammonia in methanol, unfortunately this lead to the isolation of amine \textbf{272} (Scheme 9a).

![Scheme 9a- Attempted deprotection of fluorinated nucleoside 271.](image)

It was proposed that the uracil system was causing activation of the primary fluoride of \textbf{271} increasing its lability and permitting displacement by ammonia to give \textbf{272}. Additional attempts at deprotection gave similar results, lending further credence to this hypothesis (Table 20).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K$_2$CO$_3$, MeOH</td>
<td>273 76%</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$CO$_3$, EtOH</td>
<td>274 72%</td>
</tr>
<tr>
<td>3</td>
<td>K$_2$CO$_3$, PrOH</td>
<td>Starting material</td>
</tr>
</tbody>
</table>

\textit{Table 20- Attempted deprotection of 271.}

Using potassium carbonate with methanol and ethanol furnished the deprotected methyl (273) and ethyl (274) of ethers of \textbf{270β} respectfullly (Table 20, entries 1 and 2). A final attempt using isopropanol as the solvent yielded starting material. In this case steric hindrance appears to have prevented any reaction. (Table 20, entry 3).

From these results it was clear that a different protecting strategy was needed as basic deprotection resulted in substitution of the fluoride group. Future endeavours could make use of acid sensitive trityl or acetyl protecting groups to prevent the unwanted substitution reactions.
4.2.2 Synthesis of AZT and FOT Analogues 249 and 252

The AZT analogue 249 was easily synthesised in three steps from 270β (Scheme 95).

![Scheme 95- Synthesis of AZT analogue 249](image)

Hydroxymethylated nucleoside 270β was converted to the chloride and then displaced with sodium azide as described by Seio et al to give 276 in a good yield over two steps. Cleavage of the benzoyl groups in 276 yielded the desired AZT analogue 249 in an excellent yield. With azide 276 readily available the synthesis of FOT analogue 252 was carried out (Scheme 96).
Using standard copper catalysed azide-alkyne cycloaddition conditions with 276 and 3-butyn-1-ol, triazole 277 was generated in a 54% yield.\textsuperscript{226} Having proven useful in previous efforts, DAST was used for fluorination to form 278; this was followed by basic de-protection to give the final compound 252. Yields for the final two steps were relatively low: 21% and 34% respectively. However, this was not of great concern as these steps were performed using small quantities of material (less than 10 mg of 278 was used in the de-protection) and it is believed that yields would see improvement on scale-up.

Having completed the synthesis of 252, efforts were made to develop a route more suitable for radiochemistry. \textsuperscript{[18}F\textsuperscript{]}-DAST may be synthesised; however the specific activity of this reagent is comparatively low therefore the use of \textsuperscript{[18}F\textsuperscript{]} fluoride is preferable.\textsuperscript{248} An alternative strategy was proposed involving synthesis of a tosylate analogue of 277. (Scheme 97)
Having experienced problems in forming the tosylate of 270β (Table 19 Section 4.2.1) the decision was made to introduce the tosyl functionality at earlier stage of the synthesis; as such, 3-butyn-1-ol 279 was reacted with tosyl chloride to form 280, which was used directly in the Huisgen cycloaddition with 276. Tosylate 281 was recovered in good yields, providing a suitable precursor for radiochemical synthesis of 252.

4.2.3 Synthesis of Dansylated and Dinucleoside Analogues 250 and 251

It was originally envisaged that dansylation of 270β would allow easy access to analogue 250 (Scheme 85). However, bearing in mind the previously encountered problems when attempting to functionalise the primary alcohol of 270β as well as the issues in deprotection of fluoride 271 (Scheme 94 Section 4.2.1), it was decided instead to convert 270β to the corresponding amine. It was hoped that replacement of the alcohol with an amine, followed by reaction with dansyl chloride would provide the more stable sulfonamide functionality. The required amine was accessible by reduction of azide 276 (Table 21).

Table 21- Reduction of 276 to amine 282.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;, Pd/C, MeOH, 60 °C</td>
<td>Debenzylation</td>
</tr>
<tr>
<td>2</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;, Pd/C, 1,4-dioxane, 60 °C</td>
<td>14%</td>
</tr>
<tr>
<td>3</td>
<td>PPh&lt;sub&gt;3&lt;/sub&gt;, THF-H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>74%</td>
</tr>
</tbody>
</table>

Problems were encountered when attempting reduction of 276 by hydrogenation. Heating 276 with methanol (to ensure solubility) and palladium on carbon under hydrogen gave a number of side products with displacement of the benzoyl protecting groups evident (Table 20, entry 1). Altering the solvent to 1,4-dioxane, in which 276 was fully soluble, in an effort to alleviate these problems gave a sluggish reaction that did not fully complete (Table 20, entry 2). Fortunately, reduction using Staudinger methodology provided a mild procedure for accessing the desired amine in good yields (Table 20, entry 3). With 282 now in hand synthesis of the dansylated analogue was completed. (Scheme 98)

Scheme 98- Synthesis of dansylated analogue 284.

Pleasingly reaction of 282 with dansyl chloride proceeded smoothly to furnish 283. The sulfonamide linkage also ensured that displacement by ammonia during deprotection did not occur yielding exclusively 284.

With some quantity of amine 282 remaining from endeavours towards 284, the opportunity for synthesis of a bivalent nucleoside analogous to 251 was apparent (Scheme 99).
Two equivalents of 282 were reacted with a single equivalent of CDI to generate the di-nucleoside 285. The urea linkage in 285 also proved stable to deprotection using the standard conditions to give the final product 286.

4.2.4 Synthesis of Glycosylated Nucleoside Analogue 253

The final target was the glycosylated nucleoside 253. De Kort et al. reported the synthesis of a similar analogue 258 (Scheme 86 Section 4.0.2) by glycosylation of a hydroxymethylated nucleoside using modified D-glucose. This methodology would be the basis for our synthesis. The glycosyl donor was firstly synthesised according to the procedure of Sjölin et al. (Scheme 100).

D-glucose was fully protected using benzoyl chloride to furnish 287. This was then transformed using hydrobromic acid to form the glycosyl donor 288. The next step was to use 288 for Koenigs-Knorr glycosylation of nucleoside 270β (Table 22).
Table 22- Attempted glycosylation of nucleoside 270β with 288.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AgOTf, 3Å MS, MeCN</td>
<td>Starting material</td>
</tr>
<tr>
<td>2</td>
<td>Hg(CN)₂, HgBr₂, MeCN</td>
<td>Starting material</td>
</tr>
</tbody>
</table>

In de Korts’ report glycosylation was performed using mercury bromide and mercury cyanide. In order to avoid using these highly toxic reagents and alternative protocol was identified using silver triflate as the promoter; unfortunately only starting material was recovered under these conditions (Table 21, entry 1). Returning to the Helferich reaction also only yielded starting material (Table 21, entry 2).

It was decided to investigate the use of different glycosylation methods with different donors and so acetimidate 290 was synthesised (Scheme 101).

Scheme 101- Synthesis of alternative glycosyl donor 290.

Selective deprotection of benzoyl protected glucose 287 using methylamine gave an anomeric mixture of glucopyranose 289. Further reaction of 289 with trichloroacetonitrile furnished acetimidate 290 in good yields. With 290 in hand Schmidt glycosylation of 270β could be attempted (Scheme 102).
Pleasingly, making use of TMSOTf as the promoter gave 291 in good yields. Deprotection using ammonia in methanol yielded the final product 253.

_Scheme 102- Synthesis of glycosylated nucleoside 253._
4.3  Conclusion and Further Work

Adapting the methods of Uchida and Zhang led to the development of a route permitting the facile generation of multiple gram quantities of hydroxymethylated nucleoside \( 270\beta \).\(^{242, 243}\) By performing hydroxymethylation of uracil at an early stage of the synthesis, the somewhat cumbersome hydroxymethylation of nucleosides, which tend to lead to low yields, was avoided (Scheme 103).

![Scheme 103 - Route to nucleoside \( 270\beta \).](add_diagram)

It was also demonstrated that hydroxymethylated nucleoside \( 270\beta \) can be used for the synthesis of a diverse array of structures (Figure 29).
While problems were encountered in deprotection of the fluorinated analogue 271, it is believed that altering the protecting groups to avoid the use of basic deprotection would allow access to the final product. Inspiration can be taken from the radiosynthesis of $[^{18}\text{F}]-\text{FLT}$ which makes use of the acid labile trityl protecting group (Scheme 21 Section 1.3.2.1). If these issues can be overcome radiolabelling with $[^{18}\text{F}]-\text{DAST}$ would be the logical next step. Furthermore, this synthesis could be adapted for use in generating a similarly labelled dFdc analogue, which would provide a suitable solution to issues highlighted in the conclusion of chapter three (Section 3.5) (Figure 30).

Figure 30- dFdc analogue 292.
The AZT analogue 249 could now be tested to determine its usefulness as an inhibitor of HIV reverse transcriptase. In the case of the dimer 286, the synthesis acted as a proof of concept showing how these nucleosides can be linked together. Further studies could focus on linking different nucleosides together and examining their properties as combined direct and allosteric inhibitors of HIV reverse transcriptase.

Generation of the protected azide 276 permitted the synthesis, via a Huisgen cycloaddition, of 252 an analogue of the PET tracer FOT (Scheme 104).\(^{226}\)

![Scheme 104- Synthesis of triazole nucleoside analogue.](image)

Having completed the synthesis of the cold compound and its tosylated precursor 281, future endeavours should focus on adapting this molecule for radiochemical synthesis by using \(^{18}\)F-KF to displace the tosylate.

Dansylation to form 284 provided further demonstration of the diverse range of structures that can be formed from 270β. Incorporation of other fluorophores such as rhodamine and fluorescein derivatives could be followed by an investigation of the use of these molecules for in vitro imaging (Figure 31).\(^{230}\)

![Figure 31- Fluorescein labelled nucleoside.](image)
Finally glycosylated analogue 253 was synthesised by Schmidt glycosylation of 270β using modified glucose. As previously mentioned this molecule could be used to complement studies assessing the function of 258 and its role in Kinetoplastid protozoa.
Experimental

All chemicals were used as received or purified using standard procedures. Solvents were dried by standard techniques and distilled under nitrogen before use. Tetrahydrofuran was distilled from sodium with benzophenone indicator. Toluene was distilled from sodium. Dichloromethane and methanol were distilled from calcium hydride. *N,N*-Dimethylformamide, dimethylsulfoxide, aceonte, 1,4-dioxane, acetonitrile, 1,2-dichloroethane, ethanol, *tert*-butanol, benzene and o-xylene were purchased anhydrous and used as received. All experiments were carried out in oven-dried glassware under an inert atmosphere of nitrogen or argon.

Analytical thin layer chromatography (TLC) was performed using pre-coated Merck aluminium or glass backed plates (Silicagel 60 F254). Visualization was by ultraviolet light (366 nm and 254 nm) and/or treatment with potassium permanganate or vanillin stains followed by heating as appropriate. Flash column chromatography was carried out on Merck 9385 Kieselgel 60 (230-400 mesh).

NMR spectra were recorded on a Bruker DRX-400 spectrometer using an internal deuterium lock at ambient probe temperatures. Alternatively, spectra were recorded at 500 MHz (\(^1\)H NMR) and 125 MHz (\(^{13}\)C NMR) by the NMR Service, Imperial College London, Department of Chemistry. Chemical shifts (\(\delta\)) are quoted in parts per million (ppm) and are referenced to a residual solvent peak. Coupling constants (\(J\)) are quoted in Hertz (Hz) to the nearest 0.1 Hz. Assignments were determined on the basis of unambiguous chemical shift and coupling pattern. Additional experiments (COSY, DEPT-135, HSQC, HMBC and NOESY) were used to aid assignments but are not included.

Low and high resolution mass spectrometry (EI, CI, ESI) were recorded by the Mass Spectrometry Service, Imperial College London, Department of Chemistry using a Micromass Platform II and Micromass AutoSpec-Q spectrometer.

Microanalyses were determined at London Metropolitan University Microanalysis Service.
Infra-red (IR) spectra were obtained using a Perkin-Elmer Spectrum BX II FT-IR System monitoring from 4000-700 cm$^{-1}$, with automatic background subtraction. All samples were run neat. Absorption maxima are reported in wave numbers (cm$^{-1}$).

Melting points were obtained on a Reichert-Thermovar melting point apparatus and are uncorrected.

Optical rotations were measured at 25 °C on a Perkin-Elmer 241 polarimeter with a path length of 1 dm, using a sodium lamp ($\lambda = 589$ nm, D-line); $[\alpha]_D^{25}$ values are reported in $10^1$ deg cm$^2$ g$^{-1}$ and concentrations (c) are quoted in g/100 mL.
Results and Discussion Section 2.
A solution of DIPEA (3.7 mL, 21.2 mmol) in CH$_2$Cl$_2$ (8 mL) was added dropwise to a solution of 2-azetidinone (1.00 g, 14.1 mmol) and tert-butylidimethylsilylchloride (2.44 g, 16.2 mmol) in CH$_2$Cl$_2$ (12 mL) and the reaction mixture stirred for 24 hours at room temperature. The solvent was then removed *in vacuo* and the resulting residue partitioned between Et$_2$O (30 mL) and H$_2$O (15 mL), aqueous layer extracted with Et$_2$O (3 × 30 mL). The combined organic layers were dried over MgSO$_4$ and concentrated *in vacuo* to yield the crude product. The crude product was purified using flash silica column chromatography (20% EtOAc in 40-60 petroleum ether) to give the product (2.32 g, 89%) as a colourless oil.

The data were in agreement with the published data.$^{140}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H =$ 0.23 (s, 6H$_{4+5}$), 0.95 (s, 9H$_7$), 3.06 (t, 2H$_3$, $J$=4.6Hz), 3.18 (t, 2H$_1$, $J$=4.6Hz).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta_C =$ -6.2 (C-4,5), 18.5 (C-6), 26.0 (C-7), 36.7 (C-1), 39.0 (C-3), 172.9 (C-2).

MS (ES+) m/z = 186 [M+H]$^+$. 

1-(tert-Butyldimethylsilyl)-3-(phenylthio)azetidin-2-one 142

To a solution of diisopropylamine (2.5 mL, 17.8 mmol) in THF (30 mL) at 0 °C was added 1.0 M n-BuLi in hexanes (17.8 mL, 17.8 mmol) and the resulting solution stirred for 30 minutes at 0 °C. The solution was then cooled to −78 °C and added dropwise to a −78 °C solution of β-lactam 141.
(2.2 g, 11.9 mmol) and diphenyl disulfide (2.86 g, 13.1 mmol) in THF (70 mL). The reaction mixture was allowed to slowly warm to room temperature overnight and then poured into saturated NH₄Cl (aq) (180 mL) and extracted with CH₂Cl₂ (180 mL). The organic layer was separated and washed with H₂O (3 × 60 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (5% EtOAc in 40-60 petroleum ether) to yield the product (1.64 g, 47%) as a white solid.

¹H-NMR (400 MHz, CDCl₃): δH = 0.10 (s, 3H₄/5), 0.11 (s, 3H₄/5), 0.80 (s, 9H₇), 3.02 (dd, 1H₃, J=6.4Hz, J=3.0Hz), 3.53 (m, 1H₃), 4.41 (dd, 1H₁, J=5.7Hz, J=3.0Hz), 7.31 (m, 3H-Ar), 7.55 (m, 2H-Ar).

¹³C-NMR (400 MHz, CDCl₃): δC = -6.3 (C-4,5), 18.3 (C-6), 25.7 (C-7), 44.4 (C-3), 53.3 (C-1), 128.2 (CH-Ar), 129.1 (CH-Ar), 131.4 (C-8), 133.7 (CH-Ar), 171.1 (C-2).

IR v_max (neat) = 2943, 2924, 2856, 1729 cm⁻¹

MS (ES+) m/z = 294 [M+H]⁺.

HRMS (ES+) calculated 294.1348 for C₁₅H₂₄NOSSi, observed 294.1354 [M + H]⁺.

mp (CH₂Cl₂) = 68-70 °C.

1-(tert-Butyldimethylsilyl)-3-(phenylsulfinyl)azetidin-2-one 143

To a mixture of 142 (100 mg, 0.12 mmol), Ac₂O (36 µL, 0.38 mmol) and SiO₂ (68 mg) in dichloromethane (1.7 mL) was added 30% H₂O₂ (aq) (41 µL, 0.41 mmol). The reaction mixture was stirred for 36 hours at room temperature then filtered through a sintered funnel and the collected solid washed with CH₂Cl₂ (10 mL). The filtrate was washed sequentially with saturated NaHSO₃ (aq) (10 mL), saturated NaHCO₃ (aq) (10 mL) and Brine (10 mL), then dried.
over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (20% EtOAc in 40-60 petroleum ether) to yield the product (83 mg, 79%) as a white solid in a 1.0:1.3 mixture of diastereomers. 

**1H-NMR (400 MHz, CDCl₃):** δ_H = 0.03 (s, 3H₄/₅A), 0.06 (s, 3H₄/₅A), 0.23 (s, 3H₄/₅B), 0.27 (s, 3H₄/₅B), 0.80 (s, 9H₇A), 0.97 (s, 9H₇B), 3.19 (dd, 1H₃B, J=6.7Hz, J=5.5Hz), 3.38-3.49 (m, 2H₃A), 3.67 (dd, 1H₃B, J=6.8Hz, J=2.9Hz), 4.33 (dd, 1H₁B, J=5.4Hz, J=2.9Hz), 4.72 (dd, 1H₁A, J=5.3Hz, J=3.0Hz), 7.53 (m, 6H-Ar), 7.65 (m, 2H-Ar), 7.72 (m, 2H-Ar).

**13C-NMR (400 MHz, CDCl₃):** δ_C = -6.5 (C-4/5B), -6.5 (C-4/5B), -6.3(C-4/5A), -6.2 (C-4/5A), 18.2 (C-6B), 18.5 (C-6A), 25.7 (C-7B), 25.9 (C-7A), 35.9 (C-3B), 37.8 (C-3A), 69.5 (C-1A), 71.8 (C-1B), 124.0 (CH-Ar), 125.3 (CH-Ar), 129.1 (CH-Ar), 129.4 (CH-Ar), 131.3 (CH-Ar), 131.7 (CH-Ar), 138.7 (C-8A/B), 141.9 (C-8A/B), 165.0 (C=O), 165.7 (C=O).

**IR** ν_max (neat) = 2954, 2924, 2856, 1733, 1048 cm⁻¹

**MS (ES+) m/z = 310 [M+H]^+, 332 [M+Na]^+.**

**HRMS (ES+) calculated 310.1297 for C₁₅H₂₄NO₂SSi, observed 310.1304 [M+H]^+.**

**3-Bromo-N-(4-methoxyphenyl)propionamide 145**

To a solution of para-anisidine (5.00 g, mmol) in CH₂Cl₂ (16 mL) was added 3-bromopropionyl chloride (2.15 mL, 21.4 mmol) dropwise and the resulting solution stirred for 3 hours at room temperature. The reaction mixture was then diluted with CH₂Cl₂ (100 mL), washed with H₂O (2 × 100 mL), dried over MgSO₄ and concentrated in vacuo to yield the product as a purple powder (5.15 g, 94%). The product was used directly in the next step with no further purification. The data were in agreement with the published data.¹⁴⁷
$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 2.88$ (t, $2\text{H}^1, J=6.6\text{Hz}$) 3.66 (t, $2\text{H}^2, J=6.6\text{Hz}$) 3.76 (s, $3\text{H}^8$) 6.82 (m, $2\text{H}^6$) 7.38 (m, $2\text{H}^3$) 7.87 (s, $1\text{H}^9$).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta_C = 27.4$ (C-1), 40.3 (C-2), 55.5 (C-8), 114.1 (C-6), 122.4 (C-5), 130.5 (C-4), 156.7 (C-7), 168.3 (C-3).

IR $\nu_{\text{max}}$ (neat) = 3250, 2831, 1651, 1603 cm$^{-1}$

MS (ES+) m/z = 258 [M+H]$^+$.  
HRMS (ES+) calculated 258.0130 for $C_{10}H_{13}NO_2\text{Br}$, observed 258.0135 [M+H]$^+$. 

**1-(4-Methoxyphenyl)azetidin-2-one 146**

NaH (60% in mineral oil, 1.33 g, 33.2 mmol) was washed with hexanes (2 × 20 mL) and then suspended in a CH$_2$Cl$_2$-DMF mixture (4:1, 850 mL). To this was added, slowly over a period of 6 hours, a solution of the propionamide 145 (5.20 g, 20.2 mmol) in a CH$_2$Cl$_2$-DMF mixture (4:1, 340 mL). The reaction mixture was then stirred overnight at room temperature before being quenched with saturated NH$_4$Cl (aq) (800 mL). The organic layer was separated and washed with brine (3 × 1000 mL), H$_2$O (1000 mL), dried over MgSO$_4$ and concentrated in vacuo leaving approximately 10 mL of DMF. The product precipitated upon standing and cooling yielding the $\beta$-lactam (2.42 g, 70%) as white crystals.

The data were in agreement with the published data.$^{147}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 3.09$ (t, $2\text{H}^3, J=4.4\text{Hz}$) 3.59 (t, $2\text{H}^1, J=4.4\text{Hz}$) 3.78 (s, $3\text{H}^{10}$) 6.87 (m, $2\text{H}^6$) 7.30 (m, $2\text{H}^3$).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta_C = 36.1$ (C-3), 38.1 (C-1), 55.5 (C-8), 114.4 (C-6), 117.4 (C-5), 132.3 (C-4), 156.0 (C-7), 163.9 (C-2).
**IR** \( \nu_{\text{max}} \) (neat) = 2950, 1727 cm\(^{-1} \)

**MS (ES+)** \( m/z = 178 \left[ \text{M}+\text{H} \right]^+ \).

**HRMS (ES+)** calculated 178.0868 for C\(_{10}\)H\(_{12}\)NO\(_2\), observed 178.0865 \( \left[ \text{M}+\text{H} \right]^+ \).

1-(4-Methoxyphenyl)-3-(phenylsulfanyl)azetdin-2-one 147

![Chemical structure of 1-(4-Methoxyphenyl)-3-(phenylsulfanyl)azetdin-2-one 147](image)

To a solution of diisopropylamine (3.08 mL, 22.0 mmol) in THF (50 mL) at \(-78 \, ^{\circ}\text{C} \) was added 1.1 M \( n \)-BuLi in hexanes (20 mL, 22.0 mmol) and the resulting solution stirred for 30 minutes at 0 \( ^{\circ}\text{C} \). The solution was then cooled to \(-78 \, ^{\circ}\text{C} \) and a \(-15 \, ^{\circ}\text{C} \) solution of azetidinone 146 (1.95 g, 11.0 mmol) and diphenyl disulfide (2.64 g, 12.1 mmol) in THF (42 mL) added dropwise. The reaction mixture was allowed to slowly warm to room temperature overnight, then poured into saturated NH\(_4\)Cl (aq) (200 mL) and extracted with CH\(_2\)Cl\(_2\) (300 mL). The organic layer was separated and washed with H\(_2\)O (2 \times 400 mL), dried over MgSO\(_4\) and concentrated \textit{in vacuo}. The crude product was purified using flash silica column chromatography (25% EtOAc in 40-60 petroleum ether) to yield the product (1.27 g, 40%) as a yellow solid.

\(^1\text{H-NMR (400 MHz, CDCl}_3\): \( \delta_H = 3.49 \) (dd, 1H, \( J=6.0\text{Hz}, J=2.6\text{Hz} \)) 3.81 (s, 3H) 4.01 (m, 1H) 4.55 (dd, 1H, \( J=5.4\text{Hz}, J=2.6\text{Hz} \)) 6.88 (m, 2H-Ar) 7.32 (m, 5H-Ar) 7.58 (m, 2H-Ar).

\(^{13}\text{C-NMR (400 MHz, CDCl}_3\): \( \delta_C = 46.3 \) (C-3), 50.9 (C-1), 55.5 (C-8), 114.3 (C-6), 117.7 (C-5), 128.0 (CH-Ar), 129.2 (CH-Ar), 131.4 (C-Ar), 132.1(C-Ar), 132.2 (CH-Ar), 156.3 (C-Ar), 162.5 (C-2).

**IR** \( \nu_{\text{max}} \) (neat) = 3048, 3003, 2943, 1733 cm\(^{-1} \)

**MS (ES+)** \( m/z = 286 \left[ \text{M}+\text{H} \right]^+ \).

**HRMS (ES+)** calculated 286.0902 for C\(_{16}\)H\(_{16}\)NO\(_2\)S, observed 286.0902 \( \left[ \text{M}+\text{H} \right]^+ \).
mp (CH$_2$Cl$_2$)= 82-85 °C.

3-(Benzenesulfinyl)-1-(4-Methoxyphenyl)azetidine-2-one 148

To a mixture of the sulphide 147 (800 mg, 2.8 mmol), Ac$_2$O (0.29 mL, 3.1 mmol) and SiO$_2$ (560 mg) in CH$_2$Cl$_2$ (14 mL) was added 30% H$_2$O$_2$ (aq) (0.34 mL, 3.4 mmol). The reaction mixture was stirred for 36 hours at room temperature then filtered through a celite padded frit. The filtrate was then washed sequentially with saturated NaHSO$_3$ (aq) (100 mL), saturated NaHCO$_3$ (aq) (100 mL) and brine (10 mL), then dried over MgSO$_4$ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (50% EtOAc in 40-60 petroleum ether) to give the product (670 mg, 79%) as a pale yellow solid in a 1.0:1.3 mixture of diastereomers.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H$ = 3.60 (dd, 1H$_3^A$, $J$=6.2Hz, $J$=5.4Hz) 3.77 (s, 3H$_8^B$) 3.79 (s, 3H$_8^A$) 3.84 (m, 2H$_3^B$) 4.11 (dd, 1H$_3^A$, $J$=6.3Hz, $J$=2.5Hz) 4.35 (dd, 1H$_1^A$, $J$=5.2Hz, $J$=2.5Hz) 4.58 (dd, 1H$_1^B$, $J$=4.6Hz, $J$=3.3Hz) 6.81 (m, 2H) 6.88 (m, 2H) 7.16 (m, 2H) 7.30 (m, 2H) 7.55 (m, 6H-Ar) 7.70 (m, 2H-Ar) 7.75 (m, 2H-Ar).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta_C$ = 37.5 (C-3A), 44.8 (C-3B), 55.4, 55.5 (C-8A), 67.2, 69.4 (C-1B), 114.4 (CH-Ar), 114.8 (CH-Ar), 117.4 (CH-Ar), 117.9 (CH-Ar), 123.9 (CH-Ar), 124.5 (CH-Ar), 129.1 (CH-Ar), 129.5 (CH-Ar), 130.8 (C-Ar), 131.1 (C-Ar), 131.6 (CH-Ar), 131.8 (CH-Ar), 138.9 (C-Ar), 141.6 (C-Ar), 156.1 (C-Ar), 156.5 (C-Ar), 156.7 (C=O), 157.2 (C=O).

