Synthesis of 2’-bromo-2’-fluoro-2’-deoxycytidine derivatives towards $[^{18}\text{F}]$gemcitabine

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This thesis is submitted for the degree of Doctor of Philosophy (PhD) at Cardiff University

March 2020
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References
Abstract

Gemcitabine is a frequently used chemotherapeutic agent against a range of cancers – pancreatic cancer in particular. One of its disadvantages is its poor specificity, needing to overcome many deactivation pathways in order to impart its anticancer properties. As such, a fluorine-18 labelled gemcitabine would provide in vivo pharmacokinetic information specific for each patient in a step towards personalised treatment.

Herein described is a synthetic route towards compounds that may be screened as a fluorine-18 radiolabelling precursors for the synthesis of $[^{18}\text{F}]$gemcitabine. The developed strategy centres on the synthesis of key 2-bromo-2-fluororibonolactone, which was produced in a diastereoselective fashion. The synthesised lactone was then reacted with cytosine derivatives to deliver the radiofluorination substrates.

Fluorination with fluorine-19 demonstrated the suitability of the substrates towards substitution, with initial $[^{18}\text{F}]$fluorination studies also conducted. Tissue culture studies were also carried out, in order to evaluate the bioactivity of the synthesised compounds, relative to gemcitabine.
Acknowledgements

I would like to take this opportunity to thank the many people involved with the work presented in this thesis, as many people have contributed in supporting me along the way.

Let’s start at the very beginning – my supervisor Dr. Ian Fallis, for his vast knowledge and guidance throughout this project. I know this has been a project Ian has worked on for a number of years and count myself incredibly lucky to work on such an interesting and meaningful project. Thanks for guiding me along the way, while encouraging me to push myself further than I thought possible. I would also like to Dr. Duncan Browne, who has co-supervised me and overseen the majority of my work. Over the time spent in Duncan’s research group, he has challenged me and driven me to new levels for which I cannot thank him enough, while offering new and exciting opportunities I would never have thought of doing.

The nature of this project has meant significant collaborations, which must be recognised. I would like to thank Prof. Chris Marshall, Dr. Peter Llywelyn and the rest of the Production Team and staff at PETIC in UHW for providing the opportunity to work with you all. The chance to work with $^{18}$F is rare, and the training you provided for FDG synthesis was incredible. Latterly, thank you to Dr. Steve Paisey and Dr. Matt Tredwell for their help with the radiochemistry. Thanks to Dr. Catherine Hogan for the opportunity to conduct tissue culture experiments and training me, along with Anna Richards for helping me with any other issues I had.

I must also thank my funders, who made this project possible. To Tenovus Cancer Care and Tim Banks, your support was much appreciated, and I hope this work reflects the good work you and the charity continue to do. Thanks to the KESS II scheme and team involved, notably Esther Meadows, for providing the platform for research such as this to be conducted.

A huge amount of credit must go to Dr. JieXiang Yin, who came in to help the project and really helped provide synthetic experience and a calming influence – which cannot be understated. Not only a wizard at organic chemistry (and whistling), he helped teach me so many valuable lessons (“more silica gel”) and I wish him and his young family all the very best. Some of his work is presented in this thesis (Schemes 3.03 and 3.04)
Onto my fellow DLB group members. Firstly, to Christiane and Joey. The amount of help you gave and time you both invested in me, especially at the start of my PhD, was something I didn’t really appreciate. You helped me when I needed it most, thank you so much. During my time in the group, many people have come and gone but those that have stuck around long enough to endure my singing must be recognised. So thanks to Qun, Tom, Will, Yerbol and Andy; you have all helped me at many points over the course of my PhD. Be it in the lab, the pub or at home with a cup of tea – you’ve helped keep me on the straight and narrow, while condoning my awful jokes (but really, you know they’re very good).

I would like to thank members of other groups in the department that have helped me along the way. Firstly, James, Kurt, Ben R-B, Alex and latterly Ben A., from the LCM group who offered advice in the lab, group meetings and life. Thanks to Andy, Siôn and others from Ian’s group for support when I needed it. And to all my half-way friends, for the half-hearted smiles we share.

Outside of university life, I have an amazing friendship group who always have a plan in the mix that help keep me motivated and remind me of life outside of the PhD bubble. Shout out to Mark “M.J.” Sullivan, who has had the pleasure/misery of living with me for the duration of our PhDs.

I’d like to give a special mention to my girlfriend Meg – thanks for supporting me during the good and the bad, for when I need a reminder to switch off and everything else in between. You’ve been my constant, thank you so much.

Thanks to my family for their continued support over the years. This is something we can all be proud of, and I’m so grateful for everything you’ve done for me. This one is for you.

Finally, I’d like to thank my legs for always supporting me, my arms for being by side and my fingers, because I can always count on them.
Abbreviations

Ac Acetyl
AIBN Azobisisobutyronitrile
app. Apparent
Boc tert-Butyloxycarbonyl
BOPCl Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
BSA \(N,O\)-Bis(trimethylsilyl)acetamide
Bz Benzoyl
calc. Calculated
CDI Carbonyl diimidazole
COSY Correlation spectroscopy
Cp Cyclopentadienyl
DABCO 1,4-Diazobicyclo[2.2.2]octane
DBU 1,8-Diazabicyclo(5.4.0)undec-7-ene
DCC Dicyclohexylcarbodiimide
DCE 1,2-dichloroethane
DCM Dichloromethane
DHP 3,4-Dihydropyran
DIBAL-H Diisobutylaluminium hydride
DIPEA \(N, N\)-Diisopropylethylamine
DMAP 4-Dimethylaminopyridine
DMF \(N, N\)-Dimethylformamide
DMP Dess-Martin Periodinane
DMPU \(N,N'\)-Dimethylpropyleneurea
DMSO Dimethylsulfoxide
DMTr 4,4′-Dimethoxytrityl
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<th>Definition</th>
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<td>d.r.</td>
<td>Diastereomeric ratio</td>
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<td>EDC.HCl</td>
<td>1-Ethyl-3-(3-dimethylaninopropyl)carbodiimide hydrochloride</td>
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<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
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<tr>
<td>FAC</td>
<td>1-(2'-Deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine</td>
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<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
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<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
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<tr>
<td>HOBt</td>
<td>Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>High resolution mass spectrometry</td>
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<td>Isopropyl</td>
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<td>Infrared</td>
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<td>KHMDS</td>
<td>Potassium hexamethyldisilazide</td>
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<td>LDA</td>
<td>Lithium diisopropylamine</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>Lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>LTBA</td>
<td>Lithium tri(tert-butoxy)aluminium hydride</td>
</tr>
<tr>
<td>m</td>
<td>Atomic mass of (radio)nuclide</td>
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<tr>
<td>mCPBA</td>
<td>meta-chloroperbenzoic acid</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
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<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>Ms</td>
<td>Mesyl/methanesulfonyl</td>
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<tr>
<td>MTBE</td>
<td>Methyl tert-butyl ether</td>
</tr>
<tr>
<td>(N_A)</td>
<td>Avagadro’s number</td>
</tr>
<tr>
<td>Abbreviation</td>
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</tr>
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<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>n.d.</td>
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</tr>
<tr>
<td>NDCY</td>
<td>Non-decay corrected yield</td>
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<tr>
<td>NFSI</td>
<td>N-Fluorobenzenesulfonamide</td>
</tr>
<tr>
<td>NMI</td>
<td>N-Methylimidazole</td>
</tr>
<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>Nuc.</td>
<td>Nucleophile</td>
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<tr>
<td>ONs</td>
<td>para-Nitrobenzenesulfonate (nosylate)</td>
</tr>
<tr>
<td>OTf</td>
<td>Triflate</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PLE</td>
<td>Pig liver esterase</td>
</tr>
<tr>
<td>PMBz</td>
<td>para-Methoxy benzoyl</td>
</tr>
<tr>
<td>PPy</td>
<td>4-Pyrrolidinopyridine</td>
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<td>para-Toluenesulfonic acid</td>
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<td>quant.</td>
<td>Quantitative</td>
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<td>RCC</td>
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<td>RCY</td>
<td>Radiochemical yield</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention factor</td>
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<tr>
<td>RedAl</td>
<td>Sodium bis(2-methoxyethoxy)aluminium hydride</td>
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<td>RPMI</td>
<td>Roswell Park Memorial Institute (medium)</td>
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<tr>
<td>TBAF</td>
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</tr>
<tr>
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</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>'Bu</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>TES</td>
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</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>Tetrahydropyran</td>
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<td>TIPSDCl₂</td>
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</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
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<tr>
<td>Tr</td>
<td>Trityl</td>
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1 – Introduction

1.1 – Gemcitabine

2',2'-Difluoro-2'-deoxycytidine (dFdC; 1, Figure 1.01) marketed commercially in 1988 as Gemzar® as the hydrochloride salt, is a nucleoside analogue of 2-deoxycytidine (2), with geminal fluorine atoms at the 2' position, hence the common name gemcitabine. It is a frequently used chemotherapeutic agent as a combatant against a range of cancers, such as ovarian, breast and pancreatic cancer.

Figure 1.01: Structures of gemcitabine (1) and 2-deoxycytidine (2).

Additionally, it is commonly used as a combination therapy, with platinum based compounds such as cis-platin (3) and carboplatin (4) for bladder and ovarian cancer, respectively. For pancreatic cancer, gemcitabine is typically administered as the sole chemotherapeutic agent as a first-line treatment, but has limitations in its effectiveness.

Figure 1.02: Structures of cis-platin (3) and carboplatin (4).

Gemcitabine is delivered as the prodrug into the patient, and metabolised into the active chemotherapeutic agent. Due to its highly hydrophilic nature, the drug is not actively taken up into cancer cells across the plasma lipid bilayer found in the pancreas, demonstrating poor bioavailability. Kinetic studies have illustrated that gemcitabine uptake across a cell membrane is mediated by range of transporter proteins, such as human equilibrative nucleoside transporters (hENT), specifically hENT1. It has also been
found to be transported via human concentrative nucleosides (hCNT) although to a lower extent, in particular hCNT1 which is known to favour transporting pyrimidine nucleosides.[1] With a human terminal plasma half-life of 17 minutes,[2] intracellular gemcitabine is rapidly consumed with approximately 90% of uptaken 1 being deactivated by deoxycytidine deaminase (dCDA), to the inactive 2',2'-difluoro-2'-deoxyuridine equivalent.[3]

Gemcitabine that remains intact in cells is first phosphorylated at the 5' position by deoxycytidine kinase (dCK) in the rate limiting step, to form 2',2'-difluoro-2'-deoxycytidine-5'-O-phosphate, shown in Scheme 1.01. This intermediate may also be deactivated, noted by dashed arrows, in a process facilitated by deoxycytidylate deaminase which converts it to mono-phosphorylated uridine 7. dFdCMP may be sequentially further phosphorylated to produce the corresponding diphosphate (8, dFdCDP) and triphosphate (9, dFdCTP) – both considered to be active cytotoxic metabolites in vivo. dFdCTP acts as a masked chain terminant, addition of a subsequent natural nucleotide renders the compound less susceptible to DNA repair by base pair excision,[4] inhibiting DNA strand growth and inducing apoptosis within the cell. In addition, gemcitabine also possesses a self-potentiating mechanism; dFdCDP inhibits ribonucleotide reductase (RNR), which catalyses the production of other deoxyribonucleotides needed for continued synthesis and repair of DNA. As a result of this inhibition, the concentration of DNA nucleobases and their corresponding phosphates is reduced. Ramos and co-workers go on to say that the effect of inhibiting RNR is profound, as the intracellular equilibrium is shifted leading to an overall increase in active uptake of deoxyribonucleotides, including gemcitabine, increased rates of phosphorylation, and decreased deactivation by dCDA.[5]
Scheme 1.01: Intracellular mode of action of gemcitabine, where 9 acts as a masked chain terminant.

hENT1 = human equilibrative nucleoside transporter. dCDA = deoxycytidine deaminase. dCK = deoxycytidine kinase. dCTD = deoxycytidylate deaminase.
In summary, the \textit{in vivo} release of gemcitabine is dependent on three factors:\cite{6}

i. Regulation of dCK, which is necessary for the initial phosphorylation.

ii. Regulation of enzymes, such as CDA, contributing to undesirable degradation pathways of gemcitabine.

iii. Expression of nucleoside transporters, such as hENT1, for uptake into cells.

Alternative strategies have been developed in order to overcome the observed deactivation pathways of gemcitabine. McGuigan and co-workers successfully established the field of ProTide drug delivery, in which the nucleotide is introduced with a pseudo phosphate group already installed at the 5’ position as to avoid deactivation by dCDA.\cite{7} Additionally, this strategy circumvents the rate limiting phosphorylation of gemcitabine \textit{in vivo}, and dependence on nucleoside transporters such as hENT1. One drawback of this improved activity and availability is a simultaneous loss of specific targeting.
1.1.1 – Synthesis of gemcitabine

1.1.1.1 – Original synthesis

Gemcitabine was first successfully synthesised by Hertel et al in 1998,\cite{8} with the aim of synthesising fluorinated nucleosides as potential anticancer/antiviral agents. The synthesis (Scheme 1.02) was realised by combining \( (R)-2,3-O\)-isopropylidene-D-glyceraldehyde, accessed from D-mannitol in two steps,\cite{9} with ethyl bromodifluoroacetate (10) and zinc in a Reformatsky reaction, furnishing the desired \( \beta\)-hydroxy ester in 65\% yield. Subsequent acidic deprotection of the acetal moiety and lactonisation afforded the integral 2-deoxy-2,2-difluoro-D-ribonolactone (13) (Scheme 1.02). Protection of 13 as the tert-butyl dimethyl silyl ether and reduction by DIBAL-H to yield the corresponding protected or deprotected ribofuranose (14 and 15 respectively).

Scheme 1.02: The synthesis of protected and unprotected difluorolactols (14) and (15) by zinc-based Reformatsky and lactonisation.
Ribofuranose 14 was subsequently treated with methanesulfonyl chloride to afford 16, which upon reacting with bis-TMS protected cytosine and acid hydrolysis, affords gemcitabine (Scheme 1.03, β-1). The lack of anomer selectivity observed was attributed to the gem-fluoro moiety deactivating the mesylate leaving group, leading to increased $S_N2$ character during the ring appending reaction. Consequently, the $\alpha$-anomer was formed in 40% yield (Scheme 1.03).

Scheme 1.03: The synthesis of gemcitabine (1) by mesylation and glycosylation.

The seminal work by Hertel[8] demonstrated the value of the difluoromethylene moiety in this class of chemotherapeutic agents and paved the way for alternative synthetic routes to be explored.

Building on the original zinc-based synthesis, Yasuda and co-workers investigated the use of various Lewis acids to promote the addition to unactivated protected glyceraldehydes.[10] In the absence of activator, combining ethyl difluoroiodoacetate with (R)-cyclohexyldenedeglyceraldehyde in the presence of zinc powder and triethylsilylchloride, the silyl protected product 19 was isolated in a moderate 60% yield with good selectivity for the desired anti diastereomer, in a 17:3 ratio over the syn isomer (Scheme 1.04).
Changing the latent nucleophile to ethyl bromodifluoroacetate, and screening a number of Lewis acids, they found that stoichiometric bis(cyclopentadienyl)titanium (IV) dichloride not only afforded higher yields (84%), but also further promoted the formation of the anti-diastereoisomer, in a ratio greater than 19:1. Furthermore, the observed enhanced selectivity and reactivity illustrated in Scheme 1.05 was also possible with catalytic Cp₂TiCl₂ which delivered 19 in 92% yield, albeit with slightly lower anti: syn ratio of 91:9.

The authors attribute the diastereoselectivity of the reaction to the coordination of the Lewis acid to the aldehyde oxygen, with the in situ produced nucleophile attacking from the less sterically hindered si face,[10] illustrated by the Felkin-Anh model in Figure 1.03.
Alternative strategies to installing the CF$_2$ group have had limited success outside of the Reformatsky reaction centred approach. Use of ethyl difluoroacetate with LDA and TMSCl was found to predominantly yield the self-Claisen condensation product 21, not the desired silyl enol ether 22, as shown in Scheme 1.06.

Weigel overcame this issue,$^{[11]}$ using tert-butyl thioester 23 as the latent nucleophile in conjunction with LDA as base and benzaldehyde as electrophile, affording β-hydroxy thioester 24 in 70% isolated yield (Scheme 1.07(1)). The improved selectivity was due to a less nucleophilic lithium enolate generated in situ, although the self-condensation product was detected at 10% $^{19}$F NMR yield.

Scheme 1.07: The LDA mediated reactions of S-(tert-butyl) 2,2-difluoroethanethioate (23), with benzaldehyde as electrophile (top, (1)) and TMSCl (bottom, (2)).
Conditions were also altered to include TMSCl, resulting in formation of silyl enol ether 25 as the sole product by $^{19}$F NMR. The yield of 25 was not given but was used *in situ* for further reactions, and not isolated due to its hydrolytic instability (Scheme 1.07 (2)).

With appropriate conditions for the formation of 25, 23 was combined with protected glyceraldehyde 26 furnishing 64% yield of β-hydroxy thioester 27, shown in Scheme 1.08. Inclusion of Lewis acid BF$_3$·Et$_2$O to activate aldehyde 26 increased the yield and diastereoselectivity of the reaction, preferentially forming the *anti*-isomer, an observation attributed to the steric bulk of the *tert*-butyl thioester in both instances.

**Scheme 1.08**: The LDA mediated reaction of 23 and production of β-hydroxythioester 27.
1.1.1.2 – Protecting groups

Cen and Sauve found that utilising the difluororibofuranose protected as the bis(triisopropyl)silyl ether increased the anomeric selectivity of the silyl-Hilbert-Johnson reaction (Scheme 1.09), improving Hertel's original methodology.\textsuperscript{[12]} By changing from TBDMS to TIPS, the increased sterics of the 3-O-silyl group resulted in improved β-anomer formation. In the presence of Lewis acid TMSOTf in refluxing DCE and subsequent deprotection by TMAF and acetic acid, anomerically pure gemcitabine was produced in 36% yield after HPLC purification. The undesired α-anomer was produced in a slight excess, at 42% isolated yield (overall 78% yield).

![Scheme 1.09: The Vorbruggen glycosylation reaction of 28 and TMAF mediated deprotection to yield an anomeric mixture of 1.](image)

Esters have also been employed as protecting groups for the 3- and 5-hydroxyl groups once 2-deoxy-2,2-difluororibofuranose has been synthesised. Chou \textit{et al} further developed the synthesis of gemcitabine, following Hertel’s synthesis of the requisite β-hydroxy ester, by converting intermediate 29 into its benzoyl ester; through benzoyl chloride, catalytic DMAP, and 2,6-lutidine as base in DCM.\textsuperscript{[13]} Depicted in Scheme 1.10, concomitant deprotection and cyclisation affords lactone 31, which is in turn benzoylated to afford 3,5-di-O-benzoyl-2-deoxy-2,2-difluoro-D-ribonic acid-1,4-lactone 32, from which the desired ribo-stereomer may be selectively crystallised, albeit in a low yield of 26%.
From 33, reduction by LTBA in THF/Et₂O quantitatively delivered intermediate ribofuranose as a mixture of anomers, which was then mesylated to afford 34 in quantitative yield. Subsequently, subjecting 34 to the Vorbruggen reaction and debenzoylation by ammonia in methanol furnished 1 as a near 1:1 anomeric mixture, with the α-anomer being formed in a slight excess (Scheme 1.11).

Scheme 1.10: The synthesis of key protected difluorolactone 33.

Scheme 1.11: The synthesis of 1 as an anomeric mixture by sequential reduction, glycolsylation and deprotection.
The desired β-anomer was selectively crystallised out as the hydrochloride salt firstly by recrystallisation from hot PrOH, and then tritivated with water/acetone (1:12 v/v) mixture. Chou’s method demonstrated considerable improvement in the anomeric selectivity of the silyl-Hilbert-Johnson reaction, increasing the ratio of β:α-anomer ratio from 1:4 to near 1:1. The authors noted that regardless of the diastereomeric ratio of mesylate 34 input into the reaction, the near 1:1 β:α-anomer ratio was consistently achieved. This observation was attributed to the reaction profile following a SN1 mechanism, with an oxocarbenium intermediate, shown in Scheme 1.12. Subjecting α-1 to the Vorbruggen reaction conditions resulted in no anomerisation and formation of β-1, inferring that alteration of the anomeric ratio does not occur post-nucleobase attack.

Scheme 1.12: The proposed formation of oxocarbenium 34' as reactive intermediate.

The approach of using esters for stereospecific crystallisation was capitalised on by Shen and co-workers, who developed a synthetic route which utilised the trans-cinnamoyl esters to improve selectivity of the Vorburggen reaction.\(^{[14]}\) Starting from 35 as a mix of ribo- and xylo-diastereomers following lactonisation,\(^{[8]}\) both 3- and 5-alcohol groups were acylated with trans-cinnamoyl chloride with pyridine in ethyl acetate, followed by selective crystallisation of the desired ribo-stereomer, delivering 36 in 43% (Scheme 1.13). The diastereomeric ratio of 36 was not disclosed, but Hertel’s previous work notes a 3:1 mixture of the desired configuration prior to lactonisation.\(^{[8]}\) Reduction by freshly prepared LTBA in THF and base mediated tosylation of the intermediate lactol produced 37 as a crystalline solid in 62% over two steps. Interestingly, the authors comment that the nature of the base used for the tosylation step directly affects the diastereomeric nature, although no quantification data is given.
Shen and co-workers found that they obtained a one-to-one mixture of anomers when subjecting 37 to Vorbruggen’s reaction conditions, regardless of the diastereomeric ratio of the starting material. Their observation agrees with Chou’s previous investigation and conclusion that the reaction likely proceeds via a $S_N1$ mechanism, delivering $\beta$-38 in 47% yield (Scheme 1.14). Subsequent deprotection of the cinnamoyl esters and acetamide motif furnished pure gemcitabine in 80% yield.

Scheme 1.13: The synthesis of crystalline intermediate 37.

Scheme 1.14: The synthesis of anomerically pure gemcitabine from 37.

Shen’s research demonstrates that employing groups that impart greater crystallinity during the synthesis of the intermediates may allow for enhanced processing by crystallisations, leading to an overall anomerically enriched synthesis.
1.1.1.3 – Leaving group

The Vorbrüggen reaction (also known as the silyl-Hilbert-Johnson reaction) employs nucleobases that are typically protected as their silylated equivalents and used directly for the subsequent reaction with a primed ribofuranose. Of particular note is the lack of selectivity of the desired β-anomer from the silyl-Hilbert-Johnson reaction of ribofuranose and the masked nucleobase. This reaction is more challenging due to the electron withdrawing nature of the geminal difluoro motif adjacent to the anomeric position.

One such factor that may affect the anomeric selectivity is the nature of the leaving group at the anomeric position. Hertel’s original synthesis utilised the mesylate leaving group, affording the desired β-anomer in an undesired 1:4 β:α ratio at 50% overall yield, delivering an effective 10% yield of β-1, shown in Scheme 1.15.

![Scheme 1.15](image)

**Scheme 1.15**: The synthesis of gemcitabine as an anomeric mixture by glycosylation with bis-(TMS)-cytosine.

The role of different Lewis acids was reported in a patent by Kjell, in an attempt to improve β-selectivity, listed in Table 1.01. Using 2-deoxy-2,2-difluoro-D-ribofuranosyl-3,5-dibenzoyl-1-α-methane-sulfonate as model substrate in combination with bis(TMS)-cytosine, it was shown that the selectivity may be improved simply by using anisole as solvent, delivering 77% yield of an anomeric mixture, favouring the β-anomer in a 3.4 to 1 ratio.

A range of inorganic Lewis acids were subsequently screened and were found to enhance the formation of the desired β-anomer – apart from potassium nonaflate (Table 1.01, Entry 2). One drawback of this methodology was that bis(TMS)-cytosine was frequently used in greater than 10 fold excess, which is not atom economical in a drug development context, but is irrelevant for positron emission tomography (PET) studies. The varying lengths of reaction were not discussed but would presumably be based on consumption of one of the starting materials. In general, there appears to be a trend of...
lower yielding reactions delivering improved selectivity (Entries 3-8). Larger cations caesium and barium demonstrated marked β-selectivity with the sulfate salts improving the anomic selectivity to greater than 90% (Entries 6 and 7), although barium sulfate delivers β-39 in greater yield. Employing barium triflate yielded 39 in a comparable 25% yield with very good selectivity (Entry 8); however, using caesium triflate significantly improved the yield to 65% while maintaining a high degree of β-selectivity at a ratio of 7.2:1, resulting in a greater than doubling of the effective yield of β-39 to at 57%. By using potassium carbonate, the yield was slightly improved to 70% (Entry 10), while maintaining β-enrichment.

![Chemical structures](image)

**Table 1.01:** The influence of various inorganic Lewis acids on the anomic ratio of 39 from bis-(TMS)-cytosine.

<table>
<thead>
<tr>
<th>Entry</th>
<th>MX</th>
<th>Time / h</th>
<th>Overall Yield[a]</th>
<th>β-39:α-39 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1[b]</td>
<td>-</td>
<td>20</td>
<td>77%</td>
<td>3.4:1</td>
</tr>
<tr>
<td>2[c]</td>
<td>C₄F₉SO₃K</td>
<td>16</td>
<td>33%</td>
<td>3:1</td>
</tr>
<tr>
<td>3[c]</td>
<td>K₂SO₄</td>
<td>72</td>
<td>65%</td>
<td>4.7:1</td>
</tr>
<tr>
<td>4[d]</td>
<td>KOTf</td>
<td>21</td>
<td>59%[e]</td>
<td>6.7:1</td>
</tr>
<tr>
<td>5</td>
<td>TBAOTf</td>
<td>4</td>
<td>45%</td>
<td>7.1:1</td>
</tr>
<tr>
<td>6</td>
<td>BaSO₄</td>
<td>20.5</td>
<td>36%</td>
<td>11.2:1</td>
</tr>
<tr>
<td>7</td>
<td>Cs₂SO₄</td>
<td>21</td>
<td>24%</td>
<td>14.9:1</td>
</tr>
<tr>
<td>8</td>
<td>Ba(OTf)₂</td>
<td>20.5</td>
<td>25%</td>
<td>14.4:1</td>
</tr>
<tr>
<td>9</td>
<td>CsOTf</td>
<td>20.5</td>
<td>65%</td>
<td>7.2:1</td>
</tr>
<tr>
<td>10</td>
<td>K₂CO₃</td>
<td>45</td>
<td>70%</td>
<td>7.2:1</td>
</tr>
</tbody>
</table>

A complementary study was conducted by Liu et al.\textsuperscript{[16]} in which they screened a range of conditions for the reaction of 34 with bis(trimethylsilyl)-$N^\alpha$-benzoyl cytosine to form 40 stereoselectively. Initially tin (IV) chloride was employed as stoichiometric Lewis acid in refluxing chlorobenzene and delivered 40 in a combined yield of 43\% (Table 1.02, Entry 1). Changing Lewis acid to trimethylsilyl trifluoromethane sulfonate (Entry 2) inverted the anomeric selectivity in preference of $\alpha$-40 in a 20:11 fashion, in a slightly lower yield of 44\%.

![Reaction Scheme]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additives\textsuperscript{[a]}</th>
<th>Yield\textsuperscript{[b]}</th>
<th>$\beta$-40:$\alpha$-40 Ratio\textsuperscript{[c]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SnCl\textsubscript{4}</td>
<td>43%</td>
<td>4.9:1</td>
</tr>
<tr>
<td>2</td>
<td>TMSOTf</td>
<td>44%</td>
<td>11:20</td>
</tr>
<tr>
<td>3</td>
<td>SnCl\textsubscript{4}, cat. TMSOTf</td>
<td>58%</td>
<td>6.3:1</td>
</tr>
<tr>
<td>4</td>
<td>SnCl\textsubscript{4}, cat. TBAOTf</td>
<td>62%</td>
<td>6.7:1</td>
</tr>
<tr>
<td>5</td>
<td>SnCl\textsubscript{4}, cat. TBAC</td>
<td>76%</td>
<td>5.9:1</td>
</tr>
<tr>
<td>6</td>
<td>SnCl\textsubscript{4}, cat. TBAB</td>
<td>68%</td>
<td>6.6:1</td>
</tr>
<tr>
<td>7</td>
<td>SnCl\textsubscript{4}, cat. TBAI</td>
<td>82%</td>
<td>8.0:1</td>
</tr>
<tr>
<td>8\textsuperscript{[d]}</td>
<td>SnCl\textsubscript{4}, cat. TBAI</td>
<td>&lt;5%</td>
<td>-</td>
</tr>
<tr>
<td>9\textsuperscript{[e]}</td>
<td>SnCl\textsubscript{4}, cat. TBAI</td>
<td>43%</td>
<td>1.7:1</td>
</tr>
</tbody>
</table>

\textsuperscript{2.65 equiv. of silylated nucleobase. 34 $\alpha$:$\beta$ = 1.6:1. \textsuperscript{[a]} 6 equiv. of SnCl\textsubscript{4}/TMSOTf, 8 mol\% of cat. \textsuperscript{[b]} Isolated yield after chromatographic purification. \textsuperscript{[c]} Determined by $^1$H NMR. \textsuperscript{[d]} Bis(TMS)-cytosine used as nucleobase instead. \textsuperscript{[e]} 2 equiv. of SnCl\textsubscript{4}.}

\textbf{Table 1.02}: The influence of various inorganic Lewis acids on the anomeric ratio of 40 from bis-(TMS)-$N^\alpha$-Bz-cytosine.
Combining the two activators, SnCl₄ and catalytic amounts of TMSOTf, improved both the yield of 40 and the selectivity towards β-40 (Entry 3). Following from this, a range of alternative tetrabutyl ammonium salts were screened in catalytic quantities (Table 1.02, Entries 4-7).

It was found that their inclusion promoted the selective formation of β-40 in good yields, with tetrabutylammonium iodide being the most selective and highest yielding (Entry 7). The observed effect could be due to an in-situ formation of the 1-iodoribofuranose intermediate, prior to attack of the nucleoside.[16] Use of non-N⁴-functionalised cytosine did not afford the desired product (Entry 8), while decreasing the equivalents of SnCl₄ had a deleterious effect on the reaction.

Interestingly, they found that treatment of an anomeric mixture of 34 (Scheme 1.16) with tin(IV) chloride generated 1-chlororibofuranose, which may be the active electrophile in the glycosylation reaction. Notably, 41 was formed as the α-anomer predominantly in a ratio of 7.7:1, shown in Scheme 1.16. The inclusion of tetrabutylammonium iodide improved the conversion of the transformation to quantitative, without significantly affecting the anomeric ratio of 41, again potentially invoking the in-situ formation the 1-iodoribofuranose.

The tosyl leaving group has also been employed by Shen and co-workers, as discussed previously,[14] and while although not shown to improve the anomeric selectivity, did improve the crystallinity of the intermediates synthesised.

Researchers have also targeted 1-halo-ribofuranose systems directly, and not the apparent in situ generation highlighted methodologies previously mentioned. One such example was the work by Chang et al, who targeted the 1-bromo-2,2-difluororibofuranose.[17] Having successfully accessed 42 by adaptation of the Reformatsky style synthesis (not shown), sequential reduction by LTBA and phosphorylation with diphenylphosphoryl chloride in toluene with triethylamine to afforded β-43 selectively, in
77% yield over two steps and recrystallisation from 'PrOH/H₂O (3:1 v/v). Treatment of β-43 with 30% hydrogen bromide in acetic acid delivered an 82% yield of α-44, following recrystallisation from isopropanol. The overall transformation is depicted in Scheme 1.17.

Scheme 1.17: The synthesis of α-44 by sequential reduction, lactol activation and bromination. PhBz = 4-phenylbenzoyl.

Treatment of α-44 with bis-TMS-cytosine, using heptane to aid in the distillation of by product TMSBr from the reaction mixture, afforded 92% of 45 as a mixture of anomers, preferentially forming the desired β-nucleoside in a 5.5:1 ratio (Scheme 1.18). Subsequent treatment of 45 with methanolic ammonia cleaved the ester protecting groups, delivering anomerically pure gemcitabine hemihydrate, in 71% yield.

Scheme 1.18: The synthesis of anomerically pure gemcitabine from α-44.
Notably in Chang’s work, there seems to be a large degree of SN2 character to the glycosylation reaction, with a configuration inversion at the anomeric position from pure α-44 to β-45 as the major product. This is somewhat contradictory to previous reports which suggest a SN1 pathway.\textsuperscript{[13,14]}

One explanation for this observation was described in a patent by Hwang and co-workers, who were targeting the synthesis of gemcitabine via the analogous 1-iodo-2,2-difluoro-ribofuranose,\textsuperscript{[18]} shown in Scheme 1.19. They achieved the synthesis of 47 by two methods; firstly by sequentially reacting tritylated lactol 46 with mesyl chloride under basic conditions, followed by mesyl displacement by iodide, delivering 47 in 76\% yield. Their alternative methodology was an adapted Appel reaction, using iodine and triphenylphosphine in the dark, which yielded 97\% of 47. In both examples, the diastereomeric ratio of the product was omitted.

\begin{center}
\textbf{Scheme 1.19:} The comparison of synthetic methods for the production of intermediate 47.
\end{center}

Hwang found that combining iodo precursor 47 with bis-TMS-cytosine (Scheme 1.20) in the presence of ammonium persulfate as oxidant in hot acetonitrile delivered the β-anomer with remarkable selectivity, in an 18:1 fashion over the α-anomer, although no yield was noted.

\begin{center}
\textbf{Scheme 1.20:} The formation of 48 by glycosylation reaction of 47.
\end{center}
The impressive selectivity was attributed to the involvement of the 3-O-benzoyl group, in a process that may be described as neighbouring group participation. Illustrated in Scheme 1.21, 1-iodo-ribofuranose 47 may attack iodine, which is generated \textit{in situ} by the oxidation of iodide by persulfate, following iodide exclusion from 47. Subsequent formation of oxocarbenium intermediate 49 by triiodide displacement facilitates attack of the carbonyl oxygen of the 3-O-benzoyl motif onto the 1-position, forming an alternative 6-membered oxocarbenium intermediate, which provides an additional stabilising resonance form (50). Attack of bis-TMS-cytosine onto intermediate 51 results in breaking the charged heterocycle and reforming the benzoyl group, yielding β-48. The anionic assistance of the 3-O-ester group helps selectively form the beta anomer of 48. This mechanistic proposal would also support Chang’s observation,\cite{17} as their glycosylation substrate contains a 3-O-ester group that could be involved in a neighbouring group participation style process.

![Scheme 1.21: The proposed reaction mechanism explaining the observed anomeric selectivity when using 47.](image)
1.1.1.4 – Pyrimidine ring formation

While construction of the ribofuranose ring is critical for the introduction of the geminal difluoro motif, less attention is paid to the construction of the pyrimidine ring. One of the primary reasons for this is that the ring may be easily accessed and installed from a protected cytosine. If a complementary methodology were realised offering improved anomeric selectivity, it would be a useful tool.

Linclau and co-workers investigated the construction of the pyrimidine ring beginning from commercially available lactol 52. Treatment of 52 in combination with urea in the presence of para-toluenesulfonic acid and dehydrating sodium sulfate delivered 54 in 88% yield after 36 hours of reflux (Scheme 1.22).

![Scheme 1.22](image-url)

The ratio of diastereomers of 54 was noted, but the relative assignment wasn’t possible. Taking 54 as a mixture forward, reacting it with acyl chloride 55, furnished urea derivative 56 in 71% yield in a lower anomeric ratio. The anomerisation was attributed to ring opening of the ribofuranose by deprotonation of the urea motif, aided by the electron withdrawing difluoro moiety. Switching solvent to chloroform and use of zinc chloride as Lewis acid aided in maintaining the anomeric ratio from before, albeit, with a diminished yield of 45%, shown below in Scheme 1.23.

![Scheme 1.23](image-url)

**Scheme 1.23:** The synthesis of N-acrylurea derivative 56.
Acid mediated ring closure yielded uracil congener 57 quantitatively, with diminished anomeric ratio. Subsequent treatment with 2-chlorophenyl phosphorodichloridate as chlorinating agent and 1,2,4-triazole for 5 days at ambient temperature in pyridine furnished 24% of impure 58, which was then treated with methanolic ammonia for 36 hours to produce gemcitabine in 49% yield as a mixture of anomers (Scheme 1.24).

Scheme 1.24: The acid-promoted ring closure of 56, chlorination and 1,2,4-triazole attack for intermediate 57, and ammonia-mediated transformation for the synthesis of 1.
1.1.2 – Ribonolactone halogenation

One potential drawback of the aforementioned methodologies is the installation of the geminal difluoro moiety from an early stage by Reformatsky reaction. Modification of this strategy to include alternative halogens is unattractive given the susceptibility of other halogens to partake in zinc-based reactions. The opportunity to functionalise a mono- and di-fluorinated analogue of intermediate ribonolactone 61 was realised by Cen and Sauve, from protected 2-deoxy-D-riboic acid-1,4-lactone.[20] They were investigating the diastereoselective fluorination (and difluorination) of γ-lactones, accessed via enolate chemistry and appropriate electrophilic halogenating reagents. This methodology allows for installation of the desired motif via two succinctly different steps, which arguably may not be as efficient but allows for tunability and modification – which is a key attribute when designing the synthetic route of a radiolabelling precursor and associated derivatives for PET studies.

