Chemical synthesis and biological evaluation of 4′-thionucleosides

Mieke Guinan

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Abstract

Nucleoside analogues have proven to be highly successful chemotherapeutic agents in the treatment of a wide variety of cancers and viruses. Several such compounds, including Sofosbuvir and Gemcitabine are the go-to option in first-line treatments. However, these compoundss do have limitations (poor cellular uptake, low conversion to the active triphosphate metabolite, rapid degradation or clearance and development of resistance profiles in certain cell types) and the development of next generation compounds remains a topic of significant interest and necessity. This project broadly involves the chemical synthesis of thionucleoside analogues, a class of molecules which act as antimetabolites and are known to have antiproliferative activity against numerous cancers, viruses and bacteria. The thionucleoside targets herein are inspired by current nucleoside analogue drugs on the market, and they have potential for both greater potency and improved metabolic stability *in vivo.*

Abbreviations

A	Adenine
Ac_2O	Acetic anhydride
AcCl	Acyl chloride
AcOH	Acetic acid
AdoHcy	Adenosylhomocystein
$AlMe_3$	Trimethyl aluminium
AraA	Arabinoadenosine
AraC	Arabinocytidine
AZT	Azido thymididine
BAIB	(Diacetoxyiodo)benzene
BnBr	Benzyl bromide
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
BSA	*N,O*-bis(trimethylsilyl) acetamide
BxPC3	Biopsy xenograft of Pancreatic Carcinoma line-3
BzCl	Benzoyl chloride
BzOH	Benzoyl alcohol
C	Cytosine
CaF_2	Calcium fluoride
N-Cbz	*N*-benzyl chloroformate
CC_{50}	50% Cytotoxic concentration
CCHFV	Crimean-Congo haemorrhagic fever virus

CDC	United States Centre for Disease Control
CPE	Cytoprotection effect
DAST	Diethylaminosulfur trifluoride
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DENV	Dengue virus
DIAD	Diisopropyl azodicarboxylate
DIBAL	Diisobutylaluminum hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMDC	1′-(2′-deoxy-2′-*C*-methylene-β-D-erythro-pentofuranosyl)cytosine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTrCl	4,4′-Dimethoxytriphenylmethyl chloride
DNA	2′-deoxyribonucleic acid
dr	diastereomeric ratio
EC_{50}	50% effective concentration
EC_{90}	90% effective concentration
EDC·HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
FdC	2′-deoxy-2′-fluorocytidine
HCMV AD-169	Human Cytomegalovirus (Strain AD169)

HCV	Hepatitis C
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSV	Herpes simplex virus
IC_{50}	50% inhibitory concentration
IPA	Isopropylalcohol
L-FMAU	2′-fluoro-5-methyl-β-L-arabinofuranosyluracil
LiTMP	Lithium tetramethylpiperidide
m-CPBA	*meta*-Chloroperbenzoic acid
MeCN	Acetonitrile
MERS-CoV	Middle East respiratory syndrome
$MgCl_2$	Magnesium chloride
mM	Millimolar
mmol	Millimolar
MMTr	5-monomethoxytrityl
MsCl	Mesyl chloride
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinamide
nM	Nanomole

NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser Effect
PCTL	Peripheral T-cell lymphoma
PNP	Purine nucleoside phosphorylase
RNA	Ribonucleic acid
RRM1	Ribonucleotide reductase M1
SARS	Severe acute respiratory syndrome
L-selectride	Lithium Tri-sec-butylborohydride
SVR	Sustained virological response
T	Thymine
TBAF	Tetrabutylammonium flouride
TBAI	Tetrabutylammonium iodide
TBDPSCl	*t*-Butyl(chloro)diphenylsilane
TBSCl	*Tert*-butyl-dimethylsilyl chloride
TBSOTf	*Tert*-butyl-dimethylsilyl triflate
TCSCl	2,4,5-trichlorobenzenesulfonyl chloride
T-dCyd	4′-thio-2′-deoxycytidine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran

4′-thio-DMDC	4′-thio-1′-(2′-deoxy-2′-*C*-methylene-β-D-erythro-pentofuranosyl)cytosine
TIPDS	1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane
TMS	Trimethylsilyl
TMSCl	Trimethylsilyl chloride
TMSCN	Trimethylsilyl cyanide
TMSOTf	Trimethylsilyl triflate
TPSCl	Chlorotriphenylsilane
p-TSA	*para*-Toluenesulfonic acid
TrCl	Trityl chloride
TsCl	Tosyl chloride
U	Uracil
μM	Micromolar
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
v/*v*	Volume for volume
VZV	varicella-zoster virus
ZIKV	Zika virus

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Lastly, I would like to dedicate this thesis to my parents, Marie Mason and Patrick Guinan. Thank you for your unwavering support and patience, and for always cheering me on.

Publications

1. M. Guinan, G. J. Miller, D. Lynch and M. Smith, in *Carbohydrate Chemistry: Proven Synthetic Methods Volume 5*, ed. P Kosma, T. M. Wrodnigg, A. Stütz, 1st ed., 2021, ch. 28, pp. 227–232.2021.
2. M. Guinan, C. Benckendorff, M. Smith and G. J. Miller, *Molecules*, 2020, **25**, 2050.

Chapter 1: Introduction

1.1 Background

Nucleosides are the building blocks of life, comprising the base units of DNA and RNA and are involved in a broad range of intracellular processes including DNA and RNA synthesis. Nucleosides are comprised of a furanosyl sugar moiety - ribose in RNA or 2′-deoxyribose in DNA - which is linked at C1′ to a nucleobase identified as adenine (A), thymidine (T), cytosine (C) or guanine (G) in DNA, with thymidine replaced by uracil (U) in RNA (Figure 1), while nucleotides also feature a *mono*-, *di*- or *tri*-phosphate group at the 5′-hydroxyl position. The native configuration at the C1′ position is a 1,2-*trans* β-*N*-glycosidic bond, through N1 in pyrimidines and N9 in purines. The nucleobase is numbered first, followed by the ribose moiety using primed numbers as shown in Figure 1.

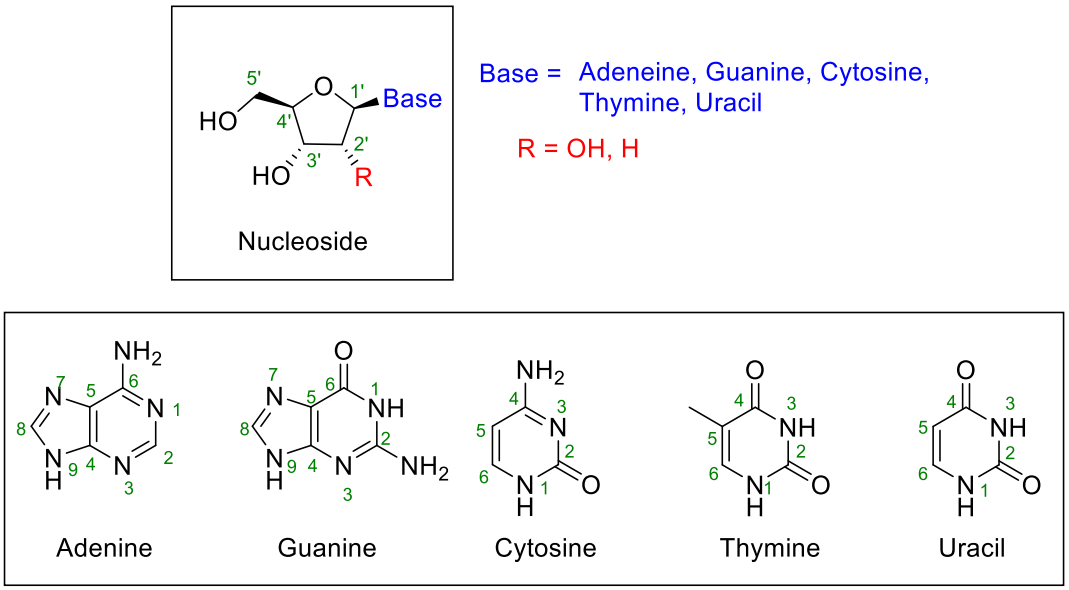


Figure 1. Structures of nucleobases adenine (A), cytosine (C), guanine (G), thymidine (T) and uracil (U); numbering system and structure of nucleosides.

The ribose ring is not planar, but rather adopts a puckered conformation - either a “half chair” twist or envelope (Figure 2).[1] Envelope conformation describes a sugar pucker where a single furanosyl carbon is displaced from the plane, whilst twist conformation describes a pucker in which two furanosyl carbons are displaced. If a furanosyl carbon is above the plane of the ring facing the nucleobase, it is denoted endo, whilst if a furanosyl carbon is below the plane of the ring, facing away from the nucleobase, it is denoted exo. The ribose moiety adopts a C2′-endo envelope conformation

in B-DNA and a C3′-endo envelope conformation in RNA. Chemical modifications to the furanosyl ring may have significant impact on the sugar pucker and, in turn, the global conformation of nucleosides and nucleotides.[2]

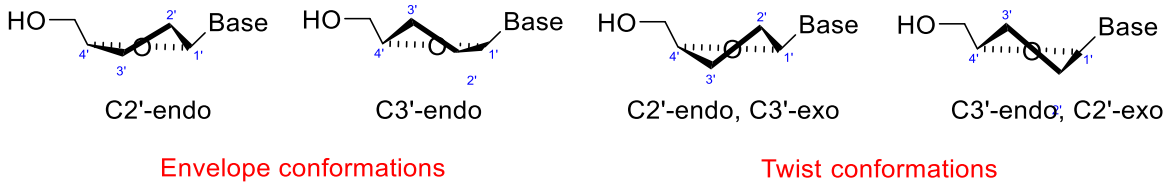


Figure 2. Conformations of ribose.

Due to their involvement in vital biological processes, naturally occurring nucleosides provide an interesting scaffold from which to develop drug candidates. Indeed, clinically relevant nucleosides have been in use since the 1950′s, with the discovery of the anticancer agents arabinosyladenosine (AraA, **1**), and 5-fluoro-2′-deoxyuridine **2** (Figure 3), sparking interest in their potential as antiviral therapeutics due to their interaction with DNA.[3] Thus, in the 1950′s and 1960′s, there was a surge of interest in the chemistry of nucleosides and nucleotides and consequently the synthetic methodologies associated with their preparation.[3] Today there are more than 30 nucleoside/nucleotide analogues on the market to treat various cancers, viruses, parasites and bacteria, with many more currently in clinical trials.[4]

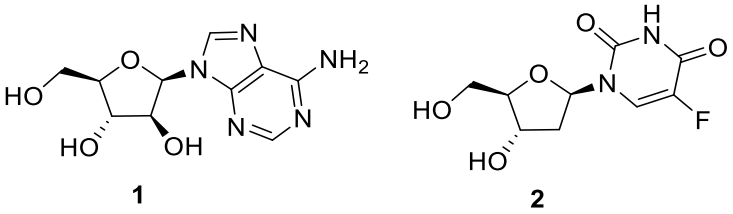


Figure 3. Structures of **1** and **2**.

Nucleoside analogues can exert their antiviral/anticancer activities in a number of ways, namely *via* incorporation into growing DNA or RNA strands ultimately resulting in chain termination, or *via* inhibition of specific viral/cellular enzymes (Figure 4).[5,6] Firstly, the nucleoside mimetics must be transported to the nucleus by specific nucleoside transporter proteins, where they are then phosphorylated by host or viral kinases to give the active mono-, di- or tri-phosphate nucleotide which exerts its chemotherapeutic activity. Degradation of nucleosides and nucleotides can occur *via*

deamination by deaminase enzymes, for example in the conversion of the cytosine nucleobase to uracil, or by reduction to their 2′-deoxy forms by ribonucleotide reductase M1 (RRM1).

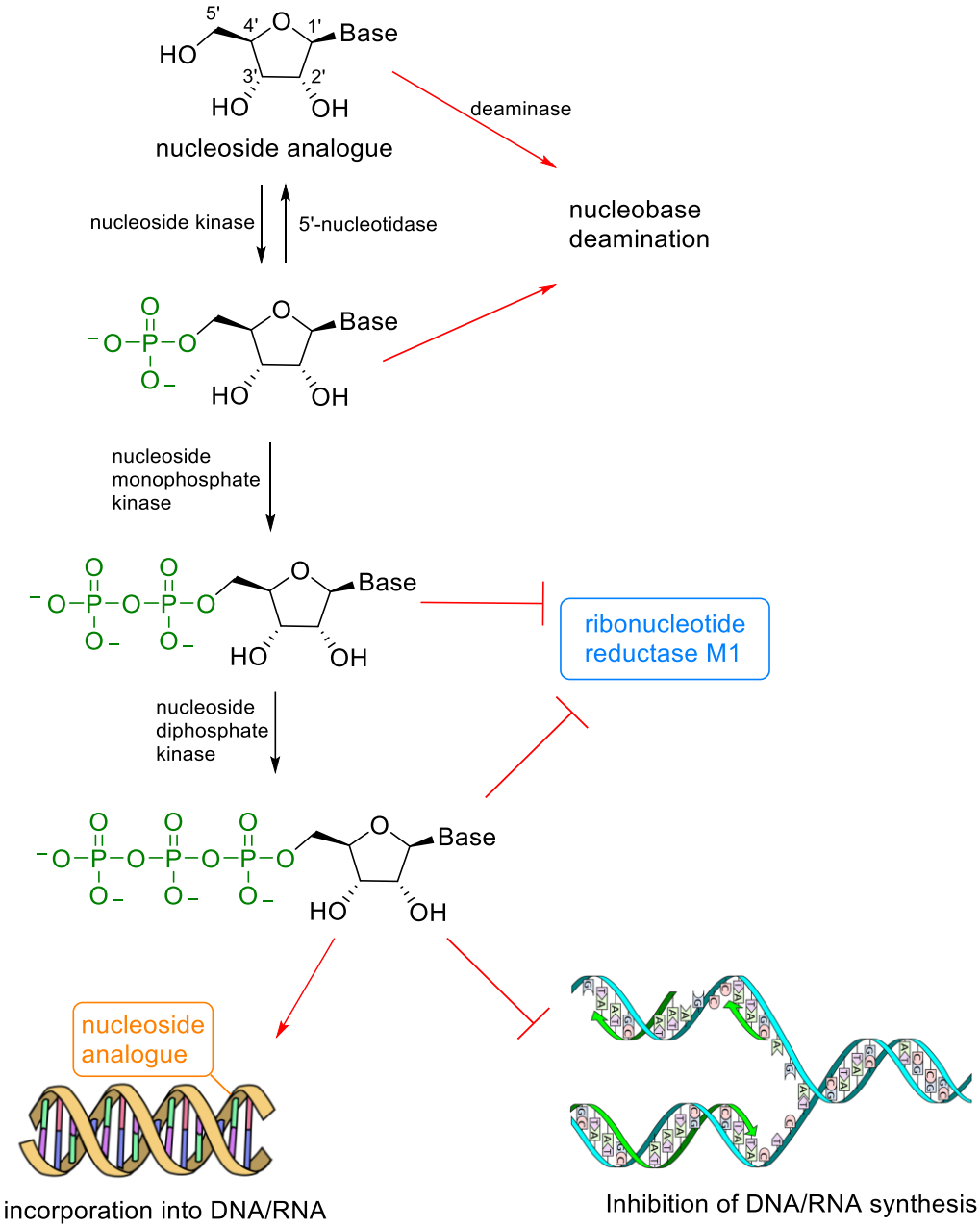


Figure 4. Mechanism of action for nucleoside analogues and their intracellular metabolism to the active triphosphate form.[5]

1.2. Historically relevant methods for nucleoside synthesis

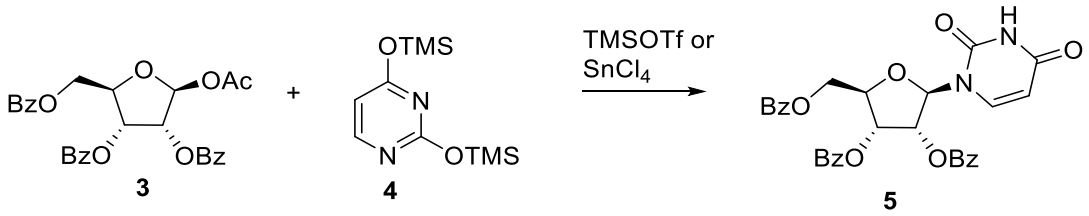
1.2.1. Formation of the *N*-glycosidic bond

A key step in nucleoside synthesis is glycosidic bond formation between a furanose and a nucleobase. This should be both stereoselective from the furanose component, to prepare the natural β-

configuration, and regioselective from the nucleobase component, such that N1 in pyrimidines and N9 in purines form part of the glycosidic linkage. This is often affected with the activation of a leaving group on the glycosyl donor, the furanosyl sugar, followed by nucleophilic substitution by a nucleobase. In this section, a selection of common *N*-glycosylation methods are discussed.