IR $\nu_{max}$ (neat) = 3057, 2944, 2834, 1766, 1746 cm$^{-1}$

MS (ES$^+$) m/z = 302 [M+H]$^+$, 365 [M+Na+MeCN]$^+$.

HRMS (ES$^+$) calculated 302.0851 for C$_{16}$H$_{15}$NO$_3$S, observed 302.0838 [M+H]$^+$.
To a solution of diisopropylamine (1.52 mL, 10.9 mmol) in THF (20 mL) at −78 °C was added 1.57 M n-BuLi in hexanes (6.90 mL, 10.9 mmol) and the resulting solution stirred for 30 minutes at 0 °C. The solution was then cooled to −78 °C and a −15 °C solution of azetidinone 146 (840 mg, 4.74 mmol) and phenyl selenium bromide (2.45 g, 10.4 mmol) in THF (20 mL) added dropwise. The reaction mixture was allowed to slowly warm to room temperature overnight and then quenched with H₂O (60 mL), extracted into Et₂O (2 × 60 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (15% EtOAc in 40-60 petroleum ether) to yield the product (1.11 g, 48%) as a yellow oil and the monoselenide 149 (168 mg, 11%) as a yellow solid.

**¹H-NMR (400 MHz, CDCl₃):** δ_H = 3.68 (s, 2H₃) 3.74 (s, 3H₈) 6.74 (m, 2H, Ar-H) 6.98 (m, 2H, Ar-H) 7.32 (m, 6H, Ar-H) 7.73 (m, 4H, Ar-H).

**¹³C-NMR (400 MHz, CDCl₃):** δ_C = 48.2 (C-1), 53.3 (C-3), 55.4 (C-8), 114.2 (CH-Ar), 117.8 (CH-Ar), 127.2 (C-Ar), 129.2 (CH-Ar), 129.4 (CH-Ar), 130.7 (C-Ar), 136.5 (CH-Ar), 156.3 (C-Ar), 163.5 (C-2).

**IR v_max (neat):** 3072, 3023, 2960, 2838, 1733 cm⁻¹

**MS (ES+) m/z =** 490 [M+H]⁺, 553 [M+Na+MeCN]⁺.

**HRMS (ES+) calculated 489.9824 for C₂₂H₂₀NO₂Se₂, observed 489.9821 [M+H]⁺.

**mp (CH₂Cl₂) =** 82-84 °C.
To a solution of diisopropylamine (0.04 mL, 0.20 mmol) in THF (0.8 mL) at −78 °C was added 2.06 M n-BuLi in hexanes (0.12 mL, 0.25 mmol) and the resulting solution stirred for 30 minutes at 0 °C. The solution was then cooled to −78 °C and a −78 °C solution of acetophenone (0.23 mL, 0.20 mmol) and the bis-selenide 151 (100 mg, 0.20 mmol) in THF (1.0 mL) added dropwise. The reaction mixture was allowed to slowly warm to room temperature overnight and then quenched with H₂O (10 mL), extracted into Et₂O (2 × 20 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (15% EtOAc in 40-60 petroleum ether) to yield the product (40 mg, 61%) as a yellow solid.

**¹H-NMR (400 MHz, CDCl₃):** δH = 3.47 (dd, 1H, J=6.2 Hz, J=2.5 Hz) 3.80 (s, 3H) 4.02 (m, 1H) 4.56 (dd, 1H, J=5.4 Hz, J=2.5 Hz) 6.85 (m, 2H, Ar-H) 7.21 (m, 2H, Ar-H) 7.30 (m, 3H, Ar-H) 7.69 (m, 2H, Ar-H)

**¹³C-NMR (400 MHz, CDCl₃):** δC = 42.6 (C-1), 46.4 (C-3), 55.4 (C-8), 114.3 (CH-Ar), 117.6, (CH-Ar) 126.2 (C-Ar), 128.5 (CH-Ar), 129.2 (CH-Ar), 131.5 (C-Ar), 135.0 (CH-Ar), 156.2 (C-Ar), 163.2 (C-2).

**IR v(max) (neat) = 3053, 2960, 1733 cm⁻¹**

**MS (ES+) m/z = 334 [M+H]^+.**

**HRMS (ES+) calculated 334.0346 for C₁₆H₁₆NO₂Se, observed 334.0338 [M+H]^+.**

**mp (Et₂O) = 82-84 °C.**
**Dimethyldioxirane**

To a 1 litre 3-necked flask containing a mixture of NaHCO₃ (45 g, 0.53 mol) in H₂O (120 mL) and acetone (100 mL) at 0 °C, was added oxone (90 g, 0.59 mol) in 3 portions during 10 minutes. The reaction mixture was stirred for 15 minutes before being allowed to warm to room temperature and the product collected by distillation (30 mbar), as a pale yellow solution (0.10 M in acetone, by titration with thioanisole) into a chilled receiving flask at –78 °C. The product was stored over anhydrous Na₂SO₄ at –25 °C.

**3-(Benzeneselenenyl)-1-(4-methoxyphenyl)azetidin-2-one**

To a solution of the selenide 149 (340 mg, 1.05 mmol) in acetone (5.3 mL) was added a 0.09 M solution of dimethyldioxirane in acetone (12.9 mL, 1.16 mmol) and the mixture stirred for 20 minutes at room temperature. The reaction mixture was then concentrated *in vacuo* and purified using flash silica column chromatography (2% MeOH in CHCl₃) to yield the product (347 mg, 98%) as a white solid in a 1.0:1.3 mixture of diastereomers.

**¹H-NMR (400 MHz, CDCl₃):** δH = 3.53 (dd, 1H³B, J=7.1Hz, J=2.0Hz) 3.59 (dd, 1H³A, J=6.4Hz, J=5.3Hz) 3.73 (s, 3H⁸B) 3.75-3.77 (m, 1H³B) 3.78 (s, 3H⁸A) 4.07 (dd, 1H³A, J=6.4Hz, J=2.2Hz) 4.42 (dd, 1H¹A, J=5.2Hz, J=2.3Hz) 4.77 (dd, 1H¹B, J=5.0Hz, J=2.0Hz) 6.72-6.75 (m, 2H-Ar) 6.85-6.89 (m, 2H-Ar) 6.94-6.96 (m, 2H-Ar) 7.27-7.29 (m, 2H-Ar) 7.42-7.48 (m, 3H-Ar) 7.55-7.60 (m, 3H-Ar) 7.75-7.79 (m, 4H-Ar).

**¹³C-NMR (400 MHz, CDCl₃):** δC = 37.5 (C-3A), 39.2 (C-3B), 55.4 (C-8B), 55.5 (C-8A), 62.0 (C-1A), 64.0 (C1-B), 114.2(CH-Ar), 114.4 (CH-Ar), 117.6 (CH-Ar), 117.9 (CH-Ar), 125.7(CH-Ar), 126.5 (CH-Ar), 129.4 (CH-Ar), 130.0 (CH-Ar), 130.4 (C-Ar), 131.1 (C-Ar), 131.8 (CH-Ar),
132.2 (CH-Ar), 135.1 (C-Ar), 139.1 (C-Ar), 156.6 (C-Ar), 156.7 (C-Ar), 157.8 (C=O), 158.2 (C=O).

\[ \text{IR } \nu_{\text{max}} (\text{neat}) = 2966, 1737 \text{ cm}^{-1} \]

\[ \text{MS (ES+) } m/z = 350 [M+H]^+, 372 [M+Na]^+. \]

\[ \text{HRMS (ESI) calculated 350.0295 for C}_{16}\text{H}_{16}\text{NO}_3\text{Se, observed 350.0293 [M+H]^+.} \]

1-(4-Methoxyphenyl)-3-(phenylselanyl)azetidin-2-one 149 and 1-(p-Methoxyphenyl)azetidine-2,3-dione 152

Selenoxide 150 (100 mg, 0.30 mmol) in toluene (3 mL) was heated in a sealed tube at 120 °C for 36 hours. The reaction mixture was then concentrated in vacuo and purified using flash silica column chromatography (100% CHCl₃) to yield selenide 149 (32 mg, 33%) and diketone 152 (17 mg, 30%). The data were in agreement with the published data.²⁵⁷

\[ \text{H-NMR (400 MHz, CDCl}_3\text{: } \delta_H = 3.84 (s, 3H^8) 4.29 (s, 2H^3) 6.96-7.00 (m, 2H^6) 7.48-7.51 (m, 2H^5).} \]

\[ \text{C-NMR (400 MHz, CDCl}_3\text{: } \delta_C = 55.6 (C-8), 59.6 (C-3), 114.8 (C-6), 119.0 (C-5), 130.7 (C-4), 158.1 (C-7), 159.3 (C-2), 190.6 (C-1).} \]

\[ \text{IR } \nu_{\text{max}} (\text{neat}) = 1814, 1741 \text{ cm}^{-1} \]

\[ \text{MS (ES+) } m/z = 192 [M+H]^+. \]

\[ \text{HRMS (ES+) calculated 192.0622 for C}_{10}\text{H}_{10}\text{NO}_3, \text{ observed 192.0613 [M+H]^+.} \]
**N-p-Methoxybenzyl-α-iminoglyoxalate 160**

To a solution of ethyl-glyoxylate (50% in toluene, 0.48 mL, 2.4 mmol) in CH₂Cl₂ (8 mL) was added dropwise a solution of *para*-anisidine (296 mg, 2.4 mmol) in CH₂Cl₂ (4 mL) and the resulting solution stirred for 30 minutes at room temperature before being treated with 4Å molecular sieves and stirred for a further 1 hour. The reaction mixture was then filtered and concentrated *in vacuo* to yield the product (419 mg, 84%) as yellow oil, which was used directly in the next step with no further purification. The data were in agreement with the published data.¹⁵⁵

**¹H-NMR (400 MHz, CDCl₃):** δ_H = 1.40 (t, 3H, J=7.1Hz) 3.84 (s, 3H) 4.41 (q, 2H, J=7.1Hz) 6.91-6.94 (m, 2H) 7.35-7.37 (m, 1H) 7.94 (s, 1H)

**¹³C-NMR (100 MHz, CDCl₃):** δ_C = 14.3 (C-9) 55.5 (C-7) 61.9 (C-8) 114.5 (C-5) 123.6 (C-4) 141.4 (C-3) 148.0 (C-2) 160.5 (C-6) 163.6 (C-1).

**Ethyl 3-(benzyloxy)-1-(4-methoxyphenyl)-4-oxoazetidine-2-carboxylate 155**

To a −78 °C solution of imine 160 (230 mg, 1.11 mmol) and NEt₃ (0.31 mL, 2.22 mmol) in CH₂Cl₂ (3 mL) was added benzyloxyacetyl chloride (0.26 mL, 1.67 mmol) dropwise. The solution was then warmed to room temperature and stirred overnight. The reaction mixture was then concentrated *in vacuo* and the crude residue purified using flash silica column
chromatography (20% EtOAc in 40-60 petroleum ether) to yield the product (201 mg, 51%) as a white solid.

The data were in agreement with the published data.\textsuperscript{154}

\textbf{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}):} $\delta_{\text{H}} = 1.25$ (t, 3H\textsuperscript{16}, $J$=7.2Hz) 3.82 (s, 3H\textsuperscript{8}) 4.28 (q, 2H\textsuperscript{15}, $J$=7.2Hz) 4.74 (d\textsuperscript{3}, 1H, $J$=5.2Hz) 4.83 (s, 2H\textsuperscript{10}) 5.04 (d, 1H, $J$=5.1Hz) 6.88-6.91 (m, 2H\textsuperscript{6}) 7.30-7.32 (m, 2H\textsuperscript{5}) 7.36-7.40 (m, 5H\textsuperscript{12,13,14})

\textbf{\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}):} $\delta_{\text{C}} = 14.2$ (C-16) 55.5 (C-8) 59.2 (C-3) 62.0 (C-15) 73.3 (C-10) 81.7 (C-1) 114.4 (CH-Ar) 118.4 (CH-Ar) 127.9 (CH-Ar), 128.2 (CH-Ar), 128.5 (CH-Ar) 130.4 (C-Ar) 136.4 (C-Ar) 156.8 (C-Ar) 162.7 (C-2) 167.6 (C-9).

\textbf{IR} $\nu_{\text{max}}$ (neat) =2962, 1742 cm\textsuperscript{-1}

\textbf{MS (ES+)} m/z = 356 [M+H]\textsuperscript{+}.

\textbf{HRMS (ES+)} calculated 356.1498 for C\textsubscript{20}H\textsubscript{22}NO\textsubscript{5}, observed 356.1506 [M+H]\textsuperscript{+}.

1,4-Bis(4'-methoxyphenyl)-1,4-diazabuta-1,3-diene 162

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{diagram.png}
\end{figure}

A mixture of 40% glyoxal (aq) (0.50 mL, 4.30 mmol), \textit{para}-anisidine (1.06 g, 8.60 mmol) and catalytic formic acid in MeOH (14 mL) were stirred at room temperature for 1 hour. The precipitated solids were collected by filtration, washed with cold MeOH and dried under vacuum to give the product (805 mg, 70%) as a yellow powder.

The data were in agreement with the published data.\textsuperscript{159}

\textbf{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}):} $\delta_{\text{H}} = 3.85$ (s, 6H\textsuperscript{11+12}) 6.93-6.98 (m, 4H) 7.32-7.36 (m, 4H) 8.42 (s, 2H\textsuperscript{1+2}).
\textbf{\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3})}: $\delta_C = 55.5$ (C-11+12), 114.6 (C6+9), 123.1 (C5+8), 143.0 (C3+4), 157.6 (C7+10), 159.8 (C1+2).

\textbf{MS (ES\textsuperscript{+}) m/z} = 269 [M+H]\textsuperscript{+}.

\textbf{HRMS (ES\textsuperscript{+})} calculated 268.1290 for C\textsubscript{16}H\textsubscript{17}N\textsubscript{2}O\textsubscript{2}, observed 269.1310 [M+H]\textsuperscript{+}.

3-(Benzyloxy)-4-formyl-1-(4-methoxyphenyl)azetidin-2-one 164

To a suspension of the di-imine 162 (690 mg, 2.57 mmol) and NE\textsubscript{t}\textsubscript{3} (0.43 mL, 3.09 mmol) in toluene (24 mL) was added a solution of benzoxyacetyl chloride (0.45 mL, 2.83 mmol) in toluene (12 mL) dropwise and the resulting solution stirred overnight at room temperature. The reaction mixture was diluted with CHCl\textsubscript{3} (100 mL), washed sequentially with saturated NaHCO\textsubscript{3} (aq) (50 mL) and H\textsubscript{2}O (2x50 mL), then dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}.

A solution of the crude lactam (350 mg, 0.84 mmol) in CHCl\textsubscript{3} (16 mL) was stirred with 5\% HCl (aq) (8.4 mL) overnight at room temperature. The reaction was then diluted with CHCl\textsubscript{3} (50 mL), washed sequentially with 1.0 M HCl (aq) (2x20 mL), H\textsubscript{2}O (20 mL) and brine (20 mL), then dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The crude material was re-crystallised from EtOAc and hexanes (1:1) to yield the product (160 mg, 61\%) as a brown solid.

The data were in agreement with the published data.\textsuperscript{158}

\textbf{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3})}: $\delta_H = 3.82$ (s, 3H\textsuperscript{9}) 4.51 (dd, 1H\textsuperscript{1}, $J$=3.9Hz, $J$=5.2Hz) 4.75 (d, 1H\textsuperscript{10}, $J$=11.6Hz) 4.86 (d, 1H\textsuperscript{10}, $J$=11.7Hz) 5.07 (d, 1H\textsuperscript{1}, $J$=5.2Hz) 6.89-6.91 (m, 2H\textsuperscript{7}) 7.28-7.30 (m, 2H\textsuperscript{8}) 7.36-7.41 (m, 5H\textsubscript{12-14}) 9.74 (d, 1H\textsuperscript{5}, $J$=3.8Hz).
$^{13}\text{C-NMR (100 MHz, CDCl}_3\text{): } \delta_C = 55.5 \text{ (C-9), 63.2 (C-3), 73.6 (C-10), 82.7 (C-1), 114.6 (CH-Ar), 118.1 \text{ (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.6(CH-Ar), 130.5 (C-Ar), 135.8 (C-Ar), 157.0 (C-Ar), 162.9 (C-2), 198.9 (C-5).} $

$\text{IR } \nu_{\text{max}} \text{ (neat) } = 2838, 1737, 1511 \text{ cm}^{-1} \text{.}$

$\text{MS (CI+)} m/z = 329 [M+\text{NH}_4]^+.$

$\text{HRMS (CI+)} \text{ calculated } 329.1501 \text{ for } C_{18}H_{21}N$_2$O$_4$, observed 329.1487 $[M+\text{NH}_4]^+$.

**Ethyl 3-hydroxy-1-(4-methoxyphenyl)-4-oxazetidine-2-carboxylate 167**

A round bottomed flask containing 10% Pd on carbon (50 mg, 0.05 mmol) was evacuated and backfilled with nitrogen three times and then evacuated and refilled with hydrogen. To this was added a solution of $\beta$-lactam 155 (106 mg, 0.30 mmol) in THF (10 mL) and the mixture stirred under hydrogen (1 atm) overnight. The reaction mixture was filtered through a celite padded frit and then concentrated in vacuo to yield the product as a white solid (77 mg, 97%).

The data were in agreement with the published data.$^{154}$

$^1\text{H-NMR (400 MHz, CDCl}_3\text{): } \delta_H = 1.31 \text{ (t, } 3H^{12}, J=7.1\text{Hz}) 3.71 \text{ (d, } 1H^2, J=9.5\text{Hz}) 3.78 \text{ (s, } 3H^{10}) 4.32 \text{ (q, } 2H^{11}, J=7.1\text{Hz}) 4.69 \text{ (d, } 1H^3, J=5.2\text{Hz}) 5.21 \text{ (dd, } 1H^1, J=9.5\text{Hz, } J=5.1\text{Hz}) 6.85-6.88 \text{ (m, } 2H^8) 7.27-7.30 \text{ (m, } 2H^7).$

$^13\text{C-NMR (100 MHz, CDCl}_3\text{): } \delta_C = 14.2 \text{ (C-12), 55.5 (C-10), 59.7 (C-3), 62.4 (C-11), 77.3 (C-1), 114.5 (C-8), 118.4 (C-7), 130.3 (C-6), 156.9 (C-9), 164.3 (C-2), 168.7 (C-4).}$

$\text{IR } \nu_{\text{max}} \text{ (neat) } = 3239, 2942, 1719 \text{ cm}^{-1} \text{.}$

$\text{MS (ES+)} m/z = 266 [M+H]^+.$
HRMS (ES+) calculated 265.1028 for C_{13}H_{16}NO_{5}, observed 266.1036 [M+H]^+.

**Ethyl 1-(4-methoxyphenyl)-4-oxo-3-[(trifluoromethane)sulfonyloxy]azetidine-2-carboxylate 165**

![Chemical Structure](image)

To a −78 °C solution of the alcohol 167 (70 mg, 0.26 mmol) and pyridine (90 µL, 1.14 mmol) in CH_{2}Cl_{2} (1.75 mL) was added Tf_{2}O (90 µL, 0.52 mmol) slowly and the mixture warmed to 0 °C and stirred for 1 hour. The reaction mixture was diluted with CH_{2}Cl_{2} (10 mL), washed with H_{2}O (2 × 10 mL), combined aqueous layers re-extracted with CH_{2}Cl_{2} (10 mL) and the combined organic layers dried over MgSO_{4} and concentrated in vacuo to yield the crude product as a yellow solid. This was used directly in the following step with no further purification.

**Ethyl 3-iodo-1-(4-methoxyphenyl)-4-oxoazetidine-2-carboxylate 166 and 170**

![Chemical Structures](image)

To a solution of triflate 165 (100 mg, 0.25 mmol) in acetone (2.5 mL) was added NaI (292 mg, 1.95 mmol) and the resulting suspension stirred for 48 hours at room temperature. The reaction mixture was then concentrated in vacuo and partitioned between Et_{2}O (10 mL) and H_{2}O (10 mL). The aqueous layer was re-extracted with Et_{2}O (10 mL), the combined organic layers dried over MgSO_{4} and then concentrated in vacuo. The crude material was purified using flash silica column chromatography (30% EtOAc in 40-60 petroleum ether) to yield the trans-β-lactam (33 mg, 35%) and the cis-β-lactam (31 mg, 33%) as pale yellow amorphous solids.
Trans-diastereomer 166

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 1.31$ (t, 3H, $J=7.2$Hz) 3.82 (s, 3H) 4.31 (m, 2H) 4.63 (d, 1H, $J=2.0$Hz) 5.07 (d, 1H, $J=1.9$Hz) 6.88-6.92 (m, 2H) 7.29-7.32 (m, 2H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 14.1$ (C-1), 15.1 (C-7), 55.5 (C-11), 62.1 (C-3), 62.6 (C-6), 114. (C-9), 118.2 (C-8), 130.6 (C-5), 157.0 (C-10), 160.4 (C-2), 168.3 (C-4).

IR: $\nu_{max}$ (neat) = 2984, 1760, 1512 cm$^{-1}$

Cis-diastereomer 170

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 1.36$ (t, 3H, $J=7.2$Hz) 3.82 (s, 3H) 4.31-4.44 (m, 2H) 4.81 (d, 1H, $J=5.7$Hz) 5.43 (d, 1H, $J=5.7$Hz) 6.89-6.92 (m, 2H) 7.26-7.30 (m, 2H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 14.3$ (C-1), 17.5 (C-7), 55.5 (C-11), 56.6 (C-3), 62.4 (C-6), 114.5 (C-9), 118.2 (C-8), 130.4 (C-5), 157.0 (C-10), 161.5 (C-2), 169.3 (C-4).

IR: $\nu_{max}$ (neat) = 2995, 1749, 1513 cm$^{-1}$

MS (ES+) m/z = 376 [M+H]$^+$.  

HRMS (ES+) calculated 376.0046 for C$_{13}$H$_{15}$NO$_4$I, observed 376.0056 [M+H]$^+$.  

3-Iodo-1-(4-methoxyphenyl)-4-oxoazetidine-2-carboxylic acid 171

A solution of β-lactam 166 (76 mg, 0.20 mmol) in THF (1 mL) was treated with potassium trimethylsilanolate (43 mg, 0.30 mmol) and the resulting suspension stirred at room temperature for 10 minutes. The reaction mixture was then concentrated in vacuo, dissolved in H$_2$O (10 mL) and washed with EtOAc (2 ×10 mL). Acidification of the aqueous layer using 1.0 M HCl (aq)
(15 mL) was followed by extraction into CH$_2$Cl$_2$ (2 x 10 mL). Combined organic layers were dried over MgSO$_4$ and concentrated under reduced pressure to yield the product (42 mg, 60%) as a yellow gum.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$H = 3.82 (s, 3H$^{10}$) 4.66 (d, 1H$^3$, J=2.0Hz) 5.13 (d, 1H$^1$, J=2.0Hz) 5.41 (br s, 1H$^6$) 6.89-6.93 (m, 2H$^8$) 7.30-7.34 (m, 2H$^7$).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$C = 14.7 (C-1), 55.6 (C-10), 61.7 (C-3), 114.7 (C-8), 118.3 (C-7), 130.4 (C-5), 157.2 (C-9), 160.5 (C-2), 171.8 (C-4).

IR $\nu_{max}$ (neat) = 3460, 2932, 1734, 1612 cm$^{-1}$

MS (ES-) m/z = 346 [M-H]$^-$.  
HRMS (ES-) calculated 345.9576 for C$_{11}$H$_9$NO$_4$I, observed 345.9588 [M-H]$^-$. 

2-Methylidenebut-3-en-1-ol 174

To a −78 °C solution of HMDS (0.64 mL, 3.04 mmol) in Et$_2$O (2.40 mL) was added 2.5 M n-BuLi (1.14 mL, 2.85 mmol) dropwise. The resulting mixture was warmed to room temperature during 30 minutes then 2-methyl-2-vinylloxirane (0.23 mL, 2.38 mmol) added and the solution refluxed for 24 hours. The reaction mixture was then cooled to room temperature poured into cold 1.0 M HCl (aq) (20 mL), stirred for 1 hour and extracted into Et$_2$O (3 × 10 mL). Combined organic layers were washed sequentially with saturated NaHCO$_3$ (aq) (10 mL) and brine (10 mL), then dried over MgSO$_4$ and concentrated in vacuo to yield the crude product as a volatile, colourless oil. This was used directly in the next step with no further purification.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$H = 1.54 (s, 1H$^6$) 4.38 (s, 2H$^1$) 5.14-5.19 (m, 2H$^5$) 5.29-5.34 (m, 2H$^4$) 6.43 (dd, 1H$^3$, J=17.9Hz, J=11.1Hz).
**13C-NMR (100 MHz, CDCl₃):** \( \delta_C = 62.6 \) (C-1), 114.1 (C-5), 115.7 (C-4), 136.3 (C-3), 145.2 (C-2).