Their preliminary findings, employing para-chloro-benzoyl ester protecting groups – which have previously found extended use in the synthesis of similar ribonolactones – had a deleterious effect on the reaction. Initial α-fluorination of 59 was attempted utilising NFSI and LiHMDS, which yielded the α,β-unsaturated γ-lactone, fluorinated at the 4- position (Scheme 1.25).

\[ \text{Scheme 1.25: The fluorination/elimination of 59 by NFSI and LiHMDS.} \]

The formation of the α,β-unsaturated-γ-fluoro-γ-lactone can be explained when considering the mechanism of the reaction (Scheme 1.26). Basic LiHMDS deprotonates the most acidic proton, at the alpha position of the lactone, to form the lithium enolate, which then cascades in an E1cB fashion, eliminating an alkoxide from the 3- position of the tetrahydrofuran ring. A second equivalent of LiHMDS can subsequently deprotonate at the γ-position of the α,β-unsaturated lactone, funnelling through to the lithium 1,2-3,4-dienolate, which in turn will cascade back around the ring to pick up the electrophilic fluorine from NFSI at the γ-position. The observation was further rationalised by considering the pK_a of the conjugate acid of the carboxylate (c.f. pK_a [H_2O] ≈ 4).
Consequently, alternative protecting groups were explored – which may be conveniently accessed from commercially available 2-deoxy-D-ribo-1,4-lactone. Therein switching to TBDMS protected alcohols, mono-fluorinated product 62 was successfully synthesised in 58% yield under the same reaction conditions. Unfortunately, 38% of the equivalent α,β-unsaturated-γ-fluoro-γ-lactone was also obtained, shown in Scheme 1.27. While demonstrating an improvement on their previous methodology, unwanted side reactions remained an issue.

Scheme 1.26: A proposed reaction mechanism for the formation of 60. R = pCl-Bz.

Scheme 1.27: The mixture of products obtained by reacting 61 with NFSI and LiHMDS.

Scheme 1.28: The diastereoselective fluorination of 64 by NFSI and LiHMDS.
The alternative reactivity was circumvented using bulkier triisopropylsilyl protected alcohols, in which no eliminated product was detected, isolating the α-fluorinated lactone \( \text{65} \) in 72% yield in diastereoselective fashion (Scheme 1.28). This was attributed to a less favourable leaving group (\( \text{pK}_a \) of silanol \( \approx 11^{\text{[21]}} \)), and the bulky silyl ether inducing a puckered ring conformation to minimise steric interactions. The puckered conformation also enforced a geometry whereby elimination of the silanoate would be reduced.\(^{[20]} \)

Alternatively, \( \text{68} \) may also be accessed via a circuitous 3-step strategy shown in Scheme 1.29 – although the author’s goal was to access the ribono stereoisomer. Initially, lactone \( \text{61} \) was sequentially treated with triethylamine and TMSOTf, then NBS to furnish a diastereomeric mixture of α-brominated lactone \( \text{66} \) in 55% isolated yield. Fluorination of \( \text{66} \) by treatment with NFSI and LiHMDS delivered \( \text{67} \) as a single diastereomer, in 55% yield. Use of catalytic azobisisobutyronitrile as radical initiator and tributyltin hydride for the radical bromide abstraction of \( \text{67} \) yielded only arabino \( \text{68} \), although no yield for the final product was reported.

![Scheme 1.29: The three-step strategy towards the synthesis of arabino 61.](image-url)
Synthesis of the ribono stereoisomer 69, illustrated by Scheme 1.30, was achieved by treating protected lactone 61 with NEt₃ and TMSOTf to yield intermediate 68 in 72% yield. Reacting 68 with NFSI and LiHMDS yielded 33% of 69, but also 61% of initial starting material 61 was recovered and may be reused.

With both mono-fluorinated diastereomers in hand, DIBAL mediated reduction afforded the corresponding lactol (Scheme 1.31) in excellent yields.

Subsequent treatment of 70 with MsCl and triethylamine quantitatively yielded the chlorinated furanose solely as the α-anomer (72, Scheme 1.32). Similarly, reacting 71 with NEt₃ and MsCl yielded chlorinated ribofuranose in quantitative yield, this time as an anomeric mixture. By comparison, reacting difluororibolactol 73 under the same conditions delivered the O-mesyl compound. This observation was ascribed to a
deactivation of $73$ by the geminal difluoro motif towards chlorination, not observed in the cases of $70$ and $71$.

Crucially, Cen and Sauve demonstrated that diastereoccontrolled $\alpha$-fluorination of suitably protected lactones was possible, while also illustrating the potential for alternative halogens to be introduced.

**Scheme 1.32**: The mesylation of $70$, $71$ and $73$ under basic conditions delivering different compounds depending on configuration at the 2-position of the lactol.
1.1.2.1 – Mixed halogen ribofuranoses

Following Cen and Sauve’s seminal work, Schinazi and co-workers reported the synthesis of 2'-bromo-2'-fluoro-nucleosides, in their phosphoramidate Protide form (discussed later), as pharmaceutical agents for the treatment of hepatitis C virus (HCV). The utilised strategy related to that developed by Cen and Sauve, whereby they initially protected 2-deoxy-D-ribone-1,4-lactone 75 using TBDPSCl and imidazole in DMF to furnish silyl ether 76 in 85% yield (Scheme 1.33). Combining 76 with NFSI and LiHMDS furnished 29% of the protected 2-fluoro arabinolactone 77 in a low yielding reaction.

**Scheme 1.33**: The synthesis of bromo-fluoro intermediate 79 from 2-deoxy-D-ribonic acid-γ-lactone (75) by fluorination-bromination strategy.
Subsequent treatment with NBS and LiHMDS yielded a near one-to-one diastereomeric mixture of the geminal dihalogenated γ-lactone (78 and 79). Taking the desired β-diastereomer forward (79), reduction by lithium tri(tert-butoxy)aluminium hydride effectively delivered lactol 80, before quantitative mesylation to produce 81.

Notably the activated lactol is observed as the O-mesyl compound thus agreeing with Cen and Sauve's observation that adjacent geminal dihalogenated ribofuranoses preferentially form compounds akin to 74 (Scheme 1.32), and not the chlorinated congener. A drawback of this strategy is evident in the product yields for the fluorination and bromination steps, with both returning target materials at isolated yields less than 30%, equating to an 8% across two steps.

Treating activated lactol 81 with in situ silylated N\(^4\)-benzoyl cytosine furnished the Vorbruggen product 83 in a combined yield of 55%. Subjecting the anomic mixture to deprotection by TBAF delivered 24% of 84 as the pure β-epimer (Scheme 1.34). Subsequent removal of the benzoyl protecting group by methanolic ammonia produced 85 in excellent yield.

**Scheme 1.34**: The synthesis of anomerically pure 85 from 81.
Following this, Schinazi and coworkers changed tact targeting the alternative diastereomer, in order to evaluate its cytotoxicity against HCV.\textsuperscript{[23]} Similarly, they commenced from a silyl ether protected ribonolactone (61, Scheme 1.30) only this time brominated first prior to electrophilic fluorination to yield 67 as the sole diastereomer, in yields of 82% and 64% respectively (Scheme 1.35). Formation of the primed lactol was achieved via sequential reduction of 67 by LTBA and activation by benzoyl chloride delivered 86 in 81% across two steps. This approach illustrated that activated lactol 86 could be achieved in 43% over 4 steps from 61, with good yields across the board.

![Scheme 1.35: The synthesis of α-bromo-β-fluoro intermediate 86 from 61 by bromination-fluorination strategy.](image)

As previously, appending 86 with $N^\delta$-benzoyl cytosine in the presence of TMSOTf under microwave irradiation yielded 87 as the coupled product, isolated as the pure β-anomer, which somewhat surprisingly had been deprotected during the Vorbruggen reaction. This observed reactivity was accredited due to increased reaction times, under microwave conditions. Like their previous report, the undesired α-epimer was formed preferentially in a 2:1 ratio.

Complete removal of the silyl ether protecting group mediated by fluoride, followed by benzoyl removal to produce free nucleoside 88 in 86% yield over two steps (Scheme 1.36).
Related to work conducted by Schinazi and co-workers, Voight et al were simultaneously investigating 2'-bromouridine derivatives for HCV treatment.\[24\] Shown in Scheme 1.37, their synthetic strategy was closely connected, where 64 was treated with NFSI and LiHMDS to deliver 71% of 65 as a single diastereomer, as observed previously – although inconsequential. Initially, electrophilic bromination was successfully achieved through NBS and LiHMDS (conditions A, Scheme 1.37) to generate 89 in 78% as an inseparable diastereomeric mixture, in favour of the desired α-fluoro-β-bromo-configuration intermediate lactone 90. Changing the source of electrophilic bromine to dibromotetrachloroethane with zinc chloride not only increased the reaction yield to 92% but crucially the diastereoselectivity. The authors state that the inclusion of ZnCl$_2$ delivers an *in situ* zinc enolate intermediate, manifesting an improved selectivity. Conversion of 65 produced *via* conditions B of Scheme 1.37, to lactol 90 by DIBAL–H proceeded well in 72% yield.

**Scheme 1.36:** The synthesis of 2'-deoxy-2'-α-bromo-2'-β-fluorocytidine (88).
After initially attempting activation and glycosylation through benzoylation and reacting with \textit{in situ} silylated $N^4$-Bz-cytosine, the desired β-anomer of the intermediate was delivered only in 5% yield, in a 1:9 ratio of the β:α epimers. Alternatively, changing the protecting groups and lactol leaving group was explored, whereby use of the same motif for both the leaving group and protecting groups facilitated a cleaner reaction. As such, reacting lactol 90 with \textit{para}-methoxybenzoyl chloride, followed by deprotection by TBAF and reprotection as the PMBz esters yielded 91 in 88% over 3 steps (Scheme 1.38).

\textbf{Scheme 1.37}: The synthesis of α-fluoro-β-bromo intermediate 90 from 64 by fluorination-bromination strategy.

\textbf{Scheme 1.38}: The activation of lactol 90 by \textit{para}-methoxybenzoyl chloride, TBAF deprotection and reprotection with \textit{para}-methoxybenzoyl chloride to form 91.
The authors contextualise the synthetic strategy towards forming 85, shown in Scheme 1.34, as impractical on multi-kilogram scale, given the need for two cryogenic reaction steps for the key halogenation steps and purification by column chromatography. Additionally, the use of TIPS as hydroxyl protecting groups – key for the halogenation reactions – was inefficient as they required replacement prior to glycosylation, in order to afford a cleaner reaction profile.

Much like Hertel’s original synthesis, the second strategy (Scheme 1.39) towards construction of 85 began with a protected glyceraldehyde (17), in combination with ethyl dibromofluoroacetate and ZnEt$_2$ as zinc source, delivering 93 as a single diastereomer in a low 25% yield. Although selectivity was poor, the product could be separated effectively by crystallisation – a factor critical in the choice of the cyclohexylidene ketal as protecting group, as other protecting groups investigated were non-crystalline. Deprotection of cyclohexylidene ketal 93 by pTSA in acetonitrile/water mixture delivered triol ester as an intermediate. The hydrolysis was driven to completion by azeotropic distillation of the reaction mixture to remove by-product cyclohexanone. Lactonisation in butyronitrile delivered crude 94, purified by crystallisation from chlorobenzene and DCM as antisolvent, affording hydrolytically unstable lactone 94 in 90% yield.

Scheme 1.39: The activation of lactol 95 by para-methoxybenzoyl chloride, TBAF deprotection and reprotction with para-methoxybenzoyl chloride to form 91.
Protection of 94 with para-methoxy benzoyl chloride, catalytic DMAP and pyridine as base, followed by reduction by LTBA yielded 74% of lactol 95, in a 1:1 ratio of diastereomers. Reacting 95 with para-methoxy benzoyl chloride delivers activated lactol 91 in 89% yield, as 3:2 mix of anomers. An important aspect of the developed methodology illustrated in Scheme 1.39 is the purification of the intermediates by crystallisation (where possible) – 95 crystallised from heptanes/EtOAc, and 91 from iPrOH – avoiding purification by column chromatography.

Prior to glycosylation, uracil (96) was protected using HMDS as TMS source with catalytic ammonium sulfate in chlorobenzene to form 97 quantitatively (Scheme 1.40). The resulting bis-TMS uracil derivative was reacted with 91 under Vorbruggen like conditions to yield 98 selectively in 51% yield. The researchers evaluated the parameters and conditions of the glycosylation reaction, revealing that lower temperatures reduced 97 reacting with a second equivalent of 91. Consequently, reaction time was increased to allow the reaction to proceed to completion. Omitting TMSOTf from the reaction mixture produced the cleanest reaction profile, using tin(IV) chloride as the sole Lewis acid. Subsequent deprotection yielded 2'-deoxy-2'-α-fluoro-2'-β-bromouridine (99, Scheme 1.41) in 82% isolated yield.

**Scheme 1.40:** The protection of uracil by HMDS with catalytic ammonium sulfate to form 97.
**Scheme 1.41**: The glycosylation of 91 with 97 and ammonia deprotection to yield 2'-deoxy-2'-α-fluoro-2'-β-bromouridine (99).
1.1.3 – Gemcitabine prodrug and ProTide strategies

As previously mentioned, one of the issues with the efficacy of gemcitabine is its hydrophilicity and hence poor uptake into fatty pancreatic cells. As such, substantial efforts have been made in order to improve the compound’s lipophilicity hence its bioavailability. Additionally, high dose treatments of orally delivered gemcitabine has been found to increase the risk of hepatotoxicity and gastrointestinal toxicity likely due to lack of selectivity in targeting specific areas of the body.

From a PET perspective, the application of this approach would also be attractive as improved bioavailability would allow for the radiolabelled agent to target the problem area with improved effectiveness and at shorter times. Moreover, utilisation of $^{18}$F PET would also offer an excellent diagnostic tool in drug development in candidate therapeutic agents and screening patients.

1.1.3.1 – LY2334737

One such example is that investigated by Eli Lilly, who focussed on modifying gemcitabine at the $N^4$ position on the cytidine ring.\textsuperscript{[25]} They targeted an amide linker in order to circumvent the deleterious effect of dCDA, while the functionality would also remain stable under enzymatic and chemical hydrolysis conditions. The compound investigated by Bender \textit{et al} contained a valproamide moiety, and was coined LY2334737 (100, Scheme 1.42). The prodrug is hydrolysed by enzyme carboxylesterase 2 (CES2) to liberate the active agent (pre-phosphorylation) and valproic acid.

The synthetic methodologies discussed always commenced from gemcitabine (hydrochloride) and explored 3 different routes by which to synthesise 100 (Scheme 1.42). The first route appended valproic acid to a bis-Boc protected gemcitabine\textsuperscript{[26]} using EDC coupling conditions, prior to TFA mediated deprotection to afford LY2334737 in 42% yield over 2 steps (38% over 3 steps). Alternatively, utilising CDI and valproic acid with a bis-TMS protected intermediate yielded 96% of 100 after work up. Interestingly, a synthetic route was developed that avoided using protecting groups, by switching solvent system and employing peptide coupling conditions (EDC, HOBt, NMM), producing 100 in excellent yield (95%). Their preliminary results demonstrate that LY2334737 is highly stable to both chemical hydrolysis, by investigating stability under a range of pHs, and enzymatic hydrolysis against small intestine homogenates.
Having successfully synthesised 100, it was screened against human colon HCT-116 cells in mice and was found to overcome deamination by dCDA and display comparable tumour reduction (% vehicle) to gemcitabine.

Further Phase I studies then commenced using LY2334737, in order to ascertain maximum tolerated dose (MTD) and dose limiting toxicities (DLTs) of the orally taken compound. The MTD of 100 was found to be 40 mg, as a standalone treatment or a combination therapy with erlotinib. Schellens and co-workers noted that only 2 out of 65 European patients suffered hematologic toxicity – a principal DLT observed when patients are treated with gemcitabine. This observation was attributed to a lower effective gemcitabine concentration over the course of LY2334737 administration vs intravenous gemcitabine regimes. It was also mentioned that dFdU was observed over the course of 100 regime, but 0.75 fold lower than gemcitabine over a two week treatment course demonstrating a marked improvement.

A second European study focussed on the potential to increase the MTD limit as the recommended dosage ahead of Phase II trials of patients with advanced/metastatic solid tumours. Patients exhibited DLTs when the dose level was increased to 100 mg; therefore 90 mg for a 21 day dose regime (followed by 7 days rest) was considered the new MTD. The new dosage was found to display linear pharmacokinetics and safety profiles of a sufficient standard. Raymond and co-workers also reported that administration schedules played a role in DLTs exhibited, in agreement with other literature findings, where administering the drug every other day (QD treatment) led to less DLTs.
Further studies investigated the MTD and found ethnicity to be a factor. Tamura and co-workers found that the MTD was lower for their work, carried out on 13 Japanese patients with advanced or metastatic solid tumours.\cite{29} Alarmingly, 3 out of 4 patients on the 40 mg course suffered DLTs such as hepatic toxicities and disseminated intravascular coagulation when on a QD treatment course. It was found that 30 mg was the MTD cut off point, attributed to lower body surface area that Japanese patients possess leading to increased area under the curve (AUC). In Tamura’s studies, the mean AUC of LY2334737 was 328 ng.h mL$^{-1}$, compared to 244 ng.h mL$^{-1}$ in Schellens’ work – although it is noted that clinical relevance of these findings is unclear.

A concurrent Phase 1b study by Adjei and co-workers into a combinative QD therapy of LY2334737 and capecitabine was halted due to Tamura’s findings,\cite{30} resulting in the MTD not being established for the investigated combination regime. They noted that QD administration of LY2334737 for 21 days, followed by 7 days rest, is not optimal for risk/benefit ratio.

Further Phase 1 investigation by Llombart and co-workers looked into using docetaxel in conjunction with 100, revealing a 30 mg QD regime of 100 and 70 mg of docetaxel gave rise to a detrimental toxicity profile, leading to an eventual MTD of 10 mg day$^{-1}$. Their finding infer a negative effect of docetaxel, despite its successful combination with gemcitabine,\cite{31} although no reason is offered for the observed impact. Llombart’s work was also suspended following Tamura’s findings, which ultimately led to further trials into LY2334737 being discontinued.

1.1.3.2 – NUC-1031 and ProTide strategies

Alternatively, improved cell membrane permeation of gemcitabine has been targeted via pre-installation of a phosphate group at the 5’ hydroxyl group of the compound. Converting the nucleoside to a monophosphate nucleotide overcomes the rate limiting step of initial phosphorylation by dCK, which in turn reduces deleterious conversion to dFdU. This strategy was termed ProTide and pioneered by McGuigan and coworkers.\cite{7,32} However, introduction of the 5’-O-monophosphate derivatives would be ineffective, showing poor efficacy due to their negative charge under physiological conditions and may be prone to dephosphorylation. As such, the ProTide strategy underwent a period of refinement and found that aryloxy phosphoramidates demonstrate the greatest selectivity for uptake into cells. These derivatives were chosen due to their tunability, illustrated in Figure 1.04 with 3’-azido-3’deoxythymidine as the nucleoside core. The modular components can be broken down into three distinct areas:
i. Carboxyl ester – modified to increase cellular uptake, by using lipophilic groups such as benzyl or 2-naphthyl. It was noted; however, that it is critical to balance the improved uptake with efficient ester hydrolysis \textit{in vivo}; use of tert-butyl esters increased incorporation into cells but was resistant to hydrolysis by PLE.\textsuperscript{[33]} Other esterases (such as cathepsin A) have been found to play a predominant role in hydrolysing the ester to the acid congener.\textsuperscript{[34]}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{The general structure of a ProTide prodrug, with $3'$-azido-$3'$-deoxythymidine (101) as model nucleobase.}
\end{figure}

ii. Amino acid side chain – demonstrates indirect effect on ester hydrolysis. Steric bulk was found to restrict hydrolysis when using cathepsin A as the esterase for isoleucine,\textsuperscript{[34]} but cathepsin G efficiently cleaved phenylalanine containing prodrugs.\textsuperscript{[35]} Interestingly, use of glycine as the amino acid yielded a less cytotoxic prodrug than cysteine – however, alanine containing prodrugs were the most active when tested against $2',3'$-didehydro-$2',3'$-dideoxythymidine.\textsuperscript{[36]} Wagner and co-workers also observed that altering the amino acid stereochemistry, from L- to D-phenylalanine, could produce up to a 200-fold increase in activity.\textsuperscript{[37]}

iii. Aryloxy motif – its primary function is as a leaving group to form the active monophosphate. Aryloxy moieties were found to demonstrate significantly greater antiviral activity over alkoxy motifs, which shows little to no meaningful antiviral activity.\textsuperscript{[38]} Substituted phenoxy groups were investigated by Siddiqui \textit{et al}, and found mildly electron withdrawing substituents, such as 4-chloro, corresponded to increased activity \textit{in vitro}.\textsuperscript{[39]} Further research typically utilised non-substituted phenyl.\textsuperscript{[40]} Moreover, the aryloxy group imparts further lipophilicity to the prodrug, with phenyl or naphthyl motifs targeted.
The mode of action is depicted in Scheme 1.43 and helps rationalise some of the aforementioned points. Once the ProTide has been taken across the cell membrane, the amino acid ester functionality is hydrolysed by esterases, such as cathepsin A,[34] to form 103 which exists as the carboxylate anion under physiological conditions. The newly formed carboxylate motif then nucleophilically attacks the phosphoramidate, releasing the aryloxy leaving group and forming mixed cyclic anhydride 104. It is interesting to note that there is little discussion surrounding the effect of the liberated aryloxy species and its potential effect on bioactivity. For example, when Ar = Ph, the cleavage of phenol/phenoxide would likely impart some toxicity given that phenol is a known toxin. Metabolite 104 is then rapidly ring opened by water, which can do so by attacking either the carbonyl or phosphorous centre, generating 105. Attack at either position yields the same product, although no cleavage of the P-N bond has been observed.[41] The final step is cleavage of the P-N bond, the rate of which has been found to correlate to bioactivity.[37] The cleavage liberates the amino acid and nucleoside monophosphate 106, and is facilitated by a phosphoramidase enzyme.[42] Thereafter 106 is converted to the diphosphate and triphosphate in turn by phosphate kinases.[42]

Scheme 1.43: The in vivo mechanism of prodrug 102 by sequential esterase and phosphoramidase cleavage process to yield 5′-O-monophosphate 106.
Given gemcitabine’s poor conversion in vivo to the active dFdCDP and dFdCTP metabolites, it was an ideal candidate to be explored under the ProTide strategy. Investigative work by McGuigan and co-workers, in collaboration with NuCanna, synthesised and evaluated a range of gemcitabine protides.\[^{6}\] Extensive research culminated in 107 (NUC-1031, Acelarin®) being identified as the most suitable candidate to pursue clinical trials with. The L-Ala-OBn phenyl protide was chosen over other candidates due to its high cytotoxicity and metabolic stability, where a mid-range half-life in human hepatocytes was targeted. 107 overcame the three parameters for gemcitabine deactivation highlighted previously (Page 3), with a key observation being that NUC-1031 is not dependent on hENT1 in order to exert its anticancer effect. The 5-O functionalisation also inhibited deleterious deamination to toxic dFdU or derivatives thereof.\[^{43}\]

The most common strategy for synthesising the aryloxy phosphoramidate begins by reacting the aryl alcohol with phosphoryl chloride before combining the product of that with the appropriate amino acid. In the context of NUC-1031, phenol is reacted with phosphoryl chloride with triethyl amine as base in anhydrous diethyl ether affording phenyl phosphorodichloridate,\[^{44}\] which is subsequently reacted with L-alanine benzyl ester hydrochloride in dry DCM using triethyl amine as base, yielding 110 in 90% over two steps after column chromatography (Scheme 1.44).

**Figure 1.05:** The structure of gemcitabine-phosphoramidate NUC-1031, 107.
Reacting aryl aminoacyl phosphorochlorodate 110 with gemcitabine affords 107 in 16% yield in a rather unselective fashion,\[^{[45]}\] with side reactions likely occurring. Later findings by Slusarczyk et al use 3-O-Boc protected gemcitabine, which affords NUC-1031 in 60% yield over 2 steps, in a more controlled approach.\[^{[6]}\] Alternative approaches involved similar construction of the aryloxy phosphoramidate; Silverman and co-workers utilised pentafluorophenol as the leaving group,\[^{[46]}\] in conjunction with catalytic dimethyl aluminium chloride as Lewis acid (Scheme 1.45). Their method avoided employing protecting groups, resulting in an improved, highly selective 5-O functionalisation of 1 and delivered an 80% yield of NUC-1031. It is interesting to note that these compounds are chiral at phosphorous and are illustrated as such through $^{31}$P NMR analysis, revealing two phosphorous environments equating to the two diastereomers formed.\[^{[46]}\] In Silverman’s case, the use of enantiopure phosphoramidate ($R_p$)-111 allowed diastereoselective formation of ($S_p$)-107 through a phosphorous S$_{N}$2-type mechanism,\[^{[46]}\] with alternative approaches dependent on selective crystallisation or HPLC separation. Although both stereogenic phosphorus centres demonstrate anti-viral activity,\[^{[47,48]}\] the chosen diastereomer can have a pronounced effect resulting in a 10-fold or greater in vitro potency,\[^{[49]}\] due to diastereospecific enzyme binding.\[^{[42]}\]

Clinical trials are ongoing using NUC-1031, although recent setbacks have appeared when using 107 in Phase II studies.\[^{[50]}\]

Scheme 1.44: The synthetic route developed by McGuigan and co-workers towards the production of 107.
Similarly, Voight et al utilised the pentafluorophenoxy leaving group in their synthesis of ABBV-168.\(^2^4\) Depicted in Scheme 1.46 is the sequential treatment of phenyl dichlorophosphate (112) with 2-amino-isobutyric acid ethyl ester hydrochloride (113) and pentafluorophenol under basic conditions, which furnished 114 in 39%, following purification by HPLC.

From 114, extensive optimisation led to identification of phenyl magnesium chloride as base, in a solvent mixture of THF and DMPU in a 2:1 ratio, which produced 115 in 71% yield. The quoted conditions suppress side reactions, such as epimerisation at the phosphorous centre and diphosphoramidation at the 5’ and 3’ hydroxyl groups. Although the product 115 doesn’t include an amino acid in the phosphoramidate motif, the research depicted in Scheme 1.47 demonstrates the potential for alternative amine moieties in ProTides, in addition to the pentafluorophenoxy leaving group to improve selectivity.
1.1.3.3 – Clavis

Unprotected 3' hydroxyl groups have been shown to be critical for enzyme mediated deamination to the uridine congener to occur, while altering the 5' substituent did not dramatically alter deamination.\[51\] Later research by Amidon and coworkers revealed that 5'-O-amino acid derivates of gemcitabine were highly resistant to deamination by CDA, compared to gemcitabine itself.\[43\] As such, synthesis of 5'-O functionalised gemcitabine was targeted by a number of pharmaceutical companies. One such example was the elaidic acid ester derivative of gemcitabine, developed by Norwegian pharmaceutical company Clavis Pharma. Their rationale for the monounsaturated aliphatic ester was the improved lipophilicity towards the treatment of solid tumours, which could be enzymatically hydrolysed in situ to gemcitabine. Such targeted moieties were part of the company’s Lipid Vector Technology, which aimed to overcome agent uptake dependence on the expression of nucleoside transporters such as hENT1.

Its synthesis was reported in 1997,\[52\] in which gemcitabine was treated with elaidic acid chloride in acidic DMF, yielding the 5'-functionalised prodrug in 30% isolated yield after column chromatography (Scheme 1.48). The approach was unselective, with a small amount of the 3'-O congener also produced.

Initial clinical studies demonstrated lower half maximal inhibitory concentration for elaidic acid derivative 116 than gemcitabine, for the four cell lines tested. The improved cytotoxicity was attributed to an improved in vivo half-life over gemcitabine, arising from a poor binding mode of the modified chemotherapeutic agent 116 to CDA. Consequently, less 116 undergoes deactivating deamination. Additionally, inclusion of the monounsaturated motif means an independence on the nucleoside transporters gemcitabine is reliant on.\[53\]
Latterly, pancreatic cancer cell lines were treated with 116,\textsuperscript{[54]} but it was found that the study on low hENT1 expression and adenocarcinoma of the pancreas (LEAP) demonstrated no difference in survival rates of patients treated with 116 in comparison to gemcitabine. The result would suggest that expression of hENT1 in cancer patients does not play a key role in effectiveness of gemcitabine as a chemotherapeutic agent and the survivability of patients.

**Scheme 1.48:** The reaction of gemcitabine with elaidic acid chloride to form 116.
1.2 – Positron emission tomography

Positron emission tomography (PET) is a non-invasive imaging technique, which allows for cross-sectional images of the subject to be taken and in turn used to construct 3D images. It has found extensive use in the field of diagnostic cancer treatment, with a range of radiopharmaceuticals employed towards neurodegenerative diseases,\textsuperscript{[55]} hypoxia\textsuperscript{[56]} and a variety of cancers such as lung,\textsuperscript{[57]} ovarian\textsuperscript{[58]} and breast.\textsuperscript{[59]} Due to the high sensitivity of PET imaging and concentrations required for image acquisition, typically in the nanomole to picomole range, it is the imaging modality of choice within clinical oncology.\textsuperscript{[60]}

1.2.1 – Principles of PET Imaging

The underpinning physical principle of PET imaging is dependent on the use of unstable radionuclides that undergo radioactive decay pathways, resulting in the emission of a positron ($\beta^+$) and a neutrino.\textsuperscript{[61]} The emission is the result of an unstable proton becoming a neutron within the radionuclide, with the total number of nucleons remaining constant. Typical $\beta^+$ decay is illustrated in Eqn. 1.01 in the context of $^{18}$F.

$$^{18}_{9}F \rightarrow ^{18}_{8}O + ^{0}_{1}e^+ + \nu_e$$

(Eqn. 1.01)

The decay process is dependent upon the radionuclide, where the number of disintegrations per time unit is related to the number of radioactive nuclei, $N$, by the decay constant $\lambda$, shown in Eqn. 1.02:

$$\frac{dN}{dt} = -\lambda N$$

(Eqn. 1.02)

The above equation is also the mathematical form of the activity, $A_t$, of a radionuclide, defined as “the number of nuclear decays occurring in a given quantity of material in a small time, interval, divided by that time interval”.

$$A_t = -\frac{dN}{dt} = \lambda N$$

(Eqn. 1.03)

The unit of activity is the Becquerel (Bq), where 1 Bq is equal to one disintegration per second. It is more commonly noted with Curie (Ci) as the non-SI unit, where 1 Ci = $3.7 \times 10^{10}$ Bq. Specific activity can also be defined as “the activity of a material
divided by the mass of the tracer”, the units of which are Bq mol\(^{-1}\). Molar activity is the activity per mole, units of Bq g\(^{-1}\) and is more commonly used (Eqn. 1.04).

\[
A_t = \frac{\lambda N}{(m \cdot N)} = \frac{\lambda N_A}{m} \tag{Eqn. 1.04}
\]

Resolving Eqn. 1.03 leads to an expression for the number of radioactive nuclei at time \(t\), if the initial number of nuclei is known, as shown in Eqn. 1.05. Indeed this expression is also true of the activity of a radionuclide (Eqn. 1.06):

\[
N_t = N_0 e^{-\lambda t} \tag{Eqn. 1.05}
\]

\[
A_t = A_0 e^{-\lambda t} \tag{Eqn. 1.06}
\]

The half-life of a radionuclide of a single radioactive decay process, is “the time required for the activity to decrease to half its value by that process”, the solution for which is illustrated in Eqns. 1.08 and 1.09:

\[
N_{t_{1/2}} / N_0 = 0.5 = e^{-\lambda t_{1/2}} \tag{Eqn. 1.08}
\]

\[
t_{1/2} = \frac{\ln (2)}{\lambda} \tag{Eqn. 1.09}
\]

In the case of fluorine-18, the half-life is 109.8 minutes, decaying to stable nuclide \(^{18}\)O (Eqn. 1.01). By contrast some (radio)nuclei may undergo alternative decay pathways, such as \(\alpha\)-decay, isomeric transition (\(\gamma\) emission) or \(\beta^+\) decay, whereby an electron is emitted such as that seen in the decay of technetium-99 (Eqn. 1.10).

\[
^{99}Tc \rightarrow ^{99}Ru + ^0_1e \tag{Eqn. 1.10}
\]

\(\beta^+\) decay is a random decay pathway, which accounts for 97% of the emission profile of \(^{18}\)F. The emitted positron has an average energy of 250 keV but may be produced at energies as high as 630 keV. The remaining 3% of the emission profile is described by electron capture, the reverse process, where an electron from the radionuclide’s inner shell combines with a proton, forming a neutron and a neutrino (Eqn. 1.11).\(^{[62]}\)

\[
^{18}_{\beta^+}F + ^0_{\gamma}e^- \rightarrow ^{18}_{\nu_e}O + ^0_{\gamma}e \tag{Eqn. 1.11}
\]
Following emission from the radionuclide, the positron travels a distance from the point of emission – typically less than 1 mm, but has a maximum mean free path of 2.4 mm in H₂O – until colliding with an electron (Figure 1.06), annihilating both particles and producing two antiparallel gamma rays with an energy of 511 keV.[63]

![Image 1.06: Principle of PET illustrating annihilation of a positron with an electron. Two collinear γ-rays are emitted of 511 keV, which are used to construct the image.](image)

The emitted γ-rays are nearly antiparallel at approximately 180° - the deviation estimated at ±0.25° which is the cause of the loss in the spatial resolution of PET detectors.[64] The γ-rays are detected using photoscintillators, whereby the origin of annihilation can be discerned along the line of response. Critically, the γ-rays need to be aligned with the scintillation crystal on the detector, otherwise no detection will be noted.

![Image 1.07: Detection of γ-rays using scintillator with photomultiplier tubes, and their arrangement on a PET scanner.](image)
Photons of energy 511 keV are absorbed by the scintillation crystal, and subsequently reemitted as lower energy photons in the visible light region or UV.[64] The lower energy photons are detected by the photomultiplier tubes and converted into an electrical signal which can be read by a computer, subsequently allowing for an image to be created. As shown in Figure 1.07, a typical PET scanner is constructed of several individual detectors, in perpendicular fashion to the scanner (z-axis) which allow for maximum spatial mapping and increased spatial resolution of the image acquired. The detector unit may also map along the z-axis by the patient bed moving, resulting in the construction of a cross-sectional image. The signals generated on opposite detectors by impact of the γ-rays must occur within a coincidence time window of 6 – 12.5 ns to be judged as coincident.[65] If within the time frame, the coincidence event is denoted a line of response connecting the two detectors, allowing for positional information to be abstracted from the annihilation event.[66]

Indeed, this is an ideal scenario – termed true coincidence – as all annihilation processes are unlikely to behave in this fashion, due to many factors. The γ-rays may be absorbed by other matter before detection by the scintillator or scattered by another medium en route.

### 1.2.2 – $^{18}$F production

Fluorine-18 is produced in a cyclotron, which accelerates particles using a magnetic current to bombard a sample. Selected examples for the production of fluorine-18 are listed in Table 1.03,[62] and demonstrate the range of molar activities available depending on the irradiation method. If $[^{18}\text{F}]F_2$ is the desired target, bombardment of neon-20 gas (containing F2) with deuterium ions delivers carrier added $[^{18}\text{F}]fluorine$ gas, containing a isotopic mixture of fluorine-18 and fluorine-19 in low molar activity.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Nuclear reaction</th>
<th>Target</th>
<th>Product</th>
<th>Molar activity (GBq μmol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$^{20}\text{Ne}(d,\alpha)^{18}\text{F}$</td>
<td>$^{20}\text{Ne}$ (200 μmol F2)</td>
<td>$[^{18}\text{F}]F_2$</td>
<td>0.04 – 0.40</td>
</tr>
<tr>
<td>2</td>
<td>$^{18}\text{O}(p,n)^{18}\text{F}$</td>
<td>$^{18}\text{O}_2$ (50 μmol F2)</td>
<td>$[^{18}\text{F}]F_2$</td>
<td>0.36 – 2.00</td>
</tr>
<tr>
<td>3</td>
<td>$^{18}\text{O}(p,n)^{18}\text{F}$</td>
<td>$H_2^{18}\text{O}$</td>
<td>$[^{18}\text{F}]F^-$</td>
<td>$4\times10^4$</td>
</tr>
</tbody>
</table>

Table 1.03: Methods of $[^{18}\text{F}]$ production.
Alternatively, the radioactive gas may also be produced via high energy proton bombardment of enriched oxygen-18 gas, again containing fluorine-19 gas, delivering $[^{18}\text{F}]\text{F}_2$ in increased specific activity. Both aforementioned production methods deliver electrophilic fluorine, which can be directly used or converted into less reactive equivalents or synthetically useful $N[^{18}\text{F}]\text{F}$ reagents.$^{[67,68]}$

The greatest activity can be obtained by high energy proton irradiation of enriched $\text{H}_2^{18}\text{O}$, which delivers $[^{18}\text{F}]\text{F}^-$ as an aqueous solution. The process is depicted in Figure 1.08. The solution is eluted over a quaternary ammonium anion (QMA) exchange cartridge, which traps the fluoride-18. The QMA exchange resin is composed of polymer-bound $\text{R}_4\text{N}^+$ salts. The eluent, $\text{H}_2^{18}\text{O}$, is collected and disposed of. The bound $[^{18}\text{F}]\text{F}^-$ is eluted from the column using an acetonitrile/water mixture (4:1 v/v) containing potassium carbonate and Kryptofix 2.2.2 ($K_{222}$), as counterion source and chelator respectively, liberating $[^{18}\text{F}]\text{KF}$. The solution of $[^{18}\text{F}]\text{KF}$ in MeCN/H$_2$O is then azeotropically dried, ready for radiochemistry. Different counterions have also been employed, leading to the development of alternative nucleophilic sources of $[^{18}\text{F}]\text{F}^-$, such as $[^{18}\text{F}]\text{TBAF}$ and $[^{18}\text{F}]\text{CsF}$.$^{[69,70]}$

![Figure 1.08: Catch and release procedure of fluoride-18 by a QMA cartridge.](image)

The reactivity of the fluoride is highly dependent on its nature and environment. Typically, dissolved metal fluorides can act as good nucleophiles, but are rendered “inert” by hydration and the nucleophilicity of fluoride is outweighed by its basicity (Figure 1.09).
The advent of phase transfer catalysts (PTC) as reagents, such as chelators like $\text{K}_{222}$ or 18-crown-6 as shown in Figure 1.10, weaken the ion pairing of fluorides, accessing fluoride with greatly improved nucleophilicity hence reactivity, often termed "naked" fluoride.\cite{71} The chelator used can be changed to match the size of the cation used in the elution mixture to maximise encapsulation of the metal ion. Equally, tetralkylammonium cations can be used without these chelators.