1.2.2. Vorbrüggen glycosylation

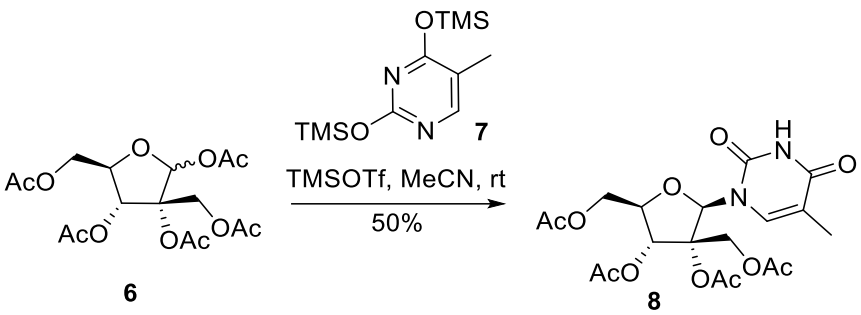
In the Vorbrüggen synthesis, a silylated nucleobase **4** reacts with the electrophilic, anomeric position in a 2,3,5-protected sugar **3** in the presence of a catalytic Lewis acid, classically $SnCl_4$ or more recently as TMSOTf (Scheme 1).[7,8]



Scheme 1. Vorbrüggen synthesis of nucleoside **5** *via* Lewis acid catalysis by TMSOTf.

The silylation of the nucleobase can be achieved with either *N*,*O*-bis(trimethylsilyl) acetamide (BSA) or hexamethyldisilazane (HMDS), although the former is preferred due to difficulties removing excess HMDS from the reaction mixture, which can cause inactivation of the Lewis acid catalyst if present.[9,10] Moreover, it has been observed that peracetylated sugars react with the nucleobase more readily than their perbenzoylated analogues.[11] The use of stoichiometric equivalents of TMSOTf over catalytic amounts of $SnCl_4$ results in higher yields of the desired nucleoside, and is also more practical to use by avoiding issues associated with toxic tin by-product residues.[8]

Monneret and colleagues employed Vorbrüggen conditions to glycosylate thymine with 2-*C*-branched ribofuranoses (Scheme 2).[12] Reaction of acetyl glycoside **6** with silylated thymine **7** in the presence of TMSOTf produced exclusively the β-nucleoside **8** in 50% yield.



Scheme 2. Synthesis of **8**.

1.2.3. Heavy metal catalysed glycosylation

Heavy metal electrophilic catalysis of glycosylation reactions was introduced by Koenigs and Knorr, and Fischer and Helferich.[13,14] Activation of the nucleobase by preparing the heavy metal salt allows displacement of a C1-halide in a 2,3,5-protected glycosyl donor. The stereochemistry of the glycosylation reaction follows the *trans* rule – the glycosylation will result in a *trans* C1′-C2′ configuration in the ribose moiety, regardless of the stereochemistry of the transient glycosyl chloride.[15] If the glycosyl chloride has a 1,2-*cis* configuration, the nucleobase will attach at C1′ *via* an S_N2/Waldon inversion, producing the desired 1′,2′-*trans* nucleosides, whilst if the glycosyl chloride has a 1,2-*trans* configuration, the nucleobase will attach at C1′ *via* a carbenium ion intermediate, thus also producing the desired 1′,2′-*trans* product (Figure 5).[3]

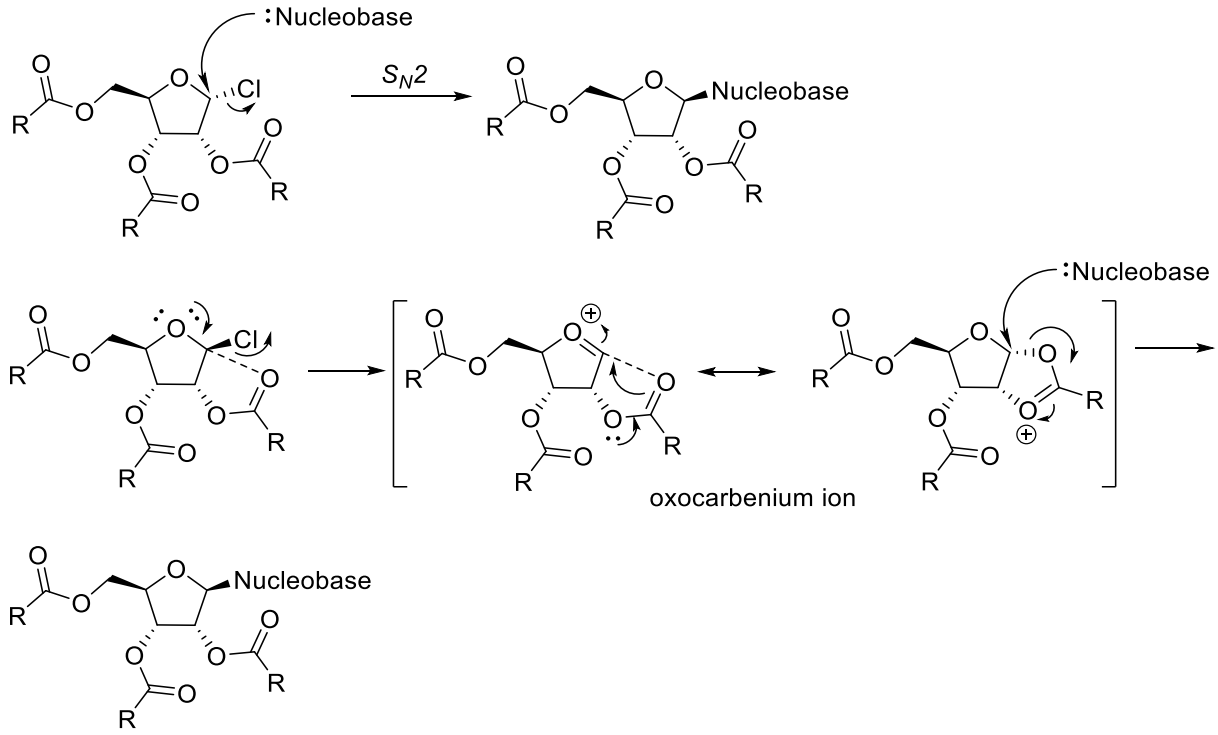
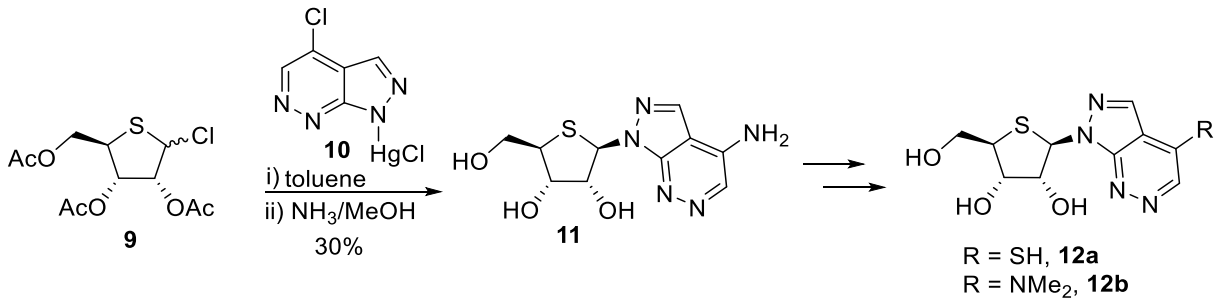


Figure 5. Illustration of the 1,2-*trans* rule.

Bobek *et al.* synthesised 4′-thionucleosides (Scheme 3) by reacting **9** with the chloromercury salt **10** in toluene followed by deacetylation by stirring the nucleoside in ammonia and methanol to give **11** in 30% overall yield.[16] The 6-substituted purine analogues **12a** – **b** were subsequently synthesised *via* nucleophilic aromatic substitution with $(H_2N)_2CS$ and $MeNH_2$ respectively.

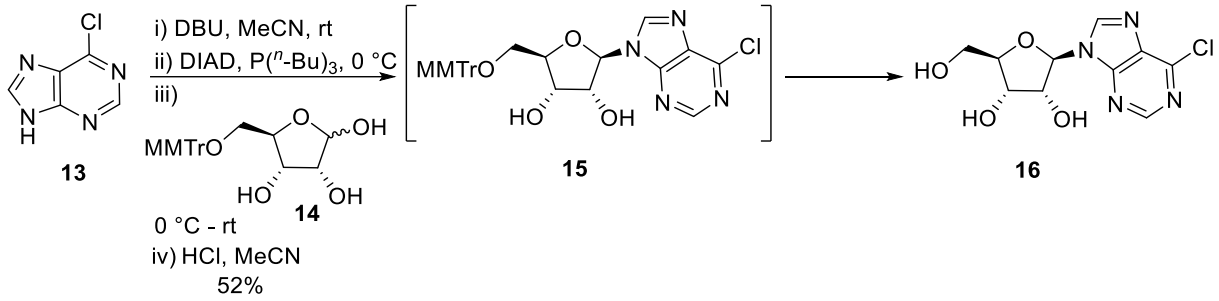


Scheme 3. Synthesis of **12a – b.**

1.2.4. Mitsunobu-type glycosylation

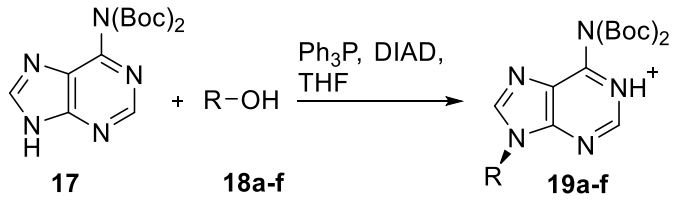
The Mitsunobu reaction allows for the direct coupling of an anomeric alcohol with a nucleobase under mild conditions *via* an S_N2 mechanism and is therefore an important alternative method of glycosylation. Downey and colleagues have examined modified Mitsunobu conditions to directly

glycosylate nucleobases with 5-monomethoxytrityl (MMTr) protected D-ribose derivatives (Scheme 4).[17,18] The 4-methoxytrityl protecting group was selected as an acid labile protecting group for the reactive primary 5-OH which served to prevent unwanted side reactions, whilst also being easily hydrolysed in the one-pot process to furnish the free nucleoside **16**. The substrate scope was expanded to include 13 different nucleobases, furnishing the deprotected β-nucleosides in 20 – 76% yield from **14**.



Scheme 4. Synthesis of **16** *via* a modified Mitsunobu type glycosylation.

The Mitsunobu reaction is a particularly useful tool in the synthesis of carbocyclic nucleosides where the ribose sugar moiety is replaced by a substituted cyclopentyl ring. [19–21] Mitsunobu-type glycosylation of adenosine has had limited application due to side reactions occurring at the C6 free amine group and the poor solubility of adenine in THF, which is the preferred solvent for Mitsunobu reactions. Yin, Li and Schneller developed a modified Mitsunobu method of preparing carbocylic adenine analogues, which employs the use of C6-Boc protected adenine **17** (Scheme 5).[22] A panel of substituted cyclopentenyl alcohols (**18a - 18f**) were reacted with **17**, furnishing the desired carbocyclic nucleosides **19a-f** in excellent yields of 85 – 96% (Table 1).



Scheme 5. Synthesis of carbocyclic adenine analogues *via* modified Mitsunobu reaction.

Entry	R-OH	Conditions	Yield

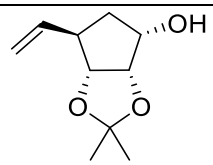
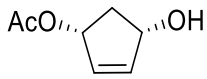
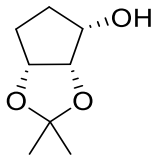
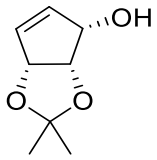
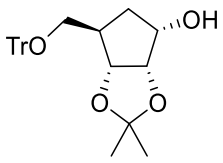
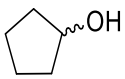
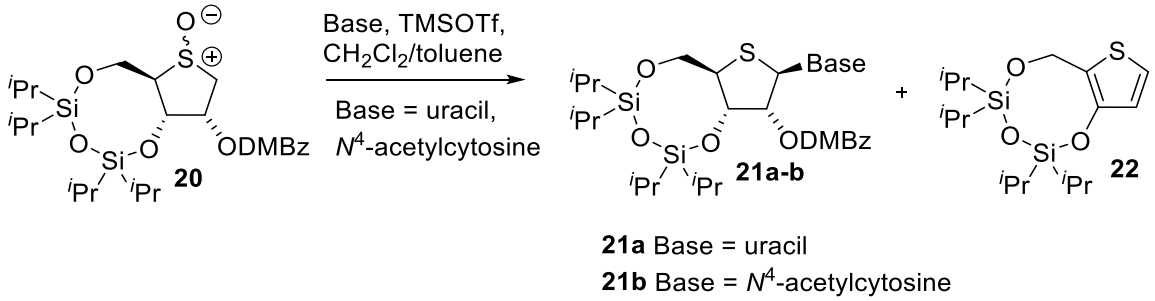
1	 **18a**	Rt, 5 h	85%
2	 **18b**	0 °C, 30 min	88%
3	 **18c**	Rt, 2 h	85%
4	 **18d**	0 °C, 30 min	93%
5	 **18e**	0 °C, 30 min	92%
6	 **18f**	0 °C, 10 min	96%

Table 1. Mitsunobu reaction of bis-Boc-adenine **4** with substituted cyclopentan(en)ols.

1.2.5. Pummerer-type glycosylation

A Pummerer-type glycosylation can be employed to prepare thionucleosides, in which the furanosyl oxygen is replaced with sulfur, from the corresponding ribosulfoxide sugar. Numerous examples of Pummerer thioglycosylations have been reported in the literature.[23–26] Naka and colleagues evaluated the Pummerer reaction of **20** with pyrimidine nucleobases, *via* treatment of **20** with TMSOTf and Et_3N in a solution of CH_2Cl_2/toluene (Scheme 6).[27] The desired β-thionucleoside product **21a** was formed stereoselectively in 57% yield. It was observed that the thiophene elimination product **22** was also formed in 8% yield due to the presence of excess Et_3N in the reaction, followed by aromatization. Thus, Et_3N was added in two portions which did not prevent formation of **22**, however led to an improved yield of 80% for **21a**, 77% for **21b**). Interestingly, Naka observed that the two diastereoisomers of the sulfoxide **20** had significant differences in reactivity. When *R*-**20** was subjected to Pummerer reaction, **21a** was obtained in 87% yield, whilst when *S*-**20** underwent Pummerer reaction, **21a** was obtained in a low yield of 27%.



Scheme 6. Synthesis of **21a – b** and **22** *via* a Pummerer-type glycosylation.

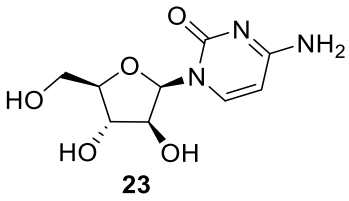
1.3. Examples of current nucleoside analogue drugs: syntheses and medicinal chemistry

Nucleoside analogues are designed such that they bear enough chemical or structural similarity to natural nucleosides to allow for their recognition by viral or cellular enzymes and their incorporation into the DNA or RNA replication cycle, however, they include modifications which lead to termination or disruption of replication. Several different approaches may be considered to modify the nucleoside scaffold, such as adding or removing substituents to the furanosyl ring or heterocyclic base, altering the atoms or the size of the ribose ring and altering the glycosidic bond. Multiple sites may be

modified in combination which results in highly diverse nucleoside structures and functions. In this section, a selection of nucleoside analogue drugs currently on the market will be discussed, including their rationale, synthesis and associated medicinal chemistry. The drugs discussed herein largely feature modifications to the ribose ring, which have in turn inspired the work in this thesis.