**Methoxy[(2-methylidenebut-3-en-1-yl)oxy]methane 175**

To a solution of the crude isoprenol 174 (700 mg, 8.3 mmol) and DIPEA (3.04 mL, 17.5 mmol) in CH₂Cl₂ (70 mL) was added chloromethyl methyl ether (1.26 mL, 16.6 mmol) dropwise and the resulting solution stirred for 16 hours at room temperature. The reaction mixture was quenched with H₂O (200 mL) and extracted with CH₂Cl₂ (2 × 150 mL). The combined organic layers were then washed with 1.0 M HCl (aq) (15-0 mL), dried over MgSO₄ and concentrated *in vacuo*. The crude material was purified by flash silica column chromatography (8% Et₂O in pentane) to yield the product (596 mg, 56%, over 2 steps) as a volatile, colourless oil.

**1H-NMR (400 MHz, CDCl₃):** \( \delta_H = 3.43 \) (s, 3H⁷) 4.28 (s, 2H¹) 4.71 (s, 2H⁶) 5.14-5.23 (m, 2H⁵) 5.31-5.36 (m, 2H⁴) 6.43 (dd, 1H³, \( J=17.8\)Hz, \( J=11.0\)Hz).

**13C-NMR (100 MHz, CDCl₃):** \( \delta_C = 55.4 \) (C-7), 66.7 (C-1), 95.7 (C-6), 114.4 (C-5), 117.5 (C-4), 136.6 (C-3), 142.2 (C-2).

**IR** \( \nu_{max} \) (neat) = 2934, 2887, 1598 cm⁻¹

**HRMS:** Did not provide spectra suitable for interpretation.
A mixture of β-lactam salt 171 (75 mg, 0.20 mmol) and diene 175 (250 mg, 1.95 mmol) was heated at 125 °C for 16 hours in a sealed tube. The reaction mixture was cooled to room temperature, diluted with EtOAc (15 mL) and washed with H₂O (15 mL). The organic layer was dried over MgSO₄ and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (10% Et₂O in pentane) to yield the product (72 mg, 58%) as a 1.0:1.0 mixture of regioisomers.

**¹H-NMR (400 MHz, CDCl₃):**  δ_H = 1.45-2.31 (m, 14H₃,4,5,6), 3.39-3.40 (m, 12H₉,14), 3.94 (br, s 4H₇), 4.08 (m, 4H₁¹), 4.64-4.67 (m, 8H₈,13), 4.96-4.98 (m, 2H₁²), 5.09-5.12 (m, 2H₁²), 5.67-5.77 (m, 2H₁).

**¹³C-NMR (100 MHz, CDCl₃):**  δ_C = 25.5 (C-3), 26.5 (C-3), 27.5 (C-4), 27.6 (C-4), 30.9 (C-6), 31.9 (C-6), 36.9 (C-5), 37.0 (C-5), 55.2 (C-9), 55.3 (C-14), 69.4 (C-11), 69.4 (C-11), 71.5 (C-7), 71.8 (C-7), 95.5 (C-8/13), 95.7 (C-8/13), 110.5 (C-12), 110.5 (C-12), 124.6 (C-1), 125.0 (C-1), 134.0 (C-2), 134.2 (C-2), 149.8 (C-10), 149.8 (C-10).

**IR v_max (neat) = 2926, 2883, 2839 cm⁻¹**

**MS (ES⁺) m/z = 279 [M+Na]⁺.**

**HRMS (ES⁺) calculated 279.1572 for C₁₄H₂₄O₄Na, observed 279.1592 [M+Na]⁺.**
1-(Adamantan-1-yl)-3-(4-methoxyphenyl)urea 180

A suspension of β-lactam salt 171 (80 mg, 0.21 mmol), 1-adamantyl isocyanate (186 mg, 1.05 mmol) and 18-crown-6 (55 mg, 0.21 mmol) in o-xylene (0.5 mL) was heated at 130 °C for 2 hours. The reaction mixture was cooled to room temperature, diluted with EtOAc (10 mL) and washed with H2O (15 mL). The organic layer was dried over MgSO4 and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (30% EtOAc in 40-60 petroleum ether) to yield the product (4 mg, 6%) as an orange solid.

The data were in agreement with the published data.258

1H-NMR (400 MHz, CDCl3): δH = 1.63-1.69 (m, 6H12), 1.95 (dd, 6H10, J=9.6Hz, J=2.9Hz), 2.02-2.10 (m, 3H11), 3.79 (s, 3H8), 4.36 (s, 1H1), 5.87 (s, 1H3), 6.83-6.87 (m, 2H-Ar), 7.14-7.18 (m, 2H-Ar).

13C-NMR (100 MHz, CDCl3): δC = 29.5 (C-11), 36.4 (C-12), 42.3 (C-10), 51.2 (C-9), 55.5 (C-8), 114.5 (C-6), 124.5 (C-5), 131.4 (C-4), 155.3 (C-7), 157.0 (C-2).

IR νmax (neat) = 3323, 2905, 1628, 1553 cm⁻¹

MS (ES+) m/z = 301 [M+H]⁺.

HRMS (ES+) calculated 301.1916 for C18H25N2O2, observed 301.1922 [M+H]⁺.
4,6-O-Benzylidene-d-glucopyranose 197

To oven dried anhydrous D-glucose (10 g, 55.5 mmol) was added catalytic para-toluenesulfonic acid (50 mg), benzaldehyde dimethyl acetal (9.2 mL, 61.1 mmol) and DMF (40 mL). The resulting suspension was heated under reduced pressure (330 mbar) at 60 °C for 30 minutes until the glucose had dissolved. The reaction was quenched by cooling and the addition of excess NEt₃ (1.5 mL), then concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (0.1% NEt₃ in EtOAc) to yield the product (7.3 g, 49%) as a white solid in a 1.0:1.5 mixture of α and β-anomers. The data were in agreement with the published data.¹⁶⁷

¹H-NMR (400 MHz, DMSO-d₆): δ_H = 3.02 (td, 1H^6α, J=7.9Hz, J=5.2Hz), 3.23-3.43 (m, 5H^3α,4α,4β,5α,6β), 3.59-3.69 (m, 3H^2α,2β,5β), 3.80 (td, 1H^3β, J=10.0Hz, J=4.8Hz), 4.09 (dd, 1H^2β, J=9.9Hz, J=4.8Hz), 4.16 (dd, 1H^2α, J=10.2Hz, J=4.0Hz), 4.43-4.47 (m, 1H^7α), 4.81 (d, 1H^9β, J=6.9Hz), 4.97-5.00 (m, 1H^7β), 5.10 (d, 1H^8β, J=5.0Hz), 5.18 (d, 1H^9α, J=5.1Hz), 5.23 (d, 1H^8α, J=4.7Hz), 5.56 (s, 1H^1β), 5.57 (s, 1H^1α), 6.55 (d, 1H^10β, J=4.8Hz), 6.84 (d, 1H^10α, J=6.6Hz), 7.36-7.40 (m, 6H-Ar), 7.43-7.46 (m, 4H-Ar).

¹³C-NMR (400 MHz, DMSO-d₆): δ_C = 62.4 (C-3β), 66.2 (C-3α), 68.5 (C-2α), 68.9 (C-2β), 70.1 (C-5α), 73.3 (C-5β), 73.4 (C-6α), 76.2 (C-6β), 81.4 (C-4α), 82.2 (C-4β), 93.6 (C-7β), 98.1 (C-7α), 101.1 (C-1α), 101.3 (C-1β),126.8 (CH-Ar), 126.8 (CH-Ar), 128.5 (CH-Ar), 129.3 (CH-Ar), 138.3 (C-Ar), 138.4 (C-Ar).

Note: missing 2 × CH-Ar due to overlapping peaks.

IR v_max (neat) = 3314, 1386 cm⁻¹

MS (ES+) m/z = 269 [M+H]⁺.

HRMS (ES+) calculated 269.1025 for C₁₃H₁₇O₆, observed 269.1035 [M+H]⁺.
1,3-Benzylidene-L-erythritol 198

A mixture of glucopyranose 197 (7.0 g, 25.9 mmol), sodium metaperiodate (11.4 g, 53.1 mmol) and sodium bicarbonate (5.8 g, 84.0 mmol) in THF-H₂O (1.0:2.3, 265 mL) was stirred at room temperature for 1 hour. The reaction was then cooled to 0 °C, sodium borohydride (2.00 g, 53.1 mmol) was added and the reaction mixture stirred for a further hour at 0 °C. The precipitate was then filtered off, the filtrate extracted with ethyl acetate (2 x 200 mL), washed with saturated Na₂SO₄ (aq)-NaHCO₃ (aq) (1:1, 2 × 200 mL) and dried over MgSO₄. The organic layer was concentrated in vacuo to yield the product (5.09 g, 94%) as a white solid. The data were in agreement with the published data.168

¹H-NMR (400 MHz, DMSO-dma): δH = 3.46-3.58 (m, 4H 2,3,4,5), 3.71-3.78 (m, 1H 5), 4.07-4.13 (m, 1H 6), 4.64 (t, 1H 7, J=5.7Hz), 5.17 (d, 1H 8, J=5.0Hz), 5.50 (s, 1H 1), 7.35-7.37 (m, 3H Ar), 7.44-7.46 (m, 2H Ar).

¹³C-NMR (400 MHz, DMSO-dma): δC = 61.3 (C-3, 5), 71.2 (C-2), 83.6 (C-4), 100.6 (C-1), 126.8 (CH-Ar), 128.4 (CH-Ar), 129.0 (CH-Ar), 138.8 (C-8).

IR νmax (neat) = 3282, 2947, 1454 cm⁻¹

MS (CI) m/z = 211 [M+H]⁺, 228 [M+NH₄]⁺.

HRMS (CI+) calculated 228.1236 for C₁₁H₁₈O₄N, observed 228.1241 [M+NH₄]⁺.
A solution of diol 198 (4.88 g, 23.2 mmol) and imidazole (1.74 g, 25.5 mmol) in anhydrous CH₂Cl₂ (430 mL) was stirred at 0 °C while TBSCl (4.37 g, 29.0 mmol) was added during 5 minutes. The resulting milky solution was stirred overnight at room temperature, then excess ethanol amine (2.5 mL) added and the mixture stirred for a further 30 minutes. The reaction mixture was diluted with H₂O (300 mL), extracted with CH₂Cl₂ (2 × 250 mL), the combined organic layers washed with brine (300 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (20% acetone in 40-60 petroleum ether) to yield the product (6.10 g, 81%) as a colourless, viscous oil. The data were in agreement with the published data.

¹H-NMR (400 MHz, CDCl₃): δH = 0.12 (s, 3H⁶/⁷), 0.13 (s, 3H⁶/⁷), 0.92 (s, 9H⁹), 3.46 (d, 1H¹⁰, J=1.2Hz), 3.62-3.67 (m, 1H³), 3.74-3.77 (m, 1H³), 3.85 (dd, 1H², J=9.6Hz, J=8.8Hz), 3.88-4.01 (m, 1H³), 4.04 (dd, 1H², J=9.7Hz, J=4.6Hz), 4.33 (dd, 1H², J=10.9Hz, J=5.3Hz), 5.50 (s, 1H¹), 7.34-7.39 (m, 3H), 7.45-7.48 (m, 2H).

¹³C-NMR (400 MHz, CDCl₃): δC = -5.6 (C-6/7), -3.6 (C-6/7), 18.6 (C-8), 25.8 (C-9), 66.3 (C-5), 66.6 (C-4), 70.5 (C-2), 78.9 (C-3), 101.1 (C-1), 126.1 (CH-Ar), 128.3 (CH-Ar), 129.0 (CH-Ar), 137.4 (C-11).

IR νmax (neat) = 3473, 2958, 2860, 1251 cm⁻¹

HRMS (ES⁺) calculated 325.1835 for C₁₇H₂₉O₄Si, observed 325.1837 [M+H]⁺.
(4S,5S)-4-((tert-Butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-yl benzoate

To a 0 °C solution of alcohol 185 (100 mg, 0.31 mmol) in toluene (3.0 mL) was added triphenylphosphine (420 mg, 1.54 mmol) and benzoic acid (188 mg, 1.54 mmol). To the resulting solution was then added DIAD (0.30 mL, 1.54 mmol), the reaction heated to reflux and stirred for 72 hours. The reaction mixture was diluted with saturated NaHCO₃ (aq) (7 mL), extracted with CH₂Cl₂ (3 × 6 mL), dried over magnesium sulphate and concentrated in vacuo. The crude product was purified using flash silica column chromatography (20% EtOAc in 40-60 petroleum ether) to yield the product (40 mg, 29%) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃): δH = -0.09 (s, 3H⁶/⁷), -0.03 (s, 3H⁶/⁷), 0.81 (s, 9H⁸), 3.81-3.89 (m, 2H²), 4.20 (dd, 1H⁴, J=13.0Hz, J=1.4Hz), 4.25 (ddd, 1H³, J=7.8Hz, J=6.0Hz, J=1.6Hz), 4.49 (dd, 1H², J=13.0Hz, J=1.3Hz), 5.12-5.14 (m, 1H¹), 5.66 (s, 1H¹), 7.36-7.42 (m, 3H-Ar), 7.44-7.48 (m, 2H-Ar), 7.53-7.60 (m, 3H-Ar), 8.13-8.15 (m, 2H-Ar).

¹³C-NMR (400 MHz, CDCl₃): δC = -5.5 (C-6/7), -5.4 (C-6/7), 18.1 (C-8), 25.7 (C-9), 61.3 (C-5), 65.4 (C-4), 69.6 (C-2), 78.2 (C-3), 101.3 (C-1), 126.2 (CH-Ar), 128.3 (CH-Ar), 128.4 (CH-Ar), 129.1 (CH-Ar), 129.8 (CH-Ar), 130.1 (C-15), 133.1 (CH-Ar), 137.9 (C-10), 166.1 (C-14).

IR νmax (neat) = 2930, 2857, 1722, 1271 cm⁻¹

MS (ESI) m/z: Did not provide spectra suitable for interpretation.
(2R,4S)-4-[[tert-Butyl(dimethyl)silyl]methyl]-2-phenyl-1,3-dioxan-5-one 201

To a solution of oxaly chloride (2.26 mL, 26.7 mmol) in CH₂Cl₂ (80 mL) at −78 °C was added DMSO (3.80 mL, 53.4 mmol). After 5 minutes a solution of alcohol 185 (5.78 g, 17.8 mmol) in CH₂Cl₂ (25 mL) was added and the reaction mixture stirred at −78 °C for 1 hour. The reaction mixture was then treated with NEt₃ (11.2 mL, 80.1 mmol), before being slowly warmed to 0 °C and stirred for 15 minutes. The solution was then poured into cold saturated NH₄Cl (aq) (250 mL), extracted with EtOAc (2 × 300 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (15% EtOAc in 40-60 petroleum ether) to yield the product (5.06 g, 88%) as a yellow oil.

¹H-NMR (400 MHz, CDCl₃): δ_H = 0.06 (s, 3H₁₋₇), 0.08 (s, 3H₁₋₇), 0.89 (s, 9H₉), 4.06-4.09 (m, 2H₅), 4.39-4.50 (m, 2H₂), 4.52-4.54 (m, 1H₄), 5.94 (s, 1H₁), 7.39-7.44 (m, 3H-Ar), 7.54-7.58 (m, 2H-Ar).

¹³C-NMR (400 MHz, CDCl₃): δ_C = -5.4 (C-6/7), -5.3 (C-6/7), 18.3 (C-8), 25.8 (C-9), 62.8 (C-5), 72.6 (C-2), 84.1 (C-4), 99.2 (C-1), 126.2 (CH-Ar), 128.4 (CH-Ar), 129.2 (CH-Ar), 137.1 (C-10), 205.2 (C-3).

IR ν_max (neat) = 2928, 2857, 1738, 1253 cm⁻¹

MS (ES+) m/z = 323 [M+H]⁺.

HRMS (ES+) calculated 323.1679 for C₁₇H₂₇O₄Si, observed 323.1669 [M+H]⁺.
(4S,5S)-4-[[tert-Butyl(dimethyl)silyl]oxymethyl]-2-phenyl-1,3-dioxan-5-ol 200

To a −78 °C solution of ketone 201 (4.50 g, 14.0 mmol) in THF (26 mL) was added 1.0 M L-selectride in THF (26.5 mL, 26.5 mmol) dropwise and the resulting solution stirred at −78 °C for 3 hours. The reaction mixture was then warmed to room temperature and treated with 10% NaOH (aq) (250 mL), the layers were separated and the aqueous layer was extracted with EtOAc (3 × 300 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (15% EtOAc in 40-60 petroleum ether) to yield the product (3.82 g, 84%) as a colourless oil.

\[ \delta_H = 0.08 \text{ (s, 3H) } \]
\[ = 0.09 \text{ (s, 3H) } \]
\[ = 0.91 \text{ (s, 9H) } \]
\[ = 2.83 \text{ (d, 1H, J=10.2Hz) } \]
\[ = 3.70-3.74 \text{ (m, 1H) } \]
\[ = 3.80 \text{ (dd, 1H, J=10.1Hz, J=5.3Hz) } \]
\[ = 3.91 \text{ (dd, 1H, J=10.1Hz, J=6.7Hz) } \]
\[ = 3.98 \text{ (ddd, 1H, J=6.7Hz, J=5.3Hz, J=1.1Hz) } \]
\[ = 4.08 \text{ (dd, 1H, J=11.9Hz, J=1.3Hz) } \]
\[ = 4.25 \text{ (dd, 1H, J=11.9Hz, J=1.9Hz) } \]
\[ = 5.59 \text{ (s, 1H) } \]
\[ = 7.35-7.40 \text{ (m, 3H-Ar) } \]
\[ = 7.48-7.51 \text{ (m, 2H-Ar) } \]

\[ \delta_C = -5.4 \text{ (C-6/7), -5.4 (C-6/7), 18.3 (C-8), 25.8 (C-9), 62.4 (C-5), 63.7 (C-4), 72.7 (C-2), 79.8 (C-3), 101.4 (C-1), 126.0 (CH-Ar), 128.2 (CH-Ar), 129.0 (CH-Ar), 137.4 (C-Ar). } \]

IR \( \nu_{max} \) (neat) = 3465, 2958, 2927, 1250 cm\(^{-1}\)

MS (ES+) m/z = 325 [M+H]\(^+\), 347 [M+Na]\(^+\).

HRMS (ESI) calculated 325.1835 for C\(_{17}\)H\(_{29}\)O\(_4\)Si, observed 325.1837 [M+H]\(^+\).
(4S,5S)-4-((tert-Butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-yl trifluoromethanesulfonate 202

To a stirred solution of alcohol 200 (300 mg, 0.92 mmol) and pyridine (0.33 mL, 4.07 mmol) in CH$_2$Cl$_2$ (6.0 mL) at −78 °C, was added trifluoromethanesulfonate anhydride (0.31 mL, 1.85 mmol) dropwise. The resulting solution was warmed to 0 °C and stirred for 2 hours. The reaction was then diluted with CH$_2$Cl$_2$ (30 mL), washed with H$_2$O (3 × 20 mL), dried over MgSO$_4$ and concentrated in vacuo to yield the crude product as an orange residue which was used directly in the proceeding step with no further purification.

tert-Butyl-dimethyl-[[2R,4S,5R]-2-phenyl-5-phenylsulfanyl-1,3-dioxan-4-yl]methoxy]silane 203

To a stirred suspension of 1.0 M $^t$BuOK in THF (1.5 mL, 1.50 mmol) and 18-crown-6 (138 mg, 0.52 mmol) in THF (1.0 mL) at −55 °C was added thiophenol (0.16 mL, 1.50 mmol). To this was added a solution of triflate 202 (470 mg, 1.03 mmol) in THF (1.2 mL). The reaction mixture was stirred for 48 hours at −55 °C and then treated with saturated NaHCO$_3$ (aq) (10 mL) and extracted into Et$_2$O (25 mL). The organic layer was washed with H$_2$O (15 mL), brine (15 mL), dried over MgSO$_4$ and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (40% CH$_2$Cl$_2$ in 40-60 petroleum ether) to yield the product (206 mg, 48%) as a colourless oil.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_{H} = 0.04$ (s, 3H$^6$/7), 0.06 (s, 3H$^6$/7), 0.09 (s, 9H$^9$), 3.51-3.57 (m, 1H$^3$), 3.76 (ddd, 1H$^4$, $J$=10.6Hz, $J$=4.0Hz, $J$=1.9Hz), 3.79 (m, 1H$^2$) 4.03 (dd, 1H$^5$, $J$=11.5Hz, $J$=....
1.9Hz), 4.10 (dd, 1H, J= 11.5 Hz, J=4.0Hz), 4.34 (dd, 1H, J=11.4Hz, J=5.1Hz), 5.20 (s, 1H), 7.24-7.36 (m, 6H-Ar), 7.46-7.52 (m, 4H-Ar).

$^{13}$C-NMR (400 MHz, CDCl₃): $\delta$C = -5.3 (C-6/7), -5.1 (C-6/7), 18.4 (C-8), 25.9 (C-9), 41.7 (C-3), 63.3 (C-5), 71.0 (C-2), 81.4 (C-4), 101.4 (C-1), 126.2 (CH-Ar), 127.5 (CH-Ar), 128.2 (CH-Ar), 128.9 (CH-Ar), 129.1 (CH-Ar), 132.3 (C-Ar), 138.0 (C-Ar).

Note: missing 1 × CH-Ar due to overlapping signals.

IR $v_{\text{max}}$ (neat) = 3031, 2956, 2927, 2855, 1252 cm⁻¹

MS (EI⁺) m/z = 417 [M+H]⁺.

HRMS (EI⁺) calculated 417.2051 for C₂₃H₃₃O₃SSi, observed 417.2057 [M+H]⁺.

[(4S,5R)-2-Phenyl-5-phenylsulfanyl-1,3-dioxan-4-yl]methanol 205

1.0 m TBAF in THF (0.79 mL, 0.79 mmol) was added dropwise to a 0 °C solution of the protected alcohol 203 (300 mg, 0.72 mmol) in THF (1.2 mL). The reaction mixture was then warmed to room temperature and stirred for a further 15 minutes at this temperature, before being poured into H₂O (10 mL) and extracted into Et₂O (2 × 30 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (15% EtOAc in 40-60 petroleum ether) to yield the product (173 mg, 79%) as an amorphous, white solid.

$^1$H-NMR (400 MHz, CDCl₃): $\delta$H = 2.07 (t, 1H, J=6.8Hz), 3.43-3.51 (m, 1H), 3.81-3.87 (m, 1H), 3.85 (ddd, 1H, J=10.5Hz, J= 5.0Hz, J= 2.6Hz), 3.98 (ddd, 1H, J=12.0Hz, J=6.8Hz, J=5.1Hz) 4.11 (ddd, 1H, J=12.0Hz, J=6.8Hz, J=2.6Hz), 4.38 (dd, 1H, J=11.4Hz, J=5.1Hz) 5.54 (s, 1H), 7.33-7.42 (m, 6H-Ar), 7.48-7.53 (m, 4H-Ar).
\( ^{13}\text{C-NMR (400 MHz, CDCl}_3\): \delta = 41.9 (C-3), 63.0 (C-5), 70.8 (C-2), 80.7 (C-4), 101.3 (C-1), 126.1 (C-3), 128.0 (C-5), 128.4 (C-2), 129.3 (C-4), 132.2 (C-1), 132.7 (C-3), 137.5 (C-5). \\

Note: missing 1 \times CH-Ar due to overlapping signals.

IR \nu_{\text{max}} \text{ (neat)} = 3348, 3071, 2975, 2858, 1138 cm\(^{-1}\)

MS (EI\(^{+}\)) m/z: 302 \[M\]^\(+\).

HRMS (EI\(^{+}\)) calculated 302.0977 for C\(_{17}\)H\(_{18}\)O\(_3\)S, observed 302.0977 \[M\]^\(+\).

\((4R,5R)-4\text{-}(Iodomethyl)-2\text{-phenyl-5-phenylsulfanyl-1,3-dioxane 206}

[Diagram of the compound]

Triphenylphosphine (290 mg, 1.12 mmol), imidazole (284 mg, 1.12 mmol) and I\(_2\) (142 mg, 1.12 mmol) were added to a solution of alcohol 205 (170 mg, 0.56 mmol) in CH\(_2\)Cl\(_2\) (10 mL). The reaction mixture was stirred for 3 hours at room temperature then concentrated in vacuo to yield the crude product, which was purified using flash silica column chromatography (2.5% EtOAc in 40-60 petroleum ether) to yield the product (147 mg, 64%) as a white solid.

\(^1\text{H-NMR (400 MHz, CDCl}_3\): \delta = 3.31 (ddd, 1H, J=11.2Hz, J=9.9Hz, J=5.0Hz), 3.56-3.66 (m, 2H), 3.81-3.90 (m, 2H), 4.33 (dd, 1H, J=11.5Hz, J=5.0Hz) 5.57 (s, 1H), 7.37-7.46 (m, 6H), 7.51-7.55 (m, 4H).

\(^{13}\text{C-NMR (400 MHz, CDCl}_3\): \delta = 7.9 (C-5), 47.0 (C-3), 70.5 (C-2), 78.7 (C-4), 101.2 (C-1), 126.1 (C-3), 128.3 (C-5), 129.1 (C-2), 129.4 (C-4), 131.5 (C-1), 133.3 (C-3), 137.4 (C-5).