![Figure 1.09: Hydration of dissolved metal fluorides.](image)

![Figure 1.10: Examples of "naked" fluoride by chelator (18-crown-6) and metal cation (left, 117) or tetrabutylammonium (centre). The structure of Kryptofix® 222 (119) is also shown for reference (right).](image)

### 1.2.3 – Application and principles of radiotracers

A range of radionuclides that undergo $\beta^+$ decay can be utilised as contrast agents for PET imaging. A representative set of radionuclides is presented in Table 1.04.

![Figure 1.11: Example radiotracers using $^{89}\text{Zr}$ (120) and $^{64}\text{Cu}$ (121).](image)
Depicted in Figure 1.11 is an example of a zirconium-89 radiotracer chelated to DOTA (120),[72] can also be used for protein labelling via a targeting vector,[73] while copper-64 has been used in combination with the ligand ATSM (121) for hypoxia imaging.[74]

While both these metal-based radiotracers have long half-lives, which would allow for image acquisition over a longer time period, their decay pathway with respect to $\beta^+$ emission is inefficient (decay pathway, Table 1.04).

By contrast, $^{18}$F has a more desirable half-life of approximately 110 minutes. As such, it makes it an ideal candidate within radiotracers as it would minimise radiation exposure to the patient, while also being appreciable on a biological timescale. Furthermore, fluorine is a commonly encountered bioisostere of the hydroxyl group, due to similar van der Waals radii and bond lengths to carbon.[75] Alternatively, nitrogen-13 and carbon-11 also undergo $\beta^+$ decay, and could be incorporated into candidate radiotracers as they will likely contain one of the two nuclides, providing an appropriate labelling strategy were in place. Critically however, is their significantly shorter half-lives. If there were a deprotection step following radiosynthesis, potentially half of the radioactivity could be lost for both radionuclides.

\textit{In vivo} tracking is the function of radiotracers, whereby a bioactive molecule mimicking its non-radiolabelled congener is introduced allowing for information regarding the biological system can be obtained. Incorporation of the radionuclide into the therapeutic agent should not significantly interfere or alter the pharmacokinetics of the metabolite.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Isotope</th>
<th>Half-life</th>
<th>$E_{\text{max}}$ $\beta^+$ / keV</th>
<th>Decay pathway / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$^{89}$Zr</td>
<td>78.4 h</td>
<td>897</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>$^{64}$Cu</td>
<td>12.7 h</td>
<td>653</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>$^{18}$F</td>
<td>109.8 min</td>
<td>633</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>$^{11}$C</td>
<td>20.4 min</td>
<td>960</td>
<td>&gt;99</td>
</tr>
<tr>
<td>4</td>
<td>$^{13}$N</td>
<td>10.0 min</td>
<td>1199</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>$^{15}$O</td>
<td>2.0 min</td>
<td>1735</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

Table 1.04: Selected examples of radionuclides that have found use within PET imaging.
This point is elegantly exploited by $[^{18}\text{F}]$fluorodeoxyglucose ($[^{18}\text{F}]$FDG, 123, Scheme 1.49), one of the most used radiotracers within the oncology field.

![Scheme 1.49: General scheme for the radiosynthesis of $[^{18}\text{F}]$FDG, 123.](image)

It was first synthesised in 1973 using $[^{18}\text{F}]$F$_2$,[76] but today it is more commonly synthesised using $[^{18}\text{F}]$KF. $[^{18}\text{F}]$FDG synthesis typically commences from mannose triflate (122), following by basic hydrolysis of the protecting acetate esters motifs (Scheme 1.01), in high yield and high molar activity. The precursor is produced from D-mannose in 16% yield over 5 steps,[77] and typically administered as a saline solution to patients. The synthesis of 123 in clinical faculties is conducted using automated instruments and disposable cartridges, using equipment such as those displayed in Figure 1.12.

![Figure 1.12: Commonly used instrument for the clinical production of $[^{18}\text{F}]$FDG, GE FASTlab 2 (left) and TRASIS AllinOne (right).](image)

![Figure 1.13: In vivo mode of action of $[^{18}\text{F}]$FDG (123).](image)
The *in vivo* activity of $[^{18}\text{F}]$FDG is key to the widespread use, illustrated in Figure 1.13. Once administered, the sugar analogue will accumulate in areas with increased metabolic activity with increased glucose demand, such as fast-growing cancer cells. It is reversibly transported into cells by glucose transporters and subsequently phosphorylated at the 6-position by hexokinase and adenosine triphosphate (ATP), producing an equivalent of adenosine diphosphate (ADP) as by-product. Once $[^{124}\text{F}]$ is formed, its anionic character means that it cannot be transported out of the cell, but equally cannot be metabolised further as it is lacking the requisite 2-OH- group for further glycolysis. As such, $[^{124}\text{F}]$ will accumulate within the sugar hungry cell, hence the radiolabelled compound will highlight the cells and where within the body they are during a PET scan.$^{[78]}$ Despite its extensive use within oncology, $[^{123}\text{F}]$ lacks specificity, hence targeting towards particular organs or diseases is difficult. As such, the development of targeted radiotherapeutics would allow for imaging with improved specificity.

Given the sensitivity of the technique, the amount of radiotracer needed to be administered to the patient is incredibly low, typically picomolar. Because the physiological concentration is low, the toxicological concerns aren’t as significant compared to the millimolar quantities required for therapeutic agents.

1.2.4 – $^{18}\text{F}$ radiochemistry

1.2.4.1 – Electrophilic fluorination

As previously discussed, one of the major pathways to access electrophilic fluorine-18 for radiochemistry is via $[^{18}\text{F}]$F$_2$ which is not widely available, nor conveniently handled or manipulated for chemistry. As such, multiple endeavours have surfaced attempting to use it indirectly for electrophilic fluorination.

Gouverneur and co-workers developed the radiosynthesis of $[^{18}\text{F}]$NFSI from the sodium dibenzenesulfonimide and $[^{18}\text{F}]$F$_2$ (Scheme 1.50),$^{[67]}$ and its application towards fluorination of silylated latent nucleophiles such as enol ethers. The method of synthesis is comparable to that which produces NFSI commercially,$^{[79]}$ and was shown to perform well in comparison to standard NFSI.

$[^{18}\text{F}]$NFSI was then successfully applied by Gouverneur to enantioselective fluorination of aldehydes (Schemes 1.50 and 1.51),$^{[80]}$ based on previous organomediated fluorination by MacMillan.$^{[81]}$
**Scheme 1.50:** The synthesis of [\(^{18}\)F]NFSI (126) and application to silylated latent nucleophiles.

**Scheme 1.51:** The organomediated enantioselective [\(^{18}\)F]-fluorination of aldehyde 133. \(^a\) RCC determined by radio-HPLC relative to [\(^{18}\)F]NFSI.
This work effectively demonstrated a merger of radiochemistry with organomediated enantioselective processes, with good radiochemical conversions and very high ee. Interestingly, all substrates were derived as their hydrazone congener, to minimise racemisation (Scheme 1.52). Radiochemical conversion is commonly used within the field, and calculated referring to the radioactivity of the active agent, in this case $^{[18}\text{F}\text{NFSI}}$.

![Scheme 1.52: The substrate scope of aldehydes explored with $^{[18}\text{F}\text{NFSI}}$. a RCC determined by radio-HPLC relative to $^{[18}\text{F}\text{NFSI}}$.

Following from this work, Teare et al. successfully synthesised $^{[18}\text{F}\text{Selectfluor bistriflate}$ as an alternative electrophilic radiofluorination agent. It was synthesised in comparable fashion to its non-radioactive analogue (Scheme 1.53), but triflate counter anions were employed due potential formation of $^{[18}\text{F}\text{BF}_4^-$. Alkylation of DABCO (139) by DCM followed by anion exchange yielded intermediate 140, with no yield noted. Fluorination of 140 by $^{[18}\text{F}\text{F}_2$ with lithium triflate delivered $^{[18}\text{F}\text{Selectfluor bistriflate}$ 141 in an average radiochemical yield of 37% across five runs.

![Scheme 1.53: The synthesis of $^{[18}\text{F}\text{Selectfluor bistriflate}$ (141) from DABCO (139).

The authors demonstrated that silyl ether 127 could be fluorinated in RCY up to 50% (not shown), but also showed that 141 could be used towards electrophilic fluorodestannylation of electron rich aromatics, in combination with silver triflate, shown in Table 1.05.
This application was illustrated for the synthesis of 6-[\(^{18}\text{F}\)]fluoro-L-DOPA,\(^{55,82}\) a commonly used imaging agent in the diagnosis and treatment of Parkinson’s disease,\(^{83}\) where it accumulates in neuroendocrine cells, allowing for imaging. It’s mechanism of action is shown in Figure 1.14.

### Table 1.05: The electrophilic radiofluorination of arylstannanes by 141.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>(\text{R}^1)</th>
<th>(\text{R}^2)</th>
<th>(\text{R}^3)</th>
<th>RCY(^{[a]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142</td>
<td>Me</td>
<td>OMe</td>
<td>Me</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>143</td>
<td>Me</td>
<td>H</td>
<td>Me</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>144</td>
<td>(^1\text{Bu})</td>
<td>H</td>
<td>H</td>
<td>14</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Decay corrected RCY after semi-preparative HPLC. Based on stock solution of xx, activity is 1:1 with \(^{[18}\text{F}\)]\text{LiF}.

**Figure 1.14:** *In vivo* mode of action of 6-[\(^{18}\text{F}\)]F-L-DOPA.

LAT1 = L-Type Amino Acid Transporter, AADC = Amino Acid Decarboxylase, VMAT = Vesicular Monoamine Transporter, DBH = Dopamine \(\beta\)-hydroxylase, DHA = Dehydroascorbic acid.
It is first transported across the cell barrier by L-type amino acid transporters, prior to decarboxylation by AADC. Further intracellular transport mediated by vesicular transporters allows storage in gastreoeenteropancreatic tumours, prior to hydroxylation by DBH to $^{18}$F-fluoronorepinephrine 147. Unlike $[^{18}F]$FDG, there is no mechanism which inhibits release of 6-$[^{18}F]$F-L-DOPA from within cells, it is due to increased storage within neuroendocrine vesicles.

6-$[^{18}F]$F-L-DOPA was prepared from precursor 148 – itself synthesised in 3 steps from L-DOPA at 21% yield – utilising the silver mediated methodology previously explored and $[^{18}F]$Selectfluor bistriflate, before deprotection by aqueous hydrobromic acid (Scheme 1.54), in acceptable radiochemical yield.

Scheme 1.54: The radiosynthesis of 145 by silver mediated, electrophilic radiofluoro-destannylation and acidic deprotection.

\[
\begin{align*}
\text{Boc} & \quad \text{SnMe}_3 & \quad \text{O} & \quad \text{OEt} & \quad \text{NHCHO} \\
\text{148} & & & & & & \\
\text{145} & & & & & & \\
& \quad \text{RCY} = 12.1\pm3.7\% \\
& \quad (n = 6)
\end{align*}
\]


1.2.4.2 – Nucleophilic fluorination

The primary method of fluorine-18 production is as fluoride-18, due to the increased molar activity. One drawback using fluoride – despite the apparent nucleophilicity\(^{[85]}\) – is the inherent basic nature of the anion (\(pK_a\) \(\text{HF}_{\text{DMSO}} \approx 15\), \(\text{HF}_{\text{water}} \approx 3.2\)), which, upon hydration in the presence of water reduces the potency of \([^{18}\text{F}]\text{F}^-\) as a nucleophile.\(^{[71]}\) As such, azeotropic drying of the aqueous dispensing solution is performed with acetonitrile and improvement of the nucleophilicity by chelators or tetraalkylammonium counterions are two methods targeted (Figures 1.09 and 1.10). Research into the functionalisation of chelators has been undertaken by Kim, which nicely demonstrates the dual capability of crown ethers to bind not only the counterion, but also dock the fluoride.\(^{[86,87]}\)

Considerations to the eluting counterion also need to be applied, where commonly used anions such as carbonate, bicarbonate and oxolate are non-nucleophilic in nature.\(^{[88]}\)

Typical nucleophilic reactions using fluoride-18 utilise dipolar aprotic solvents such as DMSO, DMF and MeCN,\(^{[89,90]}\) proceeding with \(S_N2\) like character. Tertiary alcohols such as tert-amyl alcohol have demonstrated application within nucleophilic (radio)fluorination,\(^{[71,91]}\) further demonstrating that some degree of hydrogen bonding may be beneficial.\(^{[92,93]}\)

The choice of leaving group can also be critical to effective nucleophilic displacement, with a balance between leaving group ability and stability of precursor of critical importance.\(^{[94]}\) Additionally, a better leaving group will also lead to competitive elimination under basic conditions.\(^{[88]}\) Jacobsen and Chen report the order of leaving group ability as \(\text{Cl} < \text{Br} < l < \text{OTs} < \text{OMs} < \text{O}p\text{Ns} < \text{OTf}\), with triflate being the most reactive, but greatest susceptibility to elimination.\(^{[95]}\) Sulfonates demonstrate a substantial subsection within the leaving groups available for fluoride displacement, with a range of reactivities accessible.

Other parameters that require careful modification to minimise deleterious pathways include obvious factors such as temperature, also less intuitive interactions such as the ratio of PTC to base and precursor.\(^{[88]}\)

Additional reagents may be included to improve the efficiency of the displacement. Gouverneur and co-workers utilised silver triflate in their work which employed halogens as the leaving group of -CF\(_2\) and -CHF units attached to phenols and thiophenols (Scheme 1.55).\(^{[96]}\)
Their studies allowed access to $^{[18]}\text{F}\text{SCF}_3$ and $^{[18]}\text{F}\text{OCF}_3$ motifs, which are commonly found within pharma and agrochemicals as their fluorine-19 analogues, by late stage fluorination. The strategy was applied to the synthesis of $^{[18]}\text{F}\text{riluzole}$ (150, Scheme 1.55), a drug used in the treatment of amyotrophic lateral sclerosis, more commonly known as motor neurone disease. The exploitation of the affinity of silver(I) for halides and their subsequent precipitation out of solution towards radiofluorination is an impressive strategy. Alternative metal triflates were unsuccessful in facilitating the reaction, while the silver counterion needed to be weakly coordinating such as triflate or triflimide. When concluding, they suggest that cationic intermediates are involved in the halogen exchange reaction.$^{[97]}

The developed Ag$^+$ methodology was later applied to benzylic systems, towards the formation of $^{[18]}\text{F}\text{CF}_3$ and $^{[18]}\text{F}\text{CHF}_2$ units.$^{[98]}$ Two selected examples are shown in Scheme 1.56, demonstrating the two methods employed to effectively radiofluorinate the substrates. For more difficult examples, doubling the amount of AgOTf used, from 1 to 2 equivalents, in combination with DCE as higher boiling point solvent.
1.2.5 – Radiofluorination of nucleosides

1.2.5.1 – Early-stage fluorination

$[^{18}F]$-Fluorine labelled nucleosides have been successfully synthesised as predictive biomarkers,$^{[99]}$ with varying strategies employed. One commonly utilised method is the fluorination of a ribofuranose derivative, prior to glycosylation. This strategy is termed early-stage fluorination, and has found extensive use in the synthesis of fluorinated nucleosides such as $[^{18}F]$FAC, $[^{18}F]$FMAC, $[^{18}F]$FAU derivatives and $[^{18}F]$FLT ($^{157}$, $^{158}$ and $^{161}$ respectively, Figure 1.15).$^{[100,101]}$

![Scheme 1.56: Selected examples of benzylic radiofluorination using silver triflate.](image)

![Figure 1.15: Selected structures of example $[^{18}F]$nucleosides.](image)
As depicted in Scheme 1.57, this strategy was applied in the synthesis of $[^{18}F]$FMAU by Shields and co-workers,$^{[100]}$ based upon research by Howell.$^{[102]}$ Ribofuranose 162 was subjected to radiofluorination in DMF for 5 minutes, and then converted to 1-bromo-ribofuranosyl intermediate 163. Glycosylation was conducted in chloroform, which was found to improve the anomeric selectivity over other solvents like DCM and MeCN. Final deprotection by sodium methoxide in methanol afforded the target compound in an average radiochemical yield of 42.1% (decay corrected) over 9 runs, in >98% radiochemical purity (by radio-HPLC).

The method was also applied to other uridine nucleobases, demonstrating its applicability. From these results, the synthetic methodology can be viewed as highly appealing as a generic radiofluorination approach, given it may be applied to the precursor 162, followed by selection of appropriate nucleobase prior to ring appendage. One issue surrounding the method may be the anomeric selectivity of the glycosylation, and subsequent separation of the anomers after deprotection. Due to the number of steps involved following radiofluorination, the timescale of this processes is of great importance, as the activity of the $[^{18}F]$-labelled material constantly decays and decreases. The authors claim that synthesis from initial $[^{18}F]$fluoride capture to isolated compound after HPLC purification is roughly 160 minutes, which would need to be accounted for when original $[^{18}F]$F- dispensing.

Scheme 1.57: The early stage fluorination strategy applied to $[^{18}F]$FMAU, 160.
1.2.5.2 – Late-stage fluorination

Because of the drawback surrounding the loss of activity of $[^{18}\text{F}]$fluoride associated with early-stage fluorination, late-stage fluorination offers the potential for radiopharmaceuticals with increased activity. As a result, bespoke starting materials require synthesising for the target molecule.

Alauddin and co-workers also synthesised $[^{18}\text{F}]$FMAU, but via late-stage fluorination.\textsuperscript{103,104} Radiolabelling precursor was constructed beginning from 5-methyluridine (164, Scheme 1.58) firstly by protecting the 3'- and 5'- hydroxyl groups with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and protection of the 2'-OH with TMSCl. Di-tert-butyl dicarbonate was used to protect the N\textsuperscript{3} position, before acid hydrolysis of 2'-O-TMS to afford intermediate 165. Activation of the 2'-OH by methanesulfonyl chloride, followed by fluoride mediated silyl protecting group removal and subsequent reprotuction of the 3' and 5' alcohols as their tetrahydropyranyl ethers, delivering 166 in 42% yield from 165.

Scheme 1.58: Synthesis of radiolabelling precursor 166.
**166** was fluorinated by $[^{18}\text{F}]KF$ with K$_{222}$ in acetonitrile, followed by acid hydrolysis of the THP ethers and $N^3$-Boc group (Scheme 1.59), in low radiochemical yield – lower than that of the early-stage fluorination – but high molar activities were achieved (c.f. ≥ 1.8 Ci mmol$^{-1}$)

![Scheme 1.59: Synthesis of $[^{18}\text{F}]$FMAU (160) by late-stage radiofluorination of 166.](image)

Eisenhut and co-workers reported the synthesis of 3'-deoxy-3'-$[^{18}\text{F}]$fluoro-thymidine in 2000,$^{[105]}$ starting from commercially available 5-O-(4,4'-dimethoxytrityl)thymidine (167, Scheme 1.60). Treatment of 167 with methanesulfonyl chloride activated the 3'-hydroxyl group, such that upon reacting with DBU formed anhydro compound 168.

![Scheme 1.60: Synthesis of radiolabelling precursor 168.](image)

Sequential treatment of 168 with $[^{18}\text{F}]KF$ with K$_{222}$ in anhydrous DMSO and cleavage of 5'-O-(4,4'-dimethoxy)trityl protecting group furnished $[^{18}\text{F}]$FLT (161) in an average radiochemical yield of 5.6% over 5 runs (Scheme 1.61). The method cleverly manipulates the formation of anhydro intermediate 168 such that two nucleophilic displacements at the 3'-position deliver the desired configuration of the radiofluorinated product, and not it’s diastereomer. Simultaneously, the use of 168 requires no protecting group at the $N^3$ position.
While Alauddin commented on the possible formation of the 2,2'-anhydro intermediate, they ruled it out as a competitive pathway during the radiofluorination as it was not detected by radio-HPLC, nor did it match the reference for its non-radioactive analogue.\[104\]

Recently, Cavaliere et al demonstrated that late-stage fluorination may be applied to 5-O-phosphorylated precursors,\[106\] despite the strength of P-F bonds.\[107\] The authors also targeted [\(^{18}\)F]FLT, but as the ProTide derivative such that drug delivery may be quicker after radiofluorination, bypassing the rate limiting phosphorylation of 5'-OH. Combining 3-\(\beta\)-hydroxy thymidine congener with chlorophosphoramidate 169 in the presence of NMI in anhydrous THF, followed by sequential activation of the 3'-OH by \textit{para}-nosylchloride and protection of N\(^3\) by Boc\(_2\)O to yield radiolabelling precursor 171, in 6% yield over 3 steps (Scheme 1.62).

The authors then investigated the [\(^{18}\)F]fluorination of 171 and found that [\(^{18}\)F]FLT ProTide 172 could be afforded in an average radiochemical yield of 22.5% over 5 runs (Scheme 1.63). High radiochemical purities (\(\geq 97\%\)) and specific activity of 56 GBq mol\(^{-1}\) were obtained for 172, with a total time of 130 minutes for synthesis.

\[\text{Scheme 1.61}: \text{Synthesis of [}^{18}\text{F}]\text{FLT (161) by late-stage radiofluorination of 168.}\]

\[\text{Scheme 1.62}: \text{Preparation of precursor 171 for radiolabelling.}\]
Investigators in Korea combined the aforementioned potential to execute nucleophilic fluorination substitution in protic media by synthesising $^{[18}F$FLT, with different fluoride counterions. Shown in Scheme 1.6 is the radiosynthesis of 173 by $^{[18}F$TBAF and use of tert-butanol or tert-amyl alcohol as cosolvent, followed by protecting group removal by acid treatment. $^{[18}F$FLT was synthesised in high radiochemical yield and purity (98.5±1.2%), although the number of repeats was not noted in the report. [108,109]

Scheme 1.63: $^{[18}F$Radiolabelling of precursor towards the synthesis of $^{[18}F$FLT ProTide 172.

Meyer et al employed a similar strategy to Alauddin in the construction of precursor 176 towards the synthesis of $^{[18}F$FAC, shown in Scheme 1.65. The hydroxyl motifs of cytidine (174) were sequentially protected as the silyl ethers, using TIPSDCl$_2$ at the 3' and 5' alcohol positions and trimethylsilylchloride to protect the 2'-OH. Next, di-tert-butyl dicarbonate was utilised to mask the N$^4$ position prior to 2'-O-TMS removal by para-toluene sulfonic acid to deliver intermediate 175 in 41% across 4 steps. Activation of the 2-OH by methanesulfonate chloride, tethered 3',5'-O-silyl ether removal by TBAF and re protection by dihydropyran and catalytic TsOH yielded precursor 176 in 15% yield over 3 steps, ready for radiofluorination.

Scheme 1.64: Synthesis of $^{[18}F$FLT from 173 using protic solvent and $^{[18}F$TBAF.
Noting the previous observations, the authors probe the potential formation of the 2,2'-anhydro product from precursor 176 (obtained after TBAF deprotection), by heating it to eliminate mesylate anion from the 2' position, shown in Scheme 1.66.

**Scheme 1.65**: Synthesis of radiolabelling precursor 176.

**Scheme 1.66**: Attempted formation of 178 by thermolysis of 177.
Notably, no thermolysis of the starting material was detected as analysed by $^1$H NMR. This could be ascribed to the imide-like nature of the $N^4$ position, where the lone pair of the nitrogen is involved in both amide motifs hence is has increased delocalisation and is less able to participate in 2,2'-anhydro intermediate formation.

Optimisation of the radiofluorination of 176 identified heating the azeotropically dried $[^{18}\text{F}]\text{KF}$ at 110°C in DMF as the best conditions, delivering the intermediate in 9.4±0.8% (n = 3), by radio-TLC. Lower temperatures were less efficient in inducing the $[^{18}\text{F}]$fluorination, while increasing temperature or length of reaction were deleterious to the intermediate production. Acid mediated removal of the 3',5'-O THP ethers and $N^4$-Boc moieties successfully yielded $[^{18}\text{F}]\text{FAC}$ (157) in an overall radiochemical yield of 4.9±0.6% over 8 runs (Scheme 1.67). The authors note that shorter times were investigated for the deprotection step but returned decreased yields of 157. Following isolation by semi-preparative HPLC, target compound 157 was isolated in high purity ($\geq$98%) with molar activity of $\geq$63 GBq mol$^{-1}$, delivering 0.75 – 0.86 Gbq of $[^{18}\text{F}]\text{FAC}$ after a total synthesis time of 168 minutes.

Scheme 1.67: Sequential $[^{18}\text{F}]$fluorination and acid treatment of 176 to afford $[^{18}\text{F}]\text{FAC}$ (157).
The authors probed the reason for lower radionuclide incorporation upon increased reaction heating. It was determined to be due to competitive formation of the undesired stereoisomer 179 (Figure 1.68), which suggests *in situ* formation of 2,2'-anhydro intermediate at elevated temperatures, in agreement with other reports.\[^{104,105}\]

![Figure 1.68](image_url)

**Figure 1.68:** Formation of undesired stereoisomer of [\(^{18}\)F]FAC during radiofluorination.
2 – Research aims and objectives

The ultimate aim of the research is to develop a robust, reliable synthetic method towards an appropriate radiolabelling precursor, for the synthesis of \(^{18}\text{F}\)gemcitabine (182, Scheme 2.01). The synthesis of \(^{18}\text{F}\)gemcitabine would offer critical in vivo data on patients with cancer and specifically pancreatic cancer, where the mortality rates are very high. Not only would 182 demonstrate the same pharmacokinetic characteristics as gemcitabine, but would also allow for patients to be screened to determine whether gemcitabine could be a viable treatment option. Additionally, such PET probes require significantly less material to be administered versus standard chemotherapeutics, putting the patient under less distress.

This is envisioned by fluorine-18 substitution of an appropriate leaving group from precursor 180, followed by removal of any protecting groups. Careful consideration of the chosen leaving group is required, as improved ability to leave is cancelled out by side reactions such as elimination. Additionally, the precursor would ideally be stable to air and moisture, such that sufficient amounts may be synthesised at a given time. Critically, the collaborative nature of the project allowed access to \(^{18}\text{F}\)fluoride, such that nucleophilic fluorination was the method of incorporating fluorine-18.

Ideally, a late stage radiofluorination strategy would be employed, in order to capitalise on the amount of radioactive material produced, which would require a new synthetic route towards 180. Radiofluorination at the 2' position is also challenging, given the targeted nucleophilic substitution at a tetra substituted carbon. As such, non-radioactive fluorination test reactions will also be conducted to evaluate the viability of the fluorination methods, along with synthesis of authentic, non-radioactive samples for comparison and method development.

Scheme 2.01: Proposed radiofluorination and deprotection of precursor 180 towards \(^{18}\text{F}\)gemcitabine.
3 – Results and discussion

3.1 – Synthetic route 1

3.1.1 – Disconnection strategy

The retrosynthetic design (Scheme 3.01) for the radiolabelling precursor began by disconnecting the $N^4$-functionalised cytosine nucleobase 183 – derived from cytosine (184) from the tetrahydrofuryl ring of 180, which would require an activated lactol type compound (185), with the 3’ and 5’ hydroxyl moieties appropriately protected. 185 could be accessed from the lactone, which in turn may be achieved by ring closing lactonisation. The (protected) β-hydroxy ester 187 could then be synthesised akin to Hertel’s original synthesis,[8] beginning with 2,3-O-isopropylidene-D-glyceraldehyde[110] (11) and combining with ethyl 2,2-dibromo-2-fluoro acetate (92) by way of a Reformatsky-type reaction.

Scheme 3.01: Retrosynthesis and disconnection of 180.

3.1.2 – Synthesis

Thus, drawing inspiration from Hertel’s seminal work,[8] initial investigations began with D-mannitol diacetonide 188, which was treated with 2.5 equivalents sodium periodate in
a biphasic mixture of DCM and aqueous NaHCO₃, to furnish 2,3-O-isopropylidene-D-glyceraldehyde[110] 11 in 77% yield. This reaction to cleave the vicinal diol was also scalable, producing 60 mmol after purification by distillation. It was noted that 11 was unstable when isolated and couldn’t be stored long term (greater than one month), as degradation was observed by NMR, in accordance to the literature.[110] The optical purity of the aldehyde was determined as [α] = +42° in DCM, agreeing with that reported by Ryall and coworkers,[111] confirming the correct stereochemistry was present prior to formation of lactone derivative 186.

Strategy considered for the addition to 11, such as the enolate of the corresponding functionalised ester were discounted due to competing addition reactions such as intermolecular Aldol reaction and condensation. Analogous to that described by Hertel, a Reformatsky reaction of 92 with 11 would furnish the desired β-hydroxy ester. Use of the in-situ generated organozinc intermediate would also retain the necessary ester functionality for later lactonisation. However, due to the synthetic design strategy, incorporation of the leaving group was required at this early stage. While chloride was initially mooted for the radiofluorination due to its enhanced stability, bromide would provide an improved leaving group. Moreover, iodo equivalents would be desired, allowing for easier oxidative addition of zinc, but such compounds are not commercially available and the synthesis is non-trivial.

To ensure that the active organozinc was being formed and consumed, the reaction was simplified to benzaldehyde as the electrophile and ethyl bromoacetate as the latent nucleophile. A screen of activation methods were undertaken, presented in Table 3.01.

Activation of the zinc by iodine in dioxane with sonication for 5 minutes did not produce 191 by ¹H NMR analysis (Entry 1). Pleasingly, using 1.6 equivalents of zinc with 12 mol % TMSCl in Et₂O furnished the β-hydroxy ester in 56% isolated yield (Table 3.01, Entry 3). The amount of TMSCl required was subsequently investigated (entries 4-8), but did not demonstrate any improvement, with increased amounts returning diminished yields of 191.
Results and discussion

Interestingly, the reaction proceeded in the absence of external activator when the reagents were subjected to vibratory ball milling, delivering 70% NMR yield of the β-hydroxy ester.

Satisfied that active Zn⁶ was being produced, focus returned to the original substrates for the Reformatsky reaction. As such, taking commercially available ethyl 2-bromoacetate (92) with the protected glyceraldehyde and zinc metal, and TMSCl as activator for zinc, delivered none of the target material after protic work up.

Organozinc reagents are known to be moisture sensitive and are commonly generated in situ and not isolated, so it was difficult to determine whether the active organozinc was being formed. Alternative activators such as 1,2-dibromoethane and acidic wash of the metal were also unsuccessful in making the reaction proceed.

### Table 3.01: Investigation on activators required for zinc activation for the production of 191.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Zn [equiv.]</th>
<th>Activator [equiv.]</th>
<th>Solvent</th>
<th>Time [h]</th>
<th>Yield 191 [%][a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6[^b]</td>
<td>I₂ (0.20)[^b]</td>
<td>Dioxane</td>
<td>0.083</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.6[^c]</td>
<td>TMSCl (0.12)</td>
<td>Et₂O [0.4 M]</td>
<td>16</td>
<td>(54)</td>
</tr>
<tr>
<td>3</td>
<td>1.6[^c]</td>
<td>TMSCl (0.12)</td>
<td>Et₂O [0.4 M]</td>
<td>16</td>
<td>(56)</td>
</tr>
<tr>
<td>4</td>
<td>1.6[^c]</td>
<td>TMSCl (0.05)</td>
<td>Et₂O [0.2 M]</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>1.6[^c]</td>
<td>TMSCl (0.10)</td>
<td>Et₂O [0.2 M]</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>1.6[^c]</td>
<td>TMSCl (0.15)</td>
<td>Et₂O [0.2 M]</td>
<td>16</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>1.6[^c]</td>
<td>TMSCl (0.20)</td>
<td>Et₂O [0.2 M]</td>
<td>16</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>1.6[^c]</td>
<td>TMSCl (0.25)</td>
<td>Et₂O [0.2 M]</td>
<td>16</td>
<td>41</td>
</tr>
<tr>
<td>9[^d]</td>
<td>1.6[^e]</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>70</td>
</tr>
</tbody>
</table>

Reaction conditions: benzaldehyde (1 mmol), ethyl 2-bromoacetate (1.2 equiv.), zinc (as specified), activator (as specified). [a] Yield determined by ¹H NMR using mesitylene as internal standard. Isolated yield in parentheses. [^b]Granular zinc used. [^c]20-30 mesh zinc used. [^d]Reaction conducted in mixer mill, benzaldehyde (1 mmol), ethyl 2-bromoacetate (1.2 equiv.). 30 Hz.
Specifically focussing on aldehyde 11, the lack of product formation would suggest it is not as reactive as benzaldehyde. This obstacle could be circumvented by a Lewis acid, akin to that used in the Mukaiyama aldol addition reaction. As such, employing 1.1 equivalents of bis(cyclopentadienyl) titanium (IV) dichloride with 11 to a sonicated suspension of zinc (2.4 equivalents), TMSCI (2.2 equivalents) and ethyl dibromoacetate (92, 2 equivalents) in MeCN at -40°C delivered a mixture of the β-hydroxy ester and the corresponding TMS protected ester in a combined 35% yield, separable by silica gel column chromatography. β-hydroxy ester 192 was isolated as a diastereomeric mixture, in a ratio of 86:14 by 19F NMR.

Scheme 3.03 Optimised reaction of Cp₂TiCl₂ promoted Reformatsky of β-hydroxy ester derivates 192 and 193.

This observation suggests that TMSCI may serve a dual purpose here; both as activator of zinc and alcohol protecting group. It was also observed that an alkene by-product was being formed in the reaction, which was identified as 194, resulting from a second oxidative addition of zinc and subsequent hydroxide elimination. A limitation of this approach is the diastereoselectivity of the nucleophile attack onto 11, with both syn and anti isomers possible.

Figure 3.01: Structure of alkene 194 observed during crude ¹H NMR analysis of the reaction shown in Scheme 3.03.
While the stereochemistry of the dihalo functionality does not need to be strictly defined, the configuration of the (silylated) alcohol is crucial given the need for the (R)-stereoisomer at the 3’ position of gemcitabine. Yasuda and coworkers report that using Cp₂TiCl₂ enhances the selectivity of the diastereomer formation by way of facial discrimination. While a similar concept may be proposed here, it is worth noting that the incorporation of a larger bromine atom (vs fluorine) may reduce the selectivity imparted by the sterically incumbered Lewis acid, thus explaining the lower diastereoselectivity (86:14 d.r.) observed and yield compared to those reported. Additionally, the presence of alkene 194 demonstrates the potential reactivity of the α-bromo ester functionality with zinc – a classic example in the Reformatsky and Blaise reactions.

With the desired β-hydroxy ester in hand, concomitant deprotection of the acetonide functionality and cyclisation under acidic conditions of both 192 and 193 afforded lactone 195 in 40% isolated yield, depicted in Scheme 3.04. It was noted that the unprotected lactone was susceptible to decomposition, even when stored in the fridge.

\[ \text{Scheme 3.04: Concaminant acetal deprotection and lacionisation under acidic conditions.} \]

Given the early stage nature of these reactions in the synthetic route, scale up reactions were undertaken. The oxidative cleavage of 188 was robust and could be successfully performed on 40 mmol scale yielding 8.05 g of pure aldehyde (Scheme 3.05), which was unsuitable for long-term storage. The Lewis acid aided Reformatsky reaction was unsuccessfully translated to larger scale reactions, with hydrolysis of the in-situ generated organozinc being observed by ¹H NMR. As such, an alternative synthetic strategy was explored to access the dihalogenated ribonolactone.

\[ \text{Scheme 3.05: Scale up of the oxidative cleavage of 188 by sodium periodate.} \]
3.2 – Synthetic route 2

3.2.1 – Disconnection strategy

The initial disconnection strategy shown in Scheme 3.06 was based upon incorporating the geminal di-halo functionality from one of the starting materials (92) prior to formation of the lactone. Alternatively, formation of the 2-deoxy ribonolactone 75 would provide access to its halogenated congener via enolate chemistry using electrophilic sources of the halides, akin to the work demonstrated by Cen and Sauve. This approach could allow for improved diastereoselectivity at the 2-position, depending on the addition of the halogen electrophile, and increased potential for diversification and functionalisation by this step-wise, modular approach.