1.3.1. Cytarabine

Cytarabine (AraC) **23** (Figure 6) is a chemotherapeutic agent which inhibits the growth of an extraordinary number of rodent tumours, which led to extensive clinical trials and approval as a drug in 1969.[28] In particular, **23** is used for the treatment of various leukaemias including acute myeloid, acute lymphocytic and chronic myelogenous leukaemia. It is highly potent with IC_{50} values in the nanomolar range (IC_{50} =1.0 nM (CCRF-CEM) and IC_{50} = 4.0 nM (MCF7) in human cells.[29] AraC is phosphorylated to its triphosphate form Ara-CTP *in vivo*, which then inhibits DNA polymerase, likely acting as a competitive inhibitor with deoxycytidine triphosphate.[30]

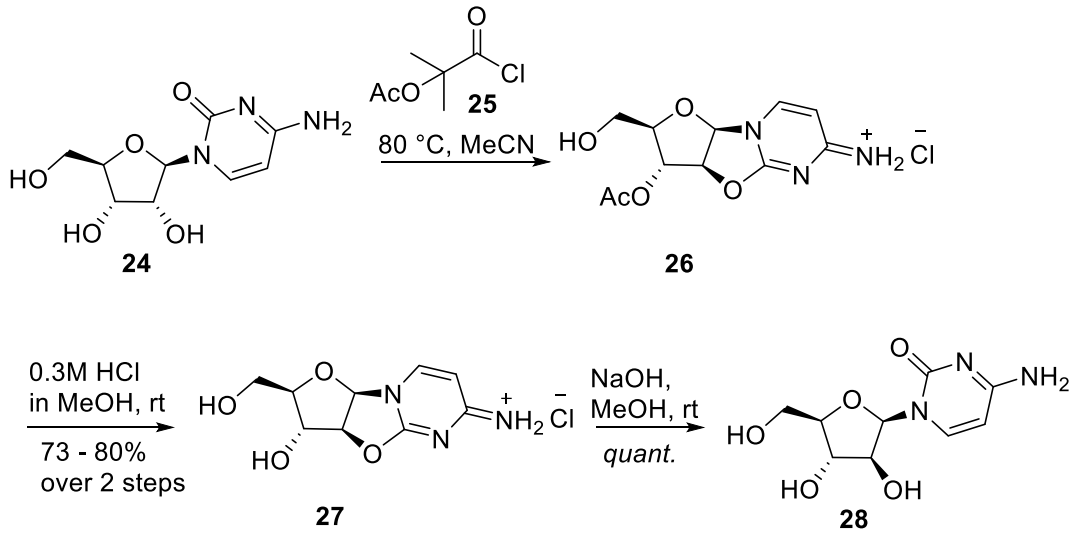


23

Figure 6. Cytarabine (AraC) **23**.

It was later found that **23** inhibits a number of DNA viruses, most notably herpes viruses as well as retroviral oncornaviruses (e.g. Rous sarcoma whose replication involves DNA synthesis *via* reverse transcriptase), and thus has intriguing potential to be examined and repurposed as an antiviral chemothereapeutic.[31]

AraC can be synthesised from cytidine **24** in three simple steps (Scheme 7). Reaction of **24** with α-acetoxyisobutyryl chloride **25** at 80 °C, prepared intermediate **26** and subsequent treatment with 0.3M methanolic HCl afforded crystalline **27** in 73-80% yield from cytidine.[32] With intermediate **27** in hand, reaction with hydroxide afforded **28** in quantitative yield.[33,34]



Scheme 7. Synthesis of **28** from **24**.

1.3.2. 2′-*C*-methylcytidine

2′-*C*-methylcytidine (**29**, Figure 7) was the first experimental inhibitor of RNA viruses to be developed and is used to treat *Flaviviridae* infections, including Hepatitis C Virus (HCV) as well as bovine viral diarrhoea virus.[35] It is converted *in vivo* to the active drug 2′-C-methylcytidine triphosphate. However, **29** has low oral bioavailability and poor absorption and is therefore delivered as Valopicitabine **30**, the 3′-*O*-valine ester prodrug which overcomes these issues by improving aqueous solubility and stability at low pH (pH 1.2).

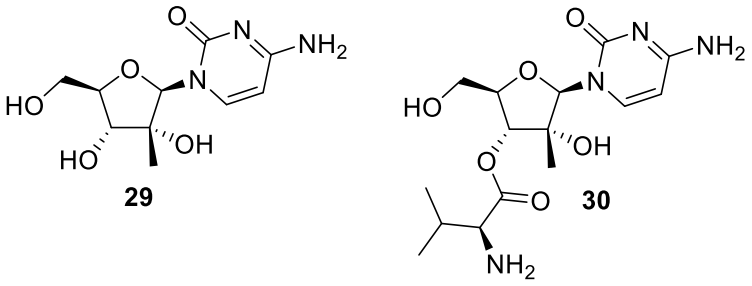
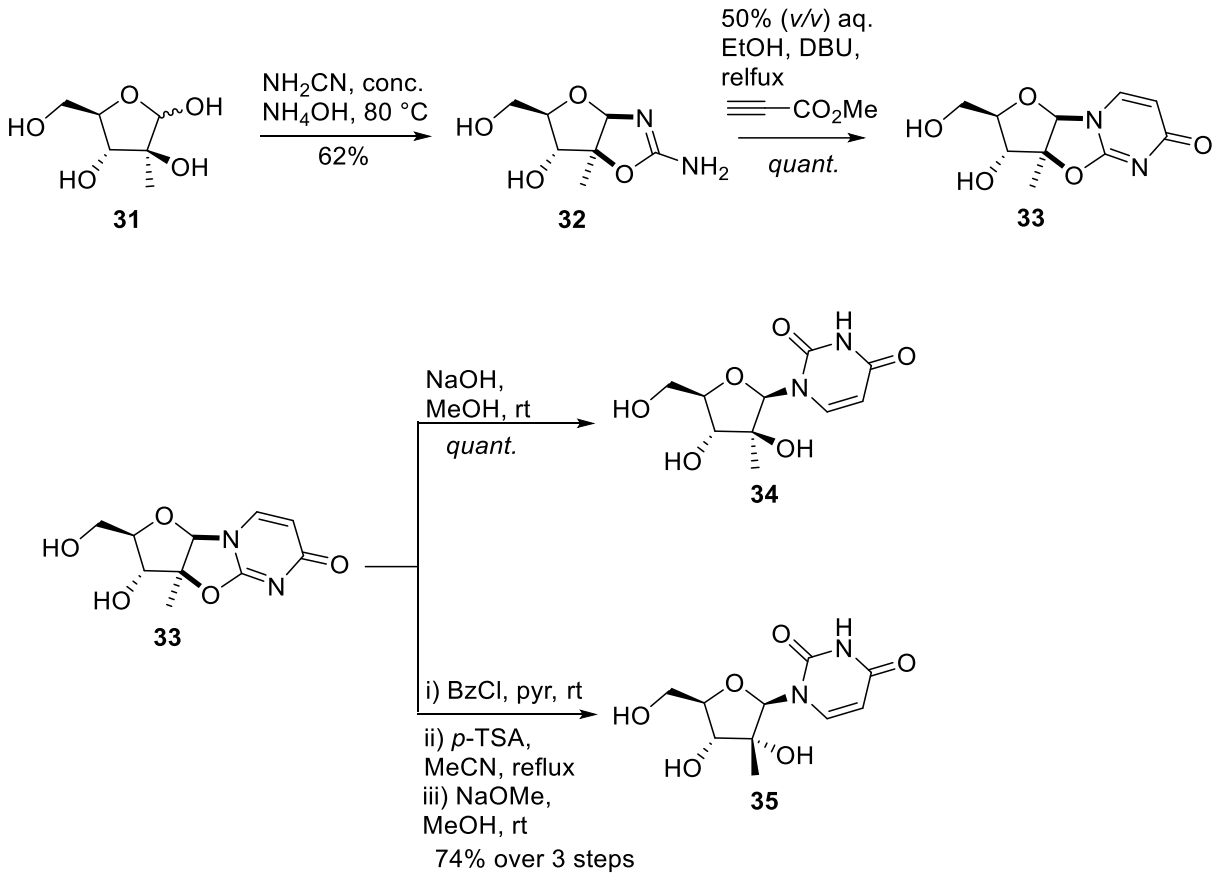


Figure 7. Structure of **29** and its prodrug **30**.

Jenkinson and colleagues reported a simple stereoselective synthesis of 2′-*C*-methyl β-pyrimidines *via* 2,2′-anhydrouridine intermediate **31** (Scheme 8).[36] Treatment of **31** (prepared in three steps from (−)-2,3-*O*-isopropylidene-D-erythronolactone) with cyanamide in conc. NH_4OH affords oxazoline **32,** which is reacted with methyl propiolate to obtain the 2,2′-anhydrouridine derivative **33**. Treatment of **33** with sodium hydroxide affords the *arabino* nucleoside **34** whereas benzoyl protection

and treatment with acid *p*-TSA affords the *ribo* 2′-*C*-methyl derivative **35** which can be converted to the corresponding cytidines *via* formation of a C4-triazole intermediate followed by ammonolysis.



Scheme 8. Synthesis of 2′-*C*-methyl nucleosides **34** and **35**.

Eyer and colleagues purchased 29 nucleoside analogues from Carbosynth Ltd. and evaluated their antiviral activity against Zika virus (ZIKV),[37] a flavivirus transmitted by mosquitos, which has become associated with severe neurological complications, including congenital malformities since the French Polynesian outbreak in 2013 and 2014.[38] Of those evaluated, five compounds **29, 35** – **38**, which bear 2′-*C*-methyl substituents showed promising anti-ZIKV activity (Figure 8). Of the five active compounds, it was observed that the identity of the nucleobase had a significant effect on antiviral activity, with **29** and **38** showing significantly reduced activity, and analogues **36** and **37** (featuring a 7-deaza moiety) were found to be particularly potent. Moreover, the five compounds were associated with little to no cytotoxicity with CC_{50} values greater than 100 μM (Table 2).

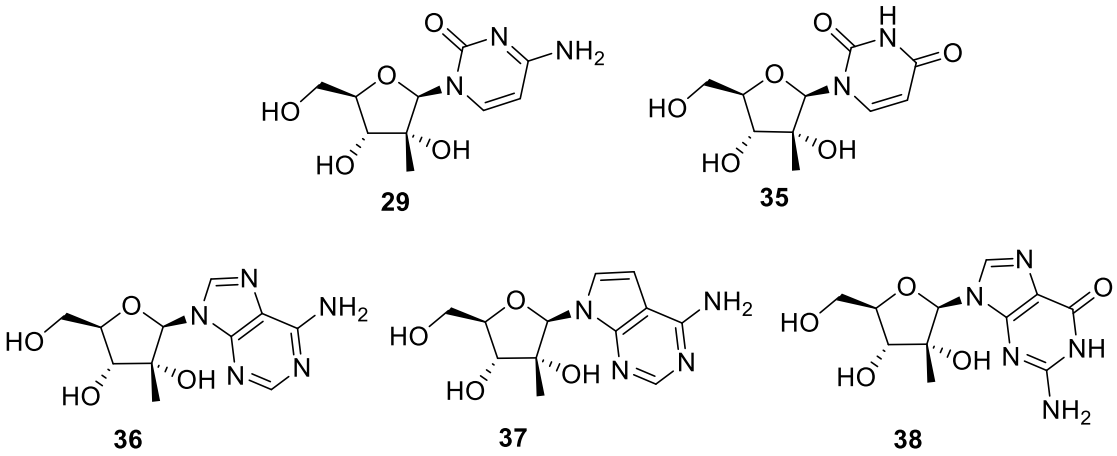


Figure 8. Structures of 2′-*C*-methyl analogues **29** and **35-38** evaluated for inhibition of Zika virus.

Entry	Compound	EC_{50} (μM)	CC_{50} (μM)
1	**29**	10.5	>100
2	**35**	45.5	>100
3	**36**	5.3	>100
4	**37**	8.9	>100
5	**38**	22.3	>100

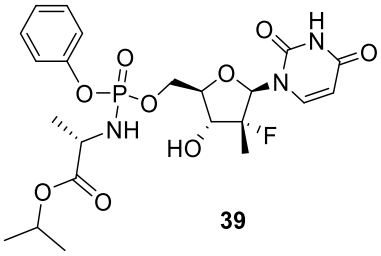
Table 2. ZIKV inhibition and cytotoxicity characteristics of selected nucleoside analogues compounds **29** and **35 – 38**.

Further, Lee and co-workers at Toku-E evaluated the antiviral activity of **29** against Dengue virus (DENV), a mosquito-borne flavivirus which currently has no vaccine nor antiviral therapy available.[39] Indeed, **29** was found to be a promising lead compound as a DENV antiviral therapy, with an IC_{50} value of 11.2 μM.

1.3.3. Sofosbuvir

Sofosbuvir **39**, is an inhibitor of HCV which was developed by Gilead Sciences (Figure 9). Sofosbuvir exerts its antiviral effect *via* inhibition of HCV NS5B viral polymerase, an essential protein for viral replication (due to its function of replicating HCV′s viral RNA) which has a highly conserved catalytic site across all HCV genotypes 1 – 4.[40,41] It undergoes hydrolytic phosphoramidate cleavage to generate the active nucleotide in the liver during first-pass metabolism.[42] During Phase II clinical trials, the use of sofosbuvir as a therapeutic for treatment-experienced patients with HCV was examined. It was found that the sustained virological response (SVR) (SVR occurs when no RNA is detected in patients'

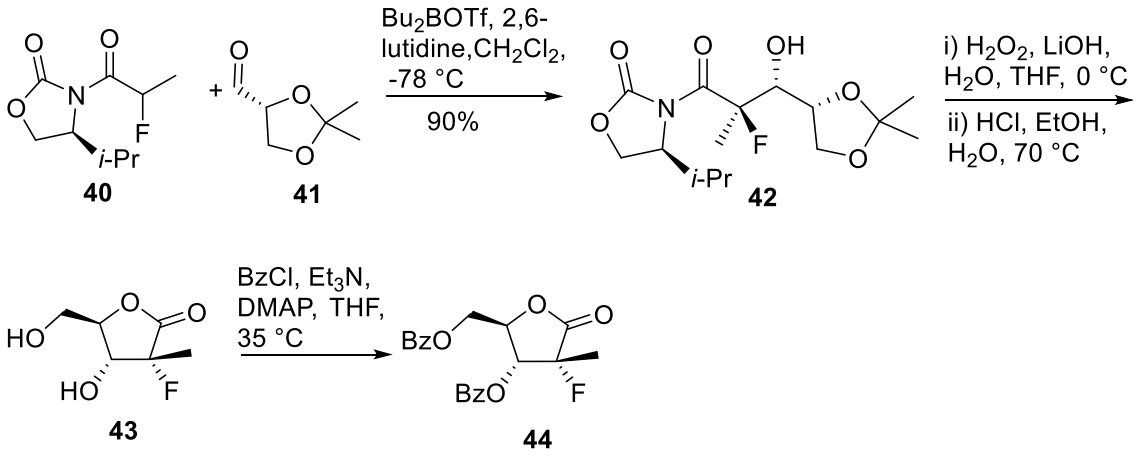
blood after 12 weeks of treatment) was 90% when **39** was used in combination with PEG-ITN-α and ribavirin, in comparison to a response rate of 60% in previous treatment regimens without sofosbuvir, indicating sofosbuvir was a superior alternative treatment.[43] In qualitative structure activity relationship studies of 2′-substituted HCV antivirals by Eldrup *et al.*, the pharmacophore was found to be quite stringent.[44] For example, increasing steric bulk above that of a methyl group at C2′ decreased the anti-HCV activity. Moreover, changing the stereo- or regiochemistry of the C2′-methyl group was not tolerated, highlighting the importance of the ribose configuration and the presence of the α-hydroxy groups at the 2′ and 3′ positions for good anti-HCV inhibitory activity.