Note: missing 1 \times CH-Ar due to overlapping signals.
\textbf{Ethyl 3-Bromopropionamidoacetate 195}

To a stirred solution of glycine-ethyl-ester hydrochloride (100 mg, 0.72 mmol) in H\textsubscript{2}O-THF (1:1, 4.0 mL) at 0 °C, was added a solution of 3-bromopropionyl (80 µL, 0.72 mmol) chloride in THF (1.0 mL) during 10 minutes, maintaining a pH between 5.5-6.5 using 1.0 M NaOH (aq). The reaction mixture was then stirred for 30 minutes, before being warmed to room temperature and stirred for a further hour. The reaction mixture was then extracted into EtOAc (25 mL), washed with brine (2 × 30 mL), dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The crude product was trituated twice with 40-60 petroleum ether (5.0 mL) to yield the product (141 mg, 83\%) as a white solid.

The data were in agreement with the published data.\textsuperscript{166}

\textbf{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3})}: \(\delta_{H} = 1.29\) (t, 3H\textsubscript{1}, \(J=7.2\)Hz), 2.84 (t, 2H\textsubscript{7}, \(J=6.7\)Hz), 3.63 (t, 2H\textsubscript{8}, \(J=6.7\)Hz), 4.06 (d, 2H\textsubscript{4}, \(J=5.0\)Hz), 4.23 (q, 2H\textsubscript{2}, \(J=7.2\)Hz), 6.18 (d, 1H\textsubscript{5}, \(J=5.1\)Hz).

\textbf{\textsuperscript{13}C-NMR (400 MHz, CDCl\textsubscript{3})}: \(\delta_{C} = 14.1\) (C-1), 26.8 (C-8), 39.3 (C-7), 41.5 (C-4), 61.7 (C-2), 169.8 (C-3), 207.1 (C-6).

\textbf{IR \(\nu_{\max}\) (neat) = 3286, 2986, 1741, 1647 cm\textsuperscript{-1}}

\textbf{MS (EI\textsuperscript{+}) m/z = 412 [M]\textsuperscript{+}.}

\textbf{HRMS (EI\textsuperscript{+}) calculated 411.9994 for C\textsubscript{17}H\textsubscript{17}I\textsubscript{2}O\textsubscript{2}S, observed 411.9989 [M]\textsuperscript{+}.}

\textbf{mp} (CH\textsubscript{2}Cl\textsubscript{2}) = 62-66 °C.
Ethyl-(2-oxazetidin-1-yl)acetate 189

Pulverised KOH (360 mg, 6.41 mmol) and tetrabutylammonium bromide (348 mg, 1.08 mmol) were suspended in a CH₂Cl₂-MeCN solution (19:1, 110 mL) and stirred vigorously at room temperature. To this was added a solution of bromide 195 (1.22 g, 5.13 mmol) in CH₂Cl₂-MeCN (19:1, 110 mL) during 6 hours. Additional KOH (360 mg, 6.41 mmol) and tetrabutylammonium bromide (348 mg, 1.08 mmol) were added and the reaction stirred overnight. The reaction mixture was then filtered and the filtrate concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (50% ethyl acetate in 40-60 petroleum ether) to yield the product (314 mg, 39% yield) as a colourless oil.

The data were in agreement with the published data.¹⁶⁶

¹H-NMR (400 MHz, CDCl₃): δ_H = 1.28 (t, 3H, J=7.2Hz), 3.03 (t, 2H, J=4.2Hz), 3.42 (t, 2H, J=4.2Hz), 3.98 (s, 2H), 4.20 (q, 2H, J=7.1Hz).

¹³C-NMR (400 MHz, CDCl₃): δ_C = 14.1 (C-1), 37.7 (C-6), 40.0 (C-5), 41.5 (C-4), 61.7 (C-2), 165.5 (C-7), 170.0 (C-3).

IR v_max (neat) = 2986, 1729 cm⁻¹

MS (ES⁺) m/z = 180 [M+Na]⁺.

HRMS (ES⁺) calculated 180.0637 for C₇H₁₁NO₃Na, observed 180.0652 [M+Na]⁺.
Results and Discussion Section 3.
1,2:5,6-Di-O-isopropylidene-D-mannitol 227

ZnCl₂ (40.3 g, 0.30 mol) was heated to a melt in vacuo (3 mbar) then cooled to room temperature under argon. To this was added anhydrous acetone (390 mL) and D-mannitol (20.0 g, 0.11 mol) and the resulting suspension stirred for 16 hours at room temperature. The reaction mixture was poured into 7.0 M K₂CO₃ (aq) (140 mL), stirred vigorously and the precipitated solid filtered and washed with acetone. The filtrate was concentrated in vacuo to remove acetone and then extracted with Et₂O (2 × 400 mL), the combined organic layers were dried over MgSO₄ and concentrated in vacuo. Hexane (400 mL) was then added, the resulting suspension stirred for 20 minutes, stored at 0 °C for 1 hour and then filtered and washed with cold hexane to yield the product (21.1 g, 73%) as a white solid.

The data were in agreement with the published data.¹⁹⁵

¹H-NMR (400 MHz, CDCl₃): δ_H = 1.36 (s, 6 H₁/₂), 1.42 (s, 6H₁/₂), 2.60 (d, 2H₇, J=6.7Hz), 3.73-3.77 (m, 2H₆), 3.97 (dd, 2H₄, J=8.5Hz, J=5.6Hz), 4.12 (dd, 2H₄, J=8.5Hz, J=6.4Hz), 4.16-4.22 (m, 2H₅).

¹³C-NMR (100 MHz, CDCl₃): δ_C = 25.2 (C-1/2), 26.7 (C-1/2), 66.7 (C-4), 71.2 (C-6), 76.3 (C-5), 109.4 (C-3).

IR v_max (neat) = 3309, 2982, 1371 cm⁻¹


HRMS (ES⁺) calculated 263.1495 for C₁₂H₂₃O₆, observed 263.1504 [M+H]⁺.
2,3-\textit{O}-Isopropylidene-\textit{d}-glyceraldehyde 225

\begin{center}
\includegraphics[width=0.5\textwidth]{image.png}
\end{center}

Silica supported sodium metaperiodate (NaIO$_4$:SiO$_2$):
NaIO$_4$ (25.7 g, 0.12 mol) was suspended in H$_2$O (50 mL) and heated to 80 °C. Silica gel (100 g) was then added followed by vigorous shaking and stirring.

To a stirred suspension of NaIO$_4$:SiO$_2$ (115 g) in CH$_2$Cl$_2$ (190 mL) at 0 °C was added a solution of protected acetonide 227 (10.0 g, 38.2 mmol) in CH$_2$Cl$_2$ (190 mL). The reaction mixture was then stirred for 30 minutes at 0 °C and then filtered. The collected solids were washed with CH$_2$Cl$_2$ and the filtrate was concentrated in vacuo. The crude product was purified by vacuum distillation (head temperature 50–54°C at 18 mbar) to give the product (6.27 g, 64%) as an unstable colourless liquid.

The data were in agreement with the published data.$^{197}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 1.41$ (s, 3H$^{1/2}$), 1.48 (s, 3H$^{1/2}$), 4.10 (dd, 1H$^4$, $J$=8.6Hz, $J$=4.8Hz), 4.20 (dd$^4$, 1H, $J$=8.6Hz, $J$=7.8Hz), 4.41 (m, 1H$^5$), 9.72 (d, 1H$^6$, $J$=1.8Hz).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 25.1$ (C-1), 26.3 (C-2), 65.6 (C-4), 79.9 (C-5), 110.1 (C-3), 201.9 (C-6).
Ethyl 3-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-difluoro-3-hydroxy-propanoate 229

To a suspension of Zn dust (8.8 g, 140 mmol) and I\textsubscript{2} (1.4 g, 0.31 mmol) in Et\textsubscript{2}O (45 mL) was added a mixture of aldehyde 225 (3.5 g, 27 mmol) and ethyl bromodifluoroacetate (4.5 mL, 35 mmol) in THF (90 mL) and Et\textsubscript{2}O (45 mL). The resulting solution was refluxed for 1 hour. The reaction mixture was cooled and poured into cold 1.0 M HCl (aq) (150 mL), extracted into Et\textsubscript{2}O (2 × 300 mL), the combined organics were washed with brine (150 mL), saturated NaHCO\textsubscript{3} (aq) (150 mL), dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The crude product was purified using flash silica column chromatography (20% EtOAc in 40-60 petroleum ether) to yield the product as a colourless oil containing a 1.0:2.3 mixture of cis (B) and trans (A) diastereomers (5.2 g, 76%).

The data were in agreement with the published data\textsuperscript{194}

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \(\delta_H = 1.36\) (m, \(18H^{1,2,10A+B}\)), \(2.69\) (d, \(1H^{11A}\), \(J=4.3Hz\)), \(2.93\) (d, \(1H^{11B}\), \(J=8.7Hz\)), \(3.88-4.40\) (m, \(12H^{4,5,6,9A+B}\)).

\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}): \(\delta_C = 13.9\) (C-10A+B), \(25.0\) (C-1A/B), \(25.3\) (C-1A/B), \(26.2\) (C-2A+B), \(63.2\) (C-9A), \(63.3\) (C-9B), \(65.5\) (d, C-4A, \(4J_{C,F}=2.6\) Hz), \(66.4\) (C-4B), \(70.7-71.0\) (m, C-6B), \(71.8\) (dd, C-6A, \(2J_{C,F}=24.9\)Hz, \(2J_{C,F}=23.0\)Hz), \(72.2\) (C-5B), \(73.4\) (C-5A), \(109.7\) (C-3A), \(110.3\) (C-3B), \(113.9\) (dd, C-7, \(J_{C,F}=254.0\), \(J_{C,F}=254.8\)Hz), \(163.0\) (C-8A/B), \(163.3\) (C-8A/B).

Note: assigning peaks for individual diastereomers was difficult due to overlapping peaks.

IR \(\nu_{\text{max}} = 3456, 2995, 1763\) cm\(^{-1}\)

MS (ES+) \(m/z = 255\) [M+H]\textsuperscript{+}.

HRMS (ES+) calculated 255.1044 for C\textsubscript{10}H\textsubscript{17}F\textsubscript{2}O\textsubscript{5}, observed 255.1038 [M+H]\textsuperscript{+}.
A solution of benzoyl chloride (0.27 mL, 2.36 mmol) in CH₂Cl₂ (2.5 mL) was added dropwise to a solution of alcohol 229 (500 mg, 1.97 mmol), 2,6-lutidine (0.45 mL, 3.93 mmol) and DMAP (151 mg, 1.24 mmol) in CH₂Cl₂ (2.5 mL) at 35 °C. The reaction mixture was then stirred for 3 hours, cooled to room temperature, diluted with CH₂Cl₂ (10 mL) and washed sequentially with 1.0 M HCl (aq) (10 mL), saturated NaHCO₃ (aq) (10 mL), H₂O (10 mL), dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (10% EtOAc in 40-60 petroleum ether) to yield the product as a colourless oil containing a 1.0:2.3 mixture of cis (B) and trans (A) diastereomers (520 mg, 74%). The data were in agreement with the published data.

¹H-NMR (400 MHz, CDCl₃): δ_H = 1.32 (m, 18H₁,₂,₁₀A+B), 4.00 (dd, 1H⁴B, J=8.9Hz, J=5.9Hz), 4.06 (dd, 1H⁴A, J=8.9Hz, J=5.8Hz), 4.12-4.19 (m, 2H⁴A+B), 4.23-4.26 (m, 4H⁹A+B), 4.50-4.54 (m, 1H⁵A), 4.55-4.60 (m, 1H⁵B), 5.70 (dd, 1H⁶B, J=13.8Hz, J_H-F=7.6Hz, J_H-F=5.8Hz), 5.83-5.91 (m, 1H⁸A), 7.44-7.49 (m, 4H-Ar), 7.58-7.63 (m, 2H-Ar), 8.06-8.10 (m, 4H-Ar).

¹³C-NMR (100 MHz, CDCl₃): δ_C = 13.8 (C-10A+B), 25.1 (C-1A), 25.4 (C-1B), 26.0 (C-2B), 26.1 (C-2A), 63.3 (C-9B), 63.4 (C-9A), 65.6 (C-4A), 65.8 (d, C-4B, J_C-F=3.5Hz), 70.8 (dd, C-6A, J_C-F=25.4Hz, J_C-F=22.8Hz), 71.1-71.7 (m, C-6B), 72.3 (C-5B), 72.5 (C-5A), 110.1 (C-3A), 110.2 (C-3B), 112.80 (t, C-7A, J_C-F=258.5Hz), 128.6 (CH-Ar), 128.7 (CH-Ar), 130.1 (CH-Ar), 130.1 (CH-Ar), 133.7 (CH-Ar), 133.9 (CH-Ar), 161.8 (C=O), 162.3 (C=O), 164.6 (C=O), 164.8 (C=O).

Note: assigning peaks for individual diastereomers was difficult, missing 2 × C-Ar and C-7B due to overlapping peaks assigning peaks.

IR ν_max = 2988, 1767, 1736 cm⁻¹

MS (Cl⁺) m/z = 359 [M+H]⁺.
HRMS (CI+) calculated 359.1306 for C_{17}H_{21}F_{2}O_{3}, observed 359.1299 [M+H]^+.

2-Deoxy-2,2-difluoro-D-pentofuranos-1-ulose-3-benzoate 231

A mixture of the propionate 230 (0.58 g, 1.63 mmol), H_{2}O (0.17 mL, 9.13 mmol) and trifluoroacetic acid (0.03 mL, 0.39 mmol) in MeCN (3.3 mL) was refluxed at 80 °C for 3 hours. Using azeotropic distillation the MeCN was removed and replaced by dry toluene and the reaction heated at 100 °C for a further hour. The mixture was then cooled to room temperature and concentrated in vacuo to yield the crude oil (0.39 g, 89%) as a 1.0:2.3 mixture of xylo (B) and ribo (A) epimers which were used directly in the next step without further purification. The data were in agreement with the published data.\(^{194}\)

Data for ribo epimer.

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta_{H} = 3.96\) (dd, 1H\(^{5}\), \(J=13.0\)Hz, \(J=3.3\)Hz) 4.11 (dd, 1H\(^{5}\), \(J=13.0\)Hz, \(J=2.7\)Hz) 4.90 (m, 1H\(^{4}\)) 5.79 (ddd, 1H, \(J=11.5\)Hz, \(3J_{H,F}=9.3\)Hz, \(3J_{H,F}=5.7\)Hz) 7.48 (m, 2H-Ar) 7.63 (m, 1H-Ar), 8.06 (m, 2H-Ar).

IR \(\nu_{\text{max}} = 3457, 2948, 1815, 1724\) cm\(^{-1}\)

MS (ES+) \(m/z = 273\ [M+H]^+\).

HRMS (ES+) calculated 273.0575 for C\(_{12}\)H\(_{11}\)F\(_{2}\)O\(_{5}\), observed 273.0577 [M+H]^+. 

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2-Deoxy-2,2-difluoro-D-ribo-pentofuranose-1-ulose-3,5-dibenzoate 228

To a solution of lactone 231 (250 mg, 0.92 mmol) in EtOAc (1.6 mL) was added pyridine (0.16 mL, 1.84 mmol) and DMAP (14 mg, 0.10 mmol) and the resulting mixture heated to 65 °C. A solution of benzoyl chloride (0.12 mL, 1.10 mmol) in EtOAc (1.6 mL) was then added and the solution stirred for 2 hours. The reaction mixture was cooled to 5 °C, filtered through a celite padded frit and concentrated in vacuo to give the crude product as a mixture of epimers (225 mg, 65%). Recrystallisation from CH$_2$Cl$_2$-Heptane yielded the desired product (70 mg, 20%) as a white solid.

The data were in agreement with the published data.$^{194}$

$[^{[\alpha]}]D_{25}^2$ (c = 1.0, CHCl$_3$) +49.6

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 4.71$ (dd, 1H, $J=12.7$Hz, $J=4.4$Hz), 4.78 (dd, 1H, $J=12.7$Hz, $J=3.9$Hz), 5.00 (m, 1H), 5.75 (ddd, 1H, $^3J_{H,F}=12.2$Hz, $^3J_{H,F}=5.9$Hz, $J=4.6$Hz), 7.45-7.53 (m, 4H-Ar), 7.59-7.70 (m, 2H-Ar), 8.03-8.10 (m, 4H-Ar).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 62.2$ (C-5), 69.5 (dd, C-1, $^2J_{C,F}=31.0$Hz, $^2J_{C,F}=15.7$Hz), 78.4 (d, C-4, $^3J_{C,F}=5.9$Hz), 111.48 (dd, C-2, $J_{C,F}=262.6$Hz, $J_{C,F}=257.9$Hz), 127.4 (C-Ar), 128.6 (CH-Ar), 128.7 (C-Ar), 128.8 (CH-Ar), 129.8 (CH-Ar), 130.2 (CH-Ar), 133.7 (CH-Ar), 134.5 (CH-Ar), 162.5 (C=O), 164.6 (C=O), 165.7 (C=O).

IR $\nu_{max}$ = 3070, 1817, 1805, 1725, 1711 cm$^{-1}$

Anal. calculated for C$_{19}$H$_{14}$F$_2$O$_6$: C, 60.64; H, 3.75. Found: C, 60.51; H, 3.65.

mp (CH$_2$Cl$_2$-Heptane) = 107-108 °C
2-Deoxy-2, 2-difluoro-D-ribofuranos-1-ulose-3,5-dibenzoate 232

To a 0 °C solution of furanose 228 (10.0 g, 26.6 mmol) in Et₂O-THF (1:1, 110 mL) was added LiAlH(OtBu)₃ (10.2 g, 40.0 mmol) portion-wise during 40 minutes and the resulting suspension warmed to room temperature and stirred for 1.5 hours. The reaction mixture was quenched by dropwise addition of MeOH (20 mL), then diluted with Et₂O (200 mL) and washed sequentially with 1.0 M HCl (aq) (200 mL), saturated NaHCO₃ (aq) (150 mL), saturated Rochelle’s salt (aq) (2 × 200 mL) and H₂O (200 mL). The organic layer was then dried over MgSO₄ and concentrated in vacuo to yield the product (10.0 g, 99.8%) as colourless oil in a 1.0:1.5 mixture of β and α anomers. This was used directly in the next step with no further purification.

The data were in agreement with the published data.¹⁰⁴

¹H-NMR (400 MHz, CDCl₃): δH = 4.45-4.49 (m, 1Hα), 4.60 (dd, 1Hα, J=12.0Hz, J=4.3Hz), 4.67-5.69 (m, 2Hβ), 4.71-4.80 (m, 2H4a,5α), 5.33-5.36 (m, 1H3β), 5.47-5.52 (m, 2H1+3α), 5.73 (td, 1H1β, JH-F=10.1Hz, J=6.2Hz), 7.38-7.50 (m, 8H-Ar), 7.54-7.66 (m, 4H-Ar), 8.04-8.10 (m, 8H-Ar).

¹³C-NMR (100 MHz, CDCl₃): δC = 63.2 (C-5α), 64.3 (C-5β), 71.3 (dd, C-1α, JₜC-F=29.0Hz, JₜC-F =16.0Hz), 72.0 (dd, C-1β, JₜC-F=36.4Hz, JₜC-F=17.6Hz), 77.2 (C-4α), 79.5 (C-4β), 95.5-96.4 (m, C-3α+β), 121.5 (t, C-2α/β, JₜC,F=261.0Hz), 122.3 (t, C-2α/β, JₜC,F=256.1Hz), 128.0 (C-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 129.6 (CH-Ar), 129.8 (CH-Ar), 129.9 (CH-Ar), 130.1 (CH-Ar), 133.0 (CH-Ar), 133.4 (CH-Ar), 133.5 (CH-Ar), 133.9 (CH-Ar), 134.0 (CH-Ar), 165.2 (C=O), 165.3 (C=O), 166.3 (C=O), 166.5 (C=O).

Note: missing 3 × C-Ar and 1 × CH-Ar due to overlapping peaks.

IR νmax (neat) = 3453, 2955, 1719 cm⁻¹

MS (ES+) m/z = 379 [M+H]⁺.

HRMS (ES+) calculated 379.0993 for C₁₉H₁₇F₂O₆, observed 379.0974 [M+H]⁺.
To a solution of hemiacetal 232 (20.0 g, 52.9 mmol) in CH₂Cl₂ (210 mL) at 0 °C was added NEt₃ (13.9 mL, 100 mmol) and methanesulfonyl chloride (7.0 mL, 90.0 mmol). The resulting solution was warmed to room temperature and stirred for 2 hours. The reaction mixture was then diluted with CH₂Cl₂ (500 mL) and washed sequentially with 1.0 M HCl (aq) (500 mL), saturated NaHCO₃ (aq) (300 mL), H₂O (500 mL), dried over MgSO₄ and concentrated in vacuo to yield the product as orange oil (21.3 g, 88%) in a 1.0:1.5 mixture of β and α anomers.

The data were in agreement with the published data.

¹H-NMR (500 MHz, CDCl₃): δH = 3.02 (s, 3H⁶β), 3.18 (s, 3H⁶α), 4.60 (dd, 1H⁵β, J=12.3Hz, J=4.4Hz), 4.64-4.69 (m, 2H⁴β,5β), 4.72-4.78 (m, 2H⁵α), 4.72-4.78 (m, 2H⁵α), 4.84 (dd, 1H⁴α, J=7.9Hz, J=4.0Hz), 5.57 (dd, 1H¹α, 3J_H-F=16.4Hz, 2J_H-F=7.1Hz), 5.94 (dt, 1H¹β, J=15.3Hz, 3J_H-F=7.1Hz), 6.03-6.05 (m, 1H³β), 6.14 (dd, 1H³α, 3J_H-F=6.0Hz, 3J_H-F=1.1Hz), 7.42-7.51 (m, 8H-Ar), 7.58-7.67 (m, 4H-Ar), 8.03-8.09 (m, 8H-Ar).

¹³C-NMR (125 MHz, CDCl₃): δC = 40.2 (C-6α), 40.3 (C-6β), 62.5 (C-5α), 63.0 (C-5β), 69.4 (dd, C-1β, 2J_C-F=26.0Hz, 2J_C-F=15.6Hz), 71.0 (dd, C-1α, 2J_C-F=36.8Hz, 2J_C-F=17.4Hz), 79.72 (d, C-4β, 3J_C-F=7.7Hz), 82.7 (C-4α), 98.8 (dd, C-3β, 2J_C-F=42.0Hz, 2J_C-F=25.0Hz), 99.6 (dd, C-3α, 2J_C-F=46.2Hz, 2J_C-F=25.0Hz), 118.3-122.9 (m, C-2α+β), 127.9 (C-Ar), 128.0 (C-Ar), 128.9 (C-Ar), 129.1 (CH-Ar), 128.5 (CH-Ar), 128.7 (CH-Ar), 128.7 (CH-Ar), 128.8 (CH-Ar), 129.8 (CH-Ar), 130.1 (CH-Ar), 130.2 (CH-Ar), 133.5 (CH-Ar), 133.7 (CH-Ar), 134.2 (CH-Ar), 134.2 (CH-Ar), 164.9 (C=O), 165.0 (C=O), 165.8 (C=O), 165.9 (C=O).

Note: missing 1 × C-Ar due overlapping peaks.

IR: vmax = 3030, 1724 cm⁻¹

HRMS: Did not provide spectra suitable for interpretation.
2′-Deoxy-2′,2′-difluoruridine-3′,5′-dibenoate 221β

Uracil (8.9 g, 79.3 mmol) and (NH₄)₂SO₄ (628 mg, 4.76 mmol) were refluxed in HMDS (220 mL) for 12 hours, then cooled to room temperature and concentrated in vacuo. The resulting residue was dissolved in DCE (150 mL), treated with TMSOTf (14.4 mL, 79.3 mmol) and a solution of mesylate 233 (21.3 g, 46.7 mmol) in DCE (80 mL) added. The mixture was heated to reflux and stirred for 24 hours, then cooled to room temperature and the solvent removed in vacuo. The resulting gum was dissolved in EtOAc (800 mL) then washed sequentially with H₂O (2 × 600 mL), saturated NaHCO₃ (aq) (600 mL) and brine (600 mL) dried over MgSO₄ and concentrated in vacuo to yield the crude product (3.92 g, 66%) as a 1.0:1.4 mixture of β and α-anomers. Recrystallisation of the crude material, once from EtOAc and once from CH₂Cl₂ yielded exclusively the β-anomer (4.65 g, 21%) as a white solid. The data were in agreement with the published data.¹⁹⁴

¹H-NMR (400 MHz, CDCl₃): δ₉ = 4.57-4.60 (m, 1H⁴), 4.69 (dd, 1H³, J=12.5Hz, J=4.3Hz), 4.83 (dd, 1H³, J=12.5Hz, J=3.1Hz), 5.62-5.68 (m, 1H¹), 5.67 (dd, 1H⁷, J=8.2Hz, J=2.1Hz), 6.39 (dd, 1H³, ³J₉,F=11.8Hz, ³J₉,F=6.3Hz), 7.39 (dd, 1H⁶, J=8.2Hz, J=2.1Hz), 7.49 (m, 4H-Ar), 7.65 (m, 2H-Ar), 8.07 (m, 4H-Ar), 8.23 (s, 1H¹⁰).

¹³C-NMR (100 MHz, CDCl₃): δC = 62.4 (C-5) 71.3 (dd, C-1, ²J₁,C=16.8Hz, ²J₁,C=4.7Hz) 78.5 (C-4) 83.1 (dd, C-3, ²J₂,C=37.5Hz, ²J₇,C=21.0Hz) 103.2 (C-7) 120.77 (dd, C-2, ²J₂,C=259.1Hz, ²J₂,F=266.6Hz) 127.7 (C-Ar) 128.7 (CH-Ar) 128.8 (CH-Ar) 129.0 (C-Ar) 129.6 (CH-Ar) 130.1 (CH-Ar) 133.7 (CH-Ar) 134.3 (CH-Ar) 139.6 (d, C-6, ⁴J₆,C=3.2Hz) 149.7 (C=O) 161.8 (C=O) 164.8 (C=O) 165.9 (C=O).

IR: νₘₙₙ = 3189, 3060, 2970, 1723, 1689 cm⁻¹

MS (ES⁺) m/z = 473 [M+H]⁺.