![Scheme 3.06: Retrosynthesis and disconnection of 186, by lactonisation (Synthetic route 1, green) or electrophilic halogenation (Synthetic route 2, purple).](image)

3.2.2 – Synthesis

3.2.2.1 – Ring construction and protection

Beginning with commercially available 2-deoxy-D-ribose 197, oxidative cyclisation with elemental bromine in the dark for 5 days furnished 2-deoxy-D-ribo-1,4-lactone 75 in 93% isolated yield, depicted in Scheme 3.07. Several previous syntheses in the literature use Ag₂CO₃ as an elegant choice of base during reaction work up – which acts to both neutralise the hydrobromic acid by-product and also precipitate AgBr, delivering pure product. It was found that using K₂CO₃ was just as effective, followed by purification of the crude mixture by silica gel column chromatography in 5% MeOH/EtOAc to afford pure 75.
Next, appropriate protecting groups for the 3- and 5-hydroxyl groups needed to be selected. This could be conveniently achieved by capping both alcohols with the same protecting group, such as a boronate ester. As shown in Scheme 3.08, three different groups were employed: (i) boronate ester (201), from 75 with phenyl boronic acid in toluene at room temp; (ii) benzylidene acetal (198), and (iii) utilising 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane with γ-lactone 75 in DMF and imidazole to yield 200. The synthesis of the analogous 1,1,3,3-tetramethyldisiloxane (199) was unsuccessful.

On balance and comparison of the three synthesised molecules it was decided that due to their low yield, continued synthesis of 198 and 199 would not be pursued.
In parallel, individual protecting groups for the hydroxyl moieties were investigated. As discussed previously, ester protecting groups have fallen foul of this ribonolactone α-functionalisation strategy due to competing elimination, rendering the requirement of bulky silanol-type protecting groups.\textsuperscript{[114]} Thus, drawing inspiration from Cen and Sauve’s work,\textsuperscript{[20]} 2-deoxy-D-ribo-1,4-lactone 75 was protected as the silyl ether, from triisopropylsilyle chloride in DMF with imidazole as base, furnishing 80% of 64 after purification by column chromatography (Scheme 3.09).

![Scheme 3.09: The formation of 64 from 75 with TIPSCI under basic conditions.](image)

It was decided that due to the enhanced yield obtained for the bis-TIPS protection, in conjunction with literature precedent surrounding its use in the functionalisation of lactones that the disiloxane protection methodology would not be further investigated.\textsuperscript{[20,23,24]}

### 3.2.2.2 – α-halogenation

In the designed synthetic strategy, the introduction of an appropriate leaving group for \textsuperscript{18}F displacement was targeted. As a start point, halides were focussed upon, as they should readily undergo the relevant substitution. As both fluoride and leaving group halide needed to be installed, it was decided that fluorination, followed by subsequent bromination would be a preliminary strategy. Depicted in Scheme 3.10 is reaction of 64 with NFSI and LiHMDS, which furnished mono-fluoro lactone 65 in 54% yield, as a single diastereomer, as previously reported.\textsuperscript{[20]}

![Scheme 3.10: The electrophilic fluorination of 64 by NFSI and LiHMDS.](image)
Results and discussion

From 65, installation of bromine via electrophilic NBS was attempted, initial efforts proceeded via silyl enol ether formation by treatment with TMSOTf and NEt₃ (Table 3.02), only to return the starting material in quantitative amount. This result suggests that the pKₐ of the α-proton is too high to be deprotonated by triethylamine, even in the presence of Lewis acidic TMSOTf. Next, stronger bases were probed. LiHMDS did not facilitate the bromination of 65 (Entry 2), potentially due to lithium-halogen exchange or bromine abstraction by LiHMDS.²⁴,¹¹⁵ It is worth noting however in Voight’s synthesis of ABBV-168 (99, Scheme 1.41), LiHMDS promoted bromination was achieved.²⁴ Switching to KHMDS proved promising, with increased formation of the gem-dihalo lactone (Table 3.02, Entry 3). The lactone was formed in a diastereomeric ratio of 9:10 of (R):(S) at the 2-position by ¹⁹F NMR,²⁴ although the configuration of the α position was unimportant at this point. Unfortunately, upon increasing the reaction time to 3 hours (Table 3.02, Entry 4), no product formation was observed.

These disappointing results forced a rethink of the α-halogenation strategy, suggesting it might be preferential to brominate first, and then fluorinate with an appropriate electrophilic agent.

Table 3.02: The screened bromination conditions of 65 by NBS.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 65[α]</th>
<th>Yield of 202[α]</th>
<th>Yield of 89[α]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NEt₃ (6 equiv.), TMSOTf (3 equiv.) then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight</td>
<td>(&gt;95%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>NBS (1.5 equiv.), LiHMDS (2 equiv.) THF, -78°C, 180 min</td>
<td>&gt;95%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>NBS (1.5 equiv.), KHMDS (2 equiv.) THF, -78°C, 15 min</td>
<td>67</td>
<td>15%</td>
<td>18%</td>
</tr>
<tr>
<td>4</td>
<td>NBS (1.5 equiv.), KHMDS (2 equiv.) THF, -78°C, 180 min</td>
<td>&gt;95%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

[α] Yield measured by ¹⁹F NMR with α,α,α-trifluorotoluene.
Utilising previously explored brominating conditions, 64 was converted efficiently into the mono-Br lactone 203, in a diastereomeric ratio of 2:1 of arabino (S)-203 to ribono (R)-203, assigned by 2D NOESY and COSY $^1$H NMR of each pure diastereomer. Conclusively, it was a through space interaction between $H^\alpha$ and $H^\beta$ that allowed one of the diastereomers to be assigned as the (R)-ribo sugar, the major product. Allowing the reaction to run overnight provides increased yield of 203, in a ratio of 1:0.7 arabino:ribo respectively (Entry 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 203$^{[a]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NE$_3$ (6 equiv.), TMSOTf (3 equiv.) then NBS (1.5 equiv.) DCM, 0°C → r.t., 3 h</td>
<td>62%</td>
</tr>
<tr>
<td>2</td>
<td>NE$_3$ (6 equiv.), TMSOTf (3 equiv.) then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight</td>
<td>70%</td>
</tr>
<tr>
<td>3</td>
<td>NE$_3$ (6 equiv.), TMSOTf (3 equiv.), then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight$^{[b]}$</td>
<td>45%</td>
</tr>
<tr>
<td>4</td>
<td>NE$_3$ (6 equiv.), TMSOTf (3 equiv.), then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight$^{[c]}$</td>
<td>55%</td>
</tr>
<tr>
<td>5</td>
<td>NE$_3$ (6 equiv.), TMSOTf (3 equiv.), then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight$^{[d]}$</td>
<td>&lt;5%$^{[d]}$</td>
</tr>
<tr>
<td>6</td>
<td>DBU (6 equiv.), TMSOTf (3 equiv.), then NBS (1.5 equiv.) DCM, 0°C → r.t., overnight</td>
<td>(&gt;95% 64)</td>
</tr>
<tr>
<td>7</td>
<td>(BrCl$_2$)$_2$ (1.3 equiv.), LiHMDS (1.5 equiv.) THF, -78°C, 4 h</td>
<td>19% (16% 64) (45% 206)</td>
</tr>
</tbody>
</table>

$^{[a]}$ Isolated yield. $^{[b]}$ Stabilised DCM used. $^{[c]}$ Reaction ran in the dark. $^{[d]}$ Analysis of crude reaction mixture. $^{[e]}$ TMSOTf added first, then NE$_3$

**Table 3.03**: The screened bromination conditions of 64.

**Figure 3.02**: The structures of brominated products from the conditions screened in Table 3.03.
The excess of (S)-203 could be rationalised by preferential si approach of NBS from the top face of the trans enolate, minimising any steric clash with the 3-O-TIPS group. Despite this, a large amount of the 2-deoxy 2-bromo ribonolactone (R)-203 is formed, which may be attributed to the re addition of bromine to the enolate, as NBS would not be considered a bulky electrophile. Alternatively a potential radical mechanism may be in play, as NBS can split homolytically and is employed in radical reactions such as the Wohl-Ziegler reaction.[116] It is noteworthy that when stabilised DCM was used (Table 3.03, Entry 3), which contains amylene as stabiliser, diminished yield of the brominated product was observed – the amylene may be acting as a bromine radical scavenger. As a control experiment, the reaction was also conducted in the dark (Entry 4) which would inhibit homolytic cleavage of NBS therefore the possibility of a radical reaction. Fortunately, this led to a good yield of the target material although lower than the optimal conditions of Entry 2 – again as a mixture of diastereomers. Markedly, adding TMSOTf before NEt3 led to a complex mixture when the crude reaction mixture was analysed by 1H NMR, with trace desired compound. This unwanted reactivity infers that initial exposure of 64 to Lewis acidic TMSOTf may lead to coordination across multiple Lewis basic sites within the starting material. Subsequent addition of base could then access a range of undesired products, such as those from ring opening of the lactone or elimination reactions.

After purification by column chromatography, the remaining isolated material is starting material 64, indicating incomplete conversion. The lack of complete conversion may either be due to insufficient NBS equivalents or a low concentration of silyl enol ether formed in situ. To probe this hypothesis, DBU was employed as a stronger base (Entry 6). Unfortunately, this control did not improve the yield of 203, returning near quantitative starting material. Use of 1,2-dibromotetrachloroethane with LiHMDS (Entry 7) successfully formed 19% isolated yield of desired compound 203 (1.5:1 arabinose:ribono ratio), and 16% of starting material 64. However, the yield was significantly diminished compared to the NEt3/TMSOTf/NBS strategy, with the majority of the isolated material being the gem-dibromo lactone 206, returned in 46% isolated yield.
Satisfied that, of the explored conditions in Table 3.02, Entry 2 was optimal for α-bromination, alternative leaving groups were investigated. By using NBS, other halides may be targeted using their respective \(N\)-halo-succinimide, illustrated in Scheme 3.11. Employing the silyl enol ether formation conditions used previously, \(N\)-chlorosuccinimide was reacted with \(\gamma\)-lactone 64, to form the \(\alpha\)-chloro congener 207, in 37% yield. Similarly, 2-deoxy-2-iodo-3,5-bis-\(O\)-TIPS-\(\gamma\)-lactone 208 was synthesised in good yield (65%).

![Scheme 3.11: The halogenation of 64 by \(N\)-halosuccinimides](image)

However, due to the lack of stability of alkyl iodides and their potential for decomposition, this synthetic pathway was discounted. Given that bromide is a better leaving group than chloride, and in the context of the goal of the project, it would potentially lead to a better candidate as a radiolabelling precursor for displacement by fluoride. Therefore, while \(\alpha\)-chloro analogue 206 was a viable option in the designed synthesis, only bromo 203 was pursued for further development.

With appropriate conditions for the formation of 203, attention then turned to fluorination. Adapting Liotta’s conditions,[117] reacting 203 with NFSI in THF at -78°C under basic conditions formed the desired ribonolactone in good yield, with no remaining starting material. (Scheme 3.12).

![Scheme 3.12: The NFSI/LiHMDS mediated fluorination of 203.](image)
Remarkably, a single diastereomer is formed (determined by $^{19}$F NMR) with the fluorine believed to be on the top face of the lactone. Due to the requirement of bulky TIPS-O protecting groups, the physical state of all but one of the functionalised lactones are sticky liquids or oils, thus absolute configuration by X-ray analysis has not been possible.

**Scheme 3.13:** The proposed reaction mechanism explaining the diastereoselective formation of 202.

Considering the reaction mechanism (Scheme 3.13) one can rationalise the formation of the single diastereomer: Following $\alpha$-deprotonation by LiHMDS, the lithium enolate could rationally be trapped by the electrophilic NFSI from both the $re$ and $si$ face of intermediate 204. However, given the steric bulk 3-$O$-TIPS group which essentially blocks the $si$ addition of fluorine, similar to what was observed for the $\alpha$-bromination. In addition, the bulky nature of NFSI will likely play a role in reinforcing preference for $re$ addition of the electrophile is the only outcome. By comparison, Voight’s studies delivered the opposite diastereomer which had notably different $^{19}$F NMR shift of -135.61 ppm, versus -127.53 ppm for 202.$^{[24]}$ Notably, no loss of the $\alpha$-Br is observed, either by bromide elimination or bromine extraction by LiHMDS.
3.2.2.3 – Lactone deprotection

While confident in the assignment of relative stereochemistry after electrophilic fluorination, and agreement with other precedent literature data,[24] absolute configuration by X-ray analysis would conclusively assign the configuration at the α-position. While protecting the 5- and 3- hydroxyl groups as their triisopropyl silyl ethers was necessary for the α functionalisation, downstream chemistry did not require such bulky groups. In addition to this, due to their lipophilic nature, most of the protected lactones discussed present themselves as viscous oils in physical appearance thus rendering them unsuitable for analysis by crystallographic methods. Deprotection of the silyl ethers and reprotuction may allow for such analysis while not hindering further synthesis.

Subjecting \textit{202} to tetramethylammonium fluoride tetrahydrate in combination with acetic acid in DMF consumed all starting material by \textsuperscript{19}F\{\textsuperscript{1}H\} NMR with reference to α,α,α-trifluorotoluene as internal standard, reaction shown in Scheme 3.14. As expected, formation of triisopropyl silyl fluoride is observed, with a peak at -185.4 ppm,\textsuperscript{[118]} at 82\% NMR yield. While the deprotection was successful, the only other environment of note was two singlets at -201.0 and -201.2 ppm, at 50\% and 30\% NMR yield respectively. Troublingly, this is a similar shift to that observed for the mono-fluorinated ribonolactone \textit{65}, whereby the two singlets arise from the two possible diastereomers of the monofluorinated compound.

\textbf{Scheme 3.14:} The TMAF mediated deprotection of \textit{202}.

This observation suggests that deprotection was successful but has potentially been accompanied by debromination. GC-MS analysis of the crude mixture revealed a peak corresponding to a mass of 230/231 m/z. This peak equates to the mass of target compound \textit{195}, demonstrating that some degree of desired product formation may have occurred, in amounts detectable by GC-MS. Crucially, the \textsuperscript{19}F NMR data didn’t match that for the previously synthesised 2-deoxy-2-bromo-2-fluoro-ribonolactone (\textit{195}), therefore the current deprotection strategy was ultimately unsuccessful.
Changing to TBAF as the fluoride source (Scheme 3.15) resulted in an increased 94% NMR yield of TIPSF. Again, all starting material was consumed, returning 23% and 48% NMR yield of the suspected monofluorinated diastereomers.

Unfortunately, it seemed although the deprotection conditions were effective in removing the bulky silyl ether protecting groups, they were not compatible with the ribonolactone, possibly leading to degradation of the resulting in situ compounds. Therefore, changing to an alternative protecting group for later reactions – and potential crystallographic analysis – was not feasible at this stage and not pursued further.

**Scheme 3.15:** The TBAF mediated deprotection of 202.
3.2.2.4 – Mesyl-O-lactol formation

With the desired gem-dihalo functionality installed, reduction of the lactone to the γ-lactol was targeted. Mild reductants were required, as ring opening of the lactone could be envisaged and such side reactions would want to be avoided. As such, 202 was treated with DIBAL-H in toluene, producing 209 in 67% isolated yield. Although successful, this strategy seemed quite wasteful given the need to use 7 equivalents of reductant. Therefore in an attempt to be more economical, akin to Chou’s synthesis,13 lithium tri-tert-butoxy aluminium hydride was utilised as the reducing agent of choice, forming lactol 209 in quantitative yield.

It was noted that the reaction proceeded more smoothly when conducted in a mixed solvent system of Et₂O/THF (4:1), resulting in a cleaner crude ¹H NMR. 209 was isolated as a 71:29 ratio of diastereomers by ¹⁹F NMR. The diastereomeric ratio remained the same even after months of storage in the fridge, despite the potential for anomerisation. Perhaps surprisingly, the diastereomeric ratio was the same using both DIBAL-H and LiAl(O'Bu)₃H. The diastereomers – shown in Figure 3.03 – are inseparable by column chromatography, so both were carried forward for the next reaction.

Table 3.04: The reduction of 202 to lactol 209.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 209[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DIBAL-H (7 equiv.) Toluene, -78°C, 2 h</td>
<td>67%</td>
</tr>
<tr>
<td>2</td>
<td>LiAl(O'Bu)₃H (1.2 equiv.) THF/Et₂O, 0°C → r.t., 4 h</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

[a] Isolated yield.

Figure 3.03: The configuration of the two diastereomers of 209.
Interestingly, the multiplicity of $^{19}$F shifts demonstrates that each diastereomer of lactol 209 has inherently different interactions with the neighbouring protons. One diastereomer exhibits a doublet of doublets at -120.62 ppm in the non-proton decoupled $^{19}$F NMR, which is the expected multiplicity given two vicinal protons, resulting in two $^3J_{F-H}$ coupling constants of 11.1 and 5.8 Hz. Interestingly, the second diastereomer only demonstrates a doublet at -127.34 ppm, with a $^3J_{F-H}$ of 12.8 Hz. This suggests that the conformation of this diastereomer shows no detectable coupling between the fluorine atom and the vicinal hydrogen atoms, potentially stemming from a near 90° dihedral angle within the strained conformation, or that the magnitude of the coupling is beyond the sensitivity of the NMR spectrometer. The 2-deoxy furanose ring is unlikely to sit as depicted in general, in an east conformation (Scheme 3.16, centre 209). The compound will preferentially sit in either a south (C2-endo/C3-exo) or north (C2-exo/C3-endo) conformation, depicted in Scheme 3.16. As a result, the fluorine at the 2-position may not interact with the vicinal hydrogen atoms, hence explaining the origin of the observed multiplicities.

Scheme 3.16: The potential extreme north/south conformations of 209.
γ-lactol \( \text{209} \) was subsequently treated with methane sulfonyl chloride in DCM with \( \text{NEt}_3 \) as base, forming the mesylated lactol \( \text{210} \) in quantitative yield, without the need for purification (Scheme 3.17). It was found that washing with water during work up of the reaction resulted in decreased product yield, causing hydrolysis of \( \text{210} \) and returning undesired starting material. Again, the compound was formed in a diastereomeric mixture, on this occasion in a ratio of 59:41. This observation suggests increased anomerisation compared to lactol \( \text{209} \), arising from the increased leaving group ability of the mesylate vs. hydroxide. Interestingly, the ratio of the diastereomers remains constant after long term storage in the fridge, demonstrating no change in equilibrium between the two diastereomers.

**Scheme 3.17**: The formation of \( \text{210} \) by basic mesylation of lactol \( \text{209} \).
3.2.2.5 – Glycosylation

With mesylated lactol 210 in hand, attention turned towards the Vorbrüggen glycosylation reaction.\textsuperscript{[119–122]} With the observed selectivity in the developed ribonofuranose synthetic methodology, the procedure could well be applied towards the synthesis of a range of nucleosides. With the focus of gemcitabine in mind, the pyrimidine cytosine was targeted for ring appendage. In order to improve the efficiency of the ring appending reaction, the \(N^4\) functionality of the cytosine nucleobase was protected as the amide (Scheme 3.18). At this point, considering the desire to improve the lipophilicity of these chemotherapeutic agents, cytosine was protected as both the \(N^4\)-acetamide and \(N^4\)-2-propylpentamide (henceforth referred to as \(N^4\)-valproamide).

![Scheme 3.18: The proposed synthesis of \(N^4\) functionalised cytosines by reacting 184 with anhydride 211.](image)

The use of valproic anhydride would provide a convenient reagent to functionalise cytosine at the \(N^4\)-position, rendering synthesis of the anhydride. Use of EDC.HCl with valproic acid formed the desired anhydride in 31% NMR yield,\textsuperscript{[123]} while DCC mediated coupling yielded no target material. In order to improve the yield of the anhydride formation, phosphorous coupling reagents were subsequently targeted. Successful anhydride formation was finally realised by combining valproic acid with diphenyl phosphoryl chloride in the presence of triethylamine,\textsuperscript{[124]} yielding 95% of 213 (Scheme 3.19). A subtle shift in the \(^1H\) NMR of the \(\alpha\)-proton from 2.38 ppm (in valproic acid) to 2.44 ppm was observed upon anhydride formation.

![Scheme 3.19: The formation of anhydride 213.](image)
Tellingly, IR spectroscopy revealed effective anhydride formation, with two carbonyl stretching frequencies observed, at 1809 and 1746 cm\(^{-1}\) (c.f. \(\tilde{\nu}_{\text{C}=\text{O}}\) (valproic acid) = 1703 cm\(^{-1}\)). These stretches are indicative of an anhydride, representing the symmetric and asymmetric carbonyl stretching frequencies, respectively.

The anhydrides were each combined with cytosine in pyridine for 24 hours and precipitated from cold water to form their respective \(N^4\)-amides in excellent yields, both isolated in 94% yield (Scheme 3.20). This simple approach would allow for a range of \(N^4\)-amido cytosines to be constructed, depending on the target molecule. It also circumvents the use of valuable downstream material for selective \(N^4\) functionalisation, such as those initially employed for the synthesis of LY2334737.\(^{[25]}\)

![Scheme 3.20: The treatment of cytosine with acetic anhydride and valproic anhydride.](image)

In line with previous literature, the Vorbrüggen reaction (also known as the silyl-Hilbert-Johnson reaction) employs nucleobases that are typically protected as their silyl ether equivalents and used directly for the subsequent reaction with a primed ribofuranose. Such protecting methodology minimises side reactions, such as those possible via the \(N^4\)-position. Given the transient nature of the TMS protecting group, and its susceptibility to moisture, the intermediates of these reactions are not commonly isolated – which begs the question, can the conversion be trusted? The simple answer is yes, as the reaction has been proved to work. But it does question the exact nature of the intermediate.

Typical conditions\(^{[125]}\) employing TMSCl as silylating agent in the presence of triethylamine in toluene did not yield the silylated pyrimidine. Changing to using HMDS as solvent in the presence of catalytic TMSCl yielded 216 in 57%. Crucially, utilising ammonium sulfate in substoichiometric quantities as weak proton source with HMDS produced \(N\)-(2-(trimethylsilyloxy)pyrimidin-4-yl) acetamide 216 in 93% isolated yield (Scheme 3.21). Interestingly, the product was isolated as the mono-TMS protected cytosine – confirmed by HRMS – and not the often reported bis-TMS. \(N^4\)-Valproyl cytosine was also subjected to the reaction conditions, isolating 217 in quantitative yield.
Results and discussion

(Also Scheme 3.21). Analogously, 218 was also isolated as the mono-TMS protected cytosine, which suggests that $N^4$-functionalised cytosine nucleobases are silylated once – contrary to that commonly reported,[13,16,126,127] but may still be true for unfunctionalised nucleobases.[8,24]

Scheme 3.21: The protection of $N^4$-amido cytosines 214 and 215 by ammonium sulfate and HMDS.

Having successfully synthesised TMS-protected $N^4$-functionalised cytosines 216 and 217, attention turned to developing its reaction with mesylated lactol 210.

In the presence of TMSOTf as Lewis acid – sometimes described as Friedel-Crafts catalyst[121] – at reflux in anhydrous DCE, 210 with 216 formed nucleoside 218 in an anomeric mixture of 6.7:1 of β:α in 76% yield (shown in Scheme 3.22). Comparably, 219 was produced from 217 and 210 in 82% but notably there is a considerable shift towards the formation of the desired β-anomer, in a ratio of 10:1. This suggests a subtle yet significant role of the amide moiety – its precise role and how the observed selectivity is imparted is unclear, and further clarification might be difficult. Upon work-up of the reaction, any unreacted $N^4$-functionalised cytosine is precipitated and recovered, allowing for it to be reused for future glycosylation reactions.

Scheme 3.22: The glycosylation of 210 with of $N^4$-amido-O-TMS-cytosines 216 and 217.

The enhanced β selectivity observed in both cases may be explained by neighbouring group participation, when considering the reaction mechanism (Scheme 3.23). A resonance form of 210, with a dissociated mesylate counter anion and an oxocarbenium
cation could be envisaged (Int'-210). This could be resonance stabilised by a lone pair of the bromine atom, thus leading to facial selectivity.

![Scheme 3.23: The possible resonance forms of 210.](image)

If resonance form (Int''-210) were present during the reaction, the incoming nucleophile would display facial selectivity towards the top face of the cationic tetrahydrofuran ring, opposite the bromonium moiety – although attack of both faces could attacked in S_N1 fashion. Straightforward displacement of the mesylate could be imagined which would allow for formation of both anomers, although because Int'-210 is an activated form of 210, this S_N2 character may be less likely. Similarly, in the case of Int'-210 the size of the bromine atom could be envisaged to play a large role in negating re face attack to the oxocarbenium – which would form the α anomer – hence preferential si face addition leading to the β anomer is observed. It is also worth noting that the 3-O-TIPS group could be influencing the selectivity too, as it is a very large steric group and would negate nucleophilic attack from the lower face of ribofuranose 210 – all of which would aid in explaining a more pronounced β selectivity.

Notably on one occasion of the reaction of 210, the products were the mono-deprotected compounds 220 and 221, shown in Scheme 3.24, which allowed separation and isolation of each anomer following purification by column chromatography.

![Scheme 3.24: The unexpected formation of 220 and 221 by glycosylation of 210 with of N^4-amido-O-TMS-cytosines 216 and 217.](image)
The reason for this deprotection remain unclear, but has been observed in previous investigations.\textsuperscript{[23]} One possible explanation may be that the conditions are particularly forcing, resulting in loss of the more facile 5-O-TIPS group. In any case, with the next step of the synthesis being the TIPS deprotection, this unusual result ultimately delivers a desired intermediate.

Notably, the anomic ratio is inverted compared to that observed under reaction conditions shown in Scheme 3.22. This was a highly unusual observation, potentially arising due to a purity issue in synthesising 216 and 217, or due to anomerisation post-glycosylation,\textsuperscript{[13]} but would require further investigation to clarify.

In an attempt to develop a higher throughput methodology, use of microwave assisted synthesis was pursued, akin to that performed by Jamison and co-workers.\textsuperscript{[128]} As such, mesyl lactol 210 was irradiated at 150°C in acetonitrile, in the absence and presence of various Lewis and Brønsted acids (Table 3.05).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 222\textsuperscript{[a]}</th>
<th>Ratio 222:219\textsuperscript{[b]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TMSOTf (10 mol %) MeCN, 150°C, 10 min (d.r. 61:39)</td>
<td>30%</td>
<td>&gt;200:1</td>
</tr>
<tr>
<td>2</td>
<td>Pyridinium triflate (10 mol %) MeCN, 150°C, 10 min (d.r. 67:5:37.5)</td>
<td>33%</td>
<td>&gt;200:1</td>
</tr>
<tr>
<td>3</td>
<td>2,6-Lutidinium triflate (10 mol %) MeCN, 150°C, 10 min (d.r. 68:32)</td>
<td>38%</td>
<td>&gt;200:1</td>
</tr>
<tr>
<td>4</td>
<td>2,4,6-Collidinium triflate (10 mol %) MeCN, 150°C, 10 min (d.r. 64:36)</td>
<td>37%</td>
<td>&gt;200:1</td>
</tr>
<tr>
<td>5</td>
<td>No catalyst MeCN, 150°C, 10 min (d.r. 69:33)</td>
<td>36%</td>
<td>&gt;200:1</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} Isolated yield. Diasteromeric ratio determined by $^{19}$F NMR of crude mixture.

\textsuperscript{[b]} Regioisomeric ratio determined by $^{19}$F NMR of crude mixture.

Table 3.05: The glycosylation of 210 with 217 under microwave irradiation with various activators.
Utilising TMSOTf in 10 mol % as Lewis acid, 219 was not detected by $^{19}$F NMR analysis. HRMS revealed the compound had the same mass as the targeted 219, but analysis of the $^1$H NMR of the isolated material revealed a different compound was formed, which was tentatively assigned as the $N^2$- nucleoside, produced in an anomeric mixture of 61:39. Employing pyridinium triflate – easily accessed from combining pyridine with triflic acid in diethyl ether – as Bronsted acid yielded 222 in a slightly improved yield of 33%. Use of bulkier, substituted pyridines increased the yield marginally again to 38% and 37% for 2,6-lutidinium triflate and 2,4,6-collidinium triflate respectively (Entries 3 and 4). The reaction was also conducted in the absence of catalyst (Entry 5), which surprisingly delivered 222 in a comparable yield of 36%. This result infers that the abstraction/displacement of the mesylate anion does not require a catalyst, implying a different reactivity – such as $S_N1$ – may be active. Considering the anomeric selectivity discussed earlier, it is highly probable that some neighbouring group participation is indeed in effect, as per Int‘-210 in Scheme 3.24.

The preferential formation of the $N^2$- product over the $N^1$- is unusual, but microwave heating allows for alternative attack from the $N^2$- position of pyrimidinone ring, as opposed to nucleophilic addition through $N^1$- (illustrated in Scheme 3.25). This example suitably demonstrates the potential of enabling technologies such as microwave chemistry allowing for development of alternative nucleosides as potential active pharmaceutical ingredients (APIs).

**Scheme 3.25:** The comparison of the reactivity of 217, depending on method employed. Formation of 219 is observed under conventional batch heating (*top*) and 222 is isolated when heating under microwave irradiation (*bottom*).
Determination of the product from the microwave glycosylation as **222** was achieved by comparison of the $^1\text{H} \text{NMR}$ spectra of the isolated $\beta$-anomer of each from their respective methods of synthesis (Table 3.06). The environment of the hydrogen atoms on the pyrimidinone ring demonstrate notable change, both in chemical shift and coupling constant, as their respective environments are inherently different. In **219**, the protons are cis-alkene in nature and in a fairly similar chemical environment, although $H^6$ is likely to be more deshielded due to its proximity to $N'$. By comparison in **222**, $H^6$ could be described as “imine-like”, explaining why it is significantly more deshielded, with a shift of 8.42 ppm. Significantly, the coupling constant between the protons is less pronounced, with $^3J_{HH} = 5.6 \text{ Hz}$, perhaps due to the relationship between $H^5$ and $H^6$, which may be described as s-cis diene, in a locked orientation.

![Structures 219 and 222](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>$H^5$ shift / ppm</th>
<th>$H^6$ shift / ppm</th>
<th>$^3J_{HH}$ / Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>219</strong></td>
<td>7.45</td>
<td>7.88</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td><strong>222</strong></td>
<td>7.89</td>
<td>8.42</td>
<td>5.6</td>
</tr>
</tbody>
</table>

**Table 3.06**: The $^1\text{H} \text{NMR}$ shifts and $^3J$ values of **219** and **222**.
3.2.2.6 – Radiolabelling precursor synthesis

Subsequently, TIPS deprotection of 218 was attempted using concentrated HCl in methanol, stirring at room temperature for 2 days, where the mono-deprotected compound β-223 precipitated out of solution while also cleaving the $N^4$-acetamide motif under acidic conditions, in 63% isolated yield. β-223 is noted as its imine tautomer due to two NH environments being observed in the $^1$H NMR in DMSO-$d_6$. Global acetylation of $O^5$ and $N^4$ positions in β-223 was achieved using acetic anhydride, catalytic 4-DMAP and triethylamine in DCM to give β-224 in 91%, depicted in Scheme 3.26.

TBAF was initially used as fluoride source to deprotect the TIPS motif but returned starting material β-224. Removal of the silyl ether protecting group was realised by combination of TMAF with AcOH in DMF, forming β-225 in 78% yield (Scheme 3.27) Subsequent bis-acetylation of β-225 yielded 91% of compound β-226, suitable as a precursor for intended radiolabelling with $^{18}$F.

Scheme 3.26: The synthesis of β-224 by deprotection of 218 and acetylation of β-223.

Scheme 3.27: The sequential deprotection of β-224 and acetylation for the synthesis of β-226 as radiolabelling precursor.
In the case of 220, while possible to partially separate the anomers during purification, it was simpler to combine the mixture and subject to TMAF mediated deprotection conditions in DMF, in combination with acetic acid (likely forming HF \textit{in situ}), yielding the desired \(\beta\)-anomer in 80\% yield after purification by column chromatography, shown in Scheme 3.28.

**Scheme 3.28:** The sequential deprotection of 219 and acetylation for the synthesis of \(\beta\)-227.

Acetylation of intermediate \(\beta\)-227 was effective in affording radiolabelling precursor \(\beta\)-228 in 90\% yield by treatment with acetic anhydride with 4-DMAP and triethylamine in DCM (Scheme 3.29). Investigation of (radio)labelling and related studies of \(\beta\)-228 is discussed in Section 3.4 (Page 107).

**Scheme 3.29:** The synthesis of radiolabelling precursor \(\beta\)-228 acetylation of \(\beta\)-227.
3.2.3 – α-hydroxy lactone formation

Halides represent a strong option as a leaving group for the targeted radiofluorination, with their installation via a range of electrophilic halogen sources, such as \(N\)-halosuccinimides. However, alternative moieties with enhanced leaving group properties exist, which may allow for greater ease of displacement by \([{^{18}}F]\)fluoride. Leaving groups such as mesylate, 4-nosylate and triflate have found use in aliphatic nucleophilic fluorination,\(^{88,129}\) which could be accessed through a 2'-fluoro-cytidine analogue. Despite the lack of literature precedent surrounding the fluorohydrin moiety that would be required, it was an interesting thought process to investigate, illustrated by the disconnection strategy shown in Scheme 3.30. Additionally, such improved leaving group ability may increase the potential for disfavoured elimination to form an \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone or other byproducts.

![Scheme 3.30: Disconnection of 229.](image_url)

Given that a robust method for forming 64 (\(R^1 = \text{TIPS}\)) was in hand, formation of α-hydroxy lactone moiety was initially targeted by electrophilic hydroxylation, using Davis’ oxaziridine as an electrophilic source of oxygen.\(^{130}\) As such, 235 was synthesised (Scheme 3.31); firstly by formation of imine 234 from benzene sulphonamide (232) and neat benzaldehyde dimethyl acetal (233), and subsequent oxidation by mCPBA using benzyl triethyl ammonium chloride as phase transfer catalyst.\(^{131}\)
As α-hydroxylation would be achieved via the enolate of lactone 64, it was treated with LiHMDS and oxaziridine 235 in the presence of TMSOTf as Lewis acid, which led to no observable desired product formation (Table 3.07, Entry 1). Omitting the Lewis acid, thereby accessing the more reactive enolate led to a different crude ¹H NMR from the reaction, with new proton environments observed at 28% NMR yield—potentially corresponding to an adjacent proton of an alcohol group. Notably, much of the oxaziridine was returned (c.f. 66% NMR yield) despite the consumption of starting material 64, with mass balance ruling out the effective formation of 236. This result also suggests that 235 may not be sufficiently electrophilic.

### Table 3.07: The conditions screened for the α-hydroxylation of 64.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Rec. of 64[a]</th>
<th>Yield of 236[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LiHMDS (1.5 equiv.), TMSOTf (1.6 equiv.) then 235 (1.7 equiv.) THF, -78°C, 2 h</td>
<td>33% (66% 235)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>LiHMDS (1.5 equiv.), 235 (1.7 equiv.) THF, -78°C, 2 h</td>
<td>9% (81% 235)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>LiHMDS (1.5 equiv.), TMSOTf (1.6 equiv.) then mCPBA (1.7 equiv.) THF, -78°C, 2 h</td>
<td>32%</td>
<td>14%</td>
</tr>
<tr>
<td>4</td>
<td>LiHMDS (1.5 equiv.), mCPBA (1.7 equiv.) THF, -78°C, 2 h</td>
<td>38%</td>
<td>-</td>
</tr>
</tbody>
</table>

[a] ¹H NMR yield using mesitylene as internal standard.
As an alternative, the Rubottom oxidation (Entries 3 and 4) was investigated. Exclusion of TMSOTf (Entry 4) – required for the formation of the silyl enol ether intermediate – did not yield 236. Interestingly, examination of the crude $^1$H NMR of Entry 3 showed a new peak at 6.17 ppm (dd, $J = 5.7, 2.0$ Hz) [and another at 5.41 ppm (tt, $J = 2.3, 1.6$ Hz)], which may be attributed to the proton adjacent to the newly installed hydroxyl group and alpha to the lactone moiety (potentially coupling to the OH). With an encouraging 14% NMR yield, attempted isolation by column chromatography yielded no desired product.

It is worth noting that the hydroxylation may have been performed on the mono-fluorinated lactone 65, as opposed to hydroxylation and subsequent fluorination, but this strategy was not explored.