39

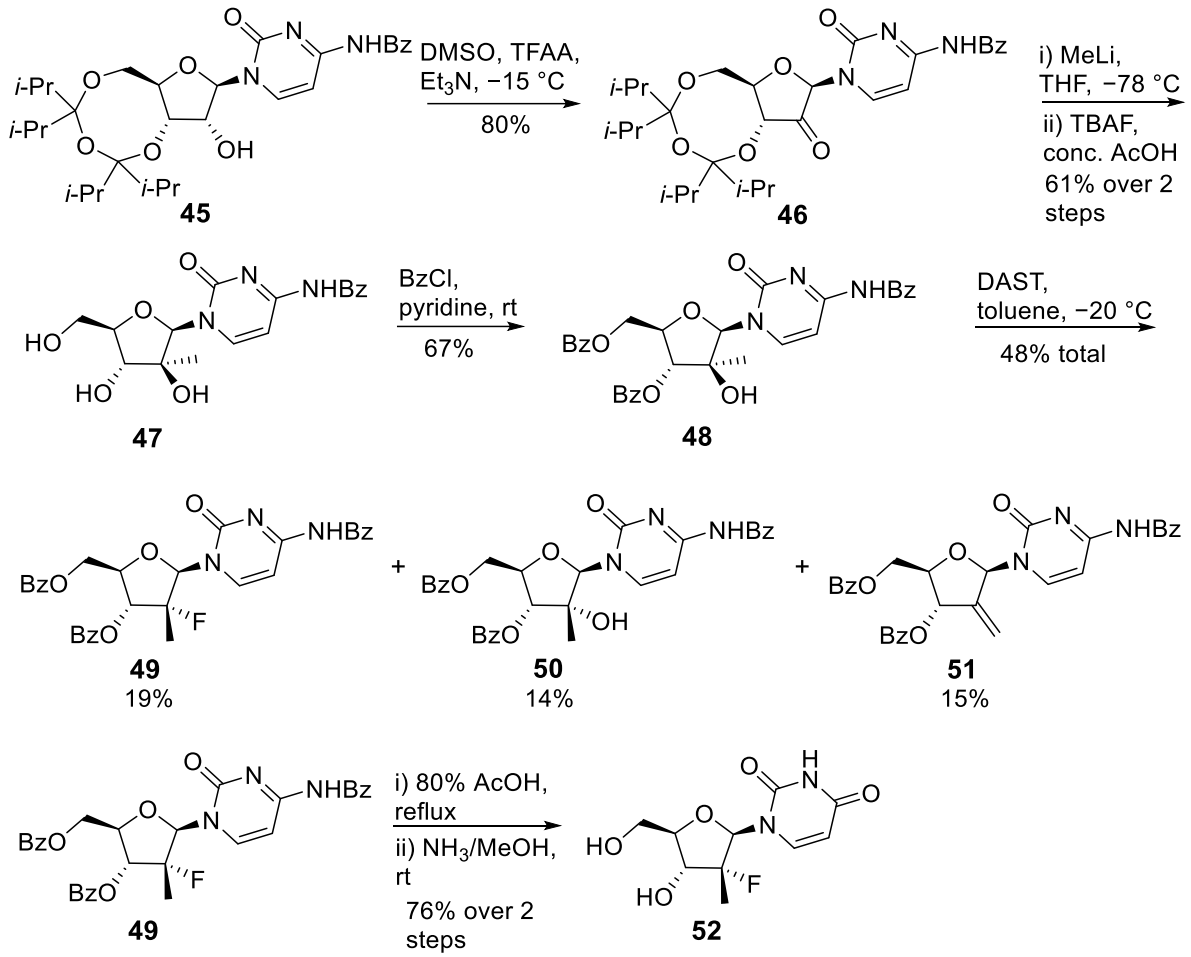
Figure 9. Structure of sofosbuvir **39**.

The synthesis of sofosbuvir has classically involved two main approaches; an early fluorination approach to access the glycosyl donor **44** or a late-stage fluorination approach onto a uridine nucleoside derivative. Hoffmann La-Roche devised an early-stage fluorination approach to synthesise **44**,[45] using a 2-fluoroester chiral auxillary **40** to enhance the diastereoselectivity of the coupling to aldehyde **41** (Scheme 9). Compound **40** was synthesised from the corresponding chiral oxazolidone and 2-fluoropropionic acid chloride. Treatment of racemic **40** with Bu_2BOTf and 2,6-lutidine afforded the corresponding boron enolate which underwent aldol-type condensation with **41** to afford **42** in 90% yield (diastereoselectivity was not provided in patent literature). Oxidative cleavage of the chiral auxillary to afford the carboxylic acid intermediate was achieved *via* reaction with wet peroxide and LiOH, and subsequently underwent acid-catalysed ring closing *via* treatment with HCl to afford the ribonolactone **43** (intermediate yields not provided in patent literature). Finally, protection of the free hydroxyl groups with benzoates *via* treatment of **43** with BzCl, Et_3N and DMAP to afford **44** in an overall yield of 51% over four steps.



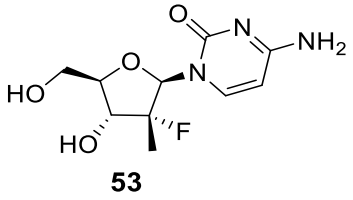
Scheme 9. Hoffmann La-Roche early stage fluorination approach for the synthesis of **44**.

Pharmasset employed a late-stage fluorination approach to synthesise protected 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine intermediate **49**,[46] a synthetic precursor to **39** (Scheme 10). Starting from **45** which was synthesised in two steps from cytidine,[47,48] the 2′-hydroxyl group was oxidised under Omura–Sharma–Swern conditions to obtain ketone **46.** Stereospecific methylation at C2′ *via* treatment with MeLi and subsequent AcOH-mediated hydrolysis of the TIPDS protecting group provided exclusively **47** in 61% yield. Reaction with BzCl afforded **48**, the key intermediate for fluorination. DAST fluorination of **48** yielded three different products: **49**, the 2′-eipermised derivative **50** and the exocyclic elimination product **51**, which were separated by column chromatography. Thus, hydrolysis of **49** to its uridine counterpart **42** was achieved *via* refluxing **49** in AcOH and subsequent deprotection *via* treatment NH_3/MeOH solution afforded the free nucleoside **42** in 5% yield over seven steps.



Scheme 10. Pharmasset late-stage fluorination approach for the synthesis of **52**.

Clark and co-workers used the methodology above to synthesise 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine **53** (Figure 10), a cytidine analogue of **52** and 2′-*C*-methylcytidine **29** which are potent inhibitors of HCV.[49] Similar to **39**, **29** is an inhibitor of HCV RNA-dependent RNA polymerase, and showed excellent inhibitory activity in a HCV replicon assay (EC_{50} = 6.50 μM) as well as no cytotoxicity (CC_{50} = > 100 μM), while **53** showed moderate inhibitory activity (EC_{50} = 19 μM) and no cytotoxicity (CC_{50} = > 100 μM).



53

Figure 10. Structure of 2′-deoxy,2′-fluoro,2′-*C*-methylcytidine **53**.

The free nucleoside **52** is not converted to the active triphosphate form *in vivo* and thus its activity and delivery relies on the inclusion of the 5′-phosphoramadate moiety. Aryoxyphosphoramidate nucleoside prodrugs are ordinarily prepared *via* early or late stage amidation (Figure 11).[50,51]

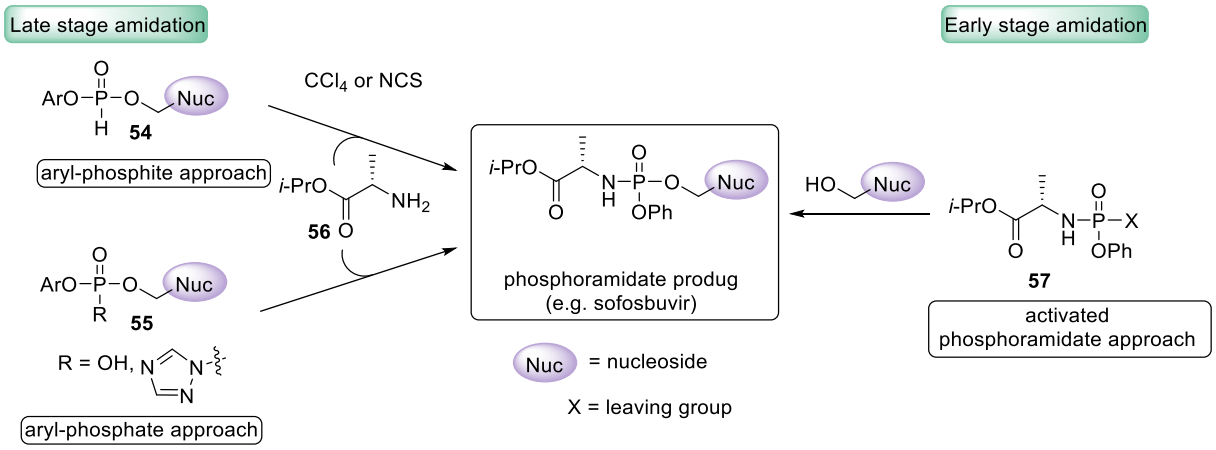
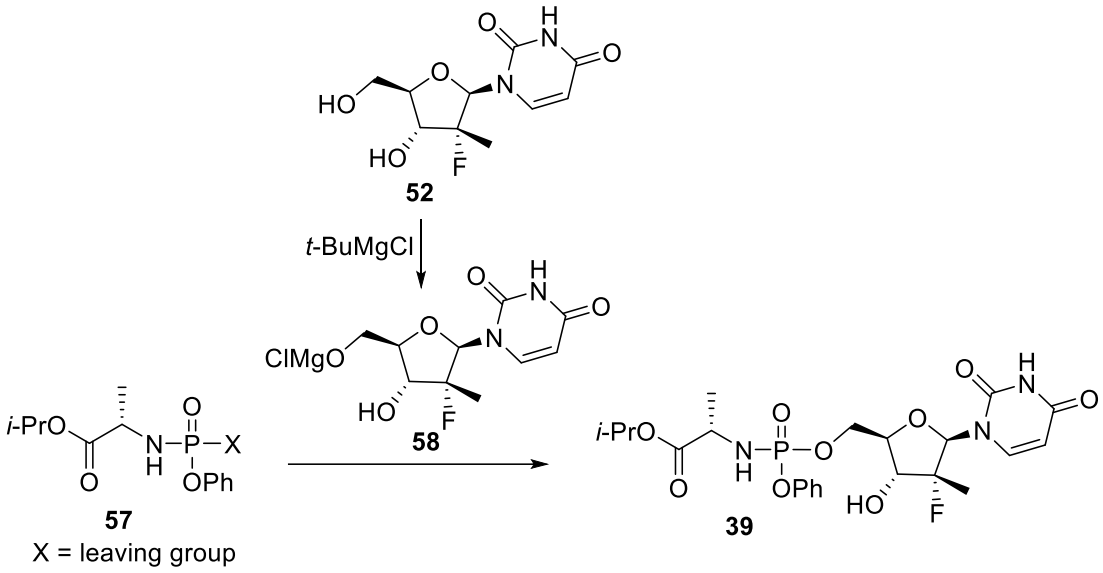


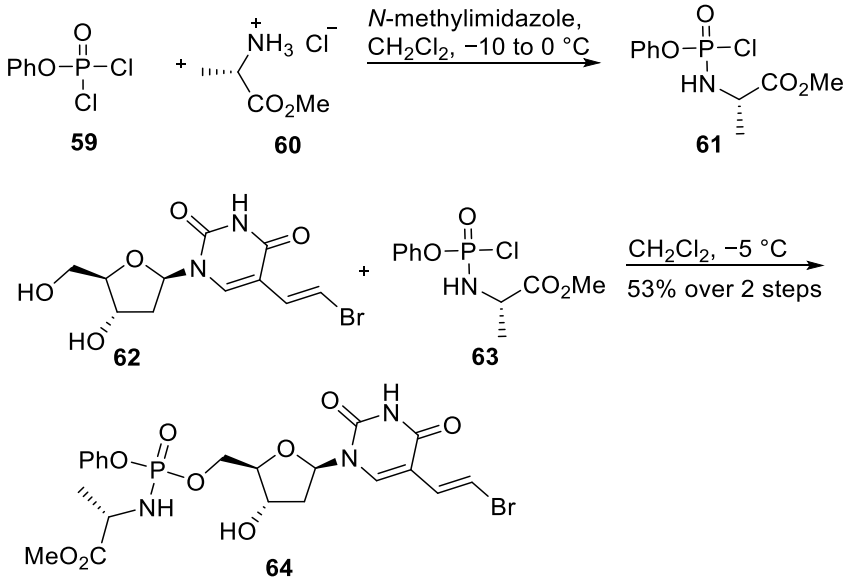
Figure 11. Methods for the preparation of phosphoramidate nucleoside prodrugs.[51,52]

The approach using aryl phosphite or phosphate adopt late-stage amino acid introduction. For the aryl phosphite approach, phosphite **54** is reacted with CCl_4 or NCS to generate the corresponding chlorophosphite electrophile *in situ* which is coupled to the amino acid alkyl ester **56** to form the P-N bond. The aryl phosphate approach is similar, using an activated phosphate **55** with a hydroxyl or triazole leaving group installed prior to coupling to the amino acid alkyl ester. Finally, the early-stage amination approach relies on the preparation of a chloride or substituted phenyl activated phosphoramidate **57** which can then be coupled with the nucleoside 5′-OH. The synthesis of **39** has historically employed an early amidation activated phosphoramidate approach using *N*-methylimidazole as a Lewis base or *t*-BuMgCl as a Brønsted base as promotors of the nucleoside 5′-OH for phosphorylation (Scheme 11).



Scheme 11. Installation of phosphoramidate in the synthesis of **39**.

Lehsten *et al.* have optimised the Lewis base approach on to multikilogram scale to prepare a number of nucleoside phosphoramidates in yields greater than 50% and more importantly in extremely high purity (>99%) (Scheme 12).[50] *N*-methylimidazole was used to activate the phosphorus chloride **59** and as a base in the protonation of the 5′-OH in **62**. Lehsten and colleagues found CH_2Cl_2 to be the best solvent and observed that reaction at temperatures between −10 °C – 0 °C were optimum as higher temperatures led to the formation of impurities, while temperatures below −10 °C did not improve the yield or purity of **64**.

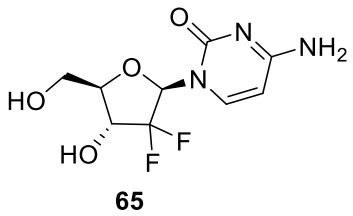


Scheme 12. Lehsten's optimised synthesis of phosphoramidate nucleoside analogues.

It is important to note that a diastereoselective approach is preferred as the absolute configuration of the phosphorus atom has a notable impact on the pharmacological activity of **39**.[53,54] The single S_p diastereomer was found to have more than 10-fold better activity (EC_{50} = 0.42 μM) in comparison to a R_p diastereoisomer (EC_{50} = 7.50 μM).

1.3.4. Gemcitabine

Gemcitabine **65** (Figure 12) is a broad-spectrum chemotherapeutic agent used to treat various cancers. It is a prodrug and is converted to the corresponding active metabolite (gemcitabine di- or tri-phosphate) *in vivo* by deoxycytidine kinase. It is generally accepted that **65** exerts its anticancer activity *via* incorporation of its triphosphate metabolite into a growing DNA strand, followed by incorporation of one more deoxynucleotide after which DNA polymerase is no longer able to proceed, ultimately resulting in cell death. Gemcitabine is used to treat breast, pancreatic, ovarian, small cell lung and bladder cancers with IC_{50} values in the nanomolar range for numerous cancer cell lines (IC_{50} = 50 nM (PANC1), 40 nM (MIAPaCa2), 18 nM (Capan2) and 12 nM (BxPC3).[55,56] In 2014, Dyall and co-workers investigated the repurposing of clinically developed drugs as potential antiviral therapies for the treatment of coronaviruses MERS-CoV and SARS-CoV.[57] In antiviral assays, gemcitabine was found to be a potent inhibitor of MERS-CoV (EC_{50} = 1.2 μM) and a had good activity against SARS-CoV (EC_{50} = 5.0 μM).