HRMS (ES⁺) calculated 473.1160 for C₂₃H₁₉N₂F₂O₇, observed 473.1150 [M+H]⁺.
2’-Deoxy-2’,2’-difluorouridine 260

To a solution of the protected nucleoside 221β (390 mg, 0.42 mmol) in MeOH (83 mL) at 0 °C was added 7.0 M NH₃ in MeOH (17 mL, 116 mmol). The resulting solution was warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue partitioned between H₂O (80 mL) and EtOAc (80 mL). The organic layer was re-extracted with H₂O (80 mL) and the combined aqueous layers concentrated in vacuo to yield the product (197 mg, 90%) as a colourless amorphous solid. The data were in agreement with the published data.¹⁰⁴

¹H-NMR (400 MHz, D₂O): δ_H = 3.75 (dd, 1H, J=13.1Hz, J=4.4Hz), 3.90 (dd, 1H, J=13.1Hz, J=2.0Hz), 3.96 (m, 1H), 4.26 (td, 1H, ³J_H-F=11.8Hz, J=8.5Hz), 5.80 (d, 1H, J=8.1Hz), 6.09 (m, 1H), 7.67 (d, 1H, J=8.1Hz).

¹³C-NMR (100 MHz, D₂O): δ_C = 59.3 (C-5), 69.2 (dd, C-1, ²J_C-F=25.6Hz, 20.7Hz) 80.46 (d, C-4, ³J_C-F=7.8Hz) 84.13 (dd, C-3, ²J_C-F=39.3Hz, 25.3Hz), 102.5 (C-7), 122.1 (dd, C-2, ⁴J_C-F= 260.6Hz, 259.5Hz), 141.4 (C-6), 151.4 (C-9), 165.9 (C-8).

IR ν_max (neat) = 3266, 1686 cm⁻¹

MS (ES+) m/z = 265 [M+H]⁺.

HRMS (ES+) calculated 265.0636 for C₉H₁₁N₂F₂O₅, observed 265.0635 [M+H]⁺.
(2R,3R,5R)-2-[{(Benzoyloxy)methyl}-4,4-difluoro-5-(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl]benzoate 235

A mixture of the protected nucleoside 221β (300 mg, 0.64 mmol), I₂ (102 mg, 0.40 mmol) and ceric ammonium nitrate (175 mg, 0.32 mmol) in MeCN (6.4 mL) were refluxed at 80 °C for 4 hours, then cooled to room temperature and stirred for 16 hours. The solvent was removed in vacuo and the resulting residue dissolved in cold EtOAc (20 mL) and washed with brine (10 mL) and 5% NaHSO₃ (aq) (5 mL). The aqueous layers were extracted with EtOAc (20 mL) and the combined organic layers were washed sequentially with 5% NaHSO₃ (aq) (5 mL), brine (15 mL) and H₂O (10 mL) dried over MgSO₄ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (4% acetone in CHCl₃) to yield the product (290 mg, 76%) as a white powder.

¹H-NMR (500 MHz, CDCl₃): δH = 4.59-4.62 (m, 1H₁), 4.76 (dd, 1H₅, J=12.6Hz, J=3.8Hz), 4.84 (dd, 1H₅, J=12.6Hz, J=2.9Hz), 5.65 (ddd, 1H₁, JH:F=14.9Hz, JH:F=4.9Hz, J=1.7Hz), 6.37 (dd, 1H₁, JH:F=12.4Hz, JH:F=5.7Hz), 7.48-7.52 (m, 4H-Ar), 7.60-7.67 (m, 2H-Ar), 7.81 (d, 1H₆, JH-F=2.3Hz), 8.08-8.10 (m, 4H-Ar), 8.28 (s, 1H₁⁰).

¹³C-NMR (125 MHz, CDCl₃): δC = 62.3 (C-5), 69.2 (C-7), 71.4 (dd, C-1, Jₐₐₐ=35.4Hz, Jₐₐ=16.5Hz), 79.0 (C-4), 83.2 (dd, C-3, Jₐₐₐ=36.0Hz, Jₐₐₐ=21.1Hz), 120.44 (dd, C-2, Jₐₐₐ=259.1Hz, 258.5Hz), 127.7 (C-Ar), 128.7 (CH-Ar), 128.8 (CH-Ar), 128.9 (C-Ar), 129.8 (CH-Ar), 130.1 (CH-Ar), 133.7 (CH-Ar), 134.4 (CH-Ar), 144.1 (C-6), 149.3 (C=O), 158.8 (C=O), 164.8 (C=O), 166.0 (C=O).

IR νₘₐₓ (neat) = 3252, 3049, 1729, 1723, 1694 cm⁻¹

MS (ES⁺) m/z = 599 [M+H]⁺.
HRMS (ES⁺) calculated 599.0127 for C₂₃H₁₈N₂F₂O₇I, observed 599.0102 [M+H]⁺.
1-[(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-ido-1,2,3,4-tetrahydropyrimidine-2,4-dione 223

To a solution of iodide 235 (280 mg, 0.47 mmol) in MeOH (95 mL) at 0 °C was added 7.0 M NH₃ in MeOH (9.4 mL, 59.3 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue partitioned between CH₂Cl₂ (50 mL) and H₂O (50 mL). The organic layer was extracted with water (2 × 50 mL) and the combined aqueous layers then concentrated under reduced pressure to yield the product (182 mg, 99%) as an off white amorphous solid.

¹H-NMR (400 MHz, MeOD-d₄):  δ_H = 3.78 (dd, 1H, J₁=12.6Hz, J₂=2.5Hz), 3.90 (dt, 1H, J₃=8.4Hz, J₄=2.4), 3.93-3.97 (m, 1H), 4.32 (td, 1H), 6.09-6.13 (m, 1H), 8.44 (s, 1H).

¹³C-NMR (100 MHz, MeOD-d₄):  δ_C = 59.9 (C-5) 69.1 (C-7) 69.6-70.0 (m, C-1) 82.8 (d, C-4, J_C,F=8.1Hz) 85.0-86.0 (m, C-3) 122.60 (dd, C-2, J_C,F=261.1Hz, J_C,F=258.6) 146.1 (C-6) 151.8 (C-9) 162.4 (C-8).

IR ν_max (neat) = 3400, 3059, 1700, 1671 cm⁻¹

MS (ES⁺) m/z = 391 [M+H]⁺.

HRMS (ES⁺) calculated 390.9603 for C₉H₁₀N₂F₂O₅I, observed 390.9605 [M+H]⁺.
1-{(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl}-5-(trimethylstannyl)-1,2,3,4-tetrahydropyrimidine-2,4-dione 224

Iodide 223 (75 mg, 0.19 mmol) was dissolved in 1,4-dioxane (3 mL) at 50 °C, then cooled to room temperature and hexamethylditin (90 µL, 0.42 mmol) and Pd(PPh₃)₄ (5 mg, 5 µmol) added. The reaction mixture was then stirred at reflux for 20 hours, cooled to room temperature and concentrated in vacuo. The crude product was purified using flash silica column chromatography (25% acetone in CH₂Cl₂) to yield the product (67 mg, 83%) as a white solid.

¹H-NMR (400 MHz, CD₃OD): δ_H = 0.25 (s, 9H¹³), [d, ²J_H⁻¹¹⁹Sn=58.8Hz, d, ²J_H⁻¹¹⁷Sn=56.3Hz] 3.75 (dd, 1H⁵, J=12.6Hz, J=2.5Hz), 3.87 (dt, 1H⁴, J=8.6Hz, J=2.5Hz), 3.92-3.95 (m, 1H⁵), 4.31 (td, 1H¹, ³J_H,F=12.5Hz , J=8.6Hz,), 6.16 (t, 1H³, ³J_H,F=7.8Hz), 7.62 (s, 1H⁶), [d, ³J¹⁹Sn-H=37.5Hz].

¹³C-NMR (100 MHz, CD₃OD): δ_C = -9.5 (C13), [d, ²J_C⁻¹¹⁷Sn=365.5Hz, d, ²J_C⁻¹¹⁹Sn=382.4Hz] 59.8 (C-5), 70.03 (t, C-1, ²J_C,F=22.6Hz), 82.22 (t, C-4, ²J_C,F=4.0Hz), 85.1-85.6 (m, C-3), 113.8 (C-7), 124.21 (t, C-2, ¹J_C,F=258.0Hz), 145.3 (C-6), 152.7 (C-9), 168.6 (C-8).

IR ν_max (neat) = 3269, 1697, 1648, 1601 cm⁻¹

HRMS Did not provide spectra suitable for interpretation.

mp (acetone) = 73-76 °C.
A mixture of Pd\(_2\)(dba)\(_3\) (124 mg, 0.12 mmol), tri(o-toly)phosphine (143 mg, 0.47 mmol) and stannane 224 (100 mg, 0.23 mmol) in DMF (30 mL) was purged with argon for 10 minutes, then treated with a solution of methyl iodide (40 mg, 0.28 mmol) in DMF (3 mL). The resulting mixture was heated at 130 °C for 10 minutes then cooled to room temperature, the catalyst removed by filtration and the filtrate concentrated in vacuo. The crude product was purified using flash silica column chromatography (7% MeOH in CHCl\(_3\)) to yield the product (39 mg, 61%) as a colourless oil.

\(^1\)H-NMR (500 MHz, MeOD-\(d_4\)):
\(\delta_H = 1.87\) (s, 3H\(^{13}\)), 3.78 (dd, 1H\(^5\), J\(_{HF} = 12.7\)Hz, J\(_{HF} = 3.1\)Hz), 3.87 (dt, 1H\(^4\), J\(_{HF} = 8.4\)Hz, J\(_{HF} = 2.7\)Hz), 3.94 (m, 1H\(^5\)), 4.30 (td, 1H\(^1\), J\(_{HF} = 12.2\)Hz, J\(_{HF} = 8.4\)Hz), 6.12 (dd, 1H\(^3\), J\(_{HF} = 9.1\)Hz, J\(_{HF} = 6.8\)Hz), 7.71 (s, 1H\(^6\)).

\(^{13}\)C-NMR (125 MHz, MeOD-\(d_4\)):
\(\delta_C = 12.4\) (C-13) 60.4 (C-5) 70.30 (dd, C-1, \(^2\)J\(_{CF} = 25.6\)Hz, \(^2\)J\(_{CF} = 20.4\)Hz), 82.5 (d, C-4, \(^3\)J\(_{CF} = 8.4\)Hz), 85.18 (dd, C-3, \(^2\)J\(_{CF} = 40.1\)Hz, \(^2\)J\(_{CF} = 25.2\)Hz), 111.7 (C-7), 124.01 (dd, C-2, J\(_{CF} = 253.1\)Hz, J\(_{CF} = 263.1\)Hz), 137.4 (C-6), 152.2 (C-9), 166.0 (C-8).

IR \(\nu_{max}\) (neat) = 3374, 1692 cm\(^{-1}\)

MS (ES+) m/z = 279 [M+H]\(^+\).
HRMS (ES+) calculated 279.0793 for C\(_{10}\)H\(_{13}\)N\(_2\)F\(_2\)O\(_5\), observed 279.0790 [M+H]\(^+\).
2',2'-Difluoro-2'-deoxycytidine-3',5'-dibenzoate 222β

Cytosine (4.82 g, 43.4 mmol) and (NH₄)₂SO₄ (0.34 g, 2.60 mmol) in HMDS (100 mL) were refluxed for 16 hours, then cooled to room temperature and concentrated in vacuo. The resulting residue was dissolved DCE (80 mL), treated with TMSOTf (7.9 mL, 43.4 mmol) and then a solution of mesylate 233 (11.8 g, 25.9 mmol) in DCE (50 mL) added. The resulting solution was heated at reflux for 48 hours then cooled to room temperature and concentrated in vacuo. The resultant residue taken up in EtOAc (150 mL), washed with H₂O (2 × 150 mL), saturated NaHCO₃ (aq) (150 mL) and then concentrated to approximately one third volume resulting in the precipitation of a white solid. This was collected by filtration, washed with cold EtOAc and then vacuum dried to give the product (4.91 g, 40%) in a 1.0:1.0 mixture of β and α-anomers. Trituration with hot MeOH allowed isolation of the insoluble β-product (1.99 g, 16%) as a white solid.

The data were in agreement with the published data.

¹H-NMR (500 MHz, DMSO-d₆): δ_H = 4.67-4.77 (m, 3H₄⁺⁵), 5.79 (d, 1H₈, J=7.5Hz), 5.80-5.84 (m, 1H₁), 6.38 (br, s, 1H₃), 7.46-7.49 (m, 2H-Ar/2H¹⁰), 7.56-7.59 (m 2H-Ar), 7.62-7.67 (m, 2H-Ar), 7.72-7.75 (m 1H-Ar), 7.94-7.96 (m, 2H-Ar), 8.04-8.06 (m, 2H-Ar).

¹³C-NMR (125 MHz, DMSO-d₆): δ_C = 63.4 (C-5), 71.7 (d, C-1, ²J_C-F=26.3Hz, ²J_C-F'=22.3Hz), 75.6 (C-4) 78.8 (t, C-3, ²J_C-F=33.4Hz) 94.99 (C-8), 121.72 (dd, C-2, ²J_C-F=268.8Hz, ²J_C-F'=254.4Hz), 128.0 (C-Ar), 128.7 (CH-Ar), 128.9 (CH-Ar), 129.1 (CH-Ar), 129.2 (CH-Ar), 129.6 (CH-Ar), 133.6 (CH-Ar), 134.2 (CH-Ar), 141.8 (C-Ar), 154.3 (C=O), 164.3 (C=O), 165.3 (C=O), 165.7 (C-7).

IR ν_max (neat) = 3366, 3176, 1719, 1631 cm⁻¹

MS (ES⁺) m/z = 472 [M+H]⁺

HRMS (ES⁺) calculated 472.1320 for C₂₃H₂₀N₃F₂O₆, observed 472.1331 [M+H]⁺.
2’-Deoxy-2’,2’-difluorocytidine 84

To a solution of protected nucleoside 222ß (600 mg, 1.27 mmol) in MeOH (120 mL) at 0 °C was added 7.0 M NH₃ in MeOH (25 mL, 178 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue partitioned between EtOAc (125 mL) and H₂O (125 mL). The organic layer was extracted with H₂O (2 × 125 mL) and the combined aqueous layers concentrated under reduced pressure to yield the product (335 mg, 100%) as an amorphous white solid.

The data were in agreement with the published data.¹⁹⁴

¹H-NMR (400 MHz, D₂O): δ_H = 3.75 (dd, 1H, J=13.1Hz, J=4.3Hz), 3.90 (dd, 1H, J=13.1Hz, J=2.2Hz), 3.92-3.96 (m, 1H), 4.23 (td, 1H, ³J_H-F=12.0Hz, J=8.7Hz), 5.96 (d, 1H, J=7.6Hz), 6.10 (t, 1H, ³J_H-F=7.9Hz), 7.64 (d, 1H, J=7.6Hz).

¹³C-NMR (100 MHz, D₂O): δ_C = 59.3 (C-5), 69.20 (t, C-1, ²J_C-F=23.3Hz), 80.02 (t, C-4, ³J_C-F =4.2Hz), 84.90 (t, C-3, ²J_C-F=31.2Hz), 96.4 (C-8), 123.5 (d, C-2, J_C-F=257.9Hz), 141.2 (C-9), 157.2 (C-6), 166.3 (C-7).

IR:ν_max (neat) = 3232, 3206, 1666, 1639 cm⁻¹

MS (ES+) m/z = 264 [M+H]^+, 286 [M+Na]^+.

HRMS (ES+) calculated 263.0796 for C₉H₁₂N₃F₂O₄, observed 264.0800 [M+H]^+. 
4-Amino-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxan-2-yl]-5-iodo-1,2-dihydropyrimidin-2-one 238

A mixture of gemcitabine 84 (123 mg, 0.47 mmol), I₂ (119 mg, 0.47 mmol) and iodic acid (83 mg, 0.47 mmol) in AcOH-H₂O-CCl₄ (8:3:2, 23 mL) was heated at 45 °C for 4 hours. The reaction mixture was then concentrated in vacuo and recrystallized from H₂O to yield the product (111 mg, 61%) as a white solid.

\(^1\)H-NMR (500 MHz, MeOD-d₄): δ_H = 3.78 (dd, 1H, J=12.7Hz, J=2.7Hz), 3.90 (td, 1H, J=8.5Hz, J=2.5Hz), 3.95 (dd, 1H, J=13.0Hz, J=2.5Hz), 4.28 (dt, 1H, \(^3\)J_H,F=12.3Hz, J=8.6Hz), 6.14-6.17 (m, 1H), 8.38 (s, 1H).

\(^13\)C-NMR (125 MHz, MeOD-d₄): δ_C = 57.6 (C-8), 60.0 (C-5), 69.7-70.1 (m, C-1), 82.5-82.6 (m, C-4), 85.7-86.2 (m, C-3), 123.89 (t, C-2, \(^2\)J_C,F=258.6Hz), 148.6 (C-9), 156.9 (C-6), 166.0 (C-7).

IR \(\nu_{max}\) (neat) = 3321, 3211, 1623 cm\(^{-1}\)

MS (ES+) m/z = 389 [M+H]⁺, 452 [M+MeCN+Na]⁺.

HRMS (ES+) calculated 389.9762 for C₉H₁₁N₃F₂O₄I, observed 389.9768 [M+H]⁺.
To a solution of iodide 238 (190 mg, 0.49 mmol) and DMAP (6.1 mg, 0.05 mmol) in pyridine (3.0 mL) was added acetic anhydride (0.41 mL, 4.40 mmol) and the resulting solution stirred for 16 hours. The reaction mixture was quenched with saturated NaHCO₃ (aq) (20 mL), extracted into EtOAc (2 × 30 mL), the combined organic layers washed with H₂O (30 mL), dried over MgSO₄ and concentrated in vacuo. The crude material was purified using flash silica column chromatography (3% acetone in CHCl₃) to yield the product (138 mg, 59%) as an amorphous solid.

**1H-NMR (500 MHz, CDCl₃):** δ_H = 2.17 (s, 3H-Ac), 2.19 (s, 3H-Ac), 2.21 (s, 3H-Ac), 4.35 (dd, 1H^1, J=8.9Hz, J=3.8Hz), 4.42-4.45 (m, 2H^5), 5.26-5.31 (m, 1H^1), 6.36-6.40 (m, 1H^3), 7.93 (s, 1H^9).

**13C-NMR (125 MHz, CDCl₃):** δ_C = 20.4 (C-Ac), 20.8 (C-Ac), 40.4 (C-8), 61.8 (C-5), 70.53 (dd, C-3, 2J_C-F=33.6Hz, 2J_C-F=18.4Hz), 78.4 (C-4), 83.3-84.5 (br, s, C-1), 149.0 (C-9), 159.1 (C=O), 168.9 (C=O), 170.2 (C=O).

Note: missing C-7, 1× C-Ac and C-2 due to a weak signal and overlapping peaks.

**IR v_{max} (neat) = 3347, 1746, 1711, 1668 cm⁻¹**

**MS (ES+) m/z = 516 [M+H]⁺, 538 [M+Na]⁺.**

**HRMS (ES+) calculated 516.0079 for C₁₅H₁₇N₃F₂O₇I, observed 516.0062 [M+H]⁺.**
Results and Discussion Section 4.
To a solution of the protected nucleoside 221β (390 mg, 0.42 mmol) in MeOH at 0 °C (83 mL) was added 7.0 M NH₃ in MeOH (17 mL, 116 mmol). The resulting solution was warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue partitioned between H₂O (80 mL) and EtOAc (80 mL). The organic layer was re-extracted with H₂O (80 mL) and the combined aqueous layers concentrated in vacuo to yield the product (197 mg, 90%) as a colourless amorphous solid. The data were in agreement with the published data.¹⁰⁴

¹H-NMR (400 MHz, D₂O): δ_H = 3.75 (dd, 1H, J=13.1Hz, J=4.4Hz), 3.90 (dd, 1H, J=13.1Hz, J=2.0Hz), 3.96 (m, 1H), 4.26 (td, 1H, ⁳J_H-F=11.8Hz, J=8.5Hz), 5.80 (d, 1H, J=8.1Hz), 6.09 (m, 1H), 7.67 (d, 1H, J=8.1Hz).

¹³C-NMR (100 MHz, D₂O): δ_C = 59.3 (C-5), 69.2 (dd, C-1, ²J_C-F=25.6Hz, 20.7Hz) 80.46 (d, C-4, ³J_C-F=7.8Hz) 84.13 (dd, C-3, ²J_C-F=39.3Hz, 25.3Hz), 102.5 (C-7), 122.1 (dd, C-2, ³J_C-F=260.6Hz, 259.5Hz), 141.4 (C-6), 151.4 (C-9), 165.9 (C-8).

IR ν_max (neat) = 3266, 1686 cm⁻¹

MS (ES+) m/z = 265 [M+H]⁺.

HRMS (ES+) calculated 265.0636 for C₉H₁₁N₂F₂O₅, observed 265.0635 [M+H]⁺.
1-[(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(hydroxymethyl)-1,2,3,4-tetrahydropyrimidine-2,4-dione 261

To a solution of nucleoside 260 (280 mg, 1.06 mmol), in H$_2$O:MeCN (7:3, 3.75 mL) was added para-formaldehyde (302 mg, 10.6 mmol) and NEt$_3$ (1.05 mL, 7.42 mmol) and the resulting solution stirred for 3 days at 60 °C. The reaction mixture was concentrated in vacuo and the crude residue purified using (15% MeOH in CH$_2$Cl$_2$) to yield the product (71 mg, 23%) as a colourless gum.

$^1$H-NMR (400 MHz, MeOD-d$_4$): $\delta_H = 3.79$ (dd, 1H$^5$, $J=12.5$Hz, $J=3.1$Hz), 3.88-3.97 (m, 2H$^4+5$), 4.30-4.36 (m, 3H$^{1+10}$), 6.15-6.19 (m, 1H$^3$), 7.87 (s, 1H$^6$).

$^{13}$C-NMR (100 MHz, MeOD-d$_4$): $\delta_C = 57.9$ (C-10), 60.5 (C-5), 70.4 (dd, C-1, $^2J_{C-F}$ 24.9Hz, $^2J_{C-F}$ =20.6Hz), 82.6 (d, C-4, $^3J_{C-F}$ =8.0Hz), 85.3 (dd, C-3, $^2J_{C-F}$ =40.2Hz, $^2J_{C-F}$ =24.8Hz), 115.6 (C-7), 125.4 (t, C-2, $J_{C-F}$=258.63Hz), 138.8 (C-6), 152.0 (C-9), 164.8 (C-8).

IR $\nu_{max}$ (neat) =3344, 2927, 1677 cm$^{-1}$

MS (ES-) m/z = 293 [M-H]$^-$.  
HRMS (ES-) calculated 293.0585 for C$_{10}$H$_{11}$N$_2$F$_2$O$_6$, observed 293.0579 [M-H]$^-$.  

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1-[(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-[(methoxymethoxy)methyl]oxolan-2-yl]-4-(methoxymethoxy)-1,2-dihydropyrimidin-2-one 262

To a solution of the nucleoside 260 (90 mg, 0.34 mmol) in DMF (2.8 mL) at 0 °C was added DIPEA (0.24 mL, 1.42 mmol) followed by dropwise addition of chloromethyl methyl ether (0.1 mL, 1.36 mmol). The resulting solution was warmed to room temperature and stirred for 16 hours. The reaction was quenched by the addition of H2O (10 mL), then extracted into EtOAc (2 × 10 mL), washed with 1.0 M HCl (aq) (10 mL) and then a mixture of H2O:brine (1:1, 2 × 20 mL). The organic layers were dried over MgSO4, concentrated in vacuo and purified using flash silica column chromatography (4% MeOH in CHCl3) to yield the product as a colourless oil (76 mg, 63%).

1H-NMR (400 MHz, CDCl3): \( \delta_H = 2.68 \) (dd, 1H\(^10\), \( J=6.2 \)Hz, \( J=1.5 \)Hz), 3.40 (s, 3H (CH\(_3\))), 3.44 (s, 3H (CH\(_3\))), 3.83 (dd, 1H\(^5\), \( J=11.5 \)Hz, \( J=3.5 \)Hz), 3.93 (dd, 1H\(^5\), \( J=11.6 \)Hz, \( J=1.8 \)Hz), 4.09 (m, 1H\(^6\)), 4.36 (m, 1H\(^7\)), 4.71 (s, 2H (CH\(_2\))), 5.37 (d, 1H, \( J=9.6 \)Hz (CH\(_2\))), 5.40 (d, 1H, \( J=9.6 \)Hz (CH\(_2\))), 5.81 (d, 1H\(^7\), \( J=8.2 \)Hz), 6.26 (dd, 1H\(^5\), \( 3J_{HF}=8.7 \)Hz, \( 3J_{HF}=7.1 \)Hz), 7.53 (d, 1H\(^6\), \( J=8.2 \)Hz).

13C-NMR (100 MHz, CDCl3): \( \delta_C = 55.6 \) (CH\(_3\), MOM), 57.9 (CH\(_3\), MOM), 64.9 (C-5), 70.5 (dd, C-1, \( 2J_{CF}=28.5 \)Hz, \( 2J_{CF}=19.2 \)Hz), 72.1 (CH\(_2\), MOM), 80.1 (d, C-4, \( 3J_{CF}=7.6 \)Hz), 84.2 (dd, C-3, \( 2J_{CF}=41.0 \)Hz, \( 2J_{CF}=22.4 \)Hz), 96.8 (CH\(_2\), MOM), 102.5 (C-7), 121.9 (dd, C-2, \( 1J_{CF}=261.8 \)Hz, \( 1J_{CF}=258.0 \)Hz), 138.1 (C-6), 151.0 (C-8), 162.2 (C-9).