Alternatively, the desired functionality may be conveniently accessed by beginning from D-ribonic-$\gamma$-lactone (231); and subsequent protection and fluorination via the previously explored enolate chemistry. As shown in Scheme 3.32, studies commenced by protecting commercially available 231 with 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane to afford 237 in 51%.\[132\]

![Scheme 3.32: The protection of 231.](image)

An orthogonal protecting group to the disilox ether moiety was deemed highly desirable for the $\alpha$-hydroxy functionality. Additionally, a non-eliminating functionality would be ideal, given the basic conditions of the electrophilic fluorination. Shown in Scheme 3.33 is the 2-$O$-functionalisation of 237; firstly, treatment with methoxymethyl chloride in DCM was unsuccessful in delivering protected lactone 238, returning the starting material in 61% isolated yield. Alternatively, Ac$_2$O was used and furnished 239 in 66% NMR yield and a diminished 24% isolated yield.
With an appropriately functionalised lactone in hand, fluorination of 239 would be targeted as the next step in this synthetic strategy.

Scheme 3.33: The attempted 2-O- derivatisation of 237.
3.3 – Scale-up of synthetic route 2

Given the target precursor for radiolabelling is chemically very similar to LY2334737 (100, Scheme 1.42), there is sufficient scope for the unprotected analogue to be investigated as a potential anticancer agent itself. Due to the amount of chemotherapy required during a course of treatment – 1000 mg m⁻² of gemcitabine is administered weekly for up to 7 weeks¹³³ – a scalable method of producing the key 2-bromo-2-fluoro-lactol 209 would be required, along with N⁴-valproyl cytosine (215).

The previously explored method of anhydride formation from reacting valproic acid with diphenylphosphoryl chloride was successfully translated to a 400 mmol scale (with respect to 212, yielding 45.4 g of anhydride at 84% yield (Scheme 3.36).

![Scheme 3.36](image)

**Scheme 3.36**: The scale up synthesis of valproic anhydride, 213.

The only modification required for the reaction was further washing with saturated aqueous NaHCO₃ to remove an acidic by-product, likely a phosphoric acid type compound, without deleterious effect on the anhydride.

Utilising valproic anhydride with cytosine in anhydrous pyridine on 83 mmol scale (with respect to cytosine) effectively afforded 215 as an impure mixture. After precipitation from cold water, the crude product contained a mixture of valproic acid as by-product and unreacted cytosine.

![Scheme 3.37](image)

**Scheme 3.37**: The combination of cytosine with valproic anhydride to form 215 on scale.
Each of these impurities were removed by selective trituration – saturated aqueous NaHCO$_3$ was used to quench remaining acid, and cytosine was removed by 0.5 M HCl – finally yielding 18.6 g of 215 at 94% yield after purification (Scheme 3.37).

As previously discussed, oxidative cyclisation of 2-deoxy-ribose was investigated and was quickly found to be scale dependent – if the reactions were conducted on a scale greater than 40 mmol, they would become inefficient, resulting in lower product yield and increased formation of undesired by-products. As such, multiple reactions could be run in parallel and then combined for work-up and purification, again utilising K$_2$CO$_3$ as neutralising agent.

Once sufficient 75 had been synthesised, different scale reactions were investigated for its protection as 64. The results are summarised in Table 3.09, but it can be seen that irrespective of scale, the reaction is highly repeatable delivering decagrams of 64.

![Scheme 3.37](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale / mmol</th>
<th>Yield of 64$^{[a]}$</th>
<th>Mass of 64 / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>75</td>
<td>12.73</td>
</tr>
<tr>
<td>2</td>
<td>86.8</td>
<td>80</td>
<td>31.00</td>
</tr>
<tr>
<td>3</td>
<td>137.3</td>
<td>78</td>
<td>47.51</td>
</tr>
<tr>
<td>4</td>
<td>75.8</td>
<td>80</td>
<td>26.90</td>
</tr>
</tbody>
</table>

$^{[a]}$ Isolated yield.

**Table 3.09:** The investigation of the effect of scale upon formation of 64.

By comparison, when scaling up the $\alpha$-bromination reaction of 64, the process was found to be more inconsistent, delivering a range of yields ranging from 35% to 70%. The reason for this variation is difficult to pinpoint conclusively, although there is somewhat of an inverse correlation between scale and yield. Alternative work up methods were also found to impair the reaction, as filtering the reaction over a plug of silica returned only 16% yield of the desired compound. Therefore, this reaction requires further refinement in order to ensure it is reproducibility and independent of scale.
Focussing on the electrophilic fluorination (Scheme 3.38), altering the stoichiometries of reagents for the reaction – increasing to 1.7 equiv. of LiHMDS and 1.9 equiv. of NFSI – allowed the reaction yield to increase to 94% when conducted on a 20 mmol scale. This result suggests that previous conditions were not forming sufficient in situ lithium enolate to react with NFSI. Even upon increasing scale, only one diastereomer is formed during this reaction.

Due to the necessity of the triisopropyl silyl protecting groups, the majority of the resulting compounds are thick, viscous oils with high boiling points. As such, purification by distillation is not possible – even when using forcing conditions such as 220°C at 10 mbar of pressure, the compounds co-distill. Kugelrohr distillation was attempted to purify crude mixtures of 202 and 203, and in both instances the reaction mixtures were not resolved.

Table 3.10: The scale-up of α-bromination reaction of 64.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale / mmol</th>
<th>Yield of 203</th>
<th>Mass of 203 / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.72</td>
<td>70</td>
<td>2.85</td>
</tr>
<tr>
<td>2</td>
<td>28.5</td>
<td>51</td>
<td>7.67</td>
</tr>
<tr>
<td>3</td>
<td>69.7</td>
<td>35</td>
<td>12.60</td>
</tr>
<tr>
<td>4(b)</td>
<td>67.5</td>
<td>16</td>
<td>5.50</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>69</td>
<td>1.74</td>
</tr>
</tbody>
</table>

[a] isolated yield, [b] Crude mixture filtered over SI plug.

Scheme 3.38: The improved fluorination of 203 when scaled-up with increased equivalents of LiHMDS and NFSI.
Remarkably the compounds were stable under these conditions, with no observable degradation by $^1$H NMR analysis. As such, the purification of the intermediates by column chromatography is a drawback of the developed methodology.

![Chemical Reaction Diagram]

Table 3.11 examines the varying scale and subsequent effect on the reduction of 202, and it can be seen that the results demonstrate a scale dependency of the reaction – the exception being Entry 2, potentially due to use of lithium tert-butoxyaluminium hydride that had degraded. The optimum conditions appear to be when the reaction is conducted on ~10 mmol scale (Entry 4), returning 5.62 g of lactol 209 quantitatively. Increasing scale further reveals a slight reduction in isolated yield (Entries 5 and 6).

Another issue upon increasing the scale of this process was the removal of the aluminium salts; which could be circumvented by use of Rochelle salts, washing with 0.5 M HCl or employing the Fieser work-up method$^{[134]}$ – but the former returned lower yields of 209.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale / mmol</th>
<th>Yield of 209[a]</th>
<th>Mass of 209 / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1[b]</td>
<td>3.9</td>
<td>70</td>
<td>1.49</td>
</tr>
<tr>
<td>2[b]</td>
<td>6.7</td>
<td>42</td>
<td>1.54</td>
</tr>
<tr>
<td>3[b]</td>
<td>8.8</td>
<td>(32% 202)</td>
<td>3.84</td>
</tr>
<tr>
<td>4[c]</td>
<td>10.6</td>
<td>quant.</td>
<td>5.62</td>
</tr>
<tr>
<td>5[c]</td>
<td>12.3</td>
<td>91</td>
<td>6.08</td>
</tr>
<tr>
<td>6[c]</td>
<td>16.3</td>
<td>87</td>
<td>7.74</td>
</tr>
</tbody>
</table>

[a] isolated yield. [b] 1.2 equiv. of LiAl(O^tBu)_3H used. [c] 1.1 equiv. of LiAl(O^tBu)_3H used.

Table 3.11: The reduction of 202 when performed on scale.
As previously, mesylation of lactol 209 proceeded smoothly independent of scale to afford 6.6 g of 210 (Scheme 3.39).

Scheme 3.39: The sequential scale up reactions towards 210 by reduction and mesylation.
3.4 – Fluorination

3.4.1 – Cold $^{19}$F fluorination

In preparation for the attempted fluorination, performing the substitution reaction with non-radioactive fluorine was targeted in order to generate & characterise a sample of the target cold material. Derivatisation of 228 was attempted, in order to access more reactive compounds that may better serve the fluorination reaction. As such, 228 was subjected to typical Finkelstein reaction conditions, to obtain the 2'-fluoro-2'-iodo congener (240). Acetone was initially employed as reaction solvent (Table 3.12, Entry 1), with an excess of potassium iodide at room temperature for 24 hours yielding none of the desired material (240), only returning 228 quantitively. Switching to 2-butanone at reflux, as higher boiling point solvent also did not produce 240 (Entry 2), nor did changing from potassium iodide to sodium iodide (Entry 3) with both attempts returning starting material, confirmed by MS and $^{19}$F NMR.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 240$^{[a]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KI (5 equiv.) Acetone, r.t., 24 h</td>
<td>- (quant. 228)</td>
</tr>
<tr>
<td>2</td>
<td>KI (5 equiv.) 2-Butanone, reflux, 46 h</td>
<td>- (quant. 228)</td>
</tr>
<tr>
<td>3</td>
<td>NaI (5 equiv.) 2-Butanone, reflux, 24 h</td>
<td>- (quant. 228)</td>
</tr>
</tbody>
</table>

$^{[a]}$ Isolated yield.

Table 3.12: The reaction of 228 under Finkelstein-like conditions.

From the results presented in Table 3.12, it can be seen that highly forcing conditions would be required to facilitate the substitution reaction, even with the aid of by-product precipitation as employed in the Finkelstein reaction. Utilising microwave chemistry could allow for this process take place, allowing for elevated temperatures to be reached in...
appreciable time, given the desired application to $^{18}$F labelling and the half-life of fluorine-18.

\[ \text{Scheme 3.40: The fluorination of 228 under } ^{18}\text{F-like conditions.} \]

Initially, precursor 228 was reacted with potassium fluoride with phase transfer catalyst K$_{222}$ in DMF, irradiated at 120°C for 30 min (Scheme 3.40) but did not successfully yield compound 241 after work up. Instead, the use of silver fluoride was tested with a two-fold target – as fluoride source and to aid with bromide abstraction, precipitating out insoluble AgBr as by-product. Replacing KF with AgF resulted in consumption of starting material 228, but no desired product formation (Table 3.13, Entry 1). Omitting chelator K$_{222}$ only returned 228 (Entry 2), while increasing time or not performing azeotropic drying of KF and K$_{222}$ (to mimic radiofluorination conditions) did not furnish 241 (Entries 3 and 4 respectively).
Returning to conventional solution chemistry, precursor 228 was reacted with silver fluoride in the presence and absence of phase transfer catalyst Kryptofix® 222 (Scheme 3.40 and 3.41 respectively). Qualitative analysis of the crude reaction mixture by HRMS revealed that, pleasingly, 241 was successfully formed under both sets of reaction conditions.

Examination of the HRMS spectrum of the fluorination reaction in the absence of K\textsubscript{222} (Figure 3.04, reaction of Scheme 3.41) reveals a fragment at 536.1251 m/z, which equates to unreacted starting material, [228+H]\textsuperscript+ – and can be identified from the splitting pattern of bromine – while the signal at 238.1556 m/z arises from N\textsuperscript{4}-valproyl cytosine (215).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 241\textsuperscript{[a]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AgF (5 equiv.), K\textsubscript{222} (1 equiv.) DMF, 120°C, 30 min</td>
<td>- (60%, 228)</td>
</tr>
<tr>
<td>2</td>
<td>AgF (5 equiv.) DMF, 120°C, 30 min</td>
<td>- (quant. 228)</td>
</tr>
<tr>
<td>3</td>
<td>AgF (5 equiv.), K\textsubscript{222} (1 equiv.) DMF, 120°C, 60 min</td>
<td>- (9% 228)</td>
</tr>
<tr>
<td>4\textsuperscript{[b]}</td>
<td>AgF (5 equiv.), K\textsubscript{222} (1 equiv.) DMF, 120°C, 30 min</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} \textsuperscript{19}F NMR yield, using α,α,α-trifluorotoluene as internal standard. \textsuperscript{[b]} No azeotropic drying.

Table 3.13: The fluorination of 228 using silver(I) fluoride under microwave irradiation.
The fragment observed at 456.2150 m/z is calculated to be from a debromohydrofluorinated derivate of the 228 (i.e. 242, [M+H]⁺). Formation of 242 may rationalised by silver mediated bromine abstraction of 228, potentially by a radical based mechanism. \[^{[135]}\]
Results and discussion

The reaction was also conducted with $K_{222}$, the HRMS spectrum of which (Figure 3.05) paints a similar picture, despite apparent increased fragmentation (which might not necessarily reflect reaction conditions). Again, fragments at 238.1557 and 536.1238 m/z correspond to $N^4$-valproyl cytosine and 228 respectively.

Scheme 3.42: The fluorination reaction of 228 by silver(I) fluoride with $K_{222}$.

Figure 3.05: HRMS spectrum of the reaction mixture of that shown in Scheme 3.42.

The reaction was also conducted with $K_{222}$, the HRMS spectrum of which (Figure 3.05) paints a similar picture, despite apparent increased fragmentation (which might not necessarily reflect reaction conditions). Again, fragments at 238.1557 and 536.1238 m/z correspond to $N^4$-valproyl cytosine and 228 respectively.
Again, the debromohydrofluorinated motif is presented as the fragment at 456.2148 m/z (calculated 455.1844 m/z, 242), along with a detectable amount of the fully protected difluorinated compound 241 ([M+H]^+ calc 474.2052 found 474.2055, see Appendix 6.3). These positive results demonstrate that fluorination is possible on the synthesised precursor 228, and that the protecting group strategy employed is also compatible.

3.4.2 – Radiofluorination

Having explored the fluorination reaction, the challenge then turned to performing the reaction radiochemically. Studies commenced by reacting 228 with azeotropically dried [¹⁸F]KF/K₂CO₃/K₂₂₂ in acetonitrile at reflux, with an initial activity of 13.9 MBq (Scheme 3.43).

Scheme 3.43: The attempted radiofluorination reaction of 228 by [¹⁸F]KF with K₂₂₂.

Analysis of the crude reaction mixture by radio-TLC (Figure 3.06) using 70% EtOAc/hexanes as eluent showed that in addition to the free fluoride signal (Figure 3.06, Region 1), a new radioactive signal was present (Region 2). Relative to the free fluoride, 37.5% of the radioactive count was from the new signal, equating to an activity of roughly 3.8 MBq from a total activity of 10.25 MBq in the crude reaction mixture post-reaction. Unfortunately, the radio-HPLC trace of the reaction media did not correspond to that of the non-radioactive sample, indicating that the reaction was not successful in forming 243, but another product. Despite the Rᵢ (radio-TLC) and retention time (radio-HPLC) having similarities the analogous ¹⁹F target compound, there is not sufficient evidence that the radioactive compound formed is 243. Due to insufficient time, further investigation into the radiofluorination reaction was not possible.
Figure 3.06: Radio-TLC trace reaction shown in Scheme 3.42.
3.4.3 – Conclusions and outlook

Radiofluorination of 228 was briefly investigated, under typical conditions employed for $^{18}$F reaction, using K$_{222}$ and MeCN to form gemcitabine prodrug 243. Results demonstrate that a new radioactive peak was detected by radio-TLC with a similar $R_f$ to the target compound, although the identity of the product could not be determined.

There is significant scope to further investigate and develop the radiofluorination chemistry applied to this system, illustrated within Scheme 3.44. With a suitable precursor in hand, besides temperature (T) and time (t), investigation of alternative solvents such as DMF and $^3$BuOH may yield the desired compound.$^{[71]}$ Similarly, utilising reagents to aid with bromine abstraction such as silver triflate or alternative Lewis acids may aid the process by increasing the $S_{N1}$ character of the reaction, given the forcing conditions required for potential $S_{N2}$ reaction to occur. Additionally a Lewis base like DABCO could be included, which may promote the formation of an activated intermediate more susceptible to radiofluorination.

Modern approaches would potentially involve the use of transition metal catalysts, such as palladium$^{[136]}$ or porphyrin ligated manganese.$^{[137,138]}$ Recent elegant developments from the Ritter lab have employed a thianthrenation strategy on aromatic substrates.$^{[139]}$ Reconsideration of the design of precursor 228 could also allow access to alternative leaving groups. Installation of an iodine at the 2' position would not only improve the leaving group ability, but access to more oxidisable motif which may further improve
radiofluorination. Similarly, sulfur and selenium groups could be introduced as these may also be oxidised, but could lead to competitive elimination reactions.

Scheme 3.45: Potential radiofluorination conditions that could be explored to synthesise 243 from 249.

Compound 228 has a great deal of potential as a precursor for radiofluorination and achieving the target of an $^{18}$F-labelled gemcitabine prodrug, while also demonstrating potential as a chemotherapeutic agent itself and will no doubt be a good platform for further work to be based from.
3.5 – Tissue culture work

Due to the structural similarity between the targeted radiolabelling precursor and \( \text{LY2334737 (100)} \), it is conceivable that 227 would also demonstrate anti-cancer properties – the gem-dihalo functionality, believed to be the source of the chemotherapeutic properties, could still induce apoptosis \textit{in vivo}.

![Figure 3.07: Structure of compounds evaluated for cytotoxicity.](image)

Based on previous in-house work, Panc 10.05 cell lines were chosen to screen 227 against, as they were found to be most sensitive cell lines to treatment with gemcitabine, as opposed to other pancreatic cancer cell lines such as Capan-2 and HPAF-II. In order to ascertain the cytotoxicity of 227, \( \text{LY2334737 (100)} \) and gemcitabine (1) were also run against Panc 10.05 as control samples. Unfortunately, synthesis of the 2'-bromo-2'-fluoro analogue of gemcitabine (244) was elusive and could not be obtained.

Each tested compound was initially dissolved in DMSO, and then diluted to the appropriate concentration. As DMSO is known to present a low risk of cytotoxicity, control samples of the respective diluted DMSO concentrations were also screened against the Panc10.05 lines.

In order to visualise the cell death, an IncuCyte S3 was used as it allowed automatic live imaging of the cell plates at specified time intervals, visualised by fluorescence microscopy. As such, Incucyte Cytotox Red Reagent was used as a fluorescent probe, which binds to the DNA of an unhealthy, permeable plasma membrane, staining the cell red (from blue) when cell death occurs. This staining is in turn detected by the microscope within the instrument. When the reagent dye is administered, a background reading of the red area is required in order to highlight the cells that have undergone apoptosis, hence the red area vs normal area.
The cell lines were cultured in growth medium after defrosting and split appropriately, and seeded in 96-well plates at a density of $2 \times 10^5$ cells mL$^{-1}$. As such, the data obtained for the tested compounds isn’t completely quantitative due to the competing growth of cells and death by cytotoxic agent.

Gemcitabine was screened as a control sample, at concentrations of 100 μM, 50 μM, 10 μM and 1 μM, and the experiment was monitored for over 2 days. It can be observed that there is a positive correlation between increasing gemcitabine concentration and cell death, indicated by amount of red area observed (Figure 3.09). Also, the rate of cell death is greater at higher dosages of gemcitabine, notably so between the higher control limits of 100 μM and 50 μM. It can also be seen that increasing concentrations of control DMSO samples induces a mild degree of apoptosis initially, before peaking at 20 hours for DMSO 100 μM sample, which implies cell growth.

**Figure 3.08**: Red area normalised to phase area (%) as a function of time for gemcitabine and DMSO control against Panc10.05.
This is reinforced upon examination of the confluency (%) vs time (h) graph, which clearly displays an increase in confluency – the degree or amount of cells present – of the untreated sample (Gem 0 μM), indicating cell growth and that the cells weren’t sufficiently confluent before the beginning of the experiment. However even when Gem 1 μM is employed, it is sufficiently cytotoxic to overcome cell growth over the course of the experiment.

Satisfied that the conditions employed for gemcitabine were suitable, 100 and 227 were also screened at the same concentrations.

**Figure 3.09**: Confluency (%) as a function of time for gemcitabine against Panc10.05.
As with gemcitabine, area of red dye normalised against background fluorescence was measured as a function of time, shown in Figure 3.10. With the inconsistent data trends at the beginning of the experiment, likely associated with equilibration and introduction to the instrument environment, it is difficult to interpret. In general for all the data sets presented in Figure 3.10, there are no clear indications of an increase in red dye – hence cell death – as time increases. One of few conclusions that can be drawn is that come the end of the experiment, the two data sets demonstrating the greatest degree of apoptosis are the 100 μM and 50 μM of 227, inferring that these are the most cytotoxic conditions explored. Analysis of cell confluence as a function of time demonstrates clearly that during the period of the experiment that cell growth continues (confluence increases), even in the presence of cytotoxic agents 100 and 227. This infers that the cells weren’t incubated for long enough prior to the beginning of the experiment, or that insufficient cells were seeded prior to initial incubation – the latter being more likely. After a period of equilibration (cf. 2 hours), the higher concentrations of LY2334737 (50 μM and 100 μM slowly decrease below a confluency of around 30% over the duration of the experiment. These two sample sets demonstrate marked inhibited cell growth, hence inferred cytotoxicity, compared with the remaining sample set given cell growth.

Figure 3.10: Red area normalised to phase area (%) as a function of time for LY2334737 (100), 227 and DMSO control against Panc10.05.
Pleasingly, the next best performer can be seen to be the 100 μM sample of 227, over the course of the experiment. Under these conditions, the compound performs comparably, if slightly poorer, than the 50 μM Lilly (LY2334737) data set up until 20 hours or so. At that point, the trend plateaus and confluency increases, indicating that cell growth is greater than cell death. This data set comes between 50 μM and 10 μM of LY2334737, indicates a fair degree of cytotoxicity. Marginal cytotoxicity for 50 μM 2'-Br-LY2334737 may also be observed, between the beginning of the experiment and 10 hours, but thereafter cell growth is clearly visible as confluency increases dramatically. Analysis of the confluence percentage shows that the 10 μM LY2334737 follows a similar trend to the 100 μM 2'-Br-LY2334737 data set, but at a greater percentage of confluence. This infers that the sample sets may be of similar cytotoxicity, but also that 100 μM 2'-Br-LY2334737 may demonstrate enhanced apoptosis than 10 μM LY2334737 and be a viable alternative as a chemotherapeutic. Unfortunately, due to the experiment conditions, no further meaningful conclusions may be drawn from the data set, with increasing confluency overwhelming the data set.

**Figure 3.11:** Confluence (%) as a function of time for LY2334737 (100) and 227 against Panc10.05.
3.5.1 – Conclusions and outlook

Initial experiments into the potential use of 227 as a chemotherapeutic agent were conducted, using gemcitabine (1) and LY2334737 (100) as control samples, against Panc10.05 cancer cell lines. Cells were cultured and treated with a range of concentrations of the investigated compounds, and apoptosis monitored over the course of the experiment. It can be seen that use of 227 inhibits cell growth, inferring cell death.

This is a positive result, as it demonstrates that the synthesised compound indeed has chemotherapeutic properties, which may be a milder alternative to the hepatotoxic LY2334737. More compelling evidence is required – including repeating the experiment to obtain more robust data and allowing for sufficient cell seeding and confluence prior to beginning an experiment. Synthesis and isolation of the nonfunctionalised 244 would also provide a direct comparison to gemcitabine, hence the influence of the bromine atom at the 2’ position. Additionally, screening alternative pancreatic cancer cells such as HPAF-II, may reveal complementary properties of 227 to the existing anti-cancer drugs for pancreatic cancer whereby increasing the options available for cancer patient treatment, while non-cancer cells should also be investigated.

While the most desirable goal would have been for 227 to show improved cytotoxicity over 100, this result illustrates that variation at the 2’ position of 2’-deoxycytidine analogues leads to different cytotoxic properties. These findings may pave the way for the synthesis of a library of anti-cancer compounds of varying cytotoxicity, which will aid a physician in treating a patient. From a synthetic viewpoint, accessing the stereoisomer of 227 (245, Figure 3.12) would provide a useful insight into the effect of the gem-dihalo functionality arrangement, which may be accessed via a different synthetic methodology such as that by Ide and coworkers.[24] Also given the scalable synthesis of the core...
Results and discussion

Ribonolactone functionality, introducing a different moiety with known biological activity, such as an azido analogue (246) or carboxylates, could influence the hydrogen bonding network within DNA and lead to a change in cytotoxicity. Similarly, modifying the amide functionality at the N4 position will likely alter the cytotoxic properties of the compound and could be manipulated to further increase the lipophilic nature of the drug hence improve efficacy. An alternative approach would utilise the protide strategy developed by McGuigan and co-workers,[32] incorporating a phosphoramidate moiety at the 5' position to overcome the rate limiting phosphorylation.[40] This tactic could allow for either the non-functionalised or N4 functionalised (248) compound to be used to improve the chances of incorporation and uptake into cancer cells.

Figure 3.13: Structures of compounds of interest that may be synthesised for possible cytotoxic activity against Panc10.05.
4 – Conclusion

The objective of the research undertaken was to develop a synthetic route towards an appropriate precursor for radiofluorination and the production of $[^{18}\text{F}]$gemcitabine (182) by late-stage fluorination. Initial modification of Hertel’s original synthesis[8] was unsuccessfully translated to a synthetically useful scale (>1 mmol). As such, the synthetic strategy was revised, targeting installation of the leaving group by enolate chemistry of lactone 64.[12,20] A scalable, diastereoselective synthetic route towards 2-bromo-2-fluoro-ribonolactone 202 has been described, being produced on decagram scale in 49% yield over 4 steps from 2-deoxy-D-ribose. The observed selectivity is attributed to the use of NFSI as fluorinating agent, in conjunction with sterically bulky TIPS protected alcohol groups, which invokes facial selectivity and promotes single diastereomer formation. This methodology is complementary to recently published research.[24] Exploration of alternative oxygen-based leaving groups was also investigated.

Glycosylation of 210 with cytosine derivatives 214 and 215 proceeded with high anomic selectivity, which were in turn successfully transformed into radiolabelling precursors 226 and 228. The multigram synthesis of 202 and 228 demonstrates notable stability, a desirable characteristic when designing precursors for radiofluorination, but hints at potential difficulty when targeting hot fluorination. Additionally, use of microwave heating allowed access to 222, a different class of nucleosides, which could themselves be tested for radiofluorination and cytotoxicity.

In preparation of fluorination, authentic cold samples were synthesised for method development and characterisation. Non-radioactive $[^{18}\text{F}]$labelling studies were conducted on precursor 228, and it was found that 228 underwent fluorination, as detected by HRMS analysis. Initial radiofluorination studies were also investigated, reacting 228 with $[^{18}\text{F}]$KF delivered a radioactive compound following analysis by radio-TLC, which unfortunately did not match the target compound. Future work would further investigate the radiofluorination of 228 (in conjunction with 226) towards $[^{18}\text{F}]$gemcitabine, in order to ascertain the suitability of 226/228 as radiolabelling precursors. Parameters to investigate may include protic solvent,[71] and additives such as AgOTf to promote fluorination of 228.[98] Synthetically, alternative leaving groups or protecting groups may also be explored for the hot fluorination if 226 and 228 are unsuitable substrates.
Cytotoxic studies were also undertaken to evaluate the bioactivity of 227, in comparison to gemcitabine (1) and LY2334737 (100). These preliminary results demonstrated anti-cancer properties, but further testing is required to determine criteria such as IC\textsubscript{50} of the synthesised compounds.
5 – Experimental

5.1 – General information

Unless stated otherwise, all reactions were performed in oven-dried glassware sealed with rubber septa under a nitrogen atmosphere and were stirred with teflon-coated magnetic stirrer bars. Dry THF, acetonitrile, toluene, DCM and diethyl ether were obtained after passing these previously degassed solvents through activated alumina columns (Mbraun, SPS-800). Dry DMF was obtained from Acros Organics. All other solvents and commercial reagents were used as supplied without further purification unless stated otherwise.

Room temperature (r.t.) refers to 20-25 °C. Temperatures of 0°C and -78°C were obtained using ice/water and CO₂ (g) / acetone baths respectively. All reactions involving heating were carried out using DrySyn blocks and a contact thermometer. In vacuo refers to the use of a rotary evaporator under reduced pressure. Microwave synthesis was carried out using a Biotage® Initiator+ Robot 60 Microwave Synthesizer.

Analytical thin layer chromatography was carried out using aluminium plates coated with silica (Kieselgel 60 F254 silica) and visualization was achieved using ultraviolet light (254 nm), followed by staining with a 1% aqueous KMnO₄ solution. Column chromatography used Kieselgel 60 silica in the solvent system stated.

Infrared spectra were recorded on a Shimadzu IRAffinity-1 Fourier Transform ATIR spectrometer as thin films using a pike miracle ATR accessory. Characteristic peaks are quoted (vmax / cm⁻¹).

¹H, ¹³C{¹H}, ¹⁹F{¹H} and ¹⁹F NMR spectra were obtained on either a Bruker Avance 400 (400 MHz ¹H, 101 MHz ¹³C, 377 MHz ¹⁹F) or a Bruker Avance 500 (500 MHz ¹H, 126 MHz ¹³C, 471 MHz ¹⁹F) spectrometer at 25 °C in the stated solvent. Chemical shifts are reported in parts per million (ppm) relative to the residual solvent signal or to internal standard (¹⁹F: α,α,α-trifluorotoluene, -62.61 ppm). All coupling constants, J, are quoted in Hz. Multiplicities are reported with the following symbols: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and multiples thereof. The abbreviation Ar is used to denote aromatic, br to denote broad and app. to denote apparent signals. Carbon shifts are reported to the nearest 0.1 ppm and the number of signals rounded to the same value is indicated in brackets. Carbons in an identical environment giving one signal are not indicated further.
The structures below denote the numbering system used when assigning $^1$H and $^{13}$C environments:

High resolution mass spectrometry (HRMS, m/z) data was acquired either at Cardiff University on a Micromass LCT spectrometer or at the EPSRC UK National Mass Spectrometry Facility at Swansea University.

Radiochemistry was conducted at the Wales Research and Diagnostic Positron Emission Tomography Imaging Centre (PETIC), University Hospital Wales, on a Trasis ALLINONE unit.

Fluorescence microscopy was conducted using a Sartorius Incucyte® S3 Live-Cell Analysis System, in conjunction with Incucyte® Cytotox Red Reagent for counting dead cells as fluorescent imaging probe.
5.2 – Synthetic route 1

2,3-O-Isopropylidene-D-glyceraldehyde (11)[9]

1,2:5,6-di-O-isopropylidene-D-mannitol (10.49 g, 40.0 mmol) was dissolved in DCM (150 mL), to which saturated aqueous NaHCO₃ (5 mL) was added, maintaining the temperature below 25°C. NaIO₄ (21.39 g, 100 mmol, 2.5 equiv.) was subsequently added portion-wise over 20 minutes, and solution was stirred for 3 hours at room temperature. Solids generated during the reaction were filtered off and washed with additional DCM, and the filtrate dried over MgSO₄. The solvent was removed under reduced pressure and purified by distillation (25 mbar, 72-74°C) to yield 11 (8.05 g, 77% yield) as colourless oil.

¹H NMR (400 MHz, CDCl₃) δ 9.71 (d, J = 1.9 Hz, 1H, CHO), 4.38 (ddd, J = 7.3, 4.7, 1.9 Hz, 1H, CH(CHO)), 4.17 (dd, J = 8.8, 7.5 Hz, 1H, CH₂), 4.10 (dd, J = 8.8, 4.7 Hz, 1H, CH₂), 1.48 (s, 3H, CH₃), 1.41 (s, 3H, CH₃).

¹³C {¹H} NMR (101 MHz, CDCl₃) δ 202.0 (C=O), 111.4 (C(CH₃)₂), 80.0 (-CH(CHO)), 65.7 (CH₂), 26.4 (CH₃), 25.3 (CH₃).

IR (cm⁻¹): 3424, 2986, 1736, 1456, 1371, 1256, 1209, 1150, 1065, 843.

HRMS (TOF ES⁺): [C₆H₁₀O₄]+ calc. 131.0708, found 131.0714

[α]D = +42° (c = 0.5, DCM, 23 °C).

Ethyl 3-hydroxy-3-phenylpropanoate (191)

To a 10 mL Retsch stainless steel jar was added zinc (2 mmol, 0.131 g), aldehyde (1 mmol), α-bromo ester (1.2 mmol) under air atmosphere. A stainless steel ball of mass 4.0 g was added and the mixture was milled at 30 Hz for 2 hours. The resulting black/grey paste mixture was transferred into a flask and the jar was rinsed with ethyl acetate (2 x 10 mL), before quenching with 2 M HCl solution (10 mL). The quenched solution was then washed and extracted with EtOAc, The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The crude mixture was purified by column chromatography (20% Ethyl Acetate/Petroleum ether) to yield 191 (140 mg, 72%) as a light yellow oil.

¹H NMR (500 MHz, CDCl₃) δ 7.43 - 7.35 (m, 4H, ArH), 7.34 - 7.27 (m, 1H, ArH), 5.16 (dd, J = 8.9, 3.9 Hz, 1H, PhCHOH), 4.21 (q, J = 7.1 Hz, 2H, OCH₂CH₃), 2.85 - 2.65 (m, 2H, CH₂), 1.29 (t, J = 7.1 Hz, 3H, OCH₂CH₃).

¹³C NMR (126 MHz, CDCl₃) δ 172.6 (C=O), 142.6 (ArC), 128.7 (ArC), 128.0 (ArC), 125.8 (ArC), 70.5 (PhCHOH), 61.0 (OCH₂), 43.5 (CH₂), 14.3 (CH₃).

Ethyl 2-bromo-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-fluoro-3-hydroxypropanoate (192) and ethyl 2-bromo-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-fluoro-3-((trimethylsilyl)oxy)propanoate (193)*

To a suspension of sonicated zinc powder (157.1 mg, 2.4 mmol, 2.4 equiv., [325 mesh]) in anhydrous MeCN (3 mL) was added ethyl dibromofluoroacetate (280 μL, 2 mmol, 2 equiv.) at room temperature. After stirring for 10 minutes, TMSCl (280 μL, 2.2 mmol, 2.2 equiv.) was added at 0°C and stirred for a further 10 minutes. After cooling to -40°C, a solution of compound 11 (130.1 mg, 1 mmol) in MeCN (1 mL) and Cp₂TiCl₂ (273.2 mg, 1.1 mmol, 1.1 equiv.) were added to the mixture. The mixture was stirred at -40°C for 1.5 h, then allowed to warm to r.t. and stirred for 1 h. Diethyl ether (10 mL) and saturated aqueous NaHCO₃ (10 mL) were added at 0°C, stirred for 5 min and filtered through celite. The filtrate was separated, washed with brine and dried over MgSO₄. Flash chromatography on silica gel (40% EtOAc/Hexane) provided a mixture of 192 (63.2 mg, 86:14 d.r.) and 193 (56.9 mg, combined 35% yield) as a colourless oil.

192: ¹H NMR (400 MHz, CDCl₃) δ 4.69 (td, J = 6.7, 3.0 Hz, 1H, CH₃), 4.51 (ddd, J = 25.1, 5.2, 3.0 Hz, 1H, CH₃OH), 4.35 (q, J = 7.1 Hz, 2H, EtCH₂), 4.07 – 4.04 (m, 2H, CH₂), 3.16 (d, J = 5.4 Hz, 1H, OH), 1.42 (s, 3H, CH₃), 1.38 (s, 3H, CH₃), 1.34 (t, J = 7.1 Hz, 3H, EtCH₂).

¹³C(¹H) NMR (101 MHz CDCl₃): δ 165.0 (d, J = 27.1 Hz, C=O), 109.0 (CH₂), 96.7 (d, J = 270.0 Hz, CFBr), 74.5 (d, J = 20 Hz, CH₂OH), 74.4 (C(CH₃)₂), 64.1 (d, J = 7.1 Hz, CH), 63.6 (EtCH₂), 26.2 (CH₃), 25.4 (CH₃), 13.8 (EtCH₂).

¹⁹F(¹H) NMR (376.5 MHz, CDCl₃): δ -133.8 (s, major), -133.1 (s, minor).

193: ¹H NMR (CDCl₃, 400 MHz): 4.75 (ddd, J = 7.6, 6.7, 2.0 Hz, 1H, CH₃), 4.63 (dd, J = 25.7, 2.0 Hz, 1H, CH₃), 4.51 (q, J = 8 Hz, 2H, EtCH₂), 3.98-3.96 (m, 2H, CH₂), 1.42 (s, 3H, CH₃), 1.38 (s, 3H, CH₃), 1.34 (t, J = 8 Hz, 3H, EtCH₂), 0.14 (s, 9H, Si(CH₃)₃).

* In the absence of MS, the structural identities of 192 and 193 cannot be conclusively assigned hence the structures proposed are tentative.

(3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)dihydrofuran-2(3H)one (195)

To a solution of 192 and 193 (0.35 mmol) in MeOH (6 mL) was added aqueous HCl (1 M, 3.8 mL, 19.0 equiv.) at room temperature. The solution was then refluxed for overnight. After cooling to room temperature, the reaction
mixture was extracted with ethyl acetate (3 x 25 mL). The organic layers were collected, dried over MgSO₄. The crude material was purified by column chromatography (100% EtOAc) to give 195 (32.0 mg, 40% yield) as a clear oily liquid.