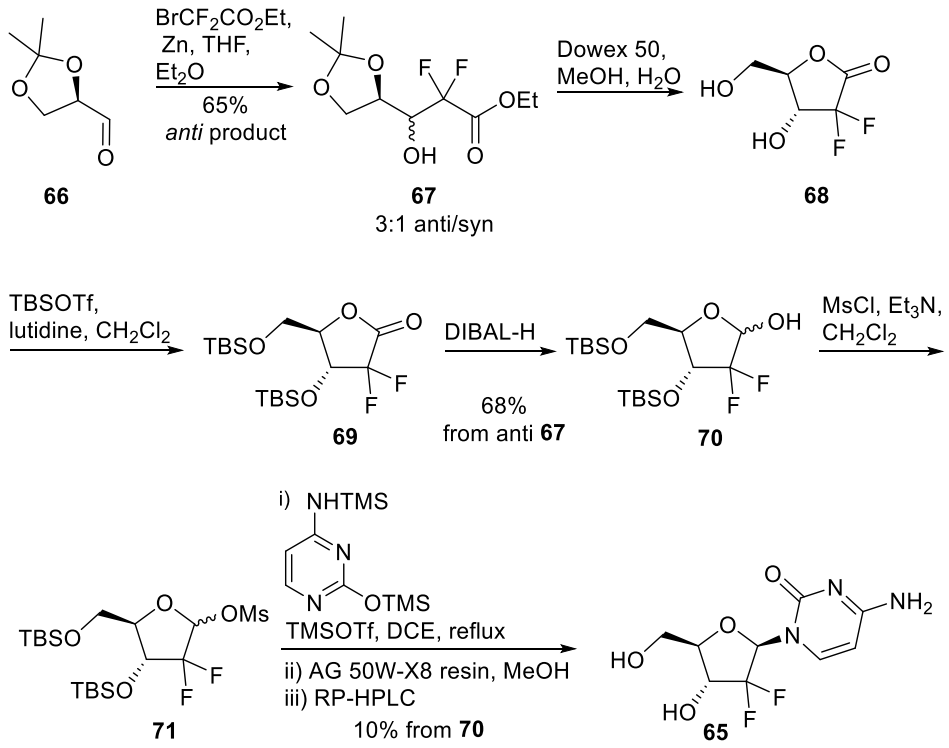


65

Figure 12. Structure of Gemcitabine **65**.

The synthesis of gemcitabine is well documented in the literature, which is covered in a comprehensive review by Linclau and co-workers.[58] The original synthesis of **65**, published by Hertel and colleagues (Scheme 13),[59] starts from enantiopure D-glyceraldehyde **66** (prepared from D-mannitol in 2-steps). Reformatsky reaction of **66** with $BrCF_2CO_2Et$ and Zn prepares **67** in a 3:1 *anti*/*syn* diastereomeric mixture. The *anti* product was obtained in 65% yield after HPLC separation of the diastereoisomers. Dowex deprotection and subsequent cyclisation of **67** furnished γ-lactone **68**.

TBS protection of the 2,3-hydroxyl groups and DIBAL-H mediated lactone reduction afforded hemiacetal **69** in 68% yield from *anti* **67**. Installation of an anomeric mesylate *via* treatment of **49** with MsCl and Et_3N afforded **50** (1:1 anomeric mixture) which was then used to glycosylate silylated cytidine *via* reaction with trimethylsilyl trifluoromethanesulfonate (TMSOTf) at reflux, followed by deprotection to obtain **65** in 50% yield. Unfortunately, the undesired α-anomer was the major diastereoisomer (α/β ratio = 4/1), and the two anomers were separated by HPLC, obtaining the β-anomer in 10% yield.

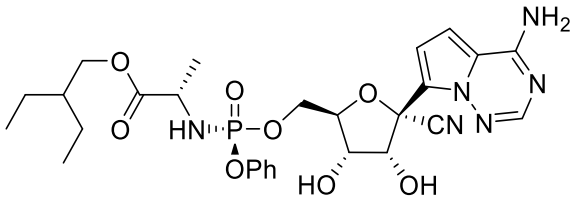


Scheme 13. Original synthesis of gemcitabine **65** by Hertel et al.

1.3.5. Remdesivir

Gilead Sciences, in collaboration with the United States Centre for Disease Control (CDC) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), evaluated roughly 1000 nucleoside analogues and their phosphate or prodrug counterparts for their therapeutic potential against RNA viruses, in a high throughput screen, primarily using a cytoprotection effect (CPE) assay.[60] From this screening, Remdesivir **72** (Figure 13), a 1′-cyano, *C*-glycosidic adenosine analogue, emerged as a promising antiviral agent, with broad spectrum antiviral activity against numerous

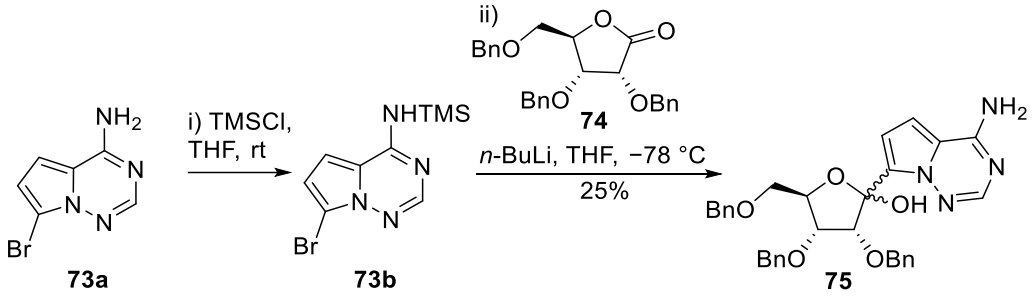
viruses including SARS, yellow fever virus, influenza A, and dengue fever virus type 2.[61] More recently, Remdesivir has garnered interest for its potential to treat COVID-19 (SARS-CoV-2), following its promising inhibition of MERS (IC_{50} = 0.76 μM) and SARS-CoV (IC_{50} = 0.07 μM).[62] In 2020, Wang and co-workers evaluated Remdesivir against the causative agent of COVID-19 (SARS-CoV-2) in *in* vitro studies and found it had excellent antiviral activity (IC_{50} = 0.77 μM) and low cytotoxicity (CC_{50} >100 mM).[63] In May 2020, Remdesivir was approved for the treatment of SARS CoV-2 in the UK, US, India and Japan.[64]



72

Figure 13. Structure of Remdesivir **72**.

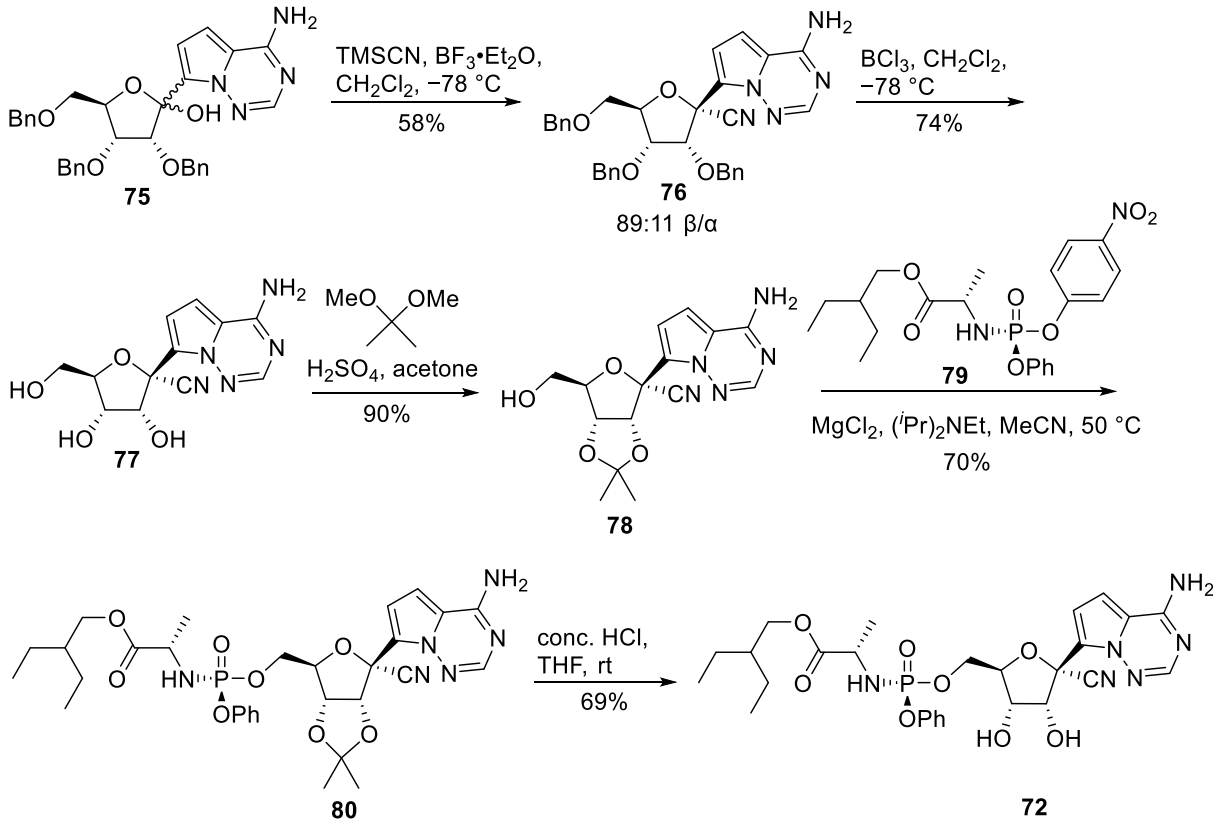
Early syntheses of Remdesivir were hindered by a low yielding *C*-glycosylation step (25%) in which modified nucleobase **73** is silylated *via* treatment with TMSCl followed by addition of **74** to prepare intermediate **75** (Scheme 14).[65,66]



Scheme 14. Key C-glycosylation step to prepare **75** in the original synthesis of Remdesivir **72**.

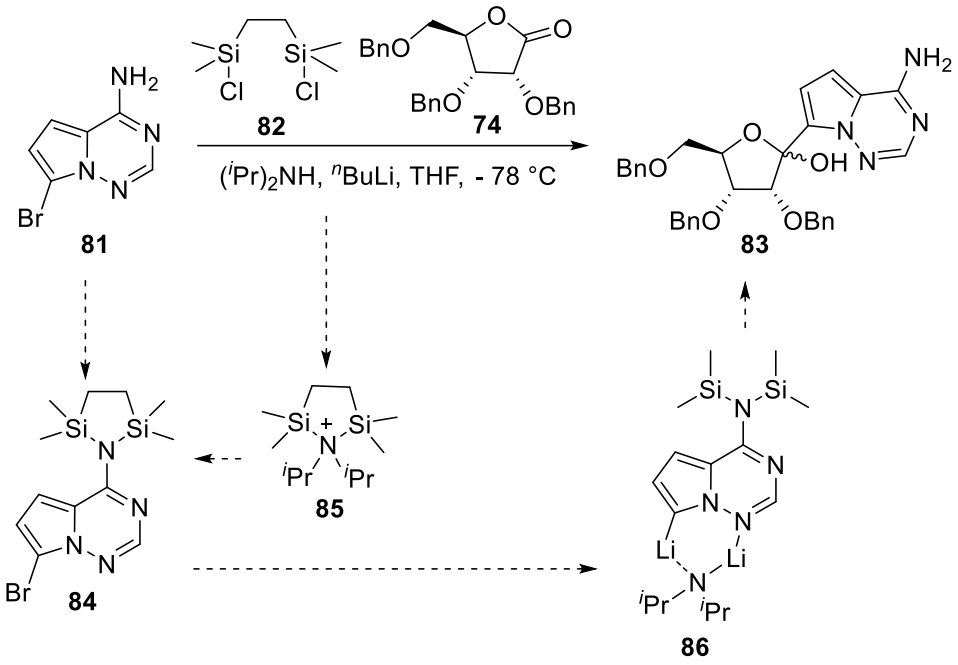
With intermediate **75** in hand, Warren and colleagues achieved the 1′-cyanation *via* treatment of **75** with TMSCN and $BF_3{\cdot}OEt_2$ (Scheme 15), obtaining **76** as an anomeric mixture, with favourable β-selectivity (89:11 β/α ratio). Reductive debenzylation with BCl_3 afforded the free nucleoside **77** which is protected at the 2′,3′-positions with an isopropylidene group and subsequently coupled to the phosphoramidoyl prodrug moiety **79** using $MgCl_2$ and $^{i}Pr_2NEt$ to afford **80** in good yield of 70% as

the desired S_p diastereoisomer, which was deprotected *via* acid hydrolysis to obtain the final product **72** in 19% overall yield from **75**.



Scheme 15. Synthesis of Remdesivir **72** by Warren and co-workers.

Increased demand for supply of Remdesivir fuelled the development of more efficient syntheses of the synthetic intermediate **75**. Xue and colleagues developed a high yielding, 100-gram scale method for preparation of intermediate **75** (Scheme 16).[67] Use of secondary amine $(^{i}Pr)_2NH$ was proposed to facilitate addition of the nucleobase **81** to lactone **74** in two ways. Firstly, activating disilane **82** *via* formation of a quaternary amine salt **85**, promoting the exchange of the silyl groups with the less active amine group on **81**. Secondly, following lithium halogen exchange, a stabilised lithium intermediate **86** could be formed which would further enhance the addition to **74**. Indeed, **75** was obtained in 74% yield which was consistent on scale up to 10 g and further scale up to 180 g furnished **75** in a slightly reduced yield of 62%.



Scheme 16. Improved synthesis of intermediate **75** developed by Xue and colleagues.

1.4. Recent developments in the synthesis and biological evaluation of novel heteroatom-substituted nucleoside analogues

There is growing interest in a class of nucleoside analogues involving the bioisosteric replacement of the furanosyl oxygen atom with heteroatoms such as sulfur, nitrogen and selenium. The differences in chemical properties such as pK_a and electronegativity and physical properties such as atomic radius affect the conformation and biological properties of the resultant nucleoside analogues.[3] While the 4′-oxo analogues have interesting biological activities, their susceptibility to degradation *via* acid-catalysed hydrolysis or phosphorylase enzymes *in vivo* calls for further chemical modifications to overcome these limitations. This section will review some noteworthy developments in the synthesis of nucleoside analogues which feature substitution of the furanosyl oxygen with other heteroatoms.

1.4.1. Azanucleosides

Azanucleosides were originally defined as nucleoside analogues where the furanosyl oxygen is replaced by nitrogen, however, this group of analogues has been extended to include nucleosides where the resultant pyrrolidine core has been replaced by other nitrogen containing rings, including heterocycles and acyclic nitrogen-containing nucleosides.[68] This class of compound have proven

successful in the treatment of cancer[69] and have also been established as having antiviral and antibacterial properties.[70] Some azanucleosides, such as Forodesine **87** (Figure 14) act as inhibitors of purine nucleoside phosphorylase (PNP) enzymes which are responsible for phosphorolytic metabolism of purine nucleosides to ribose/deoxyribose phosphate and the corresponding nucleobase. Patients with abnormally low levels of PNP possess little T-cell immunity and thus human PNP inhibitors could provide a potential treatment for T-cell lymphomas.[71]

Furthermore, some pyrrolidine-type nucleosides have displayed anti-HIV activity.[72,73] Pyrrolidine derivatives **88** and **89** are reverse transcriptase inhibitors prepared by Martínez-Montero *et al.*, with the α-derivative **89** found to have the best anti-HIV activity (EC_{50} = 36.9 μM).[72]

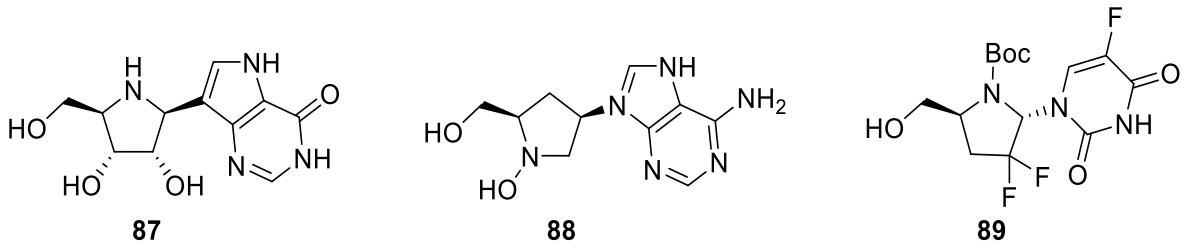
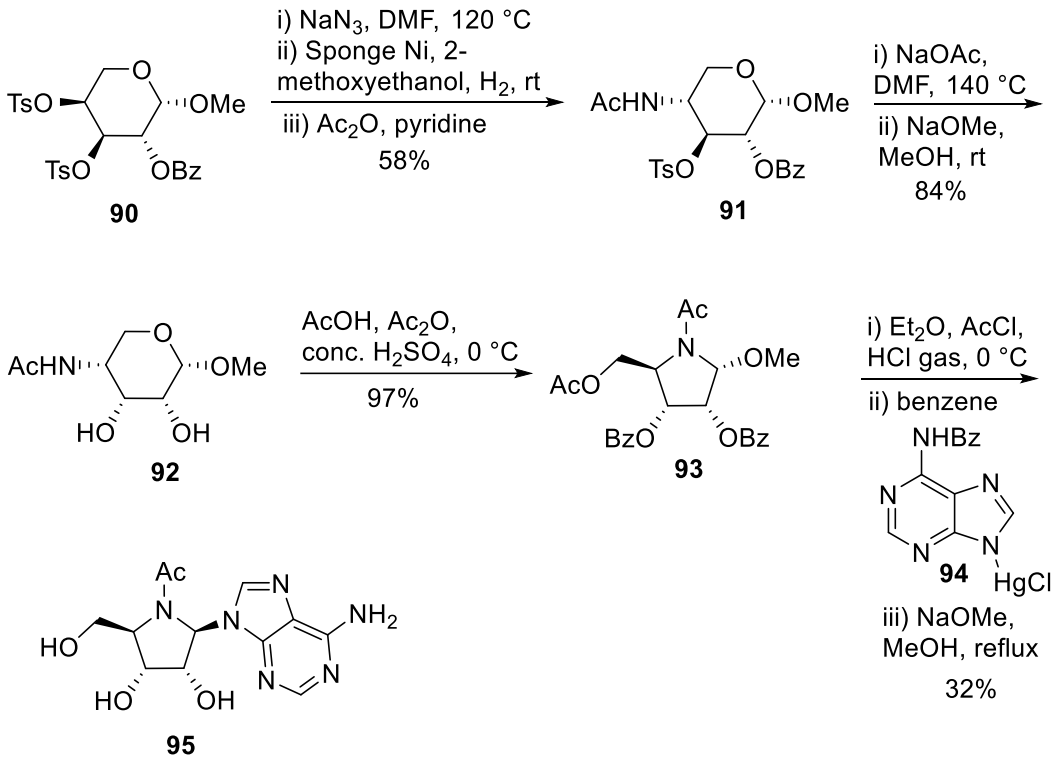


Figure 14. Structures of PNP inhibitor Forodesine **87** and the pyrrolidine mimetics **88** and **89** which act as inverse transcriptase inhibitors.