IR \( \nu_{\text{max}} \) (neat) = 3385, 2931, 1719, 1665 cm\(^{-1}\)

MS (CI+) m/z = 353 [M+H]+, 370 [M+NH\(_4\)+].

HRMS (CI+) calculated 353.1160 C\(_{13}\)H\(_{19}\)F\(_2\)N\(_2\)O\(_7\), observed 353.1165 [M+H]+.
A suspension of protected nucleoside 262 (92 mg, 0.26 mmol) and para-formaldehyde (20 mg, 0.63 mmol) in 0.5 N KOH (aq) (0.5 mL) was stirred at 60 °C for 3 days. The reaction mixture was then concentrated in vacuo and purified using flash silica column chromatography (4% MeOH in CHCl₃) to yield the starting material (25 mg, 27%) and a mixture of the product and an inseparable, unidentifiable side-product (29 mg, 29%) as a gum, which was used directly in the next step with no further purification.

**¹H-NMR (500 MHz, CDCl₃):** δ_H = 3.41 (s, 3H (CH₃)), 3.45 (s, 3H (CH₃)), 3.84 (dd, 1H, J=11.6Hz, J=3.1Hz), 3.95 (br, d, 1H, J=11.6Hz), 4.09 (dt, 1H, J=7.7Hz, J=3.0Hz), 4.37-4.46 (m, 3H), 4.73 (s, 2H (CH₂)), 5.38 (d, 1H, J=9.6Hz (CH₂)), 5.41 (d, 1H, J=9.6Hz (CH₂)), 6.27 (dd, 1H, J=9.6Hz, 3J_H-F=5.4Hz), 7.70 (s, 1H₆).

**¹³C-NMR (125 MHz, CDCl₃):** δ_C = 55.7 (CH₃, MOM) 58.1 (CH₃, MOM) 59.4 (C-10) 64.7 (C-5) 70.0 (dd, C-1, ²J_C-F =27.4Hz, ²J_C-F =19.6Hz) 72.2 (CH₂, MOM) 80.0 (d, C-4, ³J_C-F =7.9Hz) 83.9-84.6 (m, (C-3) 96.7 (CH₂, MOM) 113.6 (C-7) 124.6 (t, C-2, ¹J_C,F=261.2Hz) 135.2 (C-6) 150.7 (C-8) 162.7 (C-9).

**IR v_max (neat) = 3274, 2950, 1708, 1656 cm⁻¹**

**MS (ES+) m/z = 383 [M+H]+.**

**HRMS (ES+) calculated 383.1226 for C_{14}H_{21}N_{2}F_{2}O_{8}, observed 383.1257 [M+H]+.
To a solution of the hydroxymethylated nucleoside 263 (60 mg, 0.17 mmol) and triethylamine (70 μL, 0.52 mmol) in CH₂Cl₂ (3 mL) was added dansyl chloride (86 mg, 0.35 mmol). After stirring for 24 hours additional equivalents of triethylamine (70 μL, 0.52 mmol) and dansyl chloride (86 mg, 0.35 mmol) were added and reaction continued for a further 12 hours. The mixture was then concentrated in vacuo and purified using flash silica column chromatography (100% CHCl₃) to yield the product as yellow gum (18 mg, 17%).

**¹H-NMR (400 MHz, CDCl₃):** δ_H = 2.55-2.58 (m, 1H₂), 2.92 (s, 6H₂), 3.36 (s, 3H(CH₃)), 3.43 (s, 3H (CH₃)), 3.51 (dd, 1H, J₁=11.8Hz, J₂=2.4Hz), 3.82 (br, d, 1H, J₁=11.6Hz), 4.25 (dt, 1H, J₁=7.8Hz, J₂=2.1Hz), 4.41 (br s, 2H (CH₂)), 4.47 (d, 1H, J₁=6.6Hz), 4.58 (d, 1H, J₁=6.5Hz), 5.10-5.17 (m, 1H), 5.34 (d, 1H, J₁=9.6Hz (CH₂)), 5.38 (d, 1H, J₁=9.6Hz (CH₂)), 6.19 (t, 1H, J₁=7.7Hz), 7.25 (d, 1H, J₁=7.5Hz), 7.62 (m, 3H, J₁=7,15,18), 8.25 (d, 1H, J₁=8.6Hz), 8.33 (dd, 1H, J₁=7.3Hz, J₂=0.8Hz), 8.68 (d, 1H, J₁=8.6Hz).

**¹³C-NMR (100 MHz, CDCl₃):** δ_C = 45.42 (C-21), 55.74 (CH₃, MOM), 58.03 (CH₃, MOM), 59.31 (CH₂, MOM), 63.63 (C-5), 72.17 (CH₂, MOM), 73.71 (t, C-1, J₁,₂C,F =24.0Hz), 77.80 (C-4), 84.0-84.9 (br, s, C-3), 96.67 (C-10), 113.28 (C-7), 115.89 (C-19), 119.00 (C-16), 122.92 (C-18), 129.22 (C-15), 129.81 (C-12/13), 129.86 (C-11), 130.44 (C-12/13), 130.94 (C-17), 132.72 (C-14), 134.92 (C-6), 150.55 (C-20), 152.00 (C-8), 162.51 (C-9).

Note: missing C-2 due to overlapping signals.
IR $\nu_{\text{max}}$ (neat) = 3478, 2928, 1718, 1675 cm$^{-1}$

MS (ES+) m/z = 616 [M+H]$^+$, 638 [M+Na]$^+$.
HRMS (ES+) calculated 616.1776 for C$_{26}$H$_{32}$F$_2$N$_3$O$_{10}$S, observed 616.1767 [M+H]$^+$.

1-((6aR,8R,9aR)-9,9-Difluoro-2,2,4,4-tetraisopropyltetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)-5-iodopyrimidine-2,4(1H,3H)-dione 265

To a solution of iodide 223 (210 mg, 0.54 mmol) and imidazole (165 mg, 2.42 mmol) in DMF (1.1 mL) at 0 °C was added 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane (0.19 mL, 0.59 mmol) dropwise and the resulting solution warmed to room temperature and stirred for 16 hours. The reaction mixture was then diluted with EtOAc (20 mL), washed sequentially with brine (15 mL) and H$_2$O (15 mL), dried over MgSO$_4$ and concentrated in vacuo. The crude product was purified using flash silica column chromatography (100% CH$_2$Cl$_2$) to yield the product (302 mg, 88%) as colourless oil.

$^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta_H$ = 1.12 (m, 28H$^{11-22}$), 3.96 (ddd, 1H$^{4}$, J=9.8Hz, J=2.5Hz, J=1.4Hz), 4.06 (dd, 1H$^{5}$, J=13.6Hz, J=2.5Hz), 4.23 (d, 1H$^{5}$, J=13.4Hz), 4.34 (td, 1H$^{1}$, J=13.6Hz, J=9.6Hz), 6.10 (m, 1H$^{3}$), 7.86 (s, 1H$^{6}$), 8.28 (s, 1H$^{10}$).

$^{13}$C-NMR (100 MHz, DMSO-d$_6$): $\delta_C$ = 12.4 (C-11-14), 12.6 (C-11-14), 12.9 (C-11-14), 13.5 (C-11-14), 16.7 (C-15-22), 16.8 (C-15-22), 16.9 (C-15-22), 17.1 (C-15-22), 17.2 (C-15-22), 17.4 (C-15-22), 17.6 (C-15-22), 17.7 (C-15-22), 59.1 (C-5), 69.0 (C-1), 79.4 (C-4), 84.4 (C-3), 142.6 (C-6), 149.2 (C-9), 164.6 (C-8).

Note: missing C-2 and C-7 due to weak signals.
IR $\nu_{\text{max}}$ (neat) = 2947, 2868, 1702, 1612 cm$^{-1}$

MS (ES+) m/z = 633 [M+H]$^+$.  
HRMS (ES+) calculated 633.1125 for C$_{21}$H$_{36}$N$_2$O$_6$F$_2$Si$_2$I, observed 633.1130 [M+H]$^+$.

5-Hydroxymethyluracil 259

A suspension of uracil (2.5 g, 22.3 mmol) and para-formaldehyde (2.0 g, 66.9 mmol) in H$_2$O (75 mL) was treated with NEt$_3$ (4.6 mL, 33.4 mmol), then heated to 60 °C and stirred for 16 hours. The reaction mixture was then concentrated in vacuo, triturated with H$_2$O and EtOH (1:1, 20 mL), stirred for 1 hour at 0 °C, filtered and washed with cold EtOH to yield the product (2.3 g, 73 %) as a white solid.

The data were in agreement with the published data.$^{220}$

$^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta_H = 4.10$ (d, 2H$^7$, $J$=5.3Hz) 4.85 (t, 1H$^8$, $J$=5.3Hz) 7.24 (s, 1H$^3$) 10.73 (s, br, 1H$^3$) 11.05 (s, br, 1H$^5$).

$^{13}$C-NMR (100 MHz, DMSO-d$_6$): $\delta_C = 56.2$ (C-7), 113.2 (C-1), 138.6 (C-2), 151.8 (C-6), 164.2 (C-4).

IR $\nu_{\text{max}}$ (neat) = 3010, 1737, 1662 cm$^{-1}$

MS (CI+) m/z = 160 [M+NH$_4$]$^+$.  
HRMS (CI+) calculated 160.0722 for C$_5$H$_{10}$N$_3$O$_3$, observed 160.0715 [M+NH$_4$]$^+$. 

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5-\{\textit{tert}-Butyldiphenylsilyl\}oxy)methyl\}-uracil 267

To a solution of 5-hydroxymethyl uracil 259 (2.3 g, 16.2 mmol) and imidazole (1.4 g, 21.1 mmol) in DMF (23 mL) was added TBDPSCI (5.0 mL, 19.4 mmol) and the resulting solution stirred for 24 hours at room temperature. The reaction mixture was then concentrated \textit{in vacuo} and re-crystallised twice from MeOH:CHCl$_3$ (7:3) to yield the product as a white solid (5.0 g, 81\%).

The data were in agreement with the published data.$^{243}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 1.00$ (s, 9H$^9$), 4.36 (s, 2H$^7$), 7.27 (d, 1H$^2$, $J=5.4$Hz), 7.41-7.48 (m, 6H$^{11+13}$), 7.63-7.66 (m, 4H$^{12}$), 10.76 (d, 1H$^3$, $J=5.4$Hz), 11.13 (s, br, 1H$^5$).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta_C = 19.2$ (C-8), 27.1 (C-9), 59.3 (C-7), 114.6 (C-1) 128.4 (C-12), 130.4 (C-13), 133.2 (C-10) 135.5 (C-11), 138.6 (C-2), 151.6 (C-6), 163.9 (C-4).

IR $\nu_{\text{max}}$ (neat) = 2939, 1720, 1680 cm$^{-1}$

MS (ES+) m/z = 381 [M+H]$^+$.  
HRMS (ES+) calculated 381.1634 for C$_{21}$H$_{25}$N$_2$O$_3$Si, observed 381.1639 [M+H]$^+$.  

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(2R,3R)-2-[(Benzoyloxy)methyl]-5-{[(tert-butyldiphenylsilyl)oxy]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4,4-difluorooxolan-3-yl benzoate 269

Protected uracil 267 (1.98 g, 5.21 mmol) and (NH₄)₂SO₄ (41 mg, 0.30 mmol) were refluxed in HMDS (26 mL) for 16 hours, then cooled to room temperature and concentrated in vacuo. The resulting residue was dissolved in DCE (8 mL), TMSOTf (0.94 mL, 5.21 mmol) was added and the mixture treated with a solution of mesylates 233 (1.40 g, 3.07 mmol) in DCE (22 mL). After refluxing for 16 hours, the reaction mixture was cooled to room temperature and the solvent removed in vacuo. The resulting gum was dissolved in CH₂Cl₂ (100 mL) then washed sequentially with H₂O (2 ×100 mL), saturated NaHCO₃ (aq) (100 mL) and brine (100 mL) then dried over MgSO₄ and concentrated in vacuo to yield the crude product. The crude product was purified using flash silica column chromatography (100% CH₂Cl₂) to yield the product (2.27 g, 63%) as a colourless solid in a 1.5:1.0 mixture of anomers.

¹H-NMR (400 MHz, CDCl₃): δ_H = 1.03 (s, 9H\(^{12α}\)), 1.05 (s, 9H\(^{12β}\)), 4.53-4.72 (m, 10H\(^{4,5,10α+β}\)), 5.56-5.60 (m, 1H\(^{1β}\)), 5.82 (dt, 1H\(^{1α}\), \(^3J_H-F=10.4Hz, J=5.2Hz\)), 6.37 (dd, 1H\(^{3β}\), \(^3J_H-F=11.6Hz, J=6.5Hz\)), 6.53 (t, 1H\(^{3α}\), \(^3J_H-F=7.0Hz\)), 7.34-7.64 (m, 34H\(-Ar\)), 7.98-8.09 (m, 8H\(-Ar\)), 8.37 (s, 2H\(^{17α+β}\)).

¹³C-NMR (100 MHz, CDCl₃): δ_C = 19.2 (C-11), 19.2 (C-11), 26.7 (C-12), 26.7 (C-12), 58.7 (C-10), 58.8 (C-10), 63.1 (C-5), 63.3 (C-5) 71.6-71.9 (m, C-1α+β), 77.8 (C-4β), 81.2 (C-4α), 83.4 (C-3β), 85.1 (C-3α), 114.5 (C-7), 115.1 (C-7), 127.7-128.9 (m, C-2α+β), 127.9 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 128.7 (CH-Ar), 129.7 (CH-Ar), 129.8 (CH-Ar), 130.0 (C-Ar), 130.1 (CH-Ar), 132.5 (C-Ar), 132.6 (C-Ar), 133.5 (CH-Ar), 133.6 (CH-Ar), 134.2 (CH-Ar), 134.3 (CH-Ar), 134.7 (C-Ar), 135.3 (C-Ar) 135.4 (CH-Ar), 135.4 (CH-Ar), 149.7 (C=O), 149.7 (C=O), 161.1 (C=O), 161.2 (C=O), 164.6 (C=O), 164.6 (C=O), 165.8 (C=O), 165.9 (C=O).
Note: missing multiple CH-Ar and C-Ar due to overlapping peaks. At this point the identity of the anomers could not be ascertained α and β were arbitrarily assigned to allow easier analysis of the NMR data.

**IR** $v_{\text{max}}$ (neat) = 1721, 1689 cm$^{-1}$

**MS (ES+)** $m/z = 741$ [M+H]$^+$.  

**HRMS (ES+)** calculated 741.2444 for C$_{40}$H$_{39}$F$_2$N$_2$O$_8$Si, observed 741.2426 [M+H]$^+$.  

(2R,3R,5R)-2-[(Benzoyloxy)methyl]-4,4-difluoro-5-[5-(hydroxymethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]oxolan-3-yl benzoate 270β

![Chemical structure](image)

To a solution of protected nucleoside 269 (380 mg, 0.51 mmol) in THF (51 mL) at 0 °C was added 1.0 M TBAF in THF (0.64 mL, 0.64 mmol) dropwise and the resulting solution warmed to room temperature and stirred for 16 hours. The reaction mixture was then concentrated *in vacuo* and purified using flash silica column chromatography (3% MeOH in CHCl$_3$) to yield the product (207 mg, 81%) as a 1:1 mixture of anomers. Recrystallisation from CHCl$_3$ yielded pure β-anomer (207 mg, 40%) as colourless needles.

**$^1$H-NMR (500 MHz, DMSO-d$_6$):** $\delta_H$ = 4.15 (d, 2H$^10$, $J$=5.2Hz), 4.72 (qd, 2H$^5$, $J$=12.4Hz, $J$=4.1Hz), 4.82 (m, 1H$^4$), 5.11 (t, 1H$^{11}$, $J$=5.3Hz), 5.74-5.80 (m, 1H$^1$), 6.43 (t, 1H$^3$, $J_{H-F}$=9.4Hz), 7.47-7.51 (m, 2H-Ar), 7.56-7.60 (m, 2H-Ar), 7.61 (s, 1H$^6$), 7.63-7.74 (m, 2H-Ar), 7.97-7.98 (m, 2H-Ar), 8.05-8.07 (m, 2H-Ar), 11.74 (s, 1H$^{12}$).

**$^{13}$C-NMR (125 MHz, DMSO-d$_6$):** $\delta_C$ = 55.7 (C-10), 62.9 (C-5), 71.1-71.4 (m, C-1), 75.7 (C-4), 83.4-84.4 (br, s, C-3), 115.0 (C-7), 121.54 (t, C-2, $J_{C,F}$=261.7Hz), 127.9 (C-Ar), 128.8 (CH-Ar), 128.9 (CH-Ar), 129.0 (C-Ar), 129.2 (CH-Ar), 129.7 (CH-Ar), 133.6 (CH-Ar), 134.2 (CH-Ar), 136.5 (C-6), 150.0 (C=O), 162.2 (C=O), 164.3 (C=O), 165.4 (C=O).
IR $v_{\text{max}}$ (neat) = 3431, 1713, 1697 cm$^{-1}$

**MS (ES+) m/z = 503 [M+H]$^+$**.

**HRMS (ES+)** calculated 503.1266 for C$_{24}$H$_{21}$N$_2$F$_2$O$_8$, observed 503.1283 [M+H]$^+$.

mp (CHCl$_3$)= 160-162 °C.

1-[(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(hydroxymethyl)-1,2,3,4-tetrahydropyrimidine-2,4-dione 261

To a solution of nucleoside 270β (50 mg, 0.1 mmol) in MeOH (2.0 mL) at 0 °C was added 7.0 M NH$_3$ in MeOH (1.1 mL, 8.0 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue partitioned between H$_2$O (10 mL) and EtOAc (10 mL). The organic layer was re-extracted with H$_2$O (2 × 10 mL) and the combined aqueous layers concentrated under reduced pressure to yield the product (28 mg, 95%) as a colourless amorphous solid.

**$^1$H-NMR (400 MHz, MeOD-$d_4$):** $\delta$H = 3.79 (dd, 1H, $J=12.5$Hz, $J=3.1$Hz), 3.88-3.97 (m, 2H), 4.30-4.36 (m, 3H, 1H$^3$), 7.87 (s, 1H$^6$).

**$^{13}$C-NMR (100 MHz, MeOD-$d_4$):** $\delta$C = 57.9 (C-10), 60.5 (C-5), 70.42 (dd, C-1, $^2$J$_{C-F}$ 24.9Hz, $^2$J$_{C-F}$ =20.6Hz), 82.62 (d, C-4, $^3$J$_{C-F}$ =8.0Hz), 85.29 (dd, C-3, $^2$J$_{C-F}$ =40.2Hz, $^2$J$_{C-F}$ =24.8Hz), 115.6 (C-7), 125.39 (t, C-2, $J_{C-F}$=258.6Hz), 138.8 (C-6), 152.0 (C-9), 164.8 (C-8).

IR $v_{\text{max}}$ (neat) =3344, 2927, 1677 cm$^{-1}$

**MS (ES-) m/z = 293 [M-H]$^-$**.
HRMS (ES-) calculated 293.0585 for C_{10}H_{11}N_{2}F_{2}O_{6}, observed 293.0579 [M-H]^-. 

\((2R,3R,5R)-2-[(Benzoyloxy)methyl]-4,4-difluoro-5-[5-(fluoromethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]oxolan-3-yl benzoate 271\)

A solution of the nucleoside 270β (100 mg, 0.20 mmol) in THF (0.70 mL) was added dropwise to a −15 °C solution of DAST (28 µl, 0.21 mmol) in THF (0.55 mL), stirred at this temperature for 30 minutes and then warmed to room temperature and stirred for a further hour. The reaction mixture was then poured into cold H_{2}O (10 mL), extracted into CH_{2}Cl_{2} (10 mL), washed with H_{2}O (10 mL), dried with MgSO_{4} and concentrated in vacuo. The crude product was purified using flash silica column chromatography (2% MeOH in CH_{2}Cl_{2}) to yield the product (25 mg, 25%) as a white solid.

\(^1\)H-NMR (500 MHz, DMSO-d_{6}): δ_H = 4.70-4.84 (m, 3H\(^{5+4}\)), 5.02 (d, 2H\(^{10}\), \(^2\)J_{H-F}=48.6Hz), 5.76-5.91 (m, 1H\(^{11}\)), 6.42 (t, 1H\(^{2}\), \(^3\)J_{H-F}=7.8Hz), 7.47 (t, 2H-Ar, J=7.4Hz), 7.58 (t, 2H-Ar, J=7.3Hz), 7.63-7.75 (m, 2H-Ar), 7.93-7.96 (m, 2H-Ar), 8.02-88.06 (m, 2H-Ar), 8.07-8.09 (m, 1H\(^{6}\)), 11.96 (s, 1H\(^{11}\)).

\(^{13}\)C-NMR (125 MHz, DMSO-d_{6}): δ_C = 63.3 (C-5), 71.46 (dd, C-1, \(^2\)J_{C-F}=25.3Hz, \(^2\)J_{C-F}=22.1Hz), 75.7 (C-4), 77.15 (d, C-10, J_{C-F}=162.6Hz) 83.4-84.5 (br, s, C-3), 109.68 (d, C-7, \(^3\)J_{C-F} =18.3Hz), 121.53 (t, C-2, J_{C-F}=261.4Hz), 127.9 (C-Ar), 128.7 (CH-Ar), 128.9 (CH-Ar), 129.0 (C-Ar), 129.2 (CH-Ar), 129.7 (CH-Ar), 133.6 (CH-Ar), 134.3 (CH-Ar), 142.3 (d, C-6, \(^3\)J_{C-F} =6.9Hz), 149.8 (C=O), 161.9 (C=O), 164.3 (C=O), 165.3 (C=O).

IR ν_{max} (neat) = 1724, 1700 cm\(^{-1}\)

MS (ES-) m/z = 503 [M-H]^−.

HRMS (ES-) calculated 503.1066 for C_{24}H_{18}N_{2}F_{3}O_{7}, observed 503.1084 [M-H]^−.
mp (THF) = 152-156 °C.

1-[(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(methoxymethyl)-1,2,3,4-tetrahydropyrimidine-2,4-dione 273

To a suspension of fluoride 271 (18 mg, 0.04 mmol) in MeOH (1.0 mL) at room temperature was added K₂CO₃ (7 mg, 0.05 mmol). The reaction mixture was stirred for 30 minutes, then concentrated in vacuo and purified using flash silica column chromatography (7 % MeOH in CH₂Cl₂) to yield the product (9 mg, 76%) as a colourless gum.

¹H-NMR (400 MHz, MeOD-d₄): δ_H = 3.37 (s, 3H), 3.80 (dd, 1H, J=12.6Hz, J=2.9Hz), 3.92 (dt, 1H, J=8.4Hz, J=2.6Hz), 3.97 (br d, 1H, J=12.8Hz), 4.14 (d, 1H, J=12.4Hz), 4.20 (d, 1H, J=11.6Hz), 4.32 (td, 1H, J_H,F=12.2Hz, J=8.4Hz), 6.15-6.19 (m, 1H), 7.98 (s, 1H).

¹³C-NMR (100 MHz, MeOD-d₄): δ_C = 58.4 (C-11), 60.3 (C-5), 67.8 (C-10), 70.2 (dd, C-1, J_C,F=25.9Hz, J_C,F=19.9Hz), 82.6 (d, C-4, J_C,F=8.4Hz), 85.2 (dd, C-3, J_C,F=40.3Hz, J_C,F=24.5Hz), 112.4 (C-7), 124.0 (dd, C-2, J_C,F=259.0Hz, J_C,F=258.1Hz), 140.3 (C-6), 151.9 (C-9), 164.7 (C-8).

IR ν_max (neat) = 3363, 3072, 1682 cm⁻¹

MS (ES⁺) m/z = 309 [M+H]+.

HRMS (ES⁺) calculated 309.0898 for C₁₁H₁₅N₂F₂O₆, observed 309.0916 [M+H]⁺.
(2R,3R,5R)-5-[5-(Azidomethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-[(benzoyloxy)methyl]-4,4-difluorooxolan-3-yl benzoate 276

To a solution of nucleoside 270β (400 mg, 0.80 mmol) in 1,4-dioxane (8 mL) was added TMSCl (0.50 mL, 4.00 mmol). After 5 hours at 65 °C additional TMSCl was added (0.5 mL, 4.00 mmol) and the reaction stirred for 16 hours at this temperature. The mixture was then concentrated in vacuo, the residue re-dissolved in DMF (8 mL), sodium azide (312 mg, 4.80 mmol) added and the solution stirred for 5 hours at 65 °C. The solvents were then removed under reduced pressure and the resulting solid partitioned between EtOAc (60 mL) and brine (60 mL). The organic layer was dried using MgSO₄, concentrated in vacuo and the crude material purified using flash silica column chromatography (2% MeOH in CH₂Cl₂, dry loaded using 1,4-dioxane) to yield the product as a white solid (288 mg, 68% over 2 steps).

¹H-NMR (500 MHz, DMSO-d₆): δ_H = 4.01 (d, 1H₁⁰, J=13.6Hz), 4.05 (d, 1H₁⁰, J=13.6Hz), 4.68-4.71 (m, 1H₁), 4.76-4.82 (m, 2H₅), 5.81 (m, 1H₁), 6.40 (t, 1H₃, 3J_H-F=8.9Hz), 7.47 (t, 2H-Ar, J=7.8Hz), 7.57 (t, 2H-Ar, J=7.8Hz), 7.63-7.74 (m, 2H-Ar), 7.88 (s, 1H₆), 7.95 (dd, 2H-Ar, J=8.2Hz, J=1.1Hz), 8.04 (dd, 2H-Ar, J=8.2Hz, J=1.1Hz), 11.92 (s, 1H₁¹).