¹H NMR (400 MHz, CDCl₃) δ 4.60 (ddd, J = 15.3, 8.0, 7.4 Hz, 1H, H⁵), 4.16 – 4.08 (m, 2H, H⁵a and H⁵b), 3.92 – 3.85 (m, 1H, O⁵H), 2.97 (dd, J = 7.3, 3.8 Hz, 1H, H⁴), 2.02 (dd, J = 8.0, 4.7 Hz, 1H, O³H).

¹³C{¹H} NMR (101 MHz, CDCl₃) δ 165.0 (d, J = 27.7 Hz, C¹), 99.0 (d, J = 279.7 Hz, C²), 80.0 (d, J = 7.5 Hz, C⁴), 72.0 (d, J = 21.3 Hz, C⁵), 58.5 (C⁵).

¹⁹F{¹H} NMR (376 MHz, CDCl₃) δ -128.16 (s).

¹⁹F NMR (376 MHz, CDCl₃) δ -128.17 (dd, J = 14.5, 1.7 Hz).

* In the absence of MS, the structural identity of 195 cannot be conclusively assigned hence the structure proposed is tentative.
5.3 – Synthetic route 2

(4S,5R)-4-hydroxy-5-(hydroxymethyl)dihydrofuran-2(3H)-one (75)

Bromine (4.8 mL, 95.5 mmol, 2.5 equiv.) was added dropwise to a solution of 2-deoxy-D-ribose (5.097 g, 38 mmol) in water (50 mL), and stirred in the dark at room temperature for 5 days. The reaction was neutralised with K₂CO₃. Excess bromine and solvent were removed under reduced pressure (fitted with Na₂S₂O₃(aq) trap). The crude mixture was purified by column chromatography (0% → 5% MeOH/EtOAc) to yield 75 (4.671 g, 93% yield) as a light yellow oil.

^1^H NMR (500 MHz, DMSO-d₆) δ 5.48 (d, J = 4.1 Hz, O₃H), 5.06 (t, J = 5.4 Hz, O₅H), 4.26 (m, 2H, H₃ and H₄), 3.56 (dd, J = 12.2, 3.7 Hz, 1H, H₅a), 3.52 (dd, J = 12.2, 3.8 Hz, 1H, H₅b), 2.81 (dd, J = 17.7, 6.4 Hz, 1H, H₂a), 2.22 (dd, J = 17.7, 2.3 Hz, 1H, H₂b).

^1^3^C{^1^H} NMR (126 MHz, DMSO-d₆) δ 176.2 (C₁), 88.3 (C₄), 67.8 (C₃), 60.8 (C₅), 38.0 (C₂).

IR (cm⁻¹): 3389, 2934, 1744, 1364, 1169, 1051.

HRMS (CI): [C₅H₁₂O₄+NH₄]+= calc. 150.0761, found 150.0760.

(4aR,7aS)-2-phenyltetrahydro-6H-furo[3,2-d][1,3]dioxin-6-one (198)

A solution of 75 (663.9 mg, 5.0 mmol) with HCl (12 M, 600 μL, 7 mmol, 1.4 equiv.) in benzaldehyde (10 mL) was stirred overnight at room temperature. The mixture was concentrated in vacuo to deliver a brown solid, which was collected and washed with EtOH to deliver 198 (40.5 mg, 4% yield) as a white solid, in a diastereomeric mixture.

^1^H NMR (500 MHz, CDCl₃) δ 7.50 – 7.46 (m, 2H, ArH), 7.45 – 7.36 (m, 3H, ArH), 6.24 (s, 0.2H, PhCH₃), 5.76 (s, 1H,PhCH₃), 4.79 (ddd, J = 8.3, 3.7, 2.5 Hz, 1H, H₃), 4.75 (ddd, J = 7.5, 4.0, 2.7 Hz, 0.2H, H₃'), 4.63 – 4.55 (m, 2H, H₄, H₅a and H₅b'), 4.61 (dd, J = 13.0, 1.7 Hz, 0.2H, H₅b), 4.24 (dd, J = 13.0, 2.2 Hz, 0.2H, H₅b'), 4.23 (dd, J = 13.2, 2.1 Hz, 1H, H₅b), 3.05 (dd, J = 16.0, 2.5 Hz, 1H, H₅b'), 3.01 (obs. d, J = 2.6 Hz, 0.2H, H₅b'), 2.64 (dd, J = 16.0, 3.8 Hz, 1H, H₅b'), 2.64 – 2.59 (m, 0.2H, H₅b').

HRMS (EI+): [C₁₂H₁₂O₄] calc. 220.0736 found 220.0734.

Data is consistent with the literature.[¹⁴⁰]
(6aR,9aS)-2,2,4,4-tetraisopropyltetrahydro-8H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-8-one (200)

To a solution of 75 (1.3392 g, 10.1 mmol) and imidazole (1.7423 g, 25.6 mmol, 2.5 equiv.) in anhydrous DMF (25 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (4.8 mL, 15 mmol, 1.49 equiv.) dropwise at room temperature. After complete addition, the reaction was then stirred at r.t. for 24 h before being poured onto water (50 mL), and extracted into Et$_2$O (3 x 50 mL). The combined organics were washed with water, saturated aqueous NaHCO$_3$ and brine (3 x 40 mL each), then dried over MgSO$_4$ and concentrated in vacuo. The crude mixture was purified by column chromatography (20% Et$_2$O/Hexane) to deliver 200 (1.6323 g, 44% yield) as a thick, colourless oil.

$^1$H NMR (500 MHz, CDCl$_3$) δ 4.63 (dd, $J = 16.3, 7.9$ Hz, 1H, $H^f$), 4.21 (td, $J = 6.7, 3.6$ Hz, 1H, $H^f$), 4.14 (dd, $J = 12.3, 3.5$ Hz, 1H, $H^f$), 3.93 (dd, $J = 12.3, 6.6$ Hz, 1H, $H^f$), 2.86 (dd, $J = 17.3, 8.0$ Hz, 1H, $H^f$), 2.71 (dd, $J = 17.3, 9.2$ Hz, 1H, $H^f$), 1.12 – 1.01 (m, 28H, SiCH(CH$_3$)$_2$ and SiCH(CH$_3$)$_2$).

$^{13}$C($^1$H) NMR (126 MHz, CDCl$_3$) δ 173.1 (C’=O), 85.0 (C’), 69.8 (C’), 62.5 (C’), 38.0 (C’), 17.6 (SiCH(CH$_3$)$_2$), 17.5 (SiCH(CH$_3$)$_2$), 17.4 (2C, SiCH(CH$_3$)$_2$), 17.3 (SiCH(CH$_3$)$_2$), 17.1 (SiCH(CH$_3$)$_2$), 17.0 (2C, SiCH(CH$_3$)$_2$), 13.4 (SiCH(CH$_3$)$_2$), 13.3 (SiCH(CH$_3$)$_2$), 13.0 (SiCH(CH$_3$)$_2$), 12.7 (SiCH(CH$_3$)$_2$).

Data is consistent with the literature.$^{[141]}$

The same method was attempted synthesis of 199, but no target material was detected/synthesised.

(4aR,7aS)-2-phenyltetrahydro-6H-furo[3,2-d][1,3,2]dioxaborinin-6-one (201)

To a flame dried flask containing 75 (277.4 mg, 2.1 mmol) and phenyl boronic acid (488.1 mg, 4 mmol, 2 equiv.), was added toluene (10 mL) and the solution stirred at room temperature overnight. The reaction mixture was filtered and dried in vacuo to deliver 201 (328.8 mg, 75% yield) as an off-white solid.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.78 (d, $J = 7.2$ Hz, 2H, Ar$H^f$), 7.49 (t, $J = 7.3$ Hz, 1H, Ar$H^f$), 7.38 (t, $J = 7.4$ Hz, 2H, Ar$H^f$), 5.17 – 5.09 (m, 1H), 4.91 (d, $J = 8.3$ Hz, 1H), 4.59 (d, $J = 12.9$ Hz, 1H), 4.31 (d, $J = 13.2$ Hz, 1H), 3.07 (dd, $J = 16.2, 2.1$ Hz, 1H, $H^f$), 2.72 (dd, $J = 16.2, 3.9$ Hz, 1H, $H^f$).

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 169.0 (C’), 135.2 (ArC), 132.2 (ArC), 128.0 (ArC), 73.3 (C’), 73.2 (C’), 69.4 (C’), 35.7 (C’).

HRMS (El+): [C$_{11}$H$_{11}$O$_7$]$^{11}$B calc 218.0750 found 218.0756
(4S,5R)-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-2(3H)-one (64)

To a solution of 75 (11.4727 g, 86.84 mmol) and imidazole (35.4697 g, 521 mmol, 6 equiv.) in anhydrous DMF (100 mL) was added triisopropylsilyle chloride (75 mL, 350 mmol, 4 equiv.) dropwise via dropping funnel at 0°C. After complete addition, further DMF (75 mL) was added and the solution was warmed to room temperature and stirred for 24 h. The reaction was quenched by pouring onto water (100 mL) and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were washed successively with saturated aqueous NaHCO₃, H₂O and brine (2 x 100 mL each), dried over MgSO₄ and concentrated in vacuo. The crude mixture was purified by column chromatography (5% Et₂O/Hexane) to yield 64 (30.9951 g, 80% yield) as a colorless oil.

$^1$H NMR (500 MHz, CDCl₃) δ 4.67 (dt, $J = 6.6$, 1.9 Hz, 1H, $H_3$), 4.41 (dd, $J = 4.2$, 2.8 Hz, 1H, $H_4$), 3.93 (dd, $J = 11.3$, 3.1 Hz, 1H, $H_5a$), 3.88 (dd, $J = 11.3$, 2.4 Hz, 1H, $H_5b$), 2.88 (dd, $J = 17.6$, 6.6 Hz, 1H, $H_2a$), 2.44 (dd, $J = 17.6$, 2.0 Hz, 1H, $H_2b$), 1.14 – 1.03 (m, 42H, SiC(CH(CH₃)₂)₂ and SiCH(C(CH₃)₂)₂).

$^{13}$C{¹H} NMR (126 MHz, CDCl₃) δ 176.1 (C¹), 88.9 (C³), 70.1 (C⁵), 63.4 (C⁵), 39.7 (C⁵), 18.0 (3C, SiCH(CH₃)₂), 12.0 (2C, SiCH(CH₃)₂).

IR (film) cm⁻¹: 2943, 2866, 2359, 1788, 1460, 1385, 1165, 1125, 1098, 1067, 1013, 966, 880, 683.


(3S,4R,5R)-3-fluoro-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-2(3H)-one (65)

To a solution of 64 (791.6 mg, 1.78 mmol) and NFSI (842.3 mg, 2.67 mmol, 1.5 equiv.) in anhydrous THF (10 mL) at -78°C was added LiHMDS (2.4 mL, 1 M in THF, 1.3 equiv.) dropwise. After complete addition, the mixture was stirred at -78°C for 2 h and then quenched by saturated aqueous NH₄Cl (10 mL). The mixture was warmed to room temperature, extracted with EtOAc (3 x 10 mL), and the combined organic layers successively washed with saturated aqueous NaHCO₃ and brine (1 x 20 mL each), dried over MgSO₄, and concentrated in vacuo. The crude product was purified by flash chromatography (2.5% → 7.5% EtOAc/Hexane) to afford 65 (450.2 mg, 54%) as a colourless solid.
\[ ^1 H \text{ NMR (500 MHz, CDCl}_3\] δ 5.14 (dd, \( J = 51.3 \), 7.4 Hz, 1H, \( H^f \)), 4.94 (dt, \( J = 18.8, 7.2 \text{ Hz}, 1H, \( H^a \)), 4.19 (dt, \( J = 7.0, 2.2 \text{ Hz}, 1H, \( H^a \)), 4.11 (dt, \( J = 12.1, 2.1 \text{ Hz}, 1H, \( H^p \)), 3.94 (dd, \( J = 12.2, 2.3 \text{ Hz}, 1H, \( H^p \)), 1.16 – 1.04 (m, 42H, SiCH(CH\(_3\)_2) and SiCH(CH\(_3\)_2).

\[ ^{13}C[^1 H] \text{ NMR (126 MHz, CDCl}_3\] δ 168.9 (d, \( J = 23.2 \text{ Hz}, \( C^1 \)), 92.9 (d, \( J = 198.8 \text{ Hz}, \( C^6 \)), 82.0 (d, \( J = 10.3 \text{ Hz}, \( C^6 \)), 71.9 (d, \( J = 20.8 \text{ Hz}, \( C^6 \)), 60.4 (\( C^6 \)), 18.0 (2C, SiCH(CH\(_3\)_2), 17.9 (2C, SiCH(CH\(_3\)_2), 12.3 (SiCH(CH\(_3\)_2), 12.0 (SiCH(CH\(_3\)_2).

\[ ^{19}F \text{ NMR (376 MHz, CDCl}_3\] δ -200.84 (s).

\[ ^{19}F \text{ NMR (471 MHz, CDCl}_3\] δ -200.78 (dd, \( J = 51.5, 18.9 \text{ Hz} \)).

IR (cm\(^{-1}\)): 2943, 2864, 1809, 1464, 1336, 1236, 1142, 1107, 1070, 1040, 881, 799, 683.

HRMS (ES): [C\(_{23}H_{47}FO_4Si_2+H]^+\] calc. 463.3075 found 463.3076.

Data is consistent with the literature.[20]

(4R,5R)-3-bromo-4-((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)methyl)-dihydrofuran-2(3H)-one (203)

To a solution of 64 (3.4353 g, 7.72 mmol) and triethylamine (6.5 mL, 46.3 mmol, 6 equiv.) in anhydrous DCM (60 mL) was added TMSOTf (4.0 mL, 23.16 mmol, 3 equiv.) slowly over 10 minutes at 0°C. After complete addition, the reaction was stirred at 0°C for a further 30 minutes. NBS (2.0615 g, 11.58 mmol, 1.5 equiv.) was then added in a single portion, and the reaction stirred for 2 hours at 0°C, before warming to room temperature and stirred overnight. The reaction mixture was poured onto saturated aqueous NaHCO\(_3\) (100 mL) and extracted with DCM (3 x 50 mL). The combined organics were washed successively with water and brine (3 x 50 mL each), dried over MgSO\(_4\) and concentrated in vacuo. The residue was purified by column chromatography (5% Et\(_2\)O/Hexane) to afford 203 (2.8497 g, 70% yield) as a mixture of diastereomers as a colourless oil (arabino:ribono 2:1). Further purification by column chromatography separated the diastereomers for further analysis, and configuration assignment based on \(^1\text{H COSY and NOESY NMR. Strong spatial correlation observed between } H^f \text{ and } H^p \text{ of ribono diastereomer.}

Arabino/\( \beta \) 204 (major diastereomer):

\[ ^1 H \text{ NMR (500 MHz, CDCl}_3\] δ 4.88 (t, \( J = 4.3 \text{ Hz}, 1H, \( H^p \)), 4.38 (app. dd, \( J = 8.4, 3.9 \text{ Hz}, 1H, \( H^a \)), 4.33 (d, \( J = 4.6 \text{ Hz}, 1H, \( H^a \)), 4.06 (dd, \( J = 11.4, 4.7 \text{ Hz}, 1H, \( H^p \)), 3.97 (dd, \( J = 11.4, 3.6 \text{ Hz}, 1H, \( H^p \)), 1.15 – 1.03 (m, 42H, SiCH(CH\(_3\)_2) and SiCH(CH\(_3\)_2).

\[ ^{13}C[^1 H] \text{ NMR (126 MHz, CDCl}_3\] δ 171.1 (\( C^1 \)), 87.1 (\( C^4 \)), 76.0 (\( C^6 \)), 61.7 (\( C^6 \)), 45.0 (\( C^6 \)), 18.1 (2C, SiCH(CH\(_3\)_2), 18.0 (2C, SiCH(CH\(_3\)_2), 12.4 (SiCH(CH\(_3\)_2), 12.0 (SiCH(CH\(_3\)_2).
IR (cm\textsuperscript{-1}): 2943, 2866, 1800, 1460, 1140, 1067, 881, 683.
Appearance: Colourless oil
HRMS (TOF AP\textsuperscript{+}): [C\textsubscript{23}H\textsubscript{47}O\textsubscript{4}Si\textsubscript{2}Br+H]\textsuperscript{+} calc. 523.2275 found 523.2278

Ribono/\alpha 205 (minor diastereomer):

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 4.62 (dd, J = 5.6, 4.7 Hz, 1H, \(H^1\)), 4.57 (d, J = 5.6 Hz, 1H, \(H^2\)), 4.39 (dt, J = 4.5, 2.1 Hz, 1H, \(H^3\)), 4.10 (dd, J = 12.0, 2.3 Hz, 1H, \(H^5a\)), 3.94 (dd, J = 12.0, 2.1 Hz, 1H, \(H^5b\)), 1.13 – 1.02 (m, 42H, SiCH(CH\textsubscript{3})\textsubscript{2} and SiCH(CH\textsubscript{3})\textsubscript{2}).

\textsuperscript{13}C\textsuperscript{[\textsuperscript{1}H]} NMR (126 MHz, CDCl\textsubscript{3}) δ 171.2 (C\textsubscript{1}), 85.6 (C\textsubscript{4}), 69.2 (C\textsubscript{3}), 61.1 (C\textsubscript{5}), 46.9 (C\textsubscript{2}), 18.1 (SiCH(CH\textsubscript{3})\textsubscript{2}), 18.0 (2C, SiCH(CH\textsubscript{3})\textsubscript{2}), 12.5 (SiCH(CH\textsubscript{3})\textsubscript{2}), 12.0 (SiCH(CH\textsubscript{3})\textsubscript{2}).

IR (cm\textsuperscript{-1}): 2945, 2866, 1796, 1464, 1150, 1065, 881, 685.

HRMS (TOF ES\textsuperscript{+}): [C\textsubscript{23}H\textsubscript{46}O\textsubscript{4}Si\textsubscript{2}Br+H]\textsuperscript{+} calc. 601.1380 found 601.1392.

(4R,5R)-3,3-dibromo-4-((triisopropylsilyl)oxy)-5-((triisopropylsilyl)oxy)methyl)-dihydrofuran-2(3H)-one (206)

To a solution of 2-deoxy-3,5-di-O-(isopropylsilyl)-D-ribonolactone (904.0 mg, 2.0 mmol) and dibromotetrachloroethane (848.5 mg, 2.6 mmol, 1.3 equiv) in anhydrous THF (10 mL, [0.2 M]) at -78°C was added LiHMDS (3 mL, 3 mmol, 1.5 equiv.) slowly as to maintain T < -75°C. After complete addition, the reaction was stirred at -78°C for 4 hours, before quenching with saturated aqueous NH\textsubscript{4}Cl and warming to r.t.. The golden reaction mixture was extracted with Et\textsubscript{2}O (3 x 20 mL) and successively washed with saturated aqueous NaHCO\textsubscript{3}, water and brine (3 x 20 mL each). The crude mixture was dried over MgSO\textsubscript{4} and concentrated in vacuo. The crude mixture was purified by column chromatography (2%→3% Et\textsubscript{2}O in Petroleum ether), affording a mixture of the arabino (S) (204) and ribo (R) (205) diastereomers (3:2 ratio, 196.4 mg, 19% yield) and 206 (556.4 mg, 46% yield)

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 4.91 (d, J = 7.3 Hz, 1H, \(H^1\)), 4.18 – 4.14 (m, 2H, \(H^5a\) and \(H^5b\)), 3.95 (dd, J = 12.9, 2.4 Hz, 1H, \(H^5\)), 1.10 (m, 42H, SiCH(CH\textsubscript{3})\textsubscript{2} and SiCH(CH\textsubscript{3})\textsubscript{2}).

\textsuperscript{13}C\textsuperscript{[\textsuperscript{1}H]} NMR (126 MHz, CDCl\textsubscript{3}) δ 167.2 (C\textsubscript{1}), 83.92 (C\textsubscript{3}), 84.92 (C\textsubscript{4}), 77.80 (C\textsubscript{5}), 59.27 (C\textsubscript{2}), 58.15 (C\textsubscript{3}), 18.2 (2C, SiCH(CH\textsubscript{3})\textsubscript{2}), 18.0 (SiCH(CH\textsubscript{3})\textsubscript{2}), 17.9 (SiCH(CH\textsubscript{3})\textsubscript{2}), 12.9 (SiCH(CH\textsubscript{3})\textsubscript{2}), 12.1 (SiCH(CH\textsubscript{3})\textsubscript{2}).

IR (cm\textsuperscript{-1}): 2945, 2868, 1803, 1462, 1190, 1169, 1146, 1063, 885, 785, 685.

HRMS (TOF ES\textsuperscript{+}): [C\textsubscript{23}H\textsubscript{46}O\textsubscript{4}Si\textsubscript{2}Br+H]\textsuperscript{+} calc. 601.1380 found 601.1392.
(4R,5R)-3-chloro-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)methyl)-dihydrofuran-2(3H)-one (207)

To a solution of 64 (409.5 mg, 0.92 mmol) and triethylamine (770 µL, 5.52 mmol, 6 equiv.) in anhydrous DCM (10 mL) was added TMSOTf (480 µL, 2.76 mmol, 3 equiv.) slowly over 10 minutes at 0°C. After complete addition, the reaction was stirred at 0°C for a further 30 minutes. NCS (185.2 mg, 1.38 mmol, 1.5 equiv.) was then added in a single portion, and the reaction stirred for 1 hours at 0°C, before warming to room temperature and stirred overnight. The reaction mixture was poured onto saturated aqueous NaHCO₃ (20 mL) and extracted with DCM (3 x 20 mL). The combined organics were washed successively with water and brine (3 x 20 mL each), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (3% Et₂O/Hexane) to afford 207 (164.9 mg, 37% yield) as a mixture of diastereomers as a colourless oil (d.r. = 7:3). Assignment of diastereomers was not possible from 2D NOESY NMR. The data presented is of the separated diastereomers.

1H NMR (400 MHz, CDCl₃) δ 4.71 (dd, J = 5.5, 2.3 Hz, 1H, H₆), 4.68 (d, J = 5.5 Hz, 1H, H₅), 4.44 (app. q, J = 2.3 Hz, 1H, H₄), 4.05 (dd, J = 11.8, 2.5 Hz, 1H, H₃a), 3.92 (dd, J = 11.8, 2.0 Hz, 1H, H₃b), 1.12 (m, 42H, SiCH(CH₃)₂ and SiCH(CH₃)₉).

13C NMR (126 MHz, CDCl₃) δ 171.5 (C₁), 86.4 (C₂), 70.9 (C₃), 62.2 (C₃), 56.3 (C₇), 18.0 (4C, SiCH(CH₃)₉), 12.4 (SiCH(CH₃)₉), 11.9 (SiCH(CH₃)₉).

1H NMR (400 MHz, CDCl₃) δ 4.83 (dd, J = 6.2, 5.4 Hz, 1H, H₅), 4.38 (d, J = 6.2 Hz, 1H, H₅), 4.29 (dt, J = 5.4, 3.1 Hz, 1H, H₆), 4.08 (dd, J = 11.8, 3.4 Hz, 1H, H₃a), 3.94 (dd, J = 11.8, 3.0 Hz, 1H, H₃b), 1.12 (m, 42H, SiCH(CH₃)₂ and SiCH(CH₃)₉).

13C[¹H] NMR (126 MHz, CDCl₃) δ 170.3 (C₁), 85.3 (C₂), 75.5 (C₃), 60.9 (C₇), 58.9 (C₇), 18.1 (2C, SiCH(CH₃)₉), 18.00 (SiCH(CH₃)₉), 17.9 (SiCH(CH₃)₉), 12.4 (SiCH(CH₃)₉), 12.0 (SiCH(CH₃)₉).

HRMS (TOF AP⁺): [C₂₃H₄₇O₄Si₆Cl₃H⁺] calc. 479.2780 found 479.2783.

(4R,5R)-3-iodo-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)methyl)-dihydrofuran-2(3H)-one (208)

To a solution of 64 (423.0 mg, 0.95 mmol) and triethylamine (800 µL, 5.7 mmol, 6 equiv.) in anhydrous DCM (10 mL) was added TMSOTf (495 µL, 2.85 mmol, 3 equiv.) slowly over 10 minutes at 0°C. After complete addition, the reaction was stirred at 0°C for a further 30 minutes. NIS (321.9 mg, 1.43 mmol, 1.5 equiv.) was then added
in a single portion, and the reaction stirred for 1 hour at 0°C, before warming to room temperature and stirred overnight. The reaction mixture was poured onto saturated aqueous NaHCO₃ (25 mL) and extracted with DCM (3 x 20 mL). The combined organics were washed successively with water and brine (3 x 25 mL each), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (3% Et₂O/Hexane) to afford 208 (349.5 mg, 65% yield) as a mixture of diastereomers as a colourless solid (d.r. = 0.7:1).

¹H NMR (500 MHz, CDCl₃) δ 4.90 (t, J = 2.8 Hz, 0.7H, H²), 4.67 (d, J = 6.0 Hz, 1H, H⁴), 4.49 (td, J = 5.2, 2.6 Hz, 0.7H, H⁶), 4.43 (d, J = 2.9 Hz, 0.7H, H⁸), 4.23 (dt, J = 5.9, 2.0 Hz, 1H, H¹), 4.12 (dd, J = 12.1, 1.8 Hz, 1H, H²³), 4.07 (d, J = 5.2 Hz, 1.3H, H⁷ and H²⁵), 3.98 (app. t, J = 6.0 Hz, 1H, H²⁴), 3.94 (dd, J = 12.1, 2.1 Hz, 1H, H²⁵), 1.16 – 1.03 (m, 72H, SiCH(CH₃)₂ and SiCH(CH₃)₂).

HRMS (TOF AP⁺): [C₂₃H₄₇O₄Si₂¹²7+H]⁺ calc. 571.2136 found 571.2137.

No ¹³C[¹H] NMR recorded.

(3R,4R,5R)-3-bromo-3-fluoro-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)-methyl)dihydrofuran-2(3H)-one (202)

To a solution of 203 (5.4385 g, 10.4 mmol) and NFSI (5.7408 g, 18.2 mmol, 1.75 equiv.) in anhydrous THF (20 mL) at -78°C was added LiHMDS (15.6 mL, 1M in THF, 1.5 equiv.) dropwise. After complete addition, the mixture was stirred at -78°C for 4 h and then quenched by saturated aqueous NH₄Cl (20 mL). The mixture was warmed to room temperature, extracted with Et₂O (3 x 50 mL), and the combined organic layers successively washed with saturated aqueous NaHCO₃ and brine (3 x 20 mL each), dried over MgSO₄, and concentrated in vacuo. The crude product was purified by flash chromatography (5% Et₂O/Hexane or 40% DCM/Hexane) to afford 202 (4.525 g, 75%) as a colourless oil.

¹H NMR (500 MHz, CDCl₃) δ 4.79 (dd, J = 14.6, 7.7 Hz, 1H, H¹), 4.16 (dt, J = 12.5, 2.0 Hz, 1H, H²³), 4.05 (dt, J = 7.7, 1.6 Hz, 1H, H²⁵), 3.97 (dd, J = 12.5, 1.8 Hz, 1H, H²⁵), 1.12 (m, 42H, SiCH(CH₃)₂ and SiCH(CH₃)₂).

¹³C[¹H] NMR (126 MHz, CDCl₃) δ 165.8 (d, J = 27.9 Hz, C¹), 100.0 (d, J = 278.5 Hz, C²), 81.5 (d, J = 8.4 Hz, C³), 72.5 (d, J = 20.5 Hz, C⁴), 59.1 (C⁵), 18.0 (2C, SiCH(CH₃)₂), 17.9 (2C, SiCH(CH₃)₂), 12.6 (SiCH(CH₃)₂), 12.1 (SiCH(CH₃)₂).

¹⁹F[¹H] NMR (376 MHz, CDCl₃) δ -127.53 (s).

IR (cm⁻¹): 2943, 2870, 1813, 1462, 1192, 1134, 1065, 957, 922, 883, 795, 687, 660.

HRMS (ES): [C₂₃H₄₆BrFO₄Si₂+H]⁺ calc. 541.2180 found 541.2174.
Data is consistent with the literature.\textsuperscript{[142]}

(3R,4R,5R)-3-bromo-3-fluoro-4-(((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)-methyl)tetrahydrofuran-2-ol (209)

To a solution of lithium tri-\textit{tert}-butoxyaluminium hydride (2.97 g, 11.7 mmol, 1.1 equiv.) in anhydrous diethyl ether (90 mL) was added dropwise 202 (5.75 g, 10.62 mmol) in anhydrous THF (20 mL) over 10 minutes at 0°C. After addition, the reaction was warmed to room temperature and stirred for 3 hours. The reaction was quenched with methanol and stirred at room temperature for a further hour, before filtering over a short silica pad. The filtrate was extracted with diethyl ether (3 x 25 mL), and the combined organics washed with saturated aqueous NaHCO$_3$, water and brine (1 x 50 mL each), dried over MgSO$_4$ and concentrated \textit{in vacuo} to afford 209 (5.6244 g, 97% yield) as a colourless oil. The crude product was used for the next reaction without purification. The anomers were inseparable by column chromatography and used as a mixture for the next reaction.

$^1$H NMR (500 MHz, CDCl$_3$) δ 5.34 (dd, $J = 9.2, 0.9$ Hz, 1H, $H^\alpha$), 5.17 (dd, $J = 12.7, 5.9$, 0.6 Hz, 0.4H, $H^\beta$), 4.72 (dd, $J = 12.8, 6.6$ Hz, 1H, $H^\gamma$), 4.67 (dd, $J = 11.6, 4.6, 0.6$ Hz, 0.4H, $H^\delta$), 4.08 (td, $J = 4.6, 0.8$ Hz, 0.4H, $H^\epsilon$), 3.96 – 3.93 (app. td, $J = 7.0, 1.5$ Hz, 1H, $H^\zeta$), 3.91 – 3.86 (m, 2H, -CH$_2$OTIPS and CHO$_2$), 3.84 – 3.79 (m, 1.6H, $H^\iota$), 3.48 (d, $J = 12.7$ Hz, 0.4H, O’$H^k$), 1.21 – 1.05 (m, 59H, SiCH(CH$_3$)$_2$ and SiCH(CH$_3$)$_2$).

$^{13}$C($^1$H) NMR (126 MHz, CDCl$_3$) δ 115.6 (d, $J = 265.0$ Hz, C$^\zeta$), 112.8 (d, $J = 277.3$ Hz, C$^\iota$), 100.0 (d, $J = 21.5$ Hz, C$^\iota$), 98.8 (d, $J = 31.3$ Hz, C$^\iota$), 83.8 (d, $J = 3.8$ Hz, C$^\alpha$), 83.1 (d, $J = 8.7$ Hz, C$^\iota$), 74.6 (d, $J = 24.3$ Hz, C$^\alpha$), 72.3 (d, $J = 21.8$ Hz, C$^\delta$), 62.4 (C$^\epsilon$), 61.4 (H$^\eta$), 18.1 (2C, SiCH(CH$_3$)$_2$ and SiCH(CH$_3$)$_2$), 18.0 (2C, SiCH(CH$_3$)$_2$ 12.6 (SiCH(CH$_3$)$_2$), 12.5 (SiCH(CH$_3$)$_2$), 12.1 (SiCH(CH$_3$)$_2$), 12.0 (SiCH(CH$_3$)$_2$).

$^{19}$F ($^1$H) NMR (376 MHz, CDCl$_3$) δ -120.68 (s), -127.37 (s).


HRMS (ES): [C$_{23}$H$_{48}$BrFO$_4$Si$_2$+Na]$^+$ calc. 565.2156 found 565.2140.

\textit{Dibal-H reduction:} To a solution of 202 (6.6015 g, 12.2 mmol) in anhydrous toluene (50 mL) at -78°C was added DIBAL-H (1 M in hexanes, 86 mL, 85.4 mmol, 7 equiv.). The solution was stirred at -78°C for 2 hours, before quenching with MeOH (75 mL) and warming to rt. The mixture was then filtered over a pad of silica before washing with 0.1 M HCl. The solution was extracted with Et$_2$O (3 x 250 mL), and successively washed...
with saturated aqueous NaHCO₃, water and brine (3 x 150 mL each), dried over MgSO₄ and concentrated in vacuo. Purification by column chromatography (8% Et₂O/Hexanes) gave an impure product, which was purified again by column chromatography (50% DCM/Hexane) to yield **209** (4.4197 g, 67%) as a colourless oil, isolated as a mix of diastereomers (71:29).

Analytical data for both the products of both methods were identical.

**3'**-(3R,4R,5R)-3-bromo-3-fluoro-4-((trisopropylsilyl)oxy)-5-(((trisopropylsilyl)oxy)methyl)tetrahydrofuran-2-yl methanesulfonate (210)

To a solution of **209** (4.4197 g, 8.1 mmol), NEt₃ (1.60 mL, 11.34 mmol, 1.4 equiv.) in dry DCM (50 mL) was added methane sulfonyl chloride (760 μL, 9.82 mmol, 1.2 equiv.) slowly at 0°C. After complete addition, the reaction was warmed to room temperature and stirred overnight. The reaction mixture was then concentrated in vacuo and redissolved in EtOAc (50 mL), before washing with saturated aqueous NaHCO₃ and brine (3 x 50 mL). The solution was dried over MgSO₄ and concentrated in vacuo, to afford **210** (4.79 g, 95% yield) as a colourless oil, isolated as a diastereomeric mixture (59:41).

**¹H** NMR (400 MHz, CDCl₃) δ 6.19 (d, J = 1.6 Hz, 1H, H')OMs), 6.04 (d, J = 7.4 Hz, 0.7H, H'OMs), 4.63 – 4.52 (m, 2H, H₄ and H₅), 4.26 (dd, J = 8.5, 4.2 Hz, 0.7H, H'₆), 4.03 (dt, J = 11.7, 1.8 Hz, 1.3H, H' and H'₆), 3.94 – 3.88 (m, 2.5H, H'₈ and H'₉), 3.84 (dd, J = 11.8, 4.2 Hz, 1H, H'₉), 3.11 (s, 2H, MsCH₃), 3.07 (s, 3H, MsCH₃), 1.16 – 1.03 (m, 72H, SiCH(CH₃)₂ and SiCH(CH₃)₂).

**¹³C**[¹H] NMR (126 MHz, CDCl₃) δ 111.9 (d, J = 253.8 Hz, C'), 109.2 (d, J = 281.8 Hz, C'), 104.4 (d, J = 21.5 Hz, C'), 103.4 (d, J = 40.5 Hz, C'), 88.0 (d, J = 1.9 Hz, C'), 84.4 (d, J = 7.6 Hz, C'), 75.2 (d, J = 29.1 Hz, C'), 72.3 (d, J = 20.7 Hz, C'), 62.1 (C'), 61.8 (C'), 40.3 (MsCH₃), 40.1 (MsCH₃), 18.1 (3C, SiCH(CH₃)₂ and SiCH(CH₃)₂) 18.0 (4C, SiCH(CH₃)₂ and SiCH(CH₃)₂), 12.7 (SiCH(CH₃)₂), 12.6 (SiCH(CH₃)₂), 12.0 (SiCH(CH₃)₂) and SiCH(CH₃)₂).

**¹⁹F**[¹H] NMR (376 MHz, CDCl₃) δ -114.85 (s), -125.37 (s).

**¹⁹F** NMR (471 MHz, CDCl₃) δ -114.82 (dd, J = 16.8, 7.3 Hz, minor diastereomer), -125.34 (d, J = 12.0 Hz, major diastereomer).

IR (cm⁻¹): 2945, 2868, 2363, 1464, 1375, 1184, 1144, 1103, 1070, 951, 881, 856, 818, 681, 523.

HRMS (ES): [C₂₄H₅₀⁷⁹BrFO₆Si₂S+Na]⁺ calc. 643.1932 found 643.1931
2-Propylpentanoic/Valproic anhydride (213)

To a solution of valproic acid (212, 17.7162 g, 123 mmol, 2 equiv.), and NEt₃ (17.5 mL, 125 mmol, 2.0 equiv.) in anhydrous DCM (125 mL) was added diphenylphosphoryl chloride (12.8 mL, 61.75 mmol) dropwise at 0°C. Once addition was complete, the reaction was warmed to room temperature and stirred overnight. The reaction was quenched with cold water, organic separated and aqueous extracted with DCM (3 x 50 mL). The combined organics were with saturated aqueous NaHCO₃ (5 x 50 mL) and brine (3 x 50 mL), dried over MgSO₄ and concentrated to yield 213 (15.7978 g, 95% yield) as a colourless liquid (ρ = 0.9156 g L⁻¹)

¹H NMR (500 MHz, CDCl₃) δ 2.44 (tt, J = 8.7, 5.3 Hz, 2H, C₆H₄Pr), 1.68 – 1.60 (m, 4H, CHC₆H₄), 1.50 – 1.32 (m, 12H, CHC₆H₄ and CH₂C₆H₅CH₃), 0.91 (app. t, J = 7.3 Hz, 12H, -CH₂CH₃).

¹³C{¹H} NMR (126 MHz, CDCl₃) δ 172.1 (VpC=O), 46.4 (CH), 34.1 (CHCH₂), 20.6 (CH₂CH₂), 14.1 (CH₃).