Reist *et al.* synthesised azanucleoside **95** starting from methyl 2-*O*-benzoyl-3,4-di-*O*-(*p*-tolylsulfonyl)-β-L-arabinopyranoside **90** (Scheme 17).[74] Reaction of **90** with NaN_3 afforded the corresponding azide derivative which was subsequently reduced to the free amine and acetylated, furnishing **91** in 58% yield. Elimination of the tosylate was effected *via* reflux with NaOAc in DMF to obtain the 3-α-hydroxyl intermediate which was subsequently deprotected with NaOMe to obtain **92** in 84% yield. Acetolysis of **93** furnished the azafuranoside **93** in 97% yield with the desired D-ribo configuration at C4. Subsequently, the 1-chloro furanoside was prepared *via* treatment of **93** with AcCl and HCl gas and the nucleobase installed stereospecifically *via* condensation with the mercury chloride salt of the N^6-benzoyl purine **94**, and globally deprotected to furnish the azanucleoside **95** in 32% yield from **93**.

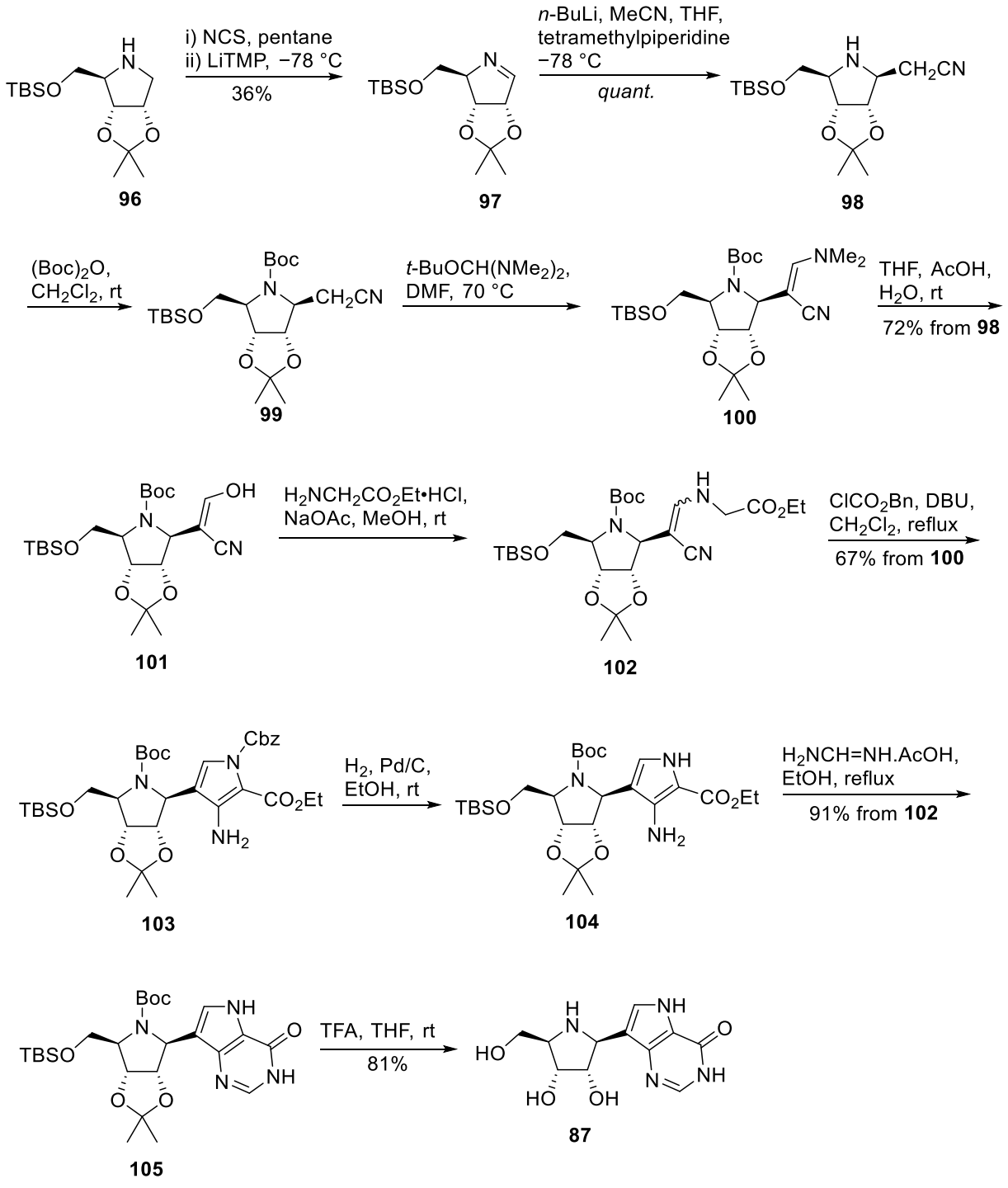


Scheme 17. Synthesis of **95**.

Forodesine **87** (Figure 14) is a guanosine analogue which acts as a highly potent PNP inhibitor (IC_{50} = 0.48 – 1.57 nM) and was recently approved for use in the treatment of relapsed/refractory peripheral T-cell lymphoma (PCTL) in Japan (April 2017).[75,76] Due to the combined substitution of the furanosyl oxygen with nitrogen and the *C*-glycosidic bond, **87** is not incorporated into DNA, acting only as a highly selective PNP inhibitor which is highly effective against T-cell malignancies with 100 – 1000 fold higher potency than previously identified inhibitors.[76,77] Also noteworthy is the adenosine mimetic of **87** which is currently under development as a broad spectrum antiviral.[78]

In 2000 Tyler *et al.* described a linear synthesis of **87** in a satisfactory 39% yield over 10 steps (Scheme 18).[79] Starting from **96**, synthesised in nine steps using established procedures from D-gulonolactone,[80] the pyrrolidine was treated with NCS, obtaining the 1-chloro glycoside which subsequently underwent elimination using lithium tetramethylpiperidide to afford imine **97**. Assembly of the nucleobase followed *via* addition of lithiated acetonitrile to afford **98**, followed by protection of the furanosyl nitrogen to furnish **99** and treatment with Bredereck′s reagent to afford enamine **100**. Acid-catalysed hydrolysis of **100** followed by reaction with ethyl glycinate furnished enamine **102**.

Treatment of **102** with excess benzyl chloroformate and DBU furnished **103** and subsequent hydrogenolysis of the *N*-Cbz group provided pyrrole **104**. Finalisation of the carbocyclic nucleobase was completed *via* treatment of **104** with formamidine acetate and acidic hydrolysis of the silicon, nitrogen and isopropylidene protecting groups to afford **87**.



Scheme 18. Synthesis of Forodesine **87**.

1.4.2. Carbocyclic nucleosides

There has been significant interest in carbocyclic nucleosides, whereby the ribose ring is replaced by a cyclopentane moiety, due to their resistance to hydrolytic metabolism by phosphorylase enzymes as well as improved biostability.[81] A broad range of antiviral carbocyclic nucleosides have been developed, including the anti-HIV reverse transcriptase inhibitors Carbovir **106** and Abacavir **107** (Figure 15). Additionally, the carbocyclic mimetic of 3-deazaadenosine **108** exhibits broad spectrum antiviral activity, *via* its inhibition of adenosylhomocysteine (AdoHcy) hydrolase, and has been reported to be 100 times more potent than other broad spectrum antivirals such as ribavirin, against measles, reovirus and parainfluenza viruses.[82]

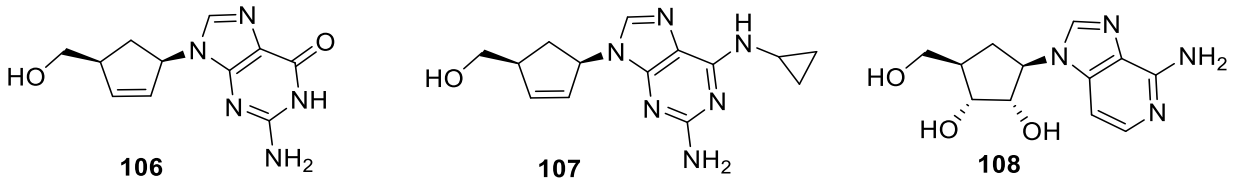


Figure 15. Structure of Carbovir **106,** Abacavir **107** and **108**.

Carbocyclic nucleoside mimetics are perhaps the most challenging class to synthesise, due to the requirement for elaborate protecting group manipulations to ensure the correct stereochemistry is achieved in the product, due to the absence of an anomeric position.

Neplanocin A **109** is a broad spectrum antiviral (Figure 16),[83,84] however it is significantly toxic to host cells and thus has little potential as a drug candidate.[85] It has been suggested that this cytotoxic effect arises from its phosphorylation by Ado kinase and concomitant metabolism by cellular enzymes.[86]

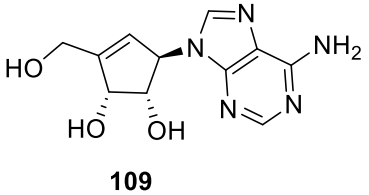
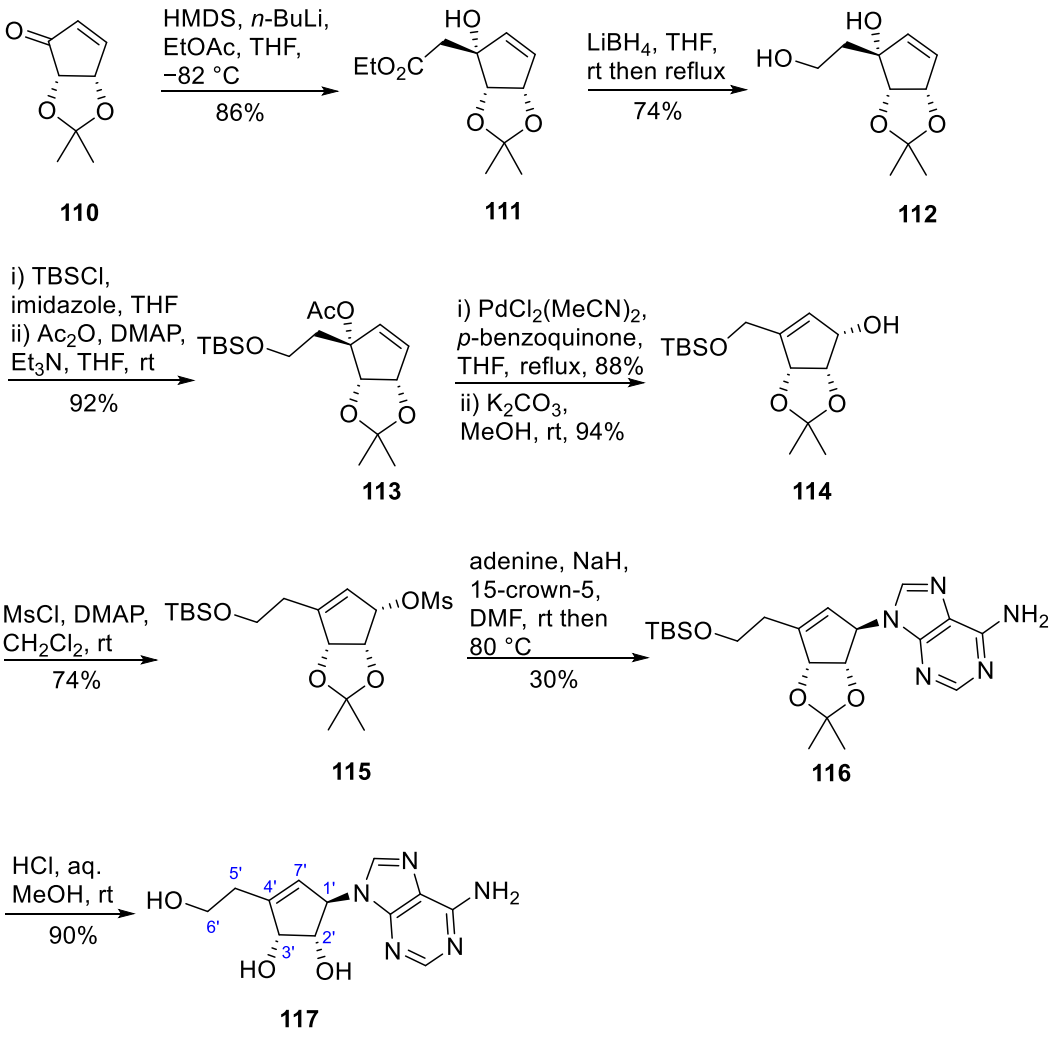


Figure 16. Structure of Neplanocin A **109**.

Based on this hypothesis, Shuto *et al* synthesised 6′-homoneplanocin A **117**, an analogue of the broad spectrum carbocyclic nucleoside Neplanocin A, which is resistant to phosphorylation by Ado kinase and deamination by adenosine deaminase but prevails as a potent antiviral through inhibition of AdoHyc hydrolase (Scheme 19).[55] It is important to note that the numbering system for carbocyclic nucleosides is slightly different, and involves numbering the longest carbon chain in the cyclopentenyl moiety and the fifth cyclopentenyl carbon last (see compound **109** in Scheme 19). The synthesis commenced with the preparation of the carbanion of EtOAc *in situ* by reaction with *n*-BuLi and HMDS which is subsequently added to commercially available lactone **110** which proceeded from the less hindered β-face stereoselectively to afford **111** in 86% yield. Reduction of **111** with $LiBH_4$ gave diol **112**, followed by acetylation and silylation of the 4- and 6- hydroxyl groups respectively to afford **113**. Allylic rearrangement of the 4-acetoxy group with catalytic $PdCl_2(MeCN)_2$ afforded **114** in 88% yield, which was subsequently treated with MsCl to prepare **115**. This then underwent S_N2 reaction with the sodium salt of adenine, followed by global deprotection to furnish the desired carbocyclic nucleoside 6′-homoneplanocin A, **117**.

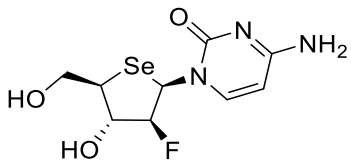


Scheme 19. Synthesis of **117**.