¹³C-NMR (125 MHz, DMSO-d₆): δ_C = 46.5 (C-10), 63.3 (C-5), 71.3-71.7 (m, C-1), 75.7 (C-4), 83.2-84.4 (br, s, C-3), 109.31 (C-7), 121.59 (t, C-2, Jₐᵣ-C₂,F =261.6Hz), 127.9 (C-Ar), 128.7(CH-Ar), 128.9 (CH-Ar), 129.0 (C-Ar), 129.2 (CH-Ar), 129.7 (CH-Ar), 133.6 (CH-Ar), 134.3 (CH-Ar), 139.9 (C-6), 149.8 (C=O), 162.4 (C=O), 164.3 (C=O), 165.4 (C=O).

IR ν_max (neat) = 3279, 2096, 1722, 1689 cm⁻¹

HRMS: Did not provide spectra suitable for interpretation.

mp (acetone) = 170-176 °C.
5-(Azidomethyl)-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2,3,4-tetrahydropyrimidine-2,4-dione 249

To a solution of the benzoyl protected azide 276 (30 mg, 60 µmol) in MeOH (3.0 mL) at 0 °C was added 7.0 M NH₃ in MeOH (0.7 mL, 4.80 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue purified using flash silica column chromatography (5% MeOH in CH₂Cl₂) to yield the product (18 mg, 94%) as an amorphous colourless solid.

¹H-NMR (500 MHz, MeOD-d₄): δ_H = 3.80 (dd, 1H₅, J=12.6Hz, J=2.9Hz), 3.90-3.93 (m, 1H₄), 3.96 (br, d, 1H₅, J=12.9Hz), 4.08 (s, 2H₁⁰), 4.32 (td, 1H₁, ³J_H-F=12.2Hz, J=8.4Hz), 6.13-6.17 (m, 1H₃), 8.05 (s, 1H₆).

¹³C-NMR (125 MHz, MeOD-d₄): δ_C = 48.2 (C-10), 60.3 (C-5), 70.18 (dd, C-1, ²J_C-F =26.5Hz, ²J_C-F J=19.4Hz) 82.77 (d, C-4, ³J_C-F =8.4Hz) 85.30 (dd, C-3, ²J_C-F =40.8Hz, ²J_C-F =23.9Hz) 110.79 (C-7) 123.96 (t, C-2, ²J_C-F=255.1Hz) 140.6 (C-6), 151.8 (C-9), 164.7 (C-8).

IR v_max (neat) = 3311, 2109, 1707, 1680 cm⁻¹

(2R,3R,5R)-5-[5-(Aminomethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-[(benzoyloxy)methyl]-4,4-difluorooxolan-3-yl benzoate 282

To a solution of the azide 276 (100 mg, 0.19 mmol) in THF:H₂O (10:1, 3.8 mL) was added triphenylphosphine (296 mg, 1.14 mmol) and the reaction mixture stirred for 16 hours at room temperature. The solvents were removed in vacuo and the crude material purified using flash silica column chromatography (5-10% MeOH in CH₂Cl₂) to yield the product (70 mg, 74%) as an amorphous white solid.

¹H-NMR (500 MHz, THF-d₈): δ_H = 3.38 (s, 2H¹⁰), 4.12 (br s, 2H¹²), 4.72 (m, 3H⁴+⁵), 5.78-5.83 (m, 1H¹), 6.38 (t, 1H³, ³J_H-F=9.1Hz), 7.42-7.66 (m, 7H-Ar), 8.00-8.14 (m, 4H-Ar).

¹³C-NMR (125 MHz, THF-d₈): δ_C = 36.8 (C-10), 61.1 (C-5) 69.90 (dd, C-1), ²J_C-F =28.5Hz, ²J_C-F =20.5Hz), 75.3 (C-4), 82.2-82.9 (br, s, C-3), 114.7 (C-7), 122.10 (t, C-2, J_C-F=259.3Hz) 126.5 (CH-Ar), 126.6 (CH-Ar), 126.8 (C-Ar), 127.6 (CH-Ar), 127.9 (C-Ar), 127.9 (CH-Ar), 131.2 (CH-Ar), 131.8 (CH-Ar), 131.6 (CH-Ar), 148.3 (C=O), 160.6 (C=O), 162.4 (C=O), 163.5 (C=O).

IR v_max (neat) = 2960, 1728, 1720 1673 cm⁻¹

MS (ES+) m/z = 502 [M+H]+.

(2R,3R,5R)-2-[(Benzoyloxy)methyl]-5-[5-[(dimethylamino)naphthalene-1-sulfonamidomethyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-4,4-difluorooxolan-3-yl benzoate 283

A room temperature solution of amine 282 (30 mg, 60 µmol) and NEt₃ (11 µl, 80 µmol) in THF (1.2 mL) was treated with dansyl chloride (19 mg, 70 µmol) and stirred for 16 hours. The reaction mixture was then concentrated in vacuo dissolved in CH₂Cl₂ (15 mL), washed with H₂O (10 mL), dried over MgSO₄ and the solvents removed in vacuo. The crude material was purified using flash silica column chromatography (2% MeOH in CH₂Cl₂) to yield the product (35 mg, 79%) as a pale green fluorescent solid.

¹H-NMR (400 MHz, CDCl₃): δH = 2.82 (s, 6H²³⁻²⁴), 3.60 (dd, 1H¹⁰, J=15.4Hz, J=6.5Hz), 3.78 (dd, 1H¹⁰, J=15.4Hz, J=6.5Hz), 4.57-4.61 (m, 1H⁴), 4.77 (dd, 1H⁵, J=12.5Hz, J=4.6Hz), 4.87 (dd, 1H⁵, J=12.5Hz, J=3.1Hz), 5.58-5.63 (m, 1H¹), 5.88 (t, 1H¹², J=6.5Hz), 6.20 (dd, 1H³, ³JH⁻⁻F=12.0Hz, ³JH⁻⁻F=6.1Hz), 7.11 (d, 2H-Ar, J=8.1Hz), 7.40 (dd, 1H-Ar, J=8.3Hz, J=7.5Hz), 7.47-7.52 (m, 5H-Ar), 7.59-7.67 (m, 2H-Ar), 8.06-8.14 (m, 6H-Ar), 8.44 (d, 1H-Ar, J=8.5Hz), 8.64 (s, 1H¹¹).

¹³C-NMR (100 MHz, CDCl₃): δC = 40.7 (C-10), 45.3 (C-23+24), 62.5 (C-5), 71.5 (dd, C-1, ²J_C⁻⁻F =34.8Hz, ²J_C⁻⁻F =16.9Hz), 78.9 (C-4), 82.6-83.4 (br, s, C-3), 110.3 (C-7), 115.2 (CH-Ar), 118.7 (CH-Ar), 123.3 (CH-Ar), 127.8 (C-Ar), 128.5 (CH-Ar), 128.8 (CH-Ar), 128.9 (CH-Ar), 128.9 (C-Ar), 129.2 (C-Ar), 129.5 (CH-Ar), 129.6 (C-Ar), 129.8 (CH-Ar), 130.2 (CH-Ar), 130.3 (CH-Ar), 133.9 (CH-Ar), 134.3 (CH-Ar), 135.5 (C-Ar), 137.3 (CH-Ar), 149.0 (C=O), 151.9 (C-Ar), 162.0 (C=O), 164.8 (C=O), 166.0 (C=O).

Note: missing C-2 due to multiple overlapping peaks.
IR $\nu_{\text{max}}$ (neat) = 3058, 1720, 1680 cm$^{-1}$

MS (ES+) m/z = 735 [M+H]$^+$.  
HRMS (ES+) calculated 735.1936 for $C_{36}H_{33}N_4F_2O_9S$, observed 735.1907 [M+H]$^+$.  

$\text{mp (CH}_2\text{Cl}_2) = 102$-107 °C.  

N-([1-(2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl]methyl)-5-(dimethylamino)naphthalene-1-sulfonamide 284  

To solution of the dansylated nucleoside 283 (30 mg, 40 µmol) in MeOH (2.0 mL) at 0 °C was added 7.0 M NH$_3$ in MeOH (0.5 mL, 3.2 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The solvents were removed in vacuo and the resultant residue purified using flash silica column chromatography (4% MeOH in CH$_2$Cl$_2$) to yield the product (16 mg, 76%) as a pale green fluorescent solid.

$^1$H-NMR (500MHz, MeOD-d$_4$): $\delta_H = 2.85$ (s, 6H$^{23+24}$), 3.83 (m, 4H$^{4,5+10}$), 3.95-3.99 (m, 1H$^5$), 4.22 (td, 1H$^1$, $^3J_{H,F}=12.1$Hz, $J=8.3$Hz), 5.90 (m, 1H$^5$), 7.23 (dd, 1H-Ar, $J=7.6$Hz, $J=0.7$Hz), 7.53 (ddd, 2H-Ar, $J=8.6$Hz, $J=7.5$Hz, $J=2.8$Hz), 7.59 (s, 1H$^6$), 8.16 (dd, 1H-Ar, $J=7.3$Hz, $J=1.2$Hz), 8.21 (td, 1H-Ar, $J=8.6$Hz, $J=0.8$Hz), 8.50 (dt, 1H-Ar, $J=8.6$Hz, $J=1.0$Hz).

$^{13}$C-NMR (125 MHz, MeOD-d$_4$): $\delta_C = 40.6$ (C-10), 45.8 (C23+24), 60.5 (C-5), 68.8-70.3 (m, C-1), 82.66 (d, C-4, $^3J_{C,F}=-7.9$Hz), 85.04 (dd, C-3, $^2J_{C,F}=39.2$Hz, $^2J_{C,F}=25.5$Hz), 111.2 (C-7), 116.4 (CH-Ar), 120.4 (CH-Ar), 123.76 (t, C-2, $^2J_{C,F}=252.8$Hz) 124.6 (CH-Ar), 129.3 (CH-Ar), 130.6 (CH-Ar), 130.7 (C-Ar), 131.0 (C-Ar), 131.2 (CH-Ar), 137.3 (C-Ar), 139.2 (C-6), 151.3 (C-9), 153.1 (C-Ar), 164.2 (C-8).
IR $\nu_{\text{max}}$ (neat) = 3338, 1677 cm$^{-1}$

MS (ES+) m/z = 527 [M+H]$^+$.  
HRMS (ES+) calculated 527.1412 for C$_{22}$H$_{25}$N$_4$F$_2$O$_7$S, observed 527.1408 [M+H]$^+$. 

$\text{mp}$ (MeOH-CH$_2$Cl$_2$) = 118-124 °C.

$\{(2R,3R)-3-(\text{Benzoyloxy})-5-[5-\{(1-\{(4R,5R)-4-(\text{benzoyloxy})-5-\{(\text{benzoyloxy})\text{methyl}\}-3,3-$

\text{difluorooxolan-2-yl}\}-2,4-$

\text{dioxo-1,2,3,4-tetrahydropyrimidin-5-yl}\text{methyl}\text{carbamoyl}\text{amino}\text{methyl})-2,4-$

\text{dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}\}-4,4-$

\text{difluorooxolan-2-yl}\text{methyl benzoate 285}$

CDI (2.3 mg, 14 µmol) was added to a solution of amine 282 (15 mg, 28 µmol) in DMF (0.3 mL) and the resulting solution stirred at room temperature for 16 hours. The reaction mixture was concentrated in vacuo and the crude material purified using flash silica column chromatography (5-10% MeOH in CH$_2$Cl$_2$) to yield the product (12 mg, 80%) as a white solid.

$^1$H-NMR (500 MHz, THF-d$_8$): $\delta$$_H$ = 3.84-3.92 (m, 4H$_{10}$), 4.63-4.67 (m, 2H$_4$), 4.71-4.79 (m, 4H$_5$), 5.87 (m, 4H$_{11}$), 6.32 (br, s, 2H$_3$), 7.40-7.43 (m, 4H$_{12}$), 7.46-7.49 (m, 4H$_{12}$), 7.53 (tt, 2H$_{12}$, $J$=7.4, $J$=1.3Hz), 7.62 (tt, 2H$_{12}$, $J$=7.5, $J$=1.3Hz), 7.69 (s, 2H$_6$), 8.03-8.09 (m, 8H$_{13}$), 10.71 (s, 2H$_{14}$).

$^{13}$C-NMR (125 MHz, THF-d$_8$): $\delta$$_C$ = 37.3 (C-10), 64.1 (C-5), 72.6-73.0 (m, C-1), 78.0 (C-4), 85.6-86.1 (br, s, C-3), 114.0 (C-7), 122.64 (t, C-2, $J_{C,F}$=261.5Hz), 129.2 (CH-Ar), 129.3 (CH-Ar), 129.6 (C-Ar), 130.4 (CH-Ar), 130.6 (C-Ar), 130.6 (CH-Ar), 133.8 (CH-Ar), 134.5 (CH-Ar), 139.1 (C-6), 151.0 (C-9), 158.6 (C-13), 163.8 (C=O), 165.1 (C=O), 166.3 (C=O).

IR $\nu_{\text{max}}$ (neat) = 1725, 1693 cm$^{-1}$
MS (ES+) m/z = 1029 [M+H]+.

HRMS (ES+) calculated 1029.2566 for C_{49}H_{41}F_{4}N_{6}O_{15}, observed 1029.2566 [M+H]+.

1,3-bis([1-[(4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl]methyl)urea 286

To a solution of dimeric nucleoside 285 (10 mg, 9.7 µmol) in MeOH (1.0 mL) at 0 °C was added 7.0 M NH₃ in MeOH (0.2 mL, 0.81 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. The reaction mixture was then concentrated in vacuo and partitioned between H₂O (5 mL) and EtOAc (5 mL). The organic layer was re-extracted with H₂O (5 mL) and the combined aqueous layers concentrated in vacuo to yield the crude product. Purification of the crude material using flash silica column chromatography (15% MeOH in CH₂Cl₂) gave the product (3 mg, 51%) as a white solid.

¹H-NMR (500 MHz, MeOD-d₄): δ_H = 3.79 (dd, 2H, J=12.6Hz, J=3.4Hz) 3.87-3.90 (m, 2H) 3.93-3.96 (m, 6H) 4.30 (td, 2H, ^3J_{H,F}=12.1Hz, J=8.3Hz) 6.11-6.13 (m, 2H) 7.80 (s, 2H).

¹³C-NMR (125 MHz, MeOD-d₄): δ_C = 37.8 (C-10), 60.6 (C-5), 70.37 (dd, C-1, ^2J_{C,F}=25.0Hz, ^2J_{C,F}=20.8Hz), 82.70 (d, C-4, ^3J_{C,F}=7.1Hz), 85.34 (dd, C-3, ^2J_{C,F}=36.3Hz, ^2J_{C,F}=28.5Hz), 113.9 (C-7), 123.92 (t, C-2, ^3J_{C,F}=252.5Hz), 138.9 (C-6), 151.9 (C-9), 160.5 (C-13), 165.1 (C-8).

IR v_max (neat) = 3350, 1683 cm⁻¹

MS (ES-) m/z = 611 [M-H].

HRMS (ES-) calculated 611.1361 for C_{21}H_{23}F_{4}N_{6}O_{11}, observed 611.1346 [M-H].
But-3-ynyl-p-toluenesulfonate 280

To a solution of 3-butyn-1-ol (0.22 mL, 2.85 mmol), NEt₃ (0.5 mL, 3.70 mmol) and 4-(dimethylamino)pyridine (34 mg, 0.28 mmol) in CH₂Cl₂ (11.5 mL) at 0 °C was added p-toluenesulfonyl chloride (598 mg, 3.14 mmol) and the resulting solution warmed to room temperature and stirred for 16 hours. 1.0 M NaOH (aq) (10 mL) was then added and the mixture stirred for a further 16 minutes. The phases were then separated and the organic layer diluted with CH₂Cl₂ (20 mL), washed sequentially with brine (20 mL) and H₂O (20 mL), then dried over MgSO₄ and concentrated in vacuo. The crude material was purified using flash silica column chromatography (30% EtOAc in 50-60 petroleum ether) to yield the product as a clear colourless oil (564 mg, 88%).

The data were in agreement with the published data.²⁵¹

¹H-NMR (400 MHz, CDCl₃): δ₁H = 1.97 (t, 1H, J=2.6Hz), 2.45 (s, 3H), 2.56 (td, 2H, J=7.1Hz, J=2.6Hz), 4.10 (t, 2H, J=7.1Hz), 7.35 (d, 2H, J=8.1Hz), 7.81 (d, 2H, J=8.2Hz).

¹³C-NMR (100 MHz, CDCl₃): δC = 19.4 (C-3), 21.6 (C-9), 67.4 (C-4), 70.7 (C-1), 78.3 (C-2), 128.0 (C-6), 129.9 (C-7), 132.8 (C-8), 145.0 (C-5).

MS (Cl⁺) m/z = 242 [M+NH₄]⁺.

HRMS (Cl⁺) calculated 242.0851 for C₁₁H₁₆NSO₃, observed 242.0850 [M+NH₄]⁺.
A solution of the azide 276 (90 mg, 0.17 mmol), ascorbic acid (30 mg, 0.17 mmol), CuSO₄·5H₂O (21 mg, 90 µmol) and alkyne 280 (46 mg, 0.20 mmol) in DMF-H₂O (9:1, 3.7 mL) was stirred for 16 hours at room temperature. The reaction mixture was concentrated in vacuo, partitioned between CH₂Cl₂ (15 mL) and H₂O (15 mL), the organic layer dried over MgSO₄ and the solvent removed under reduced pressure. The crude product was purified using flash silica column chromatography (1-2% MeOH in CH₂Cl₂) to yield the product (85 mg, 67%) as a white solid.

**1H-NMR (400 MHz, CDCl₃):** δ_H = 2.42 (s, 3H_Tos), 3.05 (t, 2H_14, J=6.8Hz), 4.27 (t, 2H_15, J=6.8Hz), 4.62 (dd, 1H_4, J=9.2Hz, J=4.6Hz), 4.75-4.83 (m, 2H_5), 5.00 (d, 1H_10, J=14.6Hz), 5.06 (d, 1H_10, J=14.6Hz), 5.66 (dd, 1H_1, 3J_H-F=16.3Hz, J=4.6Hz), 6.38 (dd, 1H_3, 3J_H-F=10.7Hz, 3J_H-F=7.1Hz), 7.32 (d, 2H-Ar, J=8.3Hz), 7.44-7.51 (m, 4H-Ar), 7.57-7.66 (m, 3H-Ar), 7.72 (s, 1H_11), 7.75 (d, 2H-Ar, J=8.3Hz), 8.06-8.09 (m, 4H-Ar), 9.70 (s, 1H_1)

**13C-NMR (100 MHz, CDCl₃):** δ_C = 21.6 (CH₃-Tos), 25.8 (C-14), 46.0 (C-10), 62.6 (C-5), 68.9 (C-15), 71.5 (dd, C-1, 2J_C,F=34.4Hz, 2J_C,F=17.0Hz), 78.8 (C-4), 83.7 (br, s, C-3), 109.6 (C-7), 120.8 (dd, C-2, J_C,F=266.4Hz, J_C,F=260.0Hz), 123.1 (CH-Ar), 127.8 (C-Ar), 127.9 (CH-Ar), 128.7 (CH-Ar), 128.8 (CH-Ar), 129.1 (C-Ar), 129.7 (CH-Ar), 129.9 (CH-Ar), 130.1 (CH-Ar), 132.8 (C-Ar), 133.7 (CH-Ar), 134.3 (CH-Ar), 140.5 (CH-Ar), 142.7 (C-Ar), 144.9 (C-Ar), 149.5 (C-Ar), 162.0 (C=O), 162.6 (C=O), 164.7 (C=O), 166.0 (C=O).

**IR** ν_max (neat) = 3068, 1723, 1693 cm⁻¹

**MS (ES+) m/z = 752 [M+H]^+, 774 [M+Na]^+.**
HRMS (ES+) calculated 752.1838 for C\textsubscript{35}H\textsubscript{32}N\textsubscript{2}F\textsubscript{5}O\textsubscript{10}S, observed 752.1846 [M+H]\(^+\).

mp (CH\textsubscript{2}Cl\textsubscript{2}) = 162-167 \degree C.

\((2R,3R,5R)-2-[(Benzoyloxy)methyl]-4,4-difluoro-5-\{[4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl]methyl\}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl\}oxolan-3-yl benzoate 277

A solution of azide 276 (80 mg, 0.15 mmol), ascorbic acid (26 mg, 0.15 mmol), CuSO\textsubscript{4}.5H\textsubscript{2}O (19 mg, 76 \mu mol) and 3-butyn-1-ol (13 \mu l, 0.17 mmol) in DMF-H\textsubscript{2}O (9:1, 3.0 mL) was stirred for 16 hours at room temperature. The reaction mixture was then concentrated \textit{in vacuo}, partitioned between CH\textsubscript{2}Cl\textsubscript{2} (15 mL) and H\textsubscript{2}O (15 mL), the organic layer dried over MgSO\textsubscript{4} and the solvent removed \textit{in vacuo}. The crude product was purified using flash silica column chromatography (5\% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to yield the product (48 mg, 54\%) as a white solid.

\(^1\text{H}-\text{NMR}\ (500\ MHz,\ DMSO-d\textsubscript{6}):\ \delta_{\text{H}} = 2.73\ (t,\ 2H\textsuperscript{14}, J=6.9Hz), 3.59\ (td,\ 2H\textsuperscript{15}, J=6.9Hz, J=5.3Hz), 4.65\ (t,\ 1H\textsuperscript{16}, J=5.3Hz), 4.64-4.82\ (m,\ 3H\textsuperscript{14-15}), 5.07-5.13\ (m,\ 2H\textsuperscript{10}), 5.79-5.88\ (m,\ 1H\textsuperscript{1}), 6.40\ (t,\ 1H\textsuperscript{3}, J\textsubscript{H-F}=8.4Hz), 7.44\ (m,\ 2H-Ar), 7.57\ (m,\ 2H-Ar), 7.62\ (m,\ 1H-Ar), 7.73\ (m,\ 1H-Ar), 7.76\ (s,\ 1H\textsuperscript{6}), 7.94\ (m,\ 2H-Ar), 8.04\ (m,\ 2H-Ar), 8.09\ (s,\ 1H\textsuperscript{12}), 11.91\ (s,\ 1H\textsuperscript{11}).

\(^{13}\text{C}-\text{NMR}\ (125\ MHz,\ DMSO-d\textsubscript{6}):\ \delta_{\text{C}} = 29.2\ (C-14), 45.7\ (C-10), 60.4\ (C-15), 63.5\ (C-5), 71.4-71.8\ (m,\ C-1), 75.8\ (C-4), 83.9-85.2\ (br,\ s,\ C-3), 109.0\ (C-7), 121.7\ (t,\ C-2, J\textsubscript{C-F}=260.9Hz), 122.5\ (C-12), 128.0\ (C-Ar), 128.8\ (CH-Ar), 129.0\ (CH-Ar), 129.1\ (C-Ar), 129.3\ (CH-Ar), 129.8\ (CH-Ar), 133.7\ (CH-Ar), 134.4\ (CH-Ar), 141.5\ (C-6), 144.4\ (C-13), 149.9\ (C=O), 162.3\ (C=O), 164.4\ (C=O), 165.5\ (C=O).

IR \nu_{\text{max}}\ (neat) = 3281, 1728, 1676\ cm\textsuperscript{-1}
MS (ES+) m/z = 598 [M+H]⁺, 620 [M+Na]⁺.
HRMS (ES+) calculated 598.1749 for C₂₈H₂₆F₂N₅O₈, observed 598.1731 [M+H]⁺.

mp (THF-Hexane) = 173-178 °C.

(2R,3R,5R)-2-[(Benzoyloxy)methyl]-4,4-difluoro-5-[[4-(2-fluoroethyl)-1H-1,2,3-triazol-1-yl]methyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl benzoate 278

To a −78 °C solution of alcohol 277 (40 mg, 70 µmol) in THF (1.4 mL) was added diethylamino sulfur trifluoride (17 µl, 0.13 mmol) and stirring continued at this temperature for 30 minutes, before being warmed to room temperature and stirred for a further hour. Cold H₂O (5 mL) was then added to the reaction and the mixture extracted into CH₂Cl₂ (2 ×10 mL). The combined organic layers were then dried over MgSO₄ and concentrated in vacuo to yield the crude product. The crude material was purified using flash silica column chromatography (4% MeOH in CH₂Cl₂) to yield the product (9 mg, 21%) as a white solid.

¹H-NMR (500 MHz, THF-d₈): δ_H = 3.02 (dt, 2H, J_H-F=22.4Hz, J=6.6Hz), 4.61 (dt, 2H, J_H-F=47.2Hz, J=6.6Hz), 4.69-4.76 (m, 3H), 5.12 (s, 2H), 5.78-5.83 (m, 1H), 6.36 (t, 1H, J=6.6Hz), 7.44-7.52 (m, 4H), 7.54-7.57 (m, 1H), 7.63-7.67 (m, 1H), 7.71 (s, 1H), 7.87 (s, 1H), 8.05-8.10 (m, 4H), 11.00 (s, 1H).

¹³C-NMR (125 MHz, THF-d₈): δ_C = 28.00 (d, C-14, J_C,F=21.8Hz), 46.5 (C-5), 63.9 (C-10), 72.5-72.9 (m, C-1), 78.4 (C-4), 83.11 (d, C-15, J_C,F=167.8Hz), 84.9-85.7 (br, C-3), 110.6 (C-7), 123.2 (C-12), 129.3 (CH-Ar), 129.4 (CH-Ar), 129.5 (C-Ar), 130.3 (CH-Ar), 130.5 (C-Ar), 130.6 (CH-Ar), 133.9 (CH-Ar), 134.6 (CH-Ar), 141.4 (C-6), 143.42 (d, C-13, J_C,F=7.5Hz), 150.7 (C-9), 163.0 (C=O), 165.1 (C=O), 166.3 (C=O).
Note: missing C-2 due to multiple overlapping signals.

**IR** $v_{\text{max}}$ (neat) = 2923, 1724, 1695 cm$^{-1}$

**MS (ES+)** $m/z = 600$ [M+H]$^+$. 
**HRMS (ES+)** calculated 600.1706 for C$_{28}$H$_{25}$F$_3$N$_5$O$_7$, observed 600.1712 [M+H]$^+$. 