IR (cm⁻¹): 2955, 2934, 2361, 1809, 1746, 1462, 1024.


N-(2-oxo-1,2-dihydropyrimidin-4-yl)acetamide (214)

Cytosine (184, 1.111 g, 10.0 mmol) and acetic anhydride (1.94 mL, 20.6 mmol) were dissolved in anhydrous pyridine (20 mL) and stirred at room temperature for 24 h. The reaction mixture was precipitated in cold water and filtered. The precipitate was washed with further cold water and dried to afford 214 (1.19 g, 78% yield) as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ 11.52 (s, 1H, N⁴H), 10.75 (s, 1H, N¹H), 7.80 (d, J = 7.0 Hz, 1H, H5), 7.09 (d, J = 7.0 Hz, 1H, H6), 2.08 (s, 3H, AcCH₃).

¹³C{¹H} NMR (126 MHz, DMSO-d₆) δ 170.9 (AcC=O), 163.3 (C⁵), 156.2 (C⁴), 147.1 (C⁶), 94.5 (C⁵), 24.3 (AcCH₃).

IR (cm⁻¹): 2972, 1703, 1609, 1593, 1501, 1460, 1427, 1371, 1308, 1211, 682, 853, 812, 779, 679, 594.

HRMS (TOF ASAP⁺): [C₆H₅N₃O₂+H]⁺ calc. 154.0617, found 154.0617.
N-(2-oxo-1,2-dihydropyrimidin-4-yl)-2-propylpentanamide (215)

Cytosine (183, 1.111 g, 10.0 mmol) and valproic anhydride (1.94 mL, 20.6 mmol) were dissolved in anhydrous pyridine (15 mL) and stirred at room temperature for 24 h. The reaction mixture was cooled and precipitated with cold water and filtered. The precipitate was washed with further cold water and dried to afford 215 (1.66 g, 70% yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-$_d$6) $\delta$ 11.55 (s, 1H, N$_4$H), 10.78 (s, 1H, N$_1$H), 7.81 (d, $J = 7.0$ Hz, 1H, $H_6$), 7.16 (d, $J = 7.0$ Hz, 1H, $H_5$), 2.60 (ddd, $J = 13.9$, 9.2, 4.8 Hz, 1H, $\text{C}_\text{H}_n\text{Pr}$), 1.51 (m, 2H, $\text{C}_2\text{H}_2$), 1.36 – 1.15 (m, 6H, $\text{C}_2\text{H}_2$), 0.85 (app. t, $J = 7.2$ Hz, 6H, $2\text{C}_3\text{H}_3$).

$^{13}$C{$^1$H} NMR (101 MHz, DMSO-$_d$6) $\delta$ 177.1 (VpC=O), 163.3 (C$_2$), 156.2 (C$_4$), 147.3 (C$_6$), 94.7 (C$_5$), 45.7 (C$_H$), 34.6 (C$_H_2$), 20.1 (CH$_2$), 14.0 (CH$_3$).

IR (cm$^{-1}$): 2957, 2934, 1705, 1614, 1497, 1449, 1418, 1296, 1219, 1136, 1096, 924, 812, 584.

HRMS (FTMS + p NSI): [C$_{12}$H$_{19}$O$_2$N$_3$+H]$^+$ calc. 238.1550 found 238.1548

N-(2-((trimethylsilyl)oxy)pyrimidin-4-yl)acetamide (216)

To a mixture of 214 (766 mg, 5 mmol) and ammonium sulfate (33.1 mg, 0.25 mmol, 0.05 equiv.) was added hexamethyldisilazane (3.14 mL, 15 mmol, 3 equiv.) and heated to reflux until a golden colour persisted (c.f. 3 hours). The mixture was then cooled to r.t. and concentrated in vacuo to afford 216 (1.05 g, 93% yield) as a white solid.

$^1$H NMR (500 MHz, DMSO-$_d$6) $\delta$ 7.80 (d, $J = 7.0$ Hz, 1H, $H_6$), 7.09 (d, $J = 7.0$ Hz, 1H, $H_5$), 5.28 (s, 1H, N$_4$H), 2.08 (s, 3H, AcCH$_3$), 0.01 (s, 9H, Si(CH$_3$)$_3$).

Unable to obtain $^{13}$C{$^1$H} NMR.

IR (cm$^{-1}$): 2978, 1697, 1686, 1607, 1501, 160, 1373, 1310, 1217, 810, 681, 564.

HRMS (TOF ASAP$^+$): [C$_9$H$_{15}$N$_3$O$_2$Si+H]$^+$ calc. 226.1012 found 226.1009

2-propyl-N-(2-((trimethylsilyl)oxy)pyrimidin-4-yl)pentanamide (217)

To a mixture of 215 (1.1865 g, 5 mmol) and ammonium sulfate (33.1 mg, 0.25 mmol, 0.05 equiv.) was added hexamethyldisilazane (3.14 mL, 15 mmol, 3 equiv.) and heated to reflux until a golden colour persisted (c.f. 3 hours). The mixture was then cooled to r.t. and concentrated in vacuo to afford 217 as a white solid (1.55 g, quant. yield).
1H NMR (500 MHz, DMSO-d6) δ 7.80 (d, J = 7.0 Hz, 1H, Hf), 7.16 (d, J = 7.0 Hz, 1H, Hf), 2.64 – 2.56 (m, 1H, CH), 1.56 – 1.46 (m, 2H, CH2), 1.36 – 1.28 (m, 2H, CH2), 1.26 – 1.17 (m, 4H, CH2), 0.85 (app. t, J = 7.3 Hz, 6H, 2CH3), 0.01 (s, 9H, Si(CH3)3).

13C{1H} NMR (126 MHz, DMSO-d6) δ 177.0 (VpC=O), 163.2 (C2), 156.2 (C1), 147.3 (C6), 94.7 (C3), 45.7 (CH), 34.6 (CH2), 20.1 (CH2), 13.9 (CH3), 1.8 (Si(CH3)3).

IR (cm⁻¹): 2980, 2363, 1690, 1626.

HRMS (TOF ASAP⁺): [C15H27N4O5Si+H]⁺ calc. 310.1951 found 310.1944

N-(1-((3R,4R,5R)-3-bromo-3-fluoro-4-((triisopropylsilyl)oxy)-5-((triisopropylsilyl)-oxy)methyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)acetamide (218)

A slurry of 216 (288.2 mg, 1.28 mmol, 1.5 equiv.) and trimethylsilyl trifluoromethanesulfonate (260 μL, 1.39 mmol, 1.63 equiv.) in anhydrous 1,2-dichloroethane (10 mL) was stirred at room temperature for 1 hour. 210 (458.5 mg, 0.853 mmol) in anhydrous 1,2-dichloroethane (8 mL) was added and the reaction was heated at reflux overnight. The mixture was then cooled to room temperature, diluted with DCM and quenched with saturated aqueous NaHCO3 (10 mL). The organic phase was washed with water (3 x 10 mL) and brine (2 x 10 mL), dried over MgSO4 and concentrated in vacuo. The residue was purified by column chromatography (60% EtOAc/Hexane) to yield 218 (1.53 g, 76% yield) as a foamy solid, in an anomic mixture (6.7:1 β:α anomer).

β-218

1H NMR (500 MHz, CDCl3) δ 9.05 (bs, 1H, N4H), 7.86 (d, J = 7.6 Hz, 1H, Hf), 7.42 (d, J = 7.6 Hz, 1H, Hf), 6.57 (d, J = 8.9 Hz, 1H, Hf'), 4.83 (dd, J = 12.9, 5.7 Hz, 1H, Hf'), 4.22 (dd, J = 9.2, 4.4 Hz, 1H, Hf'), 3.94 (ddd, J = 11.2, 4.6, 2.3 Hz, 1H, Hf')a), 3.90 (dd, J = 11.4, 2.2 Hz, 1H, Hf'b), 2.26 (s, 3H, AcCH3), 1.08 (m, 42H, SiCH(CH3)2 and SiCH(CH3)3).

13C{1H} NMR (125 MHz, CDCl3) δ 170.6 (AcC=O), 162.9 (C2), 155.0 (C1), 145.1 (C6), 116.3 (d, J = 267.5 Hz, C6'), 96.2 (C5'), 88.6 (d, J = 35 Hz, C1'), 85.0 (C4'), 75.3 (d, J = 25 Hz), 61.7 (C2'), 25.2 (AcCH3), 18.2 (SiCH(CH3)3), 18.1 (2C, SiCH(CH3)3), 18.0 (SiCH(CH3)3), 12.8 (SiCH(CH3)3), 12.6 (SiCH(CH3)3), 12.1 (SiCH(CH3)3), 12.0 (SiCH(CH3)3).

19F NMR (CDCl3, 376.5 MHz) δ -111.7 (t, J = 8.6 Hz, β), -122.1 (dd, J = 16.8, 5.0 Hz, α).

IR (cm⁻¹): 2943, 2866, 1680, 1626, 1558, 1493, 1464, 1387, 1317, 1238, 1188, 1098, 1078, 1075, 999, 953, 883, 787, 683.

HRMS (ES): [C29H33BrF3N3O5Si2+H]⁺ calc. 678.2769 found 678.2758.
N-(1-((3R,4R,5R)-3-bromo-3-fluoro-4-((triisopropylsilyl)oxy)-5-((triisopropylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-propylpenta
namide (219)

A slurry of 217 (1.81 g, 4.76 mmol, 1.5 equiv.) and trimethylsilyl trifluoromethanesulfonate (940 μL, 5.17 mmol, 1.63 equiv.) in anhydrous 1,2-dichloroethane (15 mL) was stirred at room temperature for 1 hour. 210 (2.08 g, 3.17 mmol) in anhydrous 1,2-dichloroethane (10 mL) was added and the reaction was heated to reflux overnight. The mixture was then cooled to room temperature, diluted with DCM and quenched with saturated aqueous NaHCO₃ (10 mL). The organic phase was washed with water (3 x 10 mL), brine (2 x 10 mL), then dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (60% EtOAc/Hexane) to yield 219 (2.11 g, 87% yield) as a foamy solid, in an anomeric mixture (10:1 β:α anomer).

β-219

1H NMR (500 MHz, CDCl₃) δ 7.96 (s, 1H, N₄H), 7.88 (d, J = 7.6 Hz, 1H, H₆), 7.45 (d, J = 7.6 Hz, 1H, H₅), 6.58 (d, J = 8.6 Hz, 1H, H₃), 4.84 (dd, J = 12.7, 5.9 Hz, 1H, H₃'), 4.21 (dd, J = 9.5, 3.9 Hz, 1H, H₁'), 3.95 (ddd, J = 11.4, 4.4, 2.4 Hz, 1H, H₅'a), 3.90 (dd, J = 11.5, 2.3 Hz, 1H, H₁'b), 2.30 – 2.22 (m, 1H, CH), 1.69 – 1.59 (m, 2H, CH₂), 1.52 – 1.42 (m, 2H, CH₂), 1.39 – 1.29 (m, 4H, CH₂), 1.11 (m, 4H), 0.91 (overlapping t, J = 7.3 Hz, 6H).

13C¹H NMR (125 MHz, CDCl₃) δ 176.5 (VpC=O), 162.5 (C⁵), 155.1 (C⁴), 145.3 (C⁶), 116.3 (d, J = 269.8 Hz, C²), 96.0 (C⁵'), 88.5 (d, J = 34.8 Hz, C¹'), 84.9 (d, J = 4.7 Hz, C⁴'), 75.2 (d, J = 24.5 Hz, C³'), 61.6 (C⁵'), 49.0 (CH), 35.0 (2C, CH₂), 20.8 (2C, CH₂), 18.1 (2C, SiCH(CH₃)₃), 18.0 (2C, SiCH(CH₃)₃), 14.2 (2C, CH₃), 12.5 (SiCH(CH₃)₃), 12.1 (SiCH(CH₃)₃).

19F NMR (376.5 MHz, CDCl₃) δ -112.0 (t, J = 8.1 Hz, β), -121.9 (dd, J = 17.2, 5.6 Hz, α).

IR (cm⁻¹): 1674, 1622, 1557, 1489, 1460, 1400, 1317, 1103, 1069, 883, 783, 683.

HRMS (TOF ES⁺): [C₃₅H₇₅N₆O₅FSi₂Br+H]⁺ calc. 764.3688 found 764.3694.
N-(1-((3R,4R,5R)-3-bromo-3-fluoro-5-(hydroxymethyl)-4-((triisopropylsilanyl)oxy)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)acetamide (220)

To a solution of 216 (assumed 5 mmol) in anhydrous DCE (20 mL) was added TMSOTf (780 μL, 4.5 mmol, 1.5 equiv.). The mixture was stirred at room temperature for an hour, before addition of a solution of 210 in anhydrous DCE (stock solution of 3.7354 g, 6 mmol in 10 mL; 5 mL used). After complete addition, the mixture was heated to reflux overnight. After 16 hours, the mixture was cooled to room temperature and left to stir for 48 hours. The reaction mixture was filtered to remove precipitates, and the filtrate subsequently diluted with DCM (100 mL) and washed with H₂O (3 x 100 mL), saturated aqueous NaHCO₃ and brine (1 x 100 mL each). Combined aqueous washes were back-washed with further DCM (1 x 50 mL). The combined organics were dried over MgSO₄ and concentrated in vacuo. The crude mixture was purified by column chromatography (60% EtOAc/hexanes → 100% EtOAc) to yield 220 (775.0 mg, 49% yield) as an anomic mixture (~2:3 β:α). The anomers were separated and isolated, both as white solids, and individually analysed by NMR.

β-220

1H NMR (500 MHz, CDCl₃) δ 9.73 (s, 1H, N²H), 8.22 (d, J = 7.3 Hz, 1H, Hβ), 7.46 (d, J = 7.4 Hz, 1H, Hβ), 6.69 (d, J = 3.7 Hz, 1H, H³'), 4.52 (dd, J = 15.2, 6.8 Hz, 1H, H³'), 4.11 (d, J = 12.0 Hz, 1H, H³a), 3.97 (d, J = 6.8 Hz, 1H, H³'), 3.89 (d, J = 12.1 Hz, 1H, H³b), 2.27 (s, 3H, AcCH₃), 1.20 – 1.05 (m, 21H, SiCH(CH₃)₃ and SiCH(CH₃)₃).

13C¹H NMR (126 MHz, CDCl₃) δ 171.2 (AcC=O), 163.3 (C²), 155.3 (C⁴), 145.1 (C⁶), 110.72 (d, J = 273.2 Hz, C³), 97.3 (C⁵), 90.0 (C⁴'), 82.2 (d, J = 6.5 Hz, C⁴'), 73.6 (d, J = 24.8 Hz, C³'), 59.7 (C⁵), 25.1 (AcCH₃), 18.0 (2C, SiCH(CH₃)₃), 12.6 (SiCH(CH₃)₃).

19F NMR (471 MHz, CDCl₃) δ -121.73 (s).

α-220

1H NMR (500 MHz, CDCl₃) δ 9.37 (s, 1H, N⁴H), 7.85 (d, J = 7.4 Hz, 1H, Hα), 7.47 (d, J = 7.3 Hz, 1H, Hα), 6.74 (d, J = 3.7 Hz, 1H, H⁴'), 4.78 (dd, J = 12.8, 7.1 Hz, 1H, H⁴'), 4.19 (d, J = 4.3 Hz, 1H, H⁴a), 3.94 (d, J = 12.4 Hz, 1H, H⁴b), 3.78 (d, J = 11.8 Hz, 1H, H⁵b), 2.27 (s, 3H, AcCH₃), 1.21 – 1.06 (m, 21H, SiCH(CH₃)₃ and SiCH(CH₃)₃).

13C¹H NMR (126 MHz, CDCl₃) δ 170.9 (AcC=O), 163.1 (C²), 155.3 (C⁴), 145.0 (C⁶), 116.3 (d, J = 272.0 Hz, C³), 96.4 (C⁵), 88.0 (d, J = 34.1 Hz, C⁴), 84.2 (d, J = 5.7 Hz, C⁴), 74.9 (d, J = 23.6 Hz, C³), 60.5 (C⁵), 25.2 (AcCH₃), 18.0 (SiCH(CH₃)₃), 12.5 (SiCH(CH₃)₃).

19F NMR (471 MHz, CDCl₃) δ -113.68 (app. t, J = 9.2 Hz).

1H NMR (500 MHz, DMSO) δ 11.01 (s, 1H, N⁴H), 8.05 (d, J = 7.6 Hz, 1H, Hα), 7.28 (d, J = 7.6 Hz, 1H, Hα), 6.47 (d, J = 8.1 Hz, 1H, Hβ), 5.24 (dd, J = 6.4, 4.6 Hz, 1H, O⁵H),
4.77 (dd, J = 14.2, 7.3 Hz, 1H, H^f), 4.14 (dt, J = 7.0, 3.4 Hz, 1H, H^f), 3.71 (d, J = 11.8 Hz, 1H, H^f), 3.58 (ddd, J = 12.1, 6.4, 4.0 Hz, 1H, H^f), 2.11 (s, 3H, AcCH_3), 1.17 – 1.04 (m, 21H).

^{13}C NMR (126 MHz, DMSO) δ 171.2 (AcC=O), 162.9 (C^5), 154.2 (C^6), 145.3 (C^6), 117.0 (d, J = 269.4 Hz, C^2), 95.3 (C^3), 87.3 (d, J = 34.4 Hz, C^1), 83.6 (d, J = 5.6 Hz, C^2), 74.6 (d, J = 23.6 Hz, C^5), 59.3 (C^3), 24.5 (AcCH_3), 17.7 (SiCH(CH_3)_3), 11.9 (SiCH(CH_3)_3).

^{19}F NMR (471 MHz, DMSO) δ -112.45 (dd, J = 7.1, 18.0 Hz).

HRMS (TOF AP^+): [C_{20}H_{33}N_{3}O_{2}FSiBr+H]^+ calc. 522.1435 found 522.1440.

\[N-(1-((3R,4R,5R)-3-bromo-3-fluoro-5-(hydroxymethyl)-4-((triisopropylsilyl)oxy)-tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-propylpentanamide (221)\]

To a solution of 217 (assumed 5 mmol) in anhydrous DCE (15 mL) was added TMSOTf (780 μL, 4.5 mmol, 1.5 equiv.). The mixture was stirred at room temperature for an hour, before addition of a solution of 210 in anhydrous DCE (stock solution of 3.7354 g, 6 mmol in 10 mL; 5 mL used). After complete addition, the mixture was heated to reflux overnight. After 16 hours, the mixture was cooled to room temperature and left to stir for 48 hours. The reaction mixture was diluted with DCM (100 mL) and washed with H_2O (3 x 100 mL), saturated aqueous NaHCO_3 and brine (1 x 100 mL each). Combined aqueous washes were back-washed with further DCM (1 x 50 mL). The combined organics were dried over MgSO_4 and concentrated in vacuo. The crude mixture was purified by column chromatography (80% EtO/hexanes → 100% EtO) to yield 221 (1.0622 g, 58% yield) as an anomic mixture (1:3 β:α). The anomers were separated and isolated, both as white solids, and individually analysed by NMR.

\[β-221\]

\[^1H\] NMR (500 MHz, CDCl_3) δ 8.19 (s, 1H, N^4H), 8.15 (d, J = 7.1 Hz, 1H, H^f), 7.46 (d, J = 7.6 Hz, 1H, H^f), 6.70 (d, J = 5.6 Hz, 1H, H^f), 4.54 (dd, J = 16.0, 7.0 Hz, 1H, H^f), 4.10 (dt, J = 12.2, 2.3 Hz, 1H, H^f), 4.01 – 3.95 (m, 1H, H^f), 3.91 (dd, J = 12.2, 2.9 Hz, 1H, H^f), 2.30 (td, J = 8.9, 4.5 Hz, 1H), 1.68 – 1.58 (m, 4H), 1.51 – 1.43 (m, 2H), 1.38 – 1.28 (m, 4H), 1.19 – 1.06 (m, 21H, SiCH(CH_3)_3 and SiCH(CH_2)_3), 0.90 (overlapping t, J = 7.3 Hz, 6H, CH_3).

\[^{13}C\]\[^1H\] NMR (126 MHz, CDCl_3) δ 176.4 (VpC=O), 162.5 (C^5), 155.2 (C^6), 145.2 (C^6), 96.9 (C^5), 82.2 (d, J = 6.3 Hz, C^5), 73.7 (d, J = 25.0 Hz, C^1), 59.9 (C^6), 49.1 (CH), 35.0 (d, J = 2.3 Hz, C^f), 20.8 (CH_2), 18.0 (2C, SiCH(CH_3)_3), 14.2 (SiCH(CH_3)_3), 12.6 (CH_3). N.B. C^f missing
\[^{19}\text{F NMR}\] (471 MHz, CDCl\textsubscript{3}) \(\delta -121.86\) (s).

\[^{1}\text{H NMR}\] (500 MHz, DMSO) \(\delta 11.08\) (s, 1H, N\(^4\text{H}\)), 8.43 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 7.32 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 6.56 (d, \(J = 4.5\) Hz, 1H, \(H^1\)), 5.57 (s, 1H, O\(^5\text{H}\)), 4.46 (dd, \(J = 15.3, 7.5\) Hz, 1H, \(H^3\)), 3.92 – 3.84 (m, 2H, \(H^1\) and \(H^6\)), 3.66 (d, \(J = 12.3\) Hz, 1H, \(H^6\)), 2.63 – 2.59 (m, 1H, \(CH_2\)), 1.52 (app. td, \(J = 13.7, 8.4\) Hz, 2H, \(CH_2\)), 1.38 – 1.29 (m, 2H, \(CH_2\)), 1.27 – 1.18 (m, 4H, \(CH_2\)), 1.15 – 1.04 (m, 21H, Si\(CH(CH_3)_2\)) and Si\(CH(CH_3)_2\), 0.85 (t, \(J = 7.0\) Hz, 6H, \(CH_2\)).

\[^{13}\text{C\([^{1}\text{H}]\)}\] NMR (126 MHz, DMSO) \(\delta 177.4\) (VpC=O), 162.9 (\(C^6\)), 154.3 (\(C^i\)), 144.5 (\(C^6\)), 111.0 (d, \(J = 273.3\) Hz, \(C^o\)), 95.9 (\(C^6\)), 88.6 (d, \(J = 19.1\) Hz, \(C^i\)), 81.7 (d, \(J = 6.5\) Hz, \(C^i\)), 72.8 (d, \(J = 23.7\) Hz, \(C^5\)), 58.1 (\(C^5\)), 45.8 (\(CH\)), 34.5 (\(CH_2\)), 20.0 (\(CH_2\)), 17.7 (2C, Si\(CH(CH_3)_3\)), 13.9 (2C, Si\(CH(CH_3)_3\)), 11.9 (\(CH_3\)).

\[^{19}\text{F NMR}\] (471 MHz, DMSO) \(\delta -121.85\) (s).

\textit{a-221}

\[^{1}\text{H NMR}\] (500 MHz, DMSO) \(\delta 11.04\) (s, 1H, N\(^4\text{H}\)), 8.05 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 7.34 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 6.47 (d, \(J = 8.1\) Hz, 1H, \(H^1\)), 5.23 (dd, \(J = 6.5, 4.6\) Hz, 1H, O\(^5\text{H}\)), 4.77 (dd, \(J = 14.1, 7.2\) Hz, 1H, \(H^3\)), 4.15 (dt, \(J = 6.9, 3.4\) Hz, 1H, \(H^6\)), 3.72 (d, \(J = 12.5\) Hz, 1H, \(H^6\)), 3.58 (ddd, \(J = 12.2, 6.4, 3.8\) Hz, 1H, \(H^6\)), 2.67 – 2.59 (m, 1H, \(CH_2\)), 1.58 – 1.46 (m, 2H, \(CH_2\)), 1.39 – 1.29 (m, 2H, \(CH_2\)), 1.28 – 1.19 (m, 4H, \(CH_2\)), 1.18 – 1.03 (m, 21H, Si\(CH(CH_3)_3\)) and Si\(CH(CH_3)_3\), 0.86 (overlapping t, \(J = 7.3\) Hz, 6H, \(CH_2\)).

\[^{13}\text{C\([^{1}\text{H}]\)}\] NMR (126 MHz, DMSO) \(\delta 177.4\) (VpC=O), 162.9 (\(C^6\)), 154.2 (\(C^i\)), 145.4 (\(C^6\)), 116.9 (d, \(J = 268.9\) Hz, \(C^o\)), 95.4 (\(C^6\)), 87.3 (d, \(J = 34.2\) Hz, \(C^i\)), 83.7 (\(C^6\)), 74.7 (d, \(J = 23.4\) Hz, \(C^5\)), 59.3 (\(C^5\)), 45.8 (\(CH\)), 34.5 (\(CH_2\)), 20.1 (\(CH_2\)), 17.7 (Si\(CH(CH_3)_3\)), 14.0 (Si\(CH(CH_3)_3\)), 11.9 (\(CH_3\)).

\[^{19}\text{F NMR}\] (471 MHz, DMSO) \(\delta -112.42\) (dd, \(J = 13.1, 8.8\) Hz).

\[^{1}\text{H NMR}\] (500 MHz, CDCl\textsubscript{3}) \(\delta 8.18\) (s, 1H, N\(^4\text{H}\)), 7.86 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 7.49 (d, \(J = 7.6\) Hz, 1H, \(H^6\)), 6.70 (d, \(J = 7.7\) Hz, 1H, \(H^1\)), 4.77 (dd, \(J = 13.0, 7.1\) Hz, 1H, \(H^6\)), 4.20 (dt, \(J = 6.7, 3.3\) Hz, 1H, \(H^6\)), 3.93 (d, \(J = 12.6\) Hz, 1H, \(H^6\)), 3.82 – 3.76 (m, 1H, \(H^6\)), 2.35 – 2.26 (m, 1H, \(CH_2\)), 2.17 (app. dd, \(J = 7.1, 5.2\) Hz, 1H), 1.68 – 1.60 (m, 2H), 1.48 (ddd, \(J = 13.7, 8.9, 5.9\) Hz, 2H), 1.37 – 1.30 (m, 1H), 1.11 (m, 21H, Si\(CH(CH_3)_3\)) and Si\(CH(CH_3)_3\), 0.91 (overlapping t, \(J = 7.3\) Hz, 6H, 2\(CH_2\)).

\[^{13}\text{C\([^{1}\text{H}]\)}\] NMR (126 MHz, CDCl\textsubscript{3}) \(\delta 176.4\) (VpC=O), 162.5 (\(C^6\)), 145.1 (\(C^i\)), 96.1 (\(C^6\)), 88.0 (d, \(J = 34.5\) Hz, \(C^i\)), 84.2 (\(C^i\)), 74.9 (d, \(J = 23.8\) Hz, \(C^3\)), 60.6 (\(C^5\)), 49.1 (\(CH\)), 35.0 (2C, \(CH_2\)), 29.9 (\(CH_2\)), 20.8 (2C, \(CH_2\)), 18.0 (Si\(CH(CH_3)_3\)), 14.2 (2C, Si\(CH(CH_3)_3\)), 12.5 (\(CH_3\)).

\textit{N.B.} \(C^i\) and \(C^2\) missing.

\[^{19}\text{F NMR}\] (471 MHz, CDCl\textsubscript{3}) \(\delta -113.79\) (dd, \(J = 12.0, 8.2\) Hz).

HRMS (TOF AP\textsuperscript{+}): [C\textsubscript{26}H\textsubscript{46}N\textsubscript{3}O\textsubscript{5}FSiBr+H]\textsuperscript{+} calc. 606.2374 found 606.2372.
General method: To a flame dried microwave vial with 210 (130 mg, 0.2 mmol) and catalyst (if solid, 10 mol%) was added a solution of 217 in anhydrous MeCN (0.25 M, 1 mL, 1.25 equiv.) (and catalyst, if TMSOTf, 10 mol%). The microwave vial was irradiated with a temperature gradient from 110°C to 150°C and held at 150°C for 10 minutes. After cooling to room temperature, the reaction mixture was diluted with EtOAc (10 mL), washed with H₂O (3 x 10 mL) and brine (10 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude material was purified by column chromatography (10% Et₂O/Petrol) to deliver 222.

Data for β-222, isolated from Entry 5 (no catalyst)

¹H NMR (400 MHz, CDCl₃) δ 8.42 (d, J = 5.6 Hz, 1H, H̠), 7.89 (d, J = 5.6 Hz, 1H, H̠), 7.85 (s, 1H, N⁴H), 6.69 (d, J = 1.6 Hz, 1H, H₁), 4.80 (dd, J = 12.2, 7.7 Hz, 1H, H₃), 4.00 – 3.90 (m, 2H, H₄ and H₅''), 3.84 (dd, J = 11.5, 3.3 Hz, 1H, H₅''), 2.29 – 2.21 (m, 1H, C₇H), 1.71 – 1.63 (m, 2H, CH₂), 1.48 (m, 2H, CH₂), 1.37 – 1.27 (m, 4H, CH₂), 1.17 – 1.07 (m, 21H, SiC₃H(CH₃)₃ and SiCH(C₃H₃)₃), 0.97 – 0.87 (m, 27H, CH₃ and SiCH(CH₃)₃ and SiCH(CH₃)₃).

¹³C NMR (126 MHz, CDCl₃) δ 175.8 (VpC=O), 162.9 (C⁵), 160.7 (C⁶), 158.9 (C⁴), 110.7 (d, J = 282.9 Hz, C²), 105.1 (C⁰), 100.2 (d, J = 19.0 Hz, C''), 83.4 (d, J = 7.9 Hz, C'), 72.8 (d, J = 20.6 Hz, C'), 61.8 (C⁰), 49.1 (CH), 35.2 (2C, CH₂), 20.9 (CH₂), 18.1 (2C, SiCH(CH₃)₃), 17.9 (2C, SiCH(CH₃)₃), 14.2 (2C, SiCH(CH₃)₃), 12.7 (SiCH(CH₃)₃), 11.9 (CH₃).

¹⁹F NMR (471 MHz, CDCl₃) δ -125.50 (d, J = 12.1 Hz).

HRMS (TOF AP⁺): [C₃₅H₆₆N₃O₅FSi₂Br⁺H]⁺ calc. 764.3688 found 764.3691.

α-222

¹⁹F NMR (471 MHz, CDCl₃) δ -113.90 (dd, J = 18.3, 8.8 Hz).
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<th>Entry</th>
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<th>Catalyst (10 mol%)</th>
<th>Isolated mass and yield</th>
<th>β:α ratio</th>
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<td>TMSOTf 3.5 μL</td>
<td>46.4 mg 30%</td>
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<td>2</td>
<td>B</td>
<td>127.4</td>
<td>Pyridinium triflate 4.8 mg</td>
<td>49.7 mg 33%</td>
<td>62.5:37.5 (5:3)</td>
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<td>54.6 mg 36%</td>
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**Table 5.01**: The conditions explored for the microwave-mediated glycosylation of 210 with 217, with amount of reactants, yields and anomer selectivity noted. Anomer ratio determined by $^{19}$F NMR analysis of the crude reaction mixture.
1-(2R,3R,4R,5R)-3-bromo-3-fluoro-5-(hydroxymethyl)-4-((triisopropylsilyl)oxy)-tetrahydrofuran-2-yl)-4-imino-3,4-dihydropyrimidin-2(1H)-one (β-223)

A solution of compound 218 (2.29 g, 3.37 mmol) in MeOH (22 mL) was treated with 12 M HCl (5.3 mL, 64.0 mmol, 19.0 equiv.) and stirred at room temperature over two days. White precipitate formed, which was collected by filtration and dried under high vacuum to yield β-223 (1.01 g, 63%) as a colourless solid.

1H NMR (500 MHz, DMSO-d6) δ 9.38 (broad s, 1H, N1H), 8.46 (broad s, 1H, N4H), 7.91 (d, J = 7.9 Hz, 1H, Hβ), 6.40 (d, J = 8.1 Hz, 1H, Hδ), 6.12-6.08 (m, 1H, Hα), 4.37 (dd, J = 14.0, 7.2 Hz, 1H, Hδ), 4.14 (app. dt, J = 7.1, 3.4 Hz, 1H, Hβ), 3.70 (d, J = 11.9 Hz, 1H, Hα), 3.59 (dd, J = 12.4, 4.1 Hz, 1H, H′), 1.17-1.07 (m, 21H, SiCH(CH3)2 and SiCH(CH3)3).

13C[1H] NMR (125 MHz, DMSO-d6) δ 159.8 (C5), 147.6 (C6) 143.8 (C2), 116.5 (d, J = 266.3 Hz, C3), 93.9 (C6), 87.1 (d, J = 35 Hz, C4), 83.9 (d, J = 5 Hz, C5), 74.3 (d, J = 22.5 Hz, C3), 59.3 (C5), 17.7 (SiCH(CH3)2) 11.8 (SiCH(CH3)2).

19F NMR (376.5 MHz, DMSO-d6) δ -113.5 (dd, J = 11.9, 8.5 Hz).

IR (cm⁻¹): 3354, 2976, 2899, 2363, 2334, 1734, 1653, 1558, 1508, 1456, 1418 1339, 1277, 1192, 1086, 1043, 880, 669, 519, 465, 444.

HRMS (ES): [C18H37BrFN3O4Si+H]+ calc. 480.1329 found 480.1321.

(2R,3R,4R,5R)-5-4-acetamido-2-oxopurimidin-1(2H)-yl)-4-bromo-4-fluoro-3-((triisopropylsilyl)oxy)tetrahydrofuran-2-yl)methyl acetate (β-224)

To a solution of β-223 (145.1 g, 0.3 mmol), DMAP (7.4 mg, 0.06 mmol, 0.2 equiv.) and NEt3 (252 μL, 1.8 mmol, 6 equiv.) in anhydrous DCM (4.5 mL) was added Ac2O (69 μL, 0.72 mmol, 2.4 equiv.) and stirred at room temperature overnight. The reaction was quenched and washed with H2O (3 x 30 mL) and brine (30 mL), dried over MgSO4 and concentrated in vacuo to yield β-224 (154.0 mg, 91%) as a colourless solid.

1H NMR (500 MHz, CDCl3) δ 10.09 (broad s, 1H, N4H), 7.83 (d, J = 7.7 Hz, 1H, Hδ), 7.48 (d, J = 7.7 Hz, 1H, Hδ), 6.66 (d, J = 9.0 Hz, 1H, Hβ), 4.65 (dd, J = 12.4, 5.7 Hz, 1H, Hδ), 4.24-4.38 (m, 2H, H′ and H′), 4.22-4.19 (m, 1H, Hδ), 2.30 (s, 3H, AcCH3), 2.11 (s, 3H, AcCH3), 1.19-1.09 (m, 21H, SiCH(CH3)2 and SiCH(CH3)3).

13C[1H] NMR (125 MHz, DMSO-d6) δ 170.5 (AcC=O), 166.2 (C5), 155.8 (C6), 141.0 (C6), 115.8 (d, J = 265 Hz, C3), 95.1 (C5), 87.9 (d, J = 35 Hz, C4), 76.1 (d, J = 26.3 Hz, C3), 62.3 (C6), 20.9 (AcCH3), 18.0 (SiCH(CH3)2), 12.5 (SiCH(CH3)2).
HRMS (ES): [C_{22}H_{36}BrFN_{3}O_{6}Si+H]^+ calc. 564.1541 found 564.1548.

\((2R,3R,4R,5R)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl acetate (\(\beta\)-225)

To a pre-stirred solution of tetramethylammonium fluoride tetrahydrate (297 mg, 1.8 mmol, 2 equiv.) and acetic acid (103 \(\mu\)L, 1.8 mmol, 2.0 equiv.) was added dropwise a solution of \(\beta\)-224 in anhydrous DMF (3 mL) at room temperature and stirred overnight. The reaction was quenched with \(\text{H}_2\text{O}\) (10 mL), extracted with EtOAc (3 x 20 mL), dried over MgSO\(_4\) and concentrated in vacuo to yield \(\beta\)-225 (289.2 mg, 78%) as a colourless solid.

\(^1\)H NMR (500 MHz, DMSO-\(\text{d}_6\)) \(\delta\) 11.0 (broad s, 1H, \(N\_4\)H), 8.1 (d, \(J = 7.6\) Hz, 1H, \(H\_6\)), 7.27 (d, \(J = 7.6\) Hz, 1H, \(H\_5\)), 6.86 (d, \(J = 5.7\) Hz, 1H, \(O\_3'\)H), 6.57 (d, \(J = 8.6\) Hz, 1H, \(H\_1'\)) - 4.50-4.45 (m, 1H, \(H\_3'\)), 4.35 (dd, \(J = 12.1, 2.7\) Hz, 1H, \(H\_5'b\)), 4.30 (m, 1H, \(H\_4'\)), 4.19 (dd, \(J = 12.1, 6.1\) Hz, 1H, \(H\_5'a\)), 2.12 (s, 3H, Ac\_C\_H\_3), 2.07 (s, 3H, Ac\_C\_H\_3).

\(^{13}\)C\(^{\_1\text{H}}\) NMR (125 MHz, DMSO-\(\text{d}_6\)) \(\delta\) 171.1 (Ac\_C\_=O), 170.2 (Ac\_C\_=O), 162.8 (C\_2), 154.2 (C\_4), 145.4 (C\_6), 116.9 (d, \(J = 266.7\) Hz, C\_5), 95.3 (C\_3), 87.6 (d, \(J = 34.4\) Hz, C\_1), 80.4 (d, \(J = 6.3\) Hz, C\_4), 74.0 (d, \(J = 23.6\) Hz, C\_3), 62.4 (C\_5'), 24.4 (AcCH\_3), 20.6 (AcCH\_3).