6′-Homoneplanocin A **117** was then screened against 18 viral strains and its IC_{50} and CC_{50} measured in the various virus infected cells. Neplanocin **109** was found to be especially active against arenaviruses (IC_{50} = 5.0 μg/mL for Junin virus, IC_{50} = 20 μg/mL for Tacaribe virus), human cytomegalovirus (IC_{50} = 0.15 μg/mL for HCMV AD-169; IC_{50} = 0.5 μg/mL for HCMV Davis), vaccinia virus (IC_{50} = 0.10 μg/mL) and vesicular stomatitis virus (IC_{50} = 0.70 μg/mL). Moreover, **117** proved to be significantly less cytotoxic than **109**, with CC_{50} concentrations > 400 μg/mL in E_6SM, HeLa and Vero cells, and CC_{50} > 50 μg/mL in HEL cells in comparison to concentrations of >10 μg/mL for **109**. Thus, **117** has excellent potential as an antiviral drug candidate.

1.4.3. Selenonucleosides

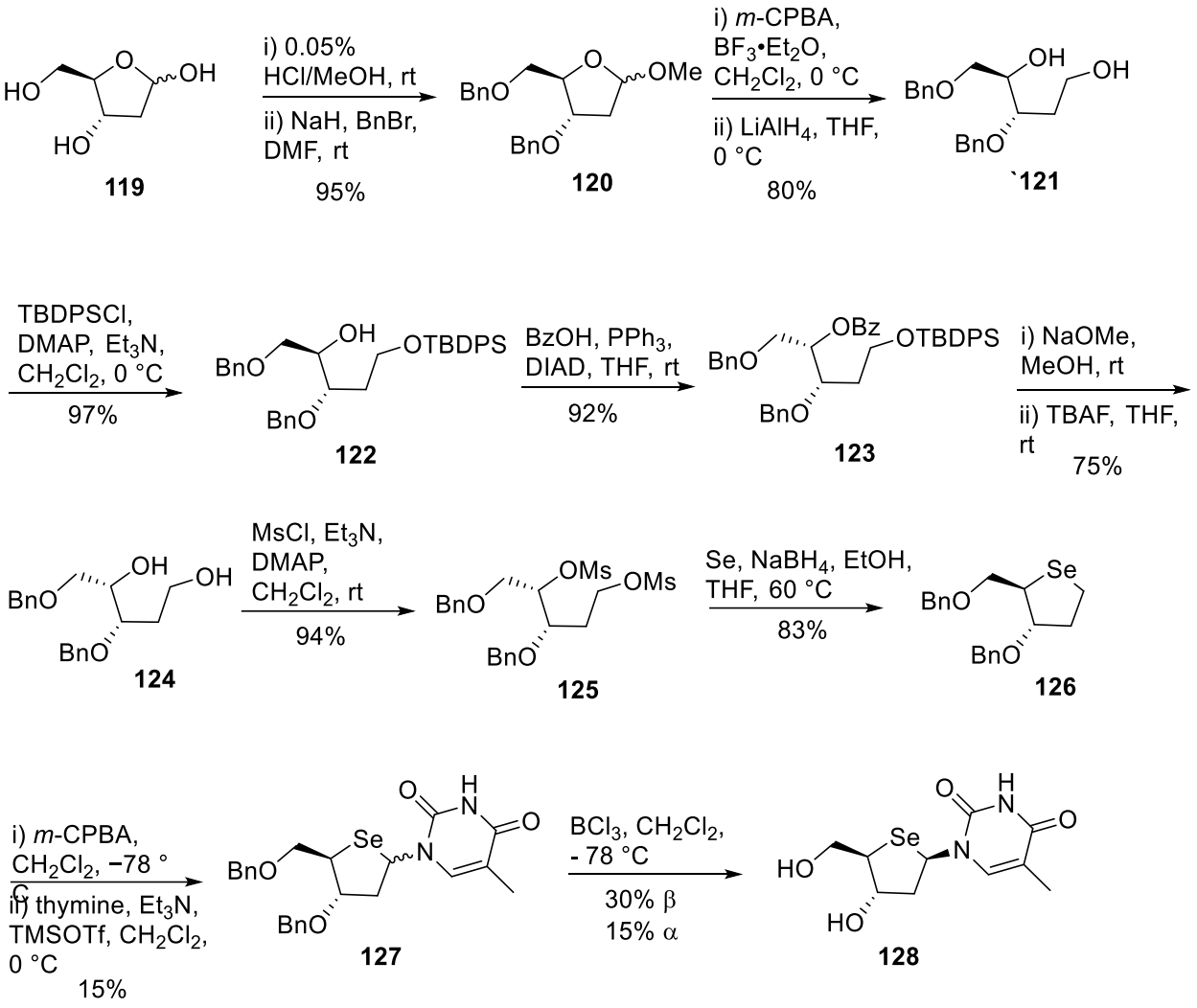
Following a similar rationale for bioisosteric placement of the 4′-oxo moiety, selenium nucleoside derivatives may also exhibit some antiviral and antineoplastic activity. The larger size of the selenium atom dictates the conformation of the sugar ring, indeed even resulting in retention of stereochemistry in DAST-mediated fluorination reactions.[87] Watts *et al.* synthesised and characterised selenonucleoside analogues of adenine, thymidine, cytidine, guanine and uridine.[88] Jeong *et al.* reported the first synthesis of 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosyl pyrimidines including **118** (Figure 17) as highly potent chemotherapeutic agents.[89]



118

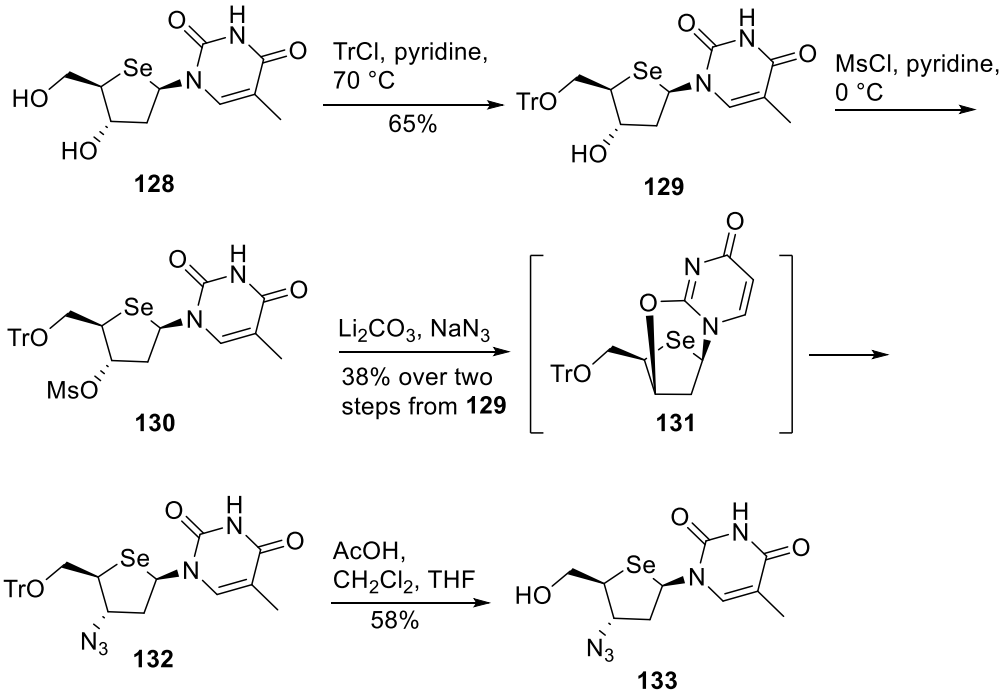
Figure 17. Structure of 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosyl cytidine **118**.

Alexander *et al.* prepared 4′-selenothymidine **128** starting from 2-deoxy ribose derivative **119** (Scheme 20).[90] The methoxy furanoside was prepared from **119** and subsequently protected with benzyl groups at the 3-*O*- and 5-*O*-positions to give **120**. Ring opening was achieved *via* treatment of **120** with *m*-CPBA and $BF_3{\cdot}Et_2O$, followed by $LiAlH_4$ to give **121**. A series of protecting group manipulations followed to prepare dimesylate derivative **125**, which was treated with NaSeH generated *in situ via* reaction of elemental selenium and $NaBH_4$ in EtOH at reflux to afford **126**. This was activated *via m*-CBPA mediated oxidation of the seleno-ether moiety and thymine installed *via* a Pummerer-type glycosylation with the selenoxide intermediate to afford **127** in a low yield of 15% over the two steps. The nucleoside was subsequently debenzylated *via* treatment with BCl_3 at –78 °C to obtain the final product **128** in 30% yield. X-ray crystallography revealed that **128** adopted the same C2′-endo/C3′-exo (South) conformation as thymine, which was interesting as it was expected that for selenonucleosides, all stereoelectronic effects driving towards the natural configurations are overwhelmed by the size of the selenium and thus sterics are driven towards non-natural conformations.[91] Indeed, the corresponding selenouridine derivative adopts a non-native C2′-endo/C3′-exo (South) conformation in comparison to uridine (C3′-endo/C2′-exo North).



Scheme 20. Synthesis of selenothymidine **128**.

With nucleoside **128** in hand, Alexander and colleagues mapped the 4′-seleno modification onto existing anti-HIV drug AZT *via* preparation of the 3′-deoxy,3′-azido derivative **133** (Scheme 21). Tritylation of the primary alcohol and preparation of the corresponding 3′-mesylate afforded **130** which was treated with Li_2CO_3 in the presence of NaN_3 to afford azide **132.** Deprotection afforded the desired nucleoside **133** in overall yield of 14% yield from **128**. Initial anti-HIV activity of **128** and **133** was measured in MT-4 cells, however, neither compound showed any anti-HIV activity.



Scheme 21. Synthesis of seleno-AZT analogue **133** from **128**.

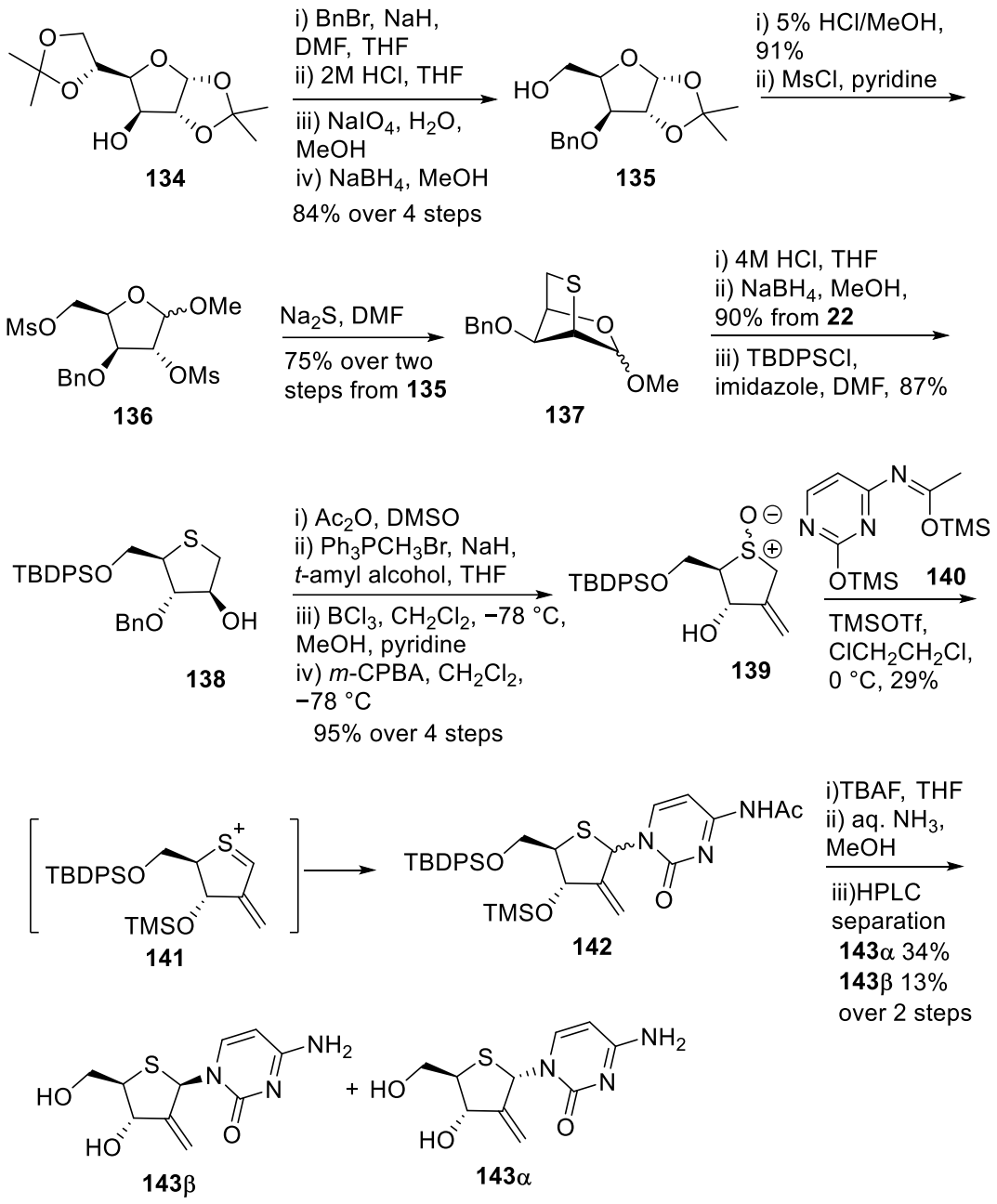
1.4.4. Thionucleosides

4′-Thiosugars are known to be of importance in biological systems, for example as chemical biology or biomedical tools,[92] and possess chemotherapeutic activity.[93,94] Furthermore, the thioaminal moiety in 4′-thionucleosides has been proven to be resistant to metabolic hydrolysis by nucleoside phosphorylase enzymes in comparison to native 4′-oxo analogues.[95] In the early 1990′s, Secrist *et al.* stimulated interest in this class of molecule with their synthesis and biological evaluation of 2′-deoxy-4′-thio pyrimidine nucleosides.[96] Since then, there has been a resurgence of research into the synthesis and antiviral activity of these molecules. However, despite numerous reported syntheses of thionucleosides and their antiviral activities, there are currently no 4′-thionucleoside analogues on the market to treat cancer or viral diseases.

1.4.4.1 Synthesis of 2′-Modified Thionucleosides

Yoshimura *et al.*, reported the synthesis and biological evaluation of 4′-thio-1′-(2′-deoxy-2′-*C*-methylene-β-D-erythro-pentofuranosyl)cytosine (4′-thio-DMDC) **28β** and 2′-deoxy-2′-fluoro-*arabino*-4′-thiocytidine **33β** as potential antitumour agents.[25,97,98] Their synthesis of **143** was complete in 18 steps from 1,2,5,6-diisopropylidene-D-glucose **20** (Scheme 22). Following a series of protecting

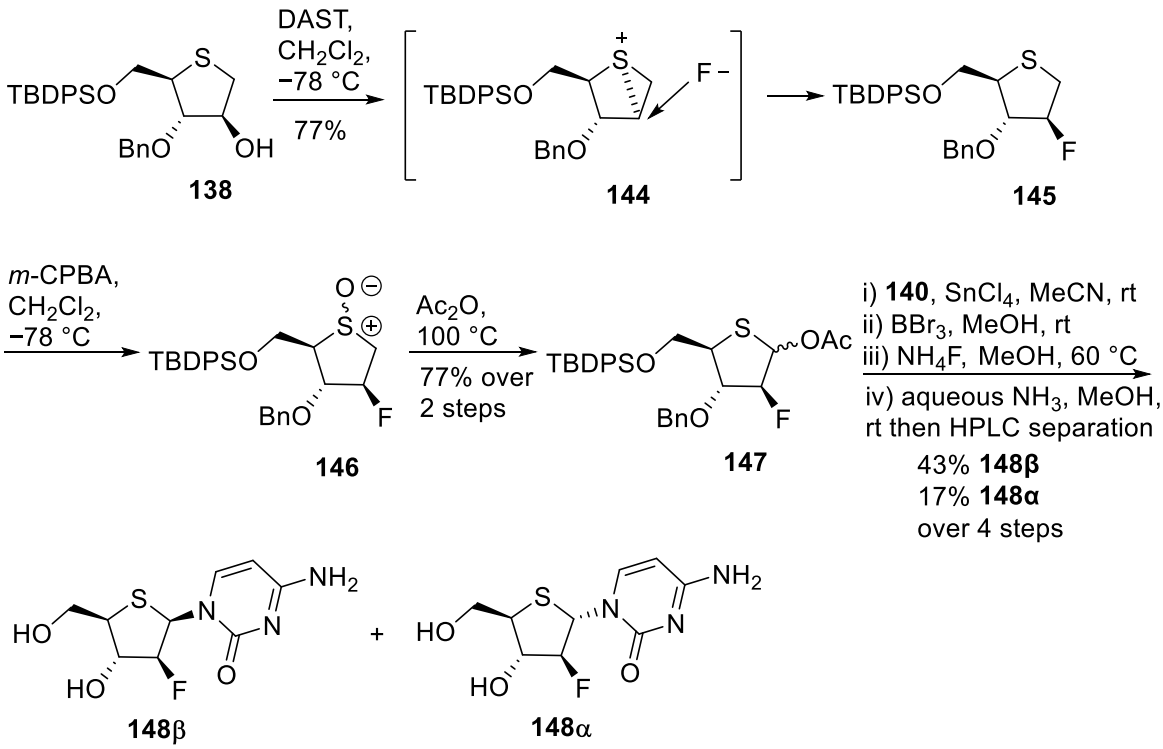
group manipulations, D-xylose derivative **136** was furnished in 76% yield from **134**. Rather than using a double inversion strategy at the C4 stereocentre, Yoshimura and colleagues mesylated the 2- and 5-hydroxyl groups, which in turn facilitated reaction with Na_2S to obtain the 2,5-bicylic intermediate **137** with the correct C4 configuration. Conversion to the thio*arabino*furanoside was followed by oxidation of the 2-hydroxyl group, and the resultant ketone subjected to Wittig reaction with Ph_3PCH_3Br to install the 2-methylene unit, followed by concomitant *m*-CPBA mediated oxidation of the thioether moiety to afford sulfoxide **139**. The cytosine nucleobase **140** was installed using a Pummerer-type thioglycosylation, *via* the sulfenium intermediate **141**, which furnished **142** as a mixture of anomers. These were deprotected to afford **143*α*** and **143β** and the desired **143β** isolated in 13% yield using HPLC separation.