**mp** (THF) = 168-172 °C.

1-[(2$R$,4$R$,5$R$)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-[[4-(2-fluoroethyl)-1H-1,2,3-triazol-1-yl]methyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione 252

To a solution of the protected nucleoside 278 (9 mg, 15 µmol) in MeOH (1.5 mL) at 0 °C was added 7.0 M NH$_3$ in MeOH (0.2 mL, 1.2 mmol) and the resulting mixture warmed to room temperature and stirred for 16 hours. The reaction mixture was then concentrated *in vacuo* and purified using flash silica column chromatography (5% MeOH in CH$_2$Cl$_2$) to yield the product (2 mg, 34%) as a white solid.

**$^1$H-NMR (400 MHz, MeOD-d$_4$):** $\delta_H = 3.07$ (dt, 2H, $^{3}J_{H,F}=24.6$Hz, $J=6.1$Hz), 3.79 (dd, 1H, $J=12.6$Hz, $J=3.1$Hz), 3.89-3.97 (m, 2H, $^{2}J_{H,F}=12.2$Hz, $J=8.3$Hz), 4.30 (td, 1H, $^{3}J_{H,F}=12.2$Hz, $J=8.3$Hz), 4.63 (dt, 1H, $^{2}J_{H,F}=47.0$Hz, $J=6.2$Hz), 5.20-5.28 (m, 2H, $^{3}J_{H,F}=9.1$Hz, $J=6.2$Hz), 7.85 (s, 1H), 8.18 (s, 1H$^2$).

**$^{13}$C-NMR (100 MHz, MeOD-d$_4$):** $\delta_C = 28.0$ (d, C-14, $^{2}J_{C,F}=21.6$Hz), 47.6 (C-10), 60.4 (C-5), 70.3 (dd, C-1, $^{2}J_{C,F}=26.6$Hz, $^{2}J_{C,F}=19.4$Hz), 82.9 (d, C-4, $^{3}J_{C,F}=8.3$Hz), 83.3 (d, C-15, $^{1}J_{C}$.
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\[ f=167.4\text{Hz}, \quad 85.3 \text{ (dd, C-3, } J_{C,F}=41.2\text{Hz, } J_{C,F}=24.7\text{Hz)}, \quad 110.0 \text{ (C-7), } \]
\[ 124.6 \text{ (C-6), } 125.17 \text{ (t, C-2, } J_{C,F}=259.1\text{Hz), } 142.0 \text{ (C-12), } 144.85 \text{ (d, C-13, } J_{C,F}=5.6\text{Hz), } 151.7 \text{ (C-9), } 164.3 \text{ (C-8).} \]

\[ \text{IR } v_{\text{max}} \text{ (neat) = } 3222, 2924, 1688 \text{ cm}^{-1} \]

\[ \text{MS (ES+)} \text{ m/z} = 392 \text{ [M+H]$.} \]

\[ \text{HRMS (ES+)} \text{ calculated 392.1182 for } C_{14}H_{17}F_{3}O_{5}, \text{ observed 392.1201 [M+H]$^+$.} \]

2,3,4,6-Tetra-\(\text{O}\)-benzoyl-\(\alpha\)-\(\text{D}\)-glucopyranosyl benzoate 287

Benzoyl chloride (3.9 mL, 33.3 mmol) was added dropwise to a 0 °C solution of D-glucose (1.0 g, 5.6 mmol) and DMAP (136 mg, 1.11 mmol) in pyridine (28 mL) and the resulting solution allowed to warm to room temperature and stirred for 16 hours. Cold H\(_2\text{O}\) (50 mL) was then added to quench the reaction and the mixture extracted into EtOAc (2 × 100 mL). The combined organic layers were washed with 1.0 M HCl (aq) (2 × 100 mL), brine (50 mL), dried over MgSO\(_4\) and concentrated in vacuo to give the crude product. The crude product was re-crystallised from EtOAc-Hexanes to yield the product (2.46 g, 63%) as a white solid.

The data were in agreement with the published data.\(^{255}\)

\[ ^{1}\text{H-NMR (400 MHz, CDCl$_3$): } \delta_H = 4.49-4.53 \text{ (m, } 1\text{H$^6$), } 4.63-4.66 \text{ (m, } 2\text{H$^{5,6}$), } 5.71 \text{ (dd, } 1\text{H$^2$, } J=10.3\text{Hz, } J=3.6\text{Hz), } 5.89 \text{ (t, } 1\text{H$^1$, } J=9.8\text{Hz), } 6.35 \text{ (t, } 1\text{H$^3$, } J=10.0\text{Hz), } 6.88 \text{ (d, } 1\text{H$^1$, } J=3.7\text{Hz), } 7.31-7.59 \text{ (m, } 13\text{H-Ar), } 7.62-7.69 \text{ (m, } 1\text{H-Ar), } 7.90-7.92 \text{ (m, } 4\text{H-Ar), } 7.96-7.98 \text{ (m, } 2\text{H-Ar), } 8.04-8.06 \text{ (m, } 2\text{H-Ar), } 8.13-8.16 \text{ (m, } 1\text{H-Ar), } 8.18-8.20 \text{ (m, } 2\text{H-Ar).} \]

\[ ^{13}\text{C-NMR (100 MHz, CDCl$_3$): } \delta_C = 62.5 \text{ (C-6), } 68.8 \text{ (C-4), } 70.4 \text{ (C-5), } 70.4 \text{ (C-2), } 70.5 \text{ (C-3), } 90.0 \text{ (C-1), } 128.4 \text{ (CH-Ar), } 128.4 \text{ (CH-Ar), } 128.7 \text{ (C-Ar), } 128.8 \text{ (CH-Ar), } 129.0 \text{ (C-Ar), } 129.5 \text{ (C-Ar), } 129.8 \text{ (CH-Ar), } 129.8 \text{ (CH-Ar), } 129.9 \text{ (CH-Ar), } 130.0 \text{ (CH-Ar), } 130.2 \text{ (CH-Ar), } 130.6 \text{ (C-Ar), } 133.2 \text{ (CH-Ar), } 133.4 \text{ (CH-Ar), } 133.5 \text{ (CH-Ar), } 133.6 \text{ (CH-Ar), } 133.7 \text{ (CH-Ar), } 134.0 \text{ (CH-Ar), } 164.4 \text{ (C=O), } 165.1 \text{ (C=O), } 165.4 \text{ (C=O), } 165.9 \text{ (C=O), } 166.1 \text{ (C=O).} \]
Note: missing 1 × C-Ar due overlapping peaks.

**IR** \( \nu_{\text{max}} \) (neat) = 2959, 1719 cm

**MS** (ES+) m/z = 723 [M+Na]⁺, 764 [M+Na+MeCN]⁺.

**HRMS** (ES+) calculated 764.2108 for C\(_{43}\)H\(_{35}\)O\(_{11}\)NaN, observed 764.2137 [M+Na+MeCN]⁺.

**2,3,4,6-Tetra-O-benzoyl-\(\alpha/\beta\)-D-glucopyranose 289**

A solution of 2.0 M methylamine in THF (5.2 mL, 10.2 mmol) was added to a solution of the protected glucose 287 (1.3 g, 1.86 mmol) in THF:MeOH (5:1, 6 mL) and the mixture stirred for 6 hours at room temperature. The reaction mixture was concentrated *in vacuo* and the crude residue purified using flash silica column chromatography (30% EtOAc in 40-60 petroleum ether) to yield the product (0.76 g, 68%) as a colourless solid in a 4:1 mixture of \(\alpha\) and \(\beta\) anomers.

The data were in agreement with the published data.²⁵⁶

**\(^{1}\)H-NMR (400 MHz, CDCl\(_3\))**: \( \delta_{\text{H}} = 3.31 \) (dd, 1H\(^{7\alpha}\), \(J=3.9\)Hz, \(J=1.1\)Hz), 4.47 (dd, 1H\(^{6\alpha}\), \(J=12.4\)Hz, \(J=4.6\)Hz), 4.51-4.55 (m, 1H\(^{6\beta}\)), 4.62-4.73 (m, 4H\(^{5+6, \alpha+\beta}\)), 5.35 (ddd, 1H\(^{2\alpha}\), \(J=10.3\)Hz, \(J=3.6\)Hz, \(J=1.0\)Hz), 5.38-5.40 (m, 1H\(^{2\beta}\)), 5.74-5.80 (m, 4H\(^{1+4, \alpha+\beta}\)), 5.95-6.02 (m, 1H\(^{3\beta}\)), 6.28 (t, 1H\(^{3\alpha}\), \(J=9.9\)Hz), 7.29-7.58 (m, 16H-Ar), 7.88-7.93 (m, 8H-Ar), 7.94-8.03 (m, 8H-Ar), 8.06-8.10 (m, 8H-Ar).

**\(^{13}\)C-NMR (100 MHz, CDCl\(_3\))**: \( \delta_{\text{C}} = 62.8 \) (C-6), 67.9 (C-5), 69.4 (C-4), 70.1 (C-3), 72.2 (C-2), 90.5 (C-1), 128.3 (CH-Ar), 128.4 (CH-Ar), 128.5 (CH-Ar), 128.9 (CH-Ar), 128.9 (C-Ar), 129.0 (C-Ar), 129.2 (C-Ar), 129.2 (C-Ar), 129.7 (CH-Ar), 129.8 (CH-Ar), 129.9 (CH-Ar), 129.9 (CH-Ar), 130.0 (CH-Ar), 133.1 (CH-Ar), 133.4 (CH-Ar), 133.5 (CH-Ar) 165.3 (C=O), 165.5 (C=O), 165.5 (C=O), 166.3 (C=O).
Note: missing 1 × C-Ar due to overlapping peaks. $^{13}$C data for α-anomer only.

IR $v_{\text{max}}$ (neat) = 3429, 2947, 1721 cm$^{-1}$

MS (ES-) m/z = 595 [M-H]$^-$.  
HRMS (ES-) calculated 595.1604 for C$_{34}$H$_{27}$O$_{10}$, observed 595.1602 [M-H]$^-$.

$O$-(2,3,4,6-Tetra-$O$-benzoyl-$\alpha$-$\beta$-glucopyranosyl) trichloroacetimidate 290

![Image of chemical structure]

1,8-Diazabicyclo[5.4.0]undec-7-ene (38 µl, 0.25 mmol) was added to a 0 °C solution of the alcohol 289 (150 mg, 0.25 mmol) and trichloroacetonitrile (0.25 mL, 2.5 mmol) in CH$_2$Cl$_2$ (1.25 mL) and the resulting solution warmed to room temperature and stirred for 1.5 hours. The reaction mixture was then concentrated in vacuo and the crude material purified using (20% EtOAc in 40-60 petroleum ether) to yield the product (136 mg, 73%) as a white foam.

The data were in agreement with the published data.$^{256}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_1$H = 4.51 (dd, 1H$^6$, $J$=12.8Hz, $J$=5.4Hz), 4.64-4.69 (m, 2H$^5$+6), 5.65 (dd, 1H$^7$, $J$=10.1Hz, $J$=3.7Hz), 5.84 (t, 1H$^4$, $J$=9.8Hz), 6.30 (t, 1H$^3$, $J$=10.1Hz), 6.86 (d, 1H$^1$, $J$=3.7Hz), 7.46 (m, 12H-Ar), 7.89 (m, 2H-Ar), 7.98 (m, 4H-Ar), 8.07 (m, 2H-Ar), 8.66 (s, 1H$^8$).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$C = 62.5 (C-6), 68.7 (C-4), 70.2 (C-3), 70.7 (C-5), 70.7 (C-2), 88.7 (C-9) 93.1 (C-1), 128.4 (CH-Ar), 128.4 (CH-Ar), 128.5 (CH-Ar), 128.5 (CH-Ar), 128.8 (CH-Ar), 128.9 (C-Ar), 129.6 (C-Ar), 129.7 (CH-Ar), 129.8 (CH-Ar), 129.9 (CH-Ar), 130.1 (C-Ar), 133.2 (CH-Ar), 133.3 (CH-Ar), 133.6 (CH-Ar), 160.5 (C-7), 165.2 (C=O), 165.4 (C=O), 165.7 (C=O), 166.1 (C=O).

Note: missing 1 × C-Ar and 1 × CH-Ar due to overlapping peaks.
IR $\nu_{\text{max}}$ \text{(neat)} = 2959, 1726, 1676 cm$^{-1}$

MS (ES+) m/z = 740 [M+H]$^+$.  
HRMS (ES+) calculated 740.0857 for C$_{36}$H$_{29}$Cl$_3$NO$_{10}$, observed 740.0889 [M+H]$^+$. 

2,3,4,6-Tetra-O-benzoyl-$\alpha$-D-glucopyranosyl bromide 288

To a solution of protected glucose 287 (1.13 g, 1.6 mmol) in acetic anhydride-acetic acid (1.0:2.7, 18 mL) was added 33% HBr in AcOH (12.7 mL, 72.4 mmol) and the resulting solution heated to 50 $^\circ$C and stirred for 4 hours. The reaction mixture was then cooled to room temperature, diluted with CH$_2$Cl$_2$ (150 mL), washed with H$_2$O (150 mL), brine (150 mL) and the organic layer dried over MgSO$_4$ and concentrated \textit{in vacuo}. The crude product was purified using (20% EtOAc in 40-60 petroleum ether) to yield the product (0.81 g, 76%) as a white solid. The data were in agreement with the published data.$^{255}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 4.54$ (dd, 1H$^6$, $J=12.5$Hz, $J=4.5$Hz), 4.70 (dd, 1H$^6$, $J=12.5$Hz, $J=2.6$Hz), 4.76 (ddd, 1H$^5$, $J=10.2$Hz, $J=4.3$Hz, $J=2.7$Hz), 5.36 (dd, 1H$^2$, $J=10.0$Hz, $J=4.1$Hz), 5.85 (t, 1H$^4$, $J=10.0$Hz), 6.29 (t, 1H$^3$, $J=9.8$Hz), 6.89 (d, 1H$^1$, $J=4.0$Hz), 7.31-7.60 (m, 12H), 7.88-7.92 (m, 2H), 7.98-8.04 (m, 4H), 8.08-8.11 (m, 2H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 62.0$ (C-6), 68.0 (C-5), 70.6 (C-4), 71.5 (C-3), 72.7 (C-2), 86.9 (C-1), 128.4 (CH-Ar), 128.5 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 128.8 (C-Ar), 129.5 (C-Ar), 129.8 (CH-Ar), 129.8 (CH-Ar), 129.9 (CH-Ar), 130.1 (CH-Ar), 133.3 (CH-Ar), 133.4 (CH-Ar), 133.6 (CH-Ar), 133.8 (CH-Ar), 165.1 (C=O), 165.3 (C=O), 165.6 (C=O), 166.0 (C=O).

Note: missing 2 × C-Ar due to overlapping peaks.

IR $\nu_{\text{max}}$ \text{(neat)} = 2959, 1721 cm$^{-1}$
MS (ES+) m/z = 580 [M-Br]+, 659 [M+H]+.

HRMS (ES+) calculated 659.0917 for C_{34}H_{28}BrO_{9}, observed 659.0938 [M+H]+.

4,5-Bis(benzoyloxy)-2-[(1-[(2R,4R,5R)-4-(benzoyloxy)-5-[(benzoyloxy)methyl]-3,3-difluorooxolan-2-yl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl]methoxy)-6-[(benzoyloxy)methyl]oxan-3-yl benzoate 291

A solution of nucleoside 270β (104 mg, 0.21 mmol) and glycosyl donor 290 (200 mg, 0.27 mmol) in MeCN (3 mL) was added to a flask containing 4Å molecular sieves and the suspension stirred for 15 minutes at room temperature, before being cooled to 0 °C and treated with TMSOTf (58µl, 0.27 mmol). The mixture was then warmed to room temperature and stirred for 16 hours. The reaction was quenched by addition of NEt$_3$ (0.1 mL), then filtered through a celite padded frit and concentrated in vacuo to give the crude product. The crude product was purified using flash silica column chromatography (40% EtOAc in 40-60 petroleum ether) to yield the product (125 mg, 55%) as a white solid.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta_H = 4.14$ (ddd, 1H, $J=9.8Hz, J=4.9Hz, J=3.3Hz$), 4.38 (dd, 1H, $J=13.1Hz, J=0.6Hz$), 4.46-4.54 (m, 2H, 5+17), 4.57-4.59 (m, 1H, 4), 4.63 (dd, 1H, $J=12.2Hz, J=3.2Hz$), 4.76-4.79 (m, 2H, 10), 4.97 (d, 1H, $J=7.9Hz$), 5.30 (dd, 1H, $J=9.8Hz, J=7.9Hz$), 5.59 (t, 1H, $J=9.8Hz$), 5.64-5.67 (m, 1H, 11), 5.89 (t, 1H, $J=9.7Hz$), 6.25-6.29 (m, 1H, 8), 7.29-7.65 (m, 19H-Ar), 7.81-7.83 (m, 2H-Ar), 7.89-7.92 (m, 4H-Ar), 8.01-8.03 (m, 2H-Ar), 8.06-8.09 (m, 4H-Ar), 8.70 (s, 1H, 11).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta_C = 62.8$ (C-10), 63.0 (C-17), 64.3 (C-5), 69.6 (C-15), 71.4-71.8 (m, C-1), 71.8 (C-13), 72.3 (C-16), 72.7 (C-14), 78.5 (C-4), 83.5-84.3 (br, s, C-3), 101.5 (C-12), 111.8 (C-7), 127.9 (C-Ar), 128.3 (CH-Ar), 128.3 (CH-Ar), 128.4 (CH-Ar), 128.7 (CH-Ar), 128.8
(C-Ar), 129.3 (C-Ar), 129.6 (C-Ar), 129.7 (CH-Ar), 129.8 (CH-Ar), 130.1 (CH-Ar), 133.2 (CH-Ar), 133.2 (CH-Ar), 133.4 (CH-Ar), 133.6 (CH-Ar), 134.2 (CH-Ar), 138.0 (CH-Ar), 149.5 (C=O), 161.5 (C=O), 164.7 (C=O), 165.0 (C=O), 165.2 (C=O), 165.7 (C=O), 166.0 (C=O), 166.1 (C=O).

Note: missing 2 × C-Ar, 5 × CH-Ar and C-2 due to overlapping peaks.

**IR** $v_{\text{max}}$ (neat) = 3261, 1721 cm$^{-1}$

**MS (ES+)** m/z = 1103 [M+Na]$^+$, 1081 [M+H]$^+$.

**HRMS (ES+)** calculated 1080.2843 for C$_{58}$H$_{47}$F$_2$N$_2$O$_{17}$, observed 1081.2866 [M+H]$^+$.

1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-[(3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy]methyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione 253

Protected nucleoside 291 (53 mg, 0.05 mmol) was dissolved in 7.0 M NH$_3$ in MeOH (3.3 mL) and allowed to stir for 16 hours at room temperature. The reaction mixture was then concentrated in vacuo and partitioned between EtOAc (10 mL) and H$_2$O (10 mL), the organic layer extracted with H$_2$O (10 mL) and the combined organic layers concentrated in vacuo. The crude material was triturated using MeOH-CH$_2$Cl$_2$ to yield the product (15 mg, 66%) as an amorphous white solid.

**$^1$H-NMR (500 MHz, D$_2$O):** $\delta_{\text{H}}$ = 3.20 (dd, 1H$^{13}$, $J$=9.3Hz, $J$=8.0Hz), 3.28-3.36 (m, 2H$^{15+16}$), 3.40 (t, 1H$^{14}$, $J$=9.0Hz), 3.64 (dd, 1H$^{17}$, $J$=12.3Hz, $J$=5.5Hz), 3.81 (dd, 1H$^{5}$, $J$=13.1Hz, $J$=4.1Hz), 3.82 (dd, 1H$^{17}$, $J$=12.3Hz, $J$=3.3Hz), 3.95 (dd, 1H$^{5}$, $J$=13.2Hz, $J$=2.3Hz), 4.01 (ddd, 1H$^{4}$, $J$=8.4Hz, $J$=4.0Hz, $J$=2.6Hz), 4.33 (td, 1H$^{1}$, $^2J_{H,F}$=11.8Hz, $J$=8.5Hz), 4.43 (d, 1H$^{12}$, $J$=8.0Hz), 4.45 (d, 1H$^{10}$, $J$=12.7Hz), 4.55 (d, 1H$^{10}$, $J$=12.7Hz), 6.14-6.17 (m, 1H$^3$), 7.88 (s, 1H$^6$).
\textsuperscript{13}C-NMR (125 MHz, D\textsubscript{2}O): $\delta$C = 59.2 (C-5), 60.7 (C-17), 64.0 (C-10), 69.1 (dd, C-1, $^2J_{C-F}$=24.6Hz, $^2J_{C-F}$=21.7Hz), 69.5 (C-14/15/16), 72.9 (C-13), 75.7 (C-14/15/16), 76.0 (C-14/15/16), 80.5 (d, C-4, $^3J_{C-F}$ =7.2Hz), 84.1 (dd, C-3, $^2J_{C-F}$ =25.3Hz, $^2J_{C-F}$ =38.5Hz), 101.4 (C-12), 110.9 (C-7), 122.1 (dd, C-2, $J_{C-F}$=259.7Hz, $J_{C-F}$ =258.6Hz), 140.7 (C-6), 151.5 (C-9), 165.0 (C-8).

IR $\nu_{max}$ (neat) =3356, 1678 \text{cm}^{-1}

MS (ES-) m/z = 595 [M+CO\textsubscript{2}H]\textsuperscript{+}.

HRMS (ES-) calculated 501.1168 for C\textsubscript{17}H\textsubscript{23}F\textsubscript{2}N\textsubscript{2}O\textsubscript{13}, observed 501.1187 [M+CO\textsubscript{2}H]\textsuperscript{+}. 

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RADIOCHEMISTRY
**Experimental Radiochemistry**

Radiosynthesis was performed on a GE TRACERlab FXc® synthesis module at the Wolfson Brain Imaging Centre (WBIC), University of Cambridge under the supervision of Dr Patrick Riss. Set-up of the synthesis module was performed by Dr Patrick Riss and Dr Valentina Ferrari.

Analytical radio-HPLC was performed on an Agilent 1100 series HPLC system (Agilent Technologies UK Ltd, Wokingham, UK), consisting of a G1312 A gradient pump and a G1314 variable wavelength UV detector. A Bioscan (Bioscan Inc., Washington DC, USA) thallium doped sodium iodide NaI(Tl) detector with Flow Count B FC 4000 analogue/digital interface were used for radioactivity detection. Lablogic Laura 4 software (LablogicSystems Ltd, Sheffield, UK) was used for data analysis and acquisition. For quality control a Chromolith RP-18e endcapped column (5 µm, 100 mm × 4.6 mm, Merck KGaA, Darmstadt, Germany) was used. The column was eluted at a flow rate of 1 mL/min using 6% EtOH in H₂O (v/v) as the mobile phase.

Semi-preparative radio-HPLC was performed using a Sykam S1122 isocratic pump, a UV detector (K2001, Knauer) and a radiodetector (built-in TRACERlab FXc®). A Phenomenex Luna RP18(2) column (10 µm, 250 mm × 10 mm), was fitted to the module. The column was eluted at a flow rate of 4.5 mL/min using 6% EtOH in H₂O (v/v) as the mobile phase. UV and radioactive traces were monitored with the TRACERlab (GE Medical Systems) software.
5-[[Methyl-\( ^{11} \text{C} \)]- 2’-deoxy-2’,2’-difluorothymidine 90*}

\[^{11} \text{C} \] CO\(_2\) was produced by the \(^{14}\text{N}(p,\alpha)^{11}\text{C}\) nuclear reaction on an aluminium target filled with \(^{14}\text{N}\) N\(_2\) containing 2% oxygen, using a GE PETtrace cyclotron. Proton energy was 13 MeV with a beam current of 40 \(\mu\)A and the duration of irradiation was 15-30 minutes. At EOB, the target gas was delivered and trapped in the GE MeI Microlab synthesiser (Figure 22 Section 3.2.1). \[^{11} \text{C} \] CH\(_3\)I was released into the reaction vessel under a stream of helium.

A mixture of Pd\(_2\)(dba)\(_3\) (0.6 mg, 0.6 \(\mu\)mol), tri(o-tolyl)phosphine (0.7 mg, 2.4 \(\mu\)mol) and stannane 224 (0.5 mg, 1.2 \(\mu\)mol) in DMF (0.17 mL) was prepared in an oven-dried, septum-equipped-vial at room temperature. The solution was purged with helium gas for 10 minutes and \[^{11} \text{C} \] CH\(_3\)I trapped in the solution at room temperature. The resulting mixture was heated at 130 °C for 5 minutes then cooled to room temperature and diluted with H\(_2\)O (250 \(\mu\)L). The mixture was filtered (13 mm, 0.22 \(\mu\)m PTFE filter) and injected onto the HPLC with a semi-preparative column. At a flow rate of 4.5 mL/min using 6% EtOH in H\(_2\)O (v/v) as the mobile phase a fraction corresponding to radiolabelled 90 was eluted at 19.5 minutes in a decay-corrected radiochemical yield of 5% (\(n=3\)).

The product was assayed for radiochemical purity by analytical HPLC. The column was eluted at 1 mL/min using 6% EtOH in H\(_2\)O (v/v), the retention time for 90 was 4.5 minutes with a radiochemical purity of 97%. 

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Supporting Data: NOESY Spectrum for 270β

Figure 32- Nucleoside 270β. Bond lengths exaggerated for clarity. H-5 corresponds to CH₂ next to benzoyl protected alcohol.

A key correlation between protons H-3 and H-4 indicates both protons occupy the same face of the molecule confirming assignment as the β-anomer. Furthermore there is no correlation apparent between protons H-3 and H-1, which would be expected in the α-anomer.
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