\(^{19}\)F\(^{\_1\text{H}}\) NMR (376.5 MHz, DMSO-\(\text{d}_6\)) \(\delta\) -113.0 (s).

IR (cm\(^{-1}\)) : 3364, 3275, 2361, 2261, 2133, 1967, 1906, 1867, 1748, 1651, 1558, 1508, 1458, 1396, 1339, 1277, 1211, 1045, 1022, 988, 823, 764, 667.

HRMS (EI): [C\_13\_H\_15\_BrFN\_3O\_6+H]^+ calc. 408.0207 found 408.0208.

\((2R,3R,4R,5R)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-2-(acetoxymethyl)-4-bromo-4-fluorotetrahydrofuran-3-yl acetate (\(\beta\)-226)

To a solution of \(\beta\)-223 (358.7 g, 0.88 mmol), DMAP (10.8 mg, 0.088 mmol, 0.1 equiv.) and NEt\(_3\) (370 \(\mu\)L, 2.64 mmol, 3 equiv.) in anhydrous DCM (4.5 mL) was added Ac\(_2\)O (105 \(\mu\)L, 1.1 mmol, 1.2 equiv.) and stirred at room temperature overnight. The reaction was quenched and washed with \(\text{H}_2\text{O}\) (3 x 30 mL) and brine (1 x 30 mL), dried over MgSO\(_4\) and concentrated in vacuo to yield \(\beta\)-226 (288.6 mg, 73%) as a colourless solid.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 10.24 (s, 1H, \(N\_4\)H), 7.76 (d, \(J = 7.7\) Hz, 1H, \(H\_6\)), 7.56 (d, \(J = 7.7\) Hz, 1H, \(H\_5\)), 6.74 (d, \(J = 10.3\) Hz, 1H, \(H\_1'\)), 5.65 (d,
To a solution of 219 (120 mg, 0.157 mmol) in anhydrous DMF (0.2M) were added acetic acid (4.0 eq) and tetramethylammonium fluoride tetrahydrate (103.9 mg, 0.629 mmol, 4 equiv.). The reaction was stirred at room temperature overnight and then was concentrated and purified by column chromatography (4% EtOH/EtOAc) to afford compound β-227 (2.11 g, 80% yield) as a white foaming solid.

$J = 14.5, 4.9 \text{ Hz, 1H, } H^3$, 4.60 (app. q, $J = 5.2 \text{ Hz, 1H, } H^1$), 4.32 (app. d, $J = 5.1 \text{ Hz, 2H, } H^4$), 2.31 (s, 3H, AcCH$_3$), 2.18 (s, 3H, AcCH$_3$), 2.13 (s, 3H, AcCH$_3$).

$^{13}$C($^1$H) NMR (125 MHz, CDCl$_3$) δ 171.5 (AcC=O) 170.5 (AcC=O), 168.5 (AcC=O), 163.7 (C$^2$), 155.0 (C$^3$), 144.0 (C$^5$), 111.6 (d, $J = 264.8 \text{ Hz, } C^3$), 97.0 (C$^6$), 89.7 (d, $J = 37.1 \text{ Hz, } C^1$), 80.6 (d, $J = 2.6 \text{ Hz, } C^4$), 74.9 (d, $J = 29.6 \text{ Hz, } C^5$), 62.2 (d, $J = 2.4 \text{ Hz, } C^5$), 25.1 (AcCH$_3$), 20.8 (2C, AcCH$_3$).

$^{19}$F NMR (376.5 MHz, CDCl$_3$) δ -111.32 (app. t, $J = 9.4 \text{ Hz}$).

IR ν(cm$^{-1}$): 1744, 1667, 1620, 1555, 1489, 1381, 1315, 1207, 1107, 1042, 953, 899, 806, 783, 733, 664, 594, 521, 478.

HRMS (EI): [C$_{15}$H$_{17}$BrFN$_3$O$_7$+H]$^+$ calc. 450.0312 found 450.0316.
To a solution of guanidine hydrochloride (96.0 mg, 1 mmol) in EtOH (1 mL) was added sodium ethoxide (68.1 mg, 1 equiv.) and diluted with further EtOH. The mixture was stirred at r.t. for 1 hour, filtered and collected.

To a solution of β-228 (53.1 mg, 0.1 mmol) in EtOH/DCM (500 μL, 9:1) [in an oven dried microwave vial] was slowly added the guanidine solution (200 μL, 1 M, 1 equiv.) at r.t.. The reaction was stirred at r.t. for 3 hours, concentrated in vacuo and purified by column chromatography to yield β-227 (32.1 mg, 71%) as a colourless solid.

To an oven dried microwave vial, β-228 (106.9 mg, 0.2 mmol) was dissolved in MeOH (1 mL) and cooled to 0°C. NH₃ (7N MeOH, 170 μL, 6 equiv) was added slowly to cooled solution and warmed to r.t.. When the reaction was complete (3 h), monitored by TLC, the reaction was concentrated in vacuo and purified by column chromatography (EtOAc) to yield β-227 (52.2 mg, 58%) as a colourless solid.

Spectroscopic data is identical for β-227 when desilylated or deacetylated.

To a solution of β-227 (2.3138 g, 5.2 mmol), DMAP (126.0 mg, 0.1 mmol, 0.2 equiv.) in anhydrous DCM (26 mL) was added NEt₃ (4.3 mL, 30.8 mmol, 6 equiv.) and Ac₂O (1.25 mL, 13 mmol, 2.5 equiv.) sequentially, and stirred at room temperature overnight. The reaction was quenched and washed with H₂O (3 x 30 mL) and brine (1 x 30 mL), dried over MgSO₄ and concentrated in vacuo. Purification by column chromatography (100% EtOAc) gave β-228 (2.1399 g, 78%) as a foaming white solid.

**1H NMR (500 MHz, CDCl₃) δ 8.12 (broad s, 1H, N₄H), 7.73 (d, J = 7.7 Hz, 1H, H₆), 7.52 (d, J = 7.6 Hz, 1H, H₅), 6.75 (d, J = 10.2 Hz, 1H, H¹), 5.67 (dd, J = 12.4, 5.0 Hz, 1H, H³), 4.56 (app. q, J = 4.9 Hz, 1H, H⁴), 4.35 - 4.26 (m, 2H, H²a and H²b), 2.30 (tt, J = 8.9, 5.3 Hz, 1H, CH₂), 2.19 (s, 3H, AcCH₃), 2.14 (s, 3H, AcCH₃), 1.68 - 1.61 (m, 2H, CH₂), 1.53 - 1.45 (m, 2H, CH₂), 1.38 - 1.30 (m, 4H, CH₂), 0.92 (overlapping t, J = 7.3 Hz, 6H, CH₃).**

**13C NMR (125 MHz, CDCl₃) δ 176.4 (Vp=C=O), 170.5 (Ac=C=O), 168.5 (Ac=C=O), 162.6 (C⁵), 154.9 (C⁴), 144.1 (C³), 111.6 (d, J = 265 Hz, C²), 96.3 (C⁵), 89.5 (d, J = 36.3 Hz, C¹), 80.4 (d, J = 3.8 Hz, C⁴), 74.9 (d, J = 27.5 Hz, C³), 62.2 (C⁵), 49.1 (CH), 35.0 (2C, CH₂), 31.1 (CH₂), 20.8 (3C, AcCH₃ and CH₂), 14.2 (2C, CH₃).**
$^{19}$F NMR (376.5 MHz, CDCl$_3$) $\delta$ -111.5 (app. t, $J = 8.3$ Hz)
IR $\nu$ (cm$^{-1}$): 3333, 2972, 2880, 1751, 1663, 1624, 1560, 1489, 1454, 1381, 1317, 1273, 1225, 1086, 1045, 880, 804, 787, 594, 432, 413.
HRMS (ES): [C$_{21}$H$_{29}$BrFN$_3$O$_7$+H]$^+$ calc. 534.1251 found 534.1241.
(E)-N-benzylidenebenzenesulfonamide (234)

A mixture of benzenesulfonamide (1.5721 g, 10.0 mmol) and benzaldehyde dimethyl acetal (1.5 mL, 10.0 mmol) was heated for 3 hours to remove methanol from the reaction by distillation. The reaction was cooled to room temperature and concentrated in vacuo. The resulting slurry mixture was dissolved in the minimum amount of DCM, and upon addition of hexane a precipitate formed. The mixture was cooled overnight to aid precipitation, and filtered to furnish 234 (999.8 mg, 41% yield) as a white solid.

$^1$H NMR (500 MHz, DMSO-d$_6$) δ 9.19 (s, 1H, -C=H=N), 8.06 – 8.02 (m, 2H, ArH), 7.99 – 7.95 (m, 2H, ArH), 7.79 – 7.70 (m, 2H, ArH), 7.67 (t, J = 7.8 Hz, 2H, ArH), 7.58 (t, J = 7.7 Hz, 2H, ArH).

$^{13}$C{[1]H} NMR (126 MHz, DMSO-d$_6$) δ 172.5 (C=H=N), 138.5 (ArC), 136.0 (ArC), 134.5 (ArC), 132.5 (ArC), 132.0 (2C, ArC), 128.0 (ArC).

IR (cm$^{-1}$): 1595, 1571, 1447, 1312, 1157, 1088, 795, 750, 683, 629, 583

HRMS (ES): [C$_{13}$H$_{11}$NO$_2$S]$^+$ calc. 245.0511 found 245.0511.

3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine [Davis' oxaziridine] (235)

To a cooled solution of 234 (492 mg, 2 mmol), benzyltriethylammonium chloride (45.7 mg, 0.2 mmol, 0.1 equiv.) in CH$_2$Cl$_2$/NaHCO$_3$ (7 mL, 1:1 v/v) was added slowly a solution of mCPBA (548 mg, 70% active, 2.2 mmol, 1.1 equiv.) in CH$_2$Cl$_2$ (6 mL) under vigorous stirring. The biphasic mixture was stirred at 0°C for 30 minutes, warmed to room temperature and partitioned. The organic phase was washed with H$_2$O (2 x 10 mL), NaSO$_3$ (1 x 10 mL), H$_2$O and brine (2 x 10 mL each), dried over MgSO$_4$ and concentrated in vacuo. The mother liquor was dissolved in the minimum amount of DCM and precipitated out by slow addition to hexane. The solids were collected by filtration to give 235 (294 mg, 56% yield) as a white solid.

$^1$H NMR (500 MHz, CDCl$_3$) δ 8.09 – 8.03 (m, 2H, ArH), 7.79 – 7.74 (m, 1H, ArH), 7.68 – 7.62 (m, 2H, ArH), 7.50 – 7.38 (m, 5H, ArH), 5.49 (s, 1H, CHN).

$^{13}$C{[1]H} NMR (126 MHz, CDCl$_3$) δ 135.16 (ArC), 134.89 (ArC), 131.60 (ArC), 130.61 (ArC), 129.54 (ArC), 129.53 (ArC), 128.91 (ArC), 128.41 (ArC), 76.45 (C-N).

IR (cm$^{-1}$): 2980, 2363, 1445, 1389, 1346, 1319, 1294, 1231, 1169, 1084, 827, 787, 760, 727, 689.

HRMS (ASAP$^+$): [C$_{13}$H$_{12}$NO$_3$S+H]$^+$ Calc. 262.0538 Found 262.0537
(6aR,9R,9aS)-9-hydroxy-2,2,4,4-tetraisopropyltetrahydro-8H-furo[3,2-\text{-}\text{f}][1,3,5,2,4]trioxadisilicon-8-one (237)\(^{[132]}\)

![Chemical Structure](image)

To a solution of D-ribo nic-\(\gamma\)-lactone (231, 593.2 mg, 4 mmol) and imidazole (1.3609, 20 mmol, 5 equiv.) in anhydrous DMF (30 mL) at 0°C was added dropwise a solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.54 mL, 4.8 mmol, 1.2 equiv.) in anhydrous DMF (10 mL) via dropping funnel. After complete addition, the reaction mixture was stirred at 0°C for 10 minutes before warming to r.t. with stirring overnight. The reaction was quenched with \(\text{H}_2\text{O}\) (40 mL) and extracted into EtOAc (3 x 30 mL). The combined organics were washed with \(\text{H}_2\text{O}\) and brine (2 x 30 mL), dried over MgSO\(_4\) and concentrated in vacuo. Purification by column chromatography yielded 237 (789.7 mg, 51% yield) as a colourless solid.

\(^1\text{H} \text{NMR}\) (500 MHz, CDCl\(_3\)) \(\delta\) 4.54 – 4.49 (m, 1H, \(H_2\)), 4.43 (td, \(J = 6.4, 3.7\) Hz, 1H, \(H_4\)), 4.24 (dd, \(J = 5.9, 2.2\) Hz, 1H, \(H_3\)), 4.15 (dd, \(J = 12.5, 3.7\) Hz, 1H, \(H_5^a\)), 3.98 (dd, \(J = 12.5, 6.2\) Hz, 1H, \(H_5^b\)), 2.93 (d, \(J = 2.5\) Hz, 1H, \(OH\)), 1.11 – 1.03 (m, 28H, Si\(\text{C}(\text{CH}_3)_2\) and Si\(\text{C}(\text{C}_3\text{H}_3)_2\)).

\(^{13}\text{C}\{^1\text{H}\} \text{NMR}\) (126 MHz, CDCl\(_3\)) \(\delta\) 171.8 (\(C_1\)), 82.8 (\(C_2\)), 70.0 (\(C_3\)), 68.6 (\(C_4\)), 61.8 (\(C_5\)), 17.5 (Si\(\text{C}(\text{CH}_3)_2\)), 17.4 (3C, Si\(\text{C}(\text{CH}_3)_2\)), 17.2 (Si\(\text{C}(\text{CH}_3)_2\)), 17.0 (2C, Si\(\text{C}(\text{CH}_3)_2\)), 16.9 (Si\(\text{C}(\text{CH}_3)_2\)), 13.4 (Si\(\text{C}(\text{CH}_3)_2\)), 13.3 (Si\(\text{C}(\text{CH}_3)_2\)), 13.0 (Si\(\text{C}(\text{CH}_3)_2\)), 12.7 (Si\(\text{C}(\text{CH}_3)_2\)).

IR (cm\(^{-1}\)): 2945, 2868, 2361, 1734, 1464, 1111, 1063, 1026, 885, 691.

HRMS (TOF AP\(^{+}\)): [C\(_{17}\)H\(_{34}\)O\(_6\)Si\(_2\)H\(^+\)] calc. 391.1972 found 391.1975.

(6aR,9R,9aR)-2,2,4,4-tetraisopropyl-8-oxotetrahydro-6H-furo[3,2-\text{-}\text{f}][1,3,5,2,4]-trioxadisilicon-9-yl acetate (239)

![Chemical Structure](image)

To a solution of 237 (98.7 mg, 0.25 mmol), DMAP (3.5 mg, 0.025 mmol, 0.1 equiv.) in anhydrous DCM (1 mL) was added NE\(_3\) (50 \(\mu\)L, 0.35 mmol, 1.4 equiv.) at 0°C, followed by dropwise addition of Ac\(_2\)O (28.5 \(\mu\)L, 0.3 mmol, 1.2 equiv.). The reaction mixture was stirred at 0°C for 30 minutes before warming to r.t. with stirring overnight. The reaction was quenched saturated aqueous NaHCO\(_3\) (1 mL), extracted with DCM (3 x 5 mL), dried over MgSO\(_4\) and concentrated in vacuo. Purification by column chromatography (10% EtOAc/Hexanes) yielded 239 (25.6 mg, 24% yield) as a colourless solid. 237 recovered (18.7 mg, 19%).
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.34 (d, $J = 6.4$ Hz, 1H, $H_2$), 4.51 (dd, $J = 7.2$, 6.6 Hz, 1H, $H_3$), 4.40 (ddd, $J = 7.4$, 5.6, 3.4 Hz, 1H, $H_3$), 4.13 (dd, $J = 12.7$, 3.4 Hz, 1H, $H_5$), 4.02 (dd, $J = 12.7$, 5.6 Hz, 1H, $H_5$), 2.15 (s, 3H, $CH_3$), 1.11 – 0.97 (m, 28H, SiCH(CH$_3$)$_2$ and SiCH(CH$_3$)$_2$).

$^{13}$C($^1$H) NMR: (101 MHz, CDCl$_3$) $\delta$ 170.1 ($C_1$), 169.1 (AcC=O), 83.6 ($C_2$), 69.0 ($C_4$), 68.9 ($C_2$), 61.5 ($C_5$), 20.4 ($CH_3$), 17.5 (SiCH(CH$_3$)$_2$), 17.4 (3C, SiCH(CH$_3$)$_2$), 17.0 (SiCH(CH$_3$)$_2$), 16.9 (2C, SiCH(CH$_3$)$_2$), 13.4 (SiCH(CH$_3$)$_2$), 13.2 (SiCH(CH$_3$)$_2$), 13.0 (SiCH(CH$_3$)$_2$), 12.8 (SiCH(CH$_3$)$_2$).

IR (cm$^{-1}$): 2945, 2868, 2361, 1749, 1464, 1231, 1030, 883, 692.

HRMS (TOF AP$^+$): [C$_{19}$H$_{36}$O$_7$Si$_2$+H]$^+$ calc. 433.2078 found 433.2080.
5.4 – Synthesis of reference compounds

\[ N-(1-((2R,4R,5R)-3,3\text{-difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl})-2-oxo-1,2-dihydropyrimidin-4-yl)-2-propylpentanamide \] [LY2334737] (100)²⁵

**Method A:** To a solution of gemcitabine hydrochloride (299.4 mg, 1 mmol) in DMF/DMSO (3:1 v/v, 10 mL) were added NMM (110 μL, 1 equiv.), HOBt (153.9 mg, 1 equiv.), valproic acid (175 μL, 1.1 equiv.) and EDC.HCl (250.3 mg, 1.3 equiv.) in order. The mixture was diluted with more DMF/DMSO (3:1 v/v, 10 mL), and heated to 55°C for 17 hours. The mixture was cooled to room temperature, and 10% NaCl solution (5 mL) and water (3 mL) were added while stirring. The mixture was subsequently extracted with EtOAc (3 x 10 mL), and the combined organic layers were washed with 15% LiCl solution (2 x 5 mL), saturated NaHCO₃ solution (10 mL) and brine (5 mL). The mixture was then concentrated *in vacuo*, and purified by flash column chromatography (80% EtOAc/Petroleum Ether 40-60) to afford 100 (97.5 mg, 25% yield) as an off-white solid.

**Method B:** To a cooled solution of gemcitabine hydrochloride (299.4 mg, 3.64 equiv.) in pyridine (6 mL) and acetonitrile (2 mL), was added TMSCl (580 μL, 4.55 equiv.) dropwise as to maintain T < 10°C. The solution was stirred at 5°C for 2.5 hours. In a second flask, valproic acid (146.6 mg, 1.0 mmol) and CDI (162.7 mg, 1.0 mmol) were dissolved in MeCN (3 mL) and stirred for 1 hour at room temperature, before adding to the cooled gemcitabine solution. The mixture was then heated to 60°C for 40 hours. The mixture was cooled to 40°C before quenching with EtOH (6mL) and stirred for 30 mins prior to dilution with water (5 mL) and heating at 50°C for a further 5 hours. The solution was then concentrated *in vacuo* yielding a golden oil, and then dissolved with EtOAc (10 mL) and water (10 mL). The pH was adjusted to 2 using H₃PO₄, before extracting with EtOAc (3 x 10 mL), and the combined organic layers were washed with saturated aqueous NaHCO₃ (2 x 20 mL) and brine (2 x 20 mL). The solution was dried over MgSO₄, and concentrated under reduced pressure, yielding the crude reaction mixture, which was purified by flash column chromatography (80—90% EtOAc/Petroleum Ether 40-60) to afford 100 (275.5 mg, 70% yield) as a white foam.
\[ ^1H \text{NMR} \ (500 \text{ MHz}, \text{DMSO}-d_6) \delta \ 11.05 \ (s, \ 1H, N^4H), \ 8.25 \ (d, \ J = 7.6 \text{ Hz}, \ 1H, H^6), \ 7.33 \ (d, \ J = 7.6 \text{ Hz}, \ 1H, H^5), \ 6.32 \ (d, \ J = 6.5 \text{ Hz}, \ 1H, OH^F), \ 6.17 \ (t, \ J = 7.4 \text{ Hz}, \ 1H, H^I), \ 5.29 \ (t, \ J = 5.4 \text{ Hz}, \ 1H, H^P), \ 4.24 - 4.14 \ (m, \ 1H, O\overline{H}^F), \ 3.89 \ (dt, \ J = 8.5, \ 3.0 \text{ Hz}, \ 1H, H^F), \ 3.84 - 3.77 \ (m, \ 1H, H^{3p}), \ 3.65 \ (ddd, \ J = 12.7, \ 5.7, \ 3.6 \text{ Hz}, \ 1H, H^F^a), \ 2.66 - 2.59 \ (m, \ 1H, CH^I), \ 1.57 - 1.38 \ (m, \ 2H, CH_2), \ 1.39 - 1.19 \ (m, \ 6H, CH_2), \ 0.85 \ (t, \ J = 7.1 \text{ Hz}, \ 6H, CH_3). \]

\[ ^{12}C \{^1H\} \text{NMR} \ (126 \text{ MHz}, \text{DMSO}-d_6) \delta \ 177.4 \ (VpC=O), \ 162.9 \ (C^5), \ 154.2 \ (C^I), \ 144.9 \ (C^6), \ 123.0 \ (t, \ J = 258.3 \text{ Hz}, \ C^2), \ 96.0 \ (C^3), \ 84.1 \ (t, \ J = 32.8 \text{ Hz}, \ C^I), \ 81.0 \ (C^4), \ 68.4 \ (t, \ J = 22.2 \text{ Hz}, \ C^3), \ 58.8 \ (C^5), \ 45.8 \ (CH), \ 34.5 \ (CH_2), \ 20.0 \ (CH_2), \ 13.9 \ (CH_3). \]

\[ ^{19}F \{^1H\} \text{NMR} \ (471 \text{ MHz}, \text{DMSO}-d_6) \delta -116.67 \ (s). \]

IR (cm\(^{-1}\)):
- 2961, 2930, 2870, 2367, 2322, 1699, 1653, 1614, 1558, 1485, 1393, 1312, 1260, 1196, 1062, 806.

HRMS(ES\(^{+}\)):
\[ [\text{C}_{17}\text{H}_{26}\text{N}_3\text{O}_5\text{F}_2+\text{H}]^+ \text{ calc. 390.1841 found 390.1854.} \]

Data is consistent with the literature.\textsuperscript{[25]}

**Method:** To a stirred solution of LY2334737 (96.8 mg, 0.25 mmol) and 4-DMAP (6.4 mg, 0.05 mmol, 0.2 equiv.) in anhydrous DCM (10 mL) was added NEt\(_3\) (210 \(\mu\)L, 1.5 mmol, 6 equiv.) and Ac\(_2\)O (60 \(\mu\)L, 0.625 mmol, 2.5 equiv.). The reaction was stirred overnight at r.t. and quenched with \(\text{H}_2\text{O}\). The mixture was extracted with DCM (3 x 10 mL), washed with \(\text{H}_2\text{O}\) and brine (3 x 20 mL each), dried over MgSO\(_4\) and concentrated in vacuo to yield \textbf{241} (105.5 mg, 89% yield) as a white solid.

\[ ^1H \text{NMR} \ (500 \text{ MHz}, \text{CDCl}_3) \delta 8.42 \ (s, \ 1H, N^4H), \ 7.77 \ (dd, \ J = 7.6, \ 1.5 \text{ Hz}, \ 1H, H^6), \ 7.53 \ (d, \ J = 7.6 \text{ Hz}, \ 1H, H^5), \ 6.46 \ (dd, \ J = 10.9, \ 6.1 \text{ Hz}, \ 1H, H^I), \ 5.28 \ (ddd, \ J = 13.2, \ 5.4, \ 3.7 \text{ Hz}, \ 1H, H^P), \ 4.42 \ (app. \ d, \ J = 4.1 \text{ Hz}, \ 2H, H^F \text{ and } H^{3p}), \ 4.34 \ (dd, \ J = 9.5, \ 4.0 \text{ Hz}, \ 1H, H^{5p}), \ 2.34 \ (tt, \ J = 7.9, \ 4.8 \text{ Hz}, \ 1H, CH^I), \ 2.19 \ (s, \ 3H, \text{Ac-CH}_3), \ 2.13 \ (s, \ 3H, \text{Ac-CH}_3), \ 1.68 - 1.58 \ (m, \ 2H, \text{CH}_2), \ 1.51 - 1.42 \ (m, \ 2H, \text{CH}_2), \ 1.36 - 1.28 \ (m, \ 4H, \text{CH}_2), \ 0.90 \ (overlapping \ t, \ J = 7.3, \ 6H, \text{CH}_3). \]

\[ ^{13}C \{^1H\} \text{NMR} \ (126 \text{ MHz}, \text{CDCl}_3) \delta 176.6 \ (VpC=O), \ 170.4 \ (Ac-C=C=O), \ 169.1 \ (Ac-C=C=O), \ 162.8 \ (C^5), \ 154.8 \ (C^I), \ 144.7 \ (C^6), \ 120.6 \ (dd, \ J = 267.0, \ 259.9 \text{ Hz}, \ C^5), \ 97.2 \ (C^6), \ 84.1 \ (dd, \ J = 39.5, \ 19.0 \text{ Hz}, \ C^I), \ 78.2 \ (d, \ J = 3.4 \text{ Hz}, \ C^6), \ 70.8 \ (dd, \ J = 34.1, \ 17.2 \text{ Hz}, \ C^3), \]

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62.0 (s, C\textsuperscript{5}), 48.9 (CH), 35.0 (2C, CH\textsubscript{2}), 20.8 (AcCH\textsubscript{3}), 20.7 (AcCH\textsubscript{3}) 20.5 (CH\textsubscript{2}), 14.2 (CH\textsubscript{3}), 14.1 (CH\textsubscript{3}).

\textsuperscript{19}F{\textsuperscript{1}H} NMR (376 MHz, CDCl\textsubscript{3}) δ -116.35 (d, \(J = 246.2\) Hz), -120.55 (d, \(J = 249.6\) Hz).

\textsuperscript{19}F NMR (471 MHz, CDCl\textsubscript{3}) δ -115.93 (dt, \(J = 246.1, 11.2\) Hz), -119.98 (d, \(J = 226.3\) Hz).

IR (cm\textsuperscript{-1}): CDCl\textsubscript{3} (film): 1753, 1676, 1624, 1555, 1483, 1389, 1315, 1215, 1125, 1055.

HRMS (TOF AP\textsuperscript{+}): [C\textsubscript{21}H\textsubscript{29}N\textsubscript{3}O\textsubscript{7}F\textsubscript{2}+H]\textsuperscript{+} calc. 474.2052 found 474.2057.

\textit{N-(1-((2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)acetamide [Gemcitabine N\textsuperscript{4}-acetate] (249)}

To a solution of gemcitabine (263.7, 1 mmol) in water (1 mL) was added a solution of acetic anhydride (150 uL, 1.5 mmol, 1.5 equiv.) in anhydrous dioxane (5 mL) at r.t.. The reaction was subsequently heated to 90 °C for 4 h. The mixture was then concentrated \textit{in vacuo}, and the crude mixture purified by column chromatography (10% EtOH/EtOAc) to yield 249 (291.3 mg, 95% yield) as a white/colourless solid.

\textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) δ 11.00 (s, 1H), 8.24 (d, \(J = 7.6\) Hz, 1H), 7.25 (d, \(J = 7.6\) Hz, 1H), 6.35 (d, \(J = 6.4\) Hz, 1H), 6.17 (t, \(J = 7.3\) Hz, 1H), 5.33 (s, 1H), 4.24 – 4.14 (m, 1H), 3.89 (d, \(J = 8.5\) Hz, 1H), 3.80 (d, \(J = 12.5\) Hz, 1H), 3.69 – 3.62 (m, 1H), 2.11 (s, 3H).

\textsuperscript{13}C{\textsuperscript{1}H} NMR (126 MHz, DMSO-d\textsubscript{6}) δ 171.7 (Ac-C=O), 163.3 (C\textsuperscript{5}), 154.7 (C\textsuperscript{4}), 145.2 (C\textsuperscript{6}), 125.84 – 120.95 (app. t, \(J = 259\) Hz, C\textsuperscript{2}), 96.3 (C\textsuperscript{5}), 84.6 (C\textsuperscript{3}), 81.5 (C\textsuperscript{1}), 68.8 (t, \(J = 22.7\) Hz, C\textsuperscript{3}), 59.2 (C\textsuperscript{5}), 24.9 (AcCH\textsubscript{3}).

\textsuperscript{19}F NMR (471 MHz, DMSO-d\textsubscript{6}) δ -116.96 (app. s).

* In the absence of MS, the structural identity of 249 cannot be conclusively assigned hence the structure proposed is tentative.

\textit{(2R,3R,5R)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-2-(acetoxymethyl)-4,4-difluorotetrahydrofuran-3-yl acetate [Gemcitabine N\textsuperscript{4},3\textsuperscript{3},5\textsuperscript{3}-O-triacetate] (250)}

To a solution of gemcitabine (263.0 mg, 1 mmol), 4-DMAP (3.7 mg, 0.03 equiv.) in pyridine (20 mL) was added acetic anhydride (570 uL, 6 mmol, 6 equiv.). The reaction mixture was stirred for 24 h at r.t., quenched with sat. aq. NaHCO\textsubscript{3} (25 mL). The mixture was extracted with Et\textsubscript{2}O (3 x 30 mL), and the combined organics were washed with water (2 x 20 mL). The
organics were concentrated in vacuo, and purified by column chromatography (2% MeOH/DCM) to yield 250 (74.0 mg, 19% yield) as a foaming solid.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 10.14 (s, 1H, N$^4$H), 7.77 (d, $J$ = 7.6 Hz, 1H, H$^6$), 7.53 (d, $J$ = 7.7 Hz, 1H, H$^5$), 6.47 – 6.40 (m, 1H, H$^1'$), 5.27 (dd, $J$ = 16.5, 5.2 Hz, 1H, H$^3'$), 4.41 (app. d, $J$ = 3.9 Hz, 2H, H$^e$ and H$^{e,a}$), 4.35 (dd, $J$ = 9.4, 4.0 Hz, 1H, H$^{e,b}$), 2.28 (s, 3H, AcCH$_3$), 2.18 (s, 3H, AcCH$_3$), 2.13 (s, 3H, AcCH$_3$).

$^{13}$C{[$^1$H]} NMR (126 MHz, CDCl$_3$) $\delta$ 171.0 (Ac-C=O), 170.4 (Ac-C=O), 169.1 (Ac-C=O), 163.5 (C$^5$), 154.8 (C$'$), 144.7 (C$^o$), 120.6 (dd, $J$ = 267.1, 259.7 Hz, C$^2$), 97.5 (C$^5$), 78.35 – 78.21 (app. t, $J$ = 3.5 Hz, C$^5'$), 70.8 (dd, $J$ = 34.1, 17.2 Hz, C$^3$), 62.0 (C$'$), 53.6 (C$^o$), 25.1 (AcCH$_3$), 20.8 (AcCH$_3$), 20.5 (AcCH$_3$).

$^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -116.23 (d, $J$ = 246.4 Hz).

IR (cm$^{-1}$): 1748, 1672, 1622, 1555, 1487, 1437, 1389, 1312, 1211, 1123, 1049, 912.

HRMS (ES$^+$): [C$_{15}$H$_{17}$F$_3$N$_3$O$_7$H]$^+$ calc. 390.1113 found 390.1124.
5.5 – Fluorination

Finkelstein

Method A
An oven dried flask containing 228 (106.7 mg, 0.2 mmol), KI (171.1 mg [KI] or NaI], 5 equiv.) and acetone (10 mL) was stirred for 24 hours at room temperature. The reaction mixture was filtered, washed with further acetone and concentrated in vacuo.

Method B
An oven dried flask containing 228 (107.9 mg, 0.2 mmol), KI (167.3 mg, 5 equiv.) and 2-butanolone (10 mL) was heated to reflux for 46 hours. The reaction mixture was cooled to r.t. filtered and concentrated in vacuo.

Method C
An oven dried flask containing 228 (53.4 mg, 0.1 mmol), NaI (77.8 mg, 5 equiv.) and 2-butanolone (4 mL) was heated to reflux for 24 hours. The reaction was quenched with H2O (5 mL), extracted into EtOAc (10 mL) and washed with H2O (3 x 10 mL). The organic phase was dried over MgSO4 and concentrated in vacuo.

Non-radioactive 19F fluorination

To an oven dried microwave vial containing KF (14.7 mg, 5 equiv) and K222 (18.8 mg, 1 equiv.) was added MeCN (200 μL) and azeotropically dried at 100°C under vacuum. Drying was repeated four further times. 228 (26.9 mg, 0.05 mmol) in solvent (300 μL) was introduced to dried KF/K222 mixture at r.t. and heated to 50°C for 15 hours. Product mixture was analysed by TLC and 19F NMR.

To an oven dried microwave vial containing metal fluoride (14.7 mg [KF], 32.0 mg [AgF], 5 equiv) and K222 (18.8 mg, 1 equiv.) was added MeCN (200 μL) and azeotropically dried at 100°C under vacuum. Drying was repeated four further times. 228 (26.9 mg, 0.05 mmol) in solvent (500 μL) was introduced to dried KF/K222 mixture at r.t. and heated at 120°C for 30/60 minutes. Product mixture was analysed by TLC and 19F NMR.
5.6 – Cell culture details

Panc 10.05 cell lines were donated by Dr. Catherine Hogan’s research group, and grown in RPMI Medium with 10% FBS. The cells were maintained in an incubator at 37°C containing 5% CO₂. The cells were split every 2-4 days (when appropriate) and medium changed at ratios of 1:1, 1:2, 1:3 or 1:5 with respect master cell stock. Growth medium was changed additionally when necessary, PBS used for washing vessels and 0.25% Trypsin-EDTA solution used to remove cells from culture vessel.

For the tested compounds, a master stock of each was prepared by dissolving an appropriate amount in 500 μL of DMSO (Cat. No. D2650-5X5ML; Hybri-Max™, sterile-filtered, BioReagent, suitable for hybridoma, ≥99.7%) prior to diluting with growth medium to create stock concentrations of 200 μM, 100 μM, 20 μM and 2 μM. (LY2334737 = 2.0 mg, 2′Br-LY2334737 = 2.3 mg, Gemcitabine = 1.3 mg). Stock solutions of tested compounds in media were stored at -20°C. Corresponding DMSO control samples were also prepared.

For experiments, cells were seeded in 96-well plates at a seeding density of 2x10⁵ cells mL⁻¹ and incubated for 24 hours after seeding to allow for adhesion to vessel surface. For each experiment, three wells were used per condition and each experiment repeated. Subsequently, each well was drained of its growth medium and resuspended in 75 μL of 500 nM IncuCyte® Cytotox Red Reagent (Cat. No. 4632) as imaging agent, and 75 μL of cytotoxic agent of known concentration introduced, resulting in an effective half concentration for both imaging and cytotoxic agent. Wells containing untreated cells were also screened as untreated controls. Plates were then incubated in IncuCyte® S3 Live-Cell Analysis System at 37°C containing 5% CO₂ for the duration of the experiment, monitored and imaged over the course of the experiment at 4 images per well per hour.
6 – Appendix

6.1 – HPLC traces of selected compounds

Precursor in 50/50 MeCN/H$_2$O with 0.1% HCO$_2$H at 40°C in a C$_{18}$ Agilent (150 mm) column. Retention time of 3 min 20 s.

Product in 50/50 MeCN/H$_2$O with 0.1% HCO$_2$H at 40°C in a C$_{18}$ Agilent (150 mm) column. Retention time of 1 min 10 s.
Product/precursor overlay trace in 50/50 MeCN/H₂O with 0.1% HCO₂H at 40°C in a C₁₈ Agilent (150 mm) column.
6.2 – Selected NMR spectra

(3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)dihydrofuran-2(3H)-one (195)
(3R,4R,5R)-3-bromo-3-fluoro-4-((triisopropylsilyl)oxy)-5-(((triisopropylsilyl)oxy)-methyl)dihydrofuran-2(3H)-one (202)
2-propyl-\(N\)-(2-((trimethylsilyl)oxy)pyrimidin-4-yl)pentanamide (217)
$N$-(1-((2R,3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-propylpentanamide (227)
This page contains a chemical structure and a 1H NMR spectrum for the compound 

$$((2R,3R,4R,5R)-3\text{-acetoxy}-4\text{-bromo}-4\text{-fluoro}-5\text{-}(2\text{-oxo}-4\text{-}(2\text{-propylpentanamido})\text{pyrimidin-1(2H)-yl})\text{tetrahydrofuran-2-yl})\text{methyl acetate (228)}}$$
6.3 – HRMS report

**Elemental Composition Report**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
93 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-21  H: 0-30  N: 0-3  O: 0-7  F: 0-2

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References