Scheme 22. Synthesis of **143β**.

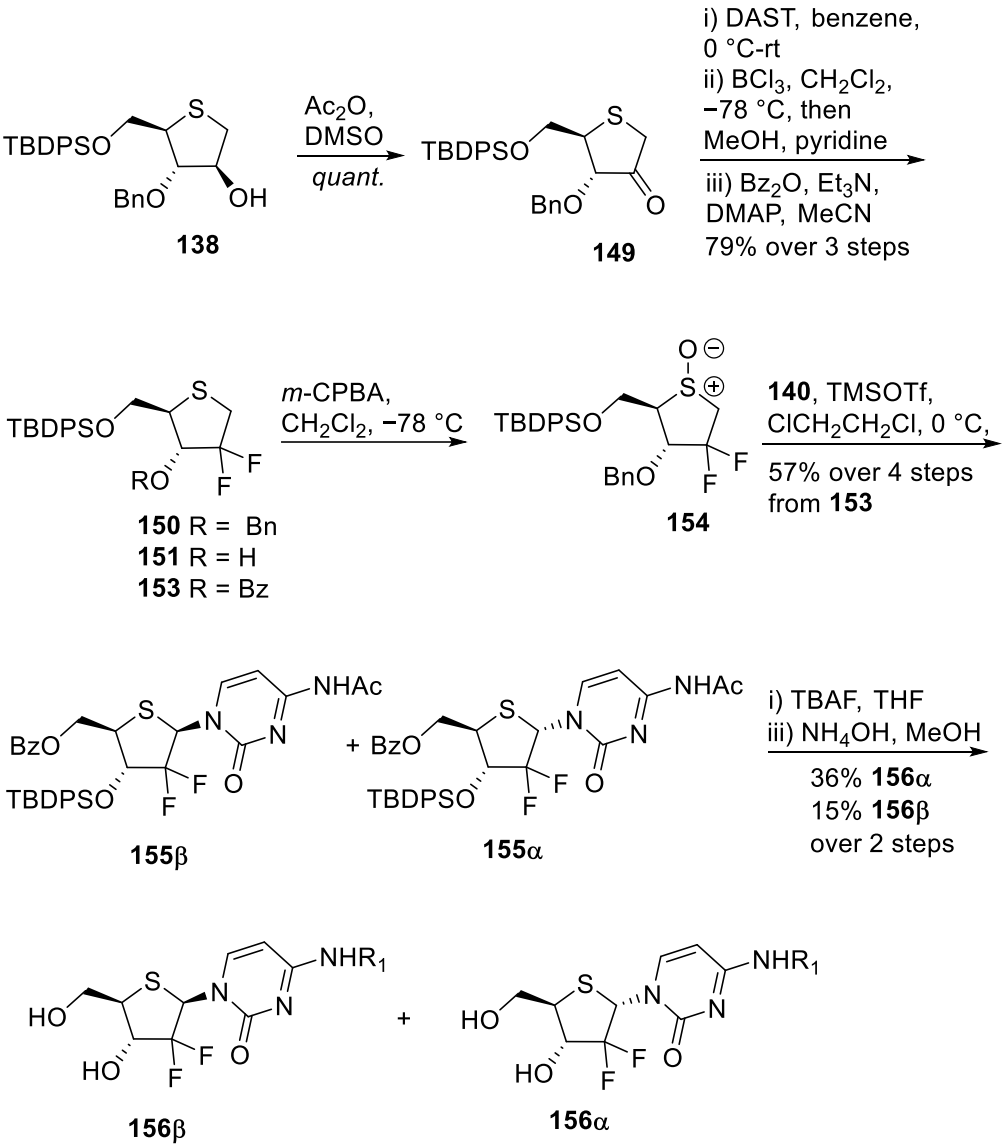
Additionally, 2′-deoxy-2′-fluoro-arabino-4′-thiocytidine **148β** was prepared from intermediate **138** (Scheme 23). DAST fluorination of **138** was effected stereospecifically through epi-sulfonium intermediate **144** which afforded **145** in 68% yield. Oxidation to the sulfoxide and concomitant Pummerer rearrangement furnished anomeric acetate **147** in 77% yield from **145**. Finally, the cytosine nucleobase was installed by employment of Vorbrüggen type glycosylation to obtain the corresponding thionucleoside as a mixture of anomers (2.9/1 α/β) in an excellent yield of 93%, the

nucleosides deprotected *via* ammoniolysis and the β-anomer separated using HPLC to obtain **148β** in 43% yield from **147**.



Scheme 23. Synthesis of **148β**.

Finally, the group synthesised 1,2′-deoxy-2′-difluoro-1′-(4′-thio-D-ribofuranose)cytosine **156β**, a thionucleoside analogue of the potent chemotherapeutic agent gemcitabine (Scheme 24). Intermediate **138** was oxidized at C2 under Albright-Goldman conditions, the ketone difluorinated using DAST, the 3-benzyl group removed *via* treatment with BCl_3 and replaced with a benzoyl group and to furnish **153** in 79% yield from **149**. *m*-CPBA mediated oxidation to the sulfoxide **154** followed by Pummerer-type glycosylation afforded thionucleosides **155α/β** which were fully deprotected to furnish the final products **156α/β** (α/β = 2.4/1) in a moderate yield of 51% from **155**.



Scheme 24. Synthesis of **156β**.

The anticancer activities of **143**, **148** and **156** were evaluated in an MTT assay and compared to Ara-C **23** and 1′-(2′-deoxy-2′-*C*-methylene-β-D-erythro-pentofuranosyl)cytosine (DMDC) (Table 3). As expected, all the derivatives which featured the unnatural α-glycosidic bond were completely inactive against T-cell leukemia CCRF-HSB-2 cells. However, the β-anomers showed considerable cytotoxic activity against the same cell line. Notably, **143β** and **148β** were highly potent against both T-cell leukemia CCRF-HSB-2 cells and human solid tumour KB cells, with IC_{50} values of 0.01 μg/mL (CCRF-HSB-2) and 0.12 μg/mL (KB cells) for **143β** 0.05 μg/mL (CCRF-HSB-2) 0.02 μg/mL (KB cells) for **148β**. Indeed, **143β** was significantly more potent in both cell lines than that of its native counterpart, DMDC, which has an IC_{50} value 2.4 times higher in CCRF-HSB-2 cells (IC_{50} = 0.02

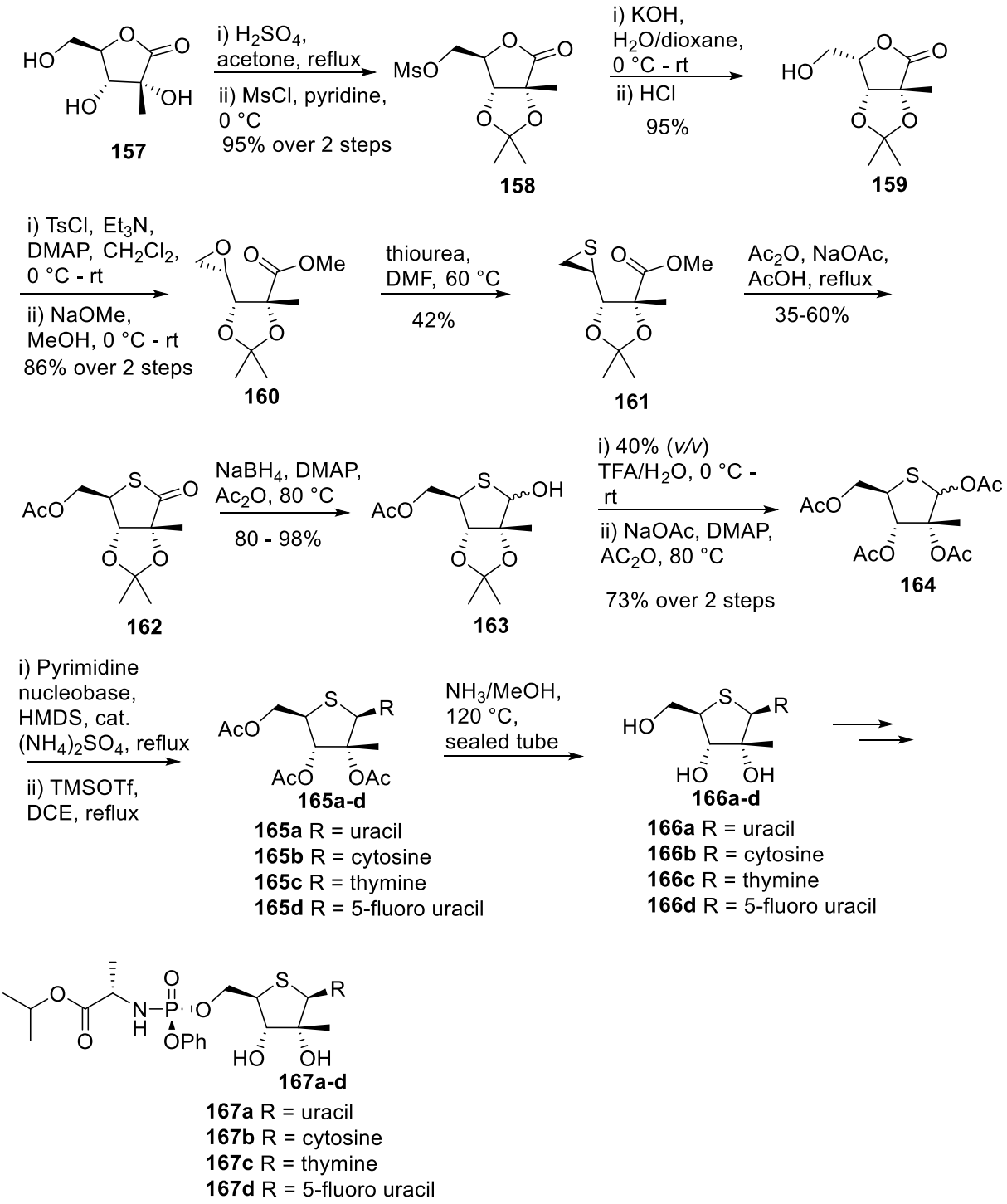
μg/mL) and 3.7 times higher in KB cells (IC_{50} = 0.44 μg/mL). Interestingly, 4′-thiogemcitabine analogue **156β** was considerably less potent compared to **65**, with a notably high IC_{50} value of 17 μg/mL in KB cells, which Yoshimura proposes may be due to reduced phosphorylation efficacy of **156β** by deoxycytidine kinase, a key enzyme which converts 2′-deoxycytidine analogues to their corresponding monophosphates.

Entry	Compound (anomer)	2′-substituent	Antineoplastic Activities IC_{50} (μg/mL)	
			CCRF-HSB-2[a]	KB cells[b]
1	143α	$=CH_2$	>10	ND[c]
2	143β	$=CH_2$	0.01	0.12
3	148α	F (arabino)	>10	ND
4	148β	F (arabino)	0.05	0.02
5	156α	F_2	>10	ND
6	156β	F_2	1.5	17
7	Ara-C 23[d]		0.05	0.26
8	DMDC[e]		0.02	0.44

Table 3. Antineoplastic activities of 2′-modified-4′-thionucleosides. [a]MTT assay[98] [b]Dye uptake method[98] [c]Not determined, [d]arabinocytidine (cytarabine), [e]1-(2-deoxy-2-C-methylene-β-D-erythro-pentofuranosyl)cytosine.

Recently, Liotta and colleagues synthesised a series of 4′-thionucleosides bearing a 2′-*C*-methyl substituent and their corresponding monophosphate prodrugs and evaluated their anti HCV activity in a cellular HCV replicon assay.[99] Following synthetic methodology published by Dukhan and colleagues,[100] the synthesis commenced with commercially available lactone **157**, which underwent standard protecting group manipulations, furnishing **158** in 95% yield (Scheme 25). The synthesis of thiofuranoses and their corresponding thionucleosides generally involves a double inversion of the stereochemistry at C4 to obtain the final thiofuranose derivatives with the correct D-ribo configuration. Base hydrolysis of the 5-mesylate **158** with KOH resulted in the formation of a

4,5-epoxide which was not isolated and was protonated and subsequently hydrolysed to the free alcohol following treatment with HCl to afford **159** with inverted stereochemistry at C4 in 95% yield. Installation of a 5-tosyl group and trans-esterification followed by intramolecular S_N2 furnished epoxide **160** in 86% yield over two steps which was then treated with thiourea, incorporating sulfur and inverting the stereochemistry at C4 to afford **161** in a low yield of 42% from **160**. Intermediate **161** was treated with acetate at reflux, which opened the thiirane, albeit in low yields due to formation of both the 5- and 6-membered thiolactones. The desired 5-membered thiolactone **162** was separated and underwent a series of standard protecting group manipulations to furnish the globally acetylated thiofuranoside **164**. Pyrimidine bases (uracil, cytosine, thymine, 5-fluorouracil) were installed using standard Vorbrüggen glycosylation procedures, followed by acetyl hydrolysis to afford 2′-*C*-methyl thionucleosides **167a** – **d**.



165a R = uracil
165b R = cytosine
165c R = thymine
165d R = 5-fluoro uracil

166a R = uracil
166b R = cytosine
166c R = thymine
166d R = 5-fluoro uracil

167a R = uracil
167b R = cytosine
167c R = thymine
167d R = 5-fluoro uracil

Scheme 25. Synthesis of 2′-C-methyl thionucleoside analogue **167a – d**.

Of the four compounds evaluated in the HCV replicon assay, only two had EC_{50} values <10 μM, with EC_{50} = 2.10 μM for **167a** and EC_{50} = 9.82 μM for **167b** respectively. However, neither compound had better anti-HCV activity than the control substance, sofosbuvir **39** (EC_{50} = 0.05 μM). The compounds were also screened for their reduction in CPE in Huh7 cells infected with Dengue

virus (DENV2 New Guinea) and Zika virus (ZIKA PRVABC59) at 5 μM concentration. Liotta found that the compounds showed 0 – 12% reduction in CPE in the Dengue virus infected cells, and up to 20% reduction in CPE in the Zika virus infected cells. However, sofosbuvir performed better than all other compounds in the screen, showing 100% reduction in CPE for Dengue virus infected cells at 5 μM and EC_{50} = 1.36 μM against Zika virus.

1.4.4.2. Synthesis of 4′-Modfied 2′-deoxy-thionucleosides

Multiple syntheses and biological activities of 4′-subsituted nucleosides have been reported in the literature.[101,102] Modifications of the 4′-position have since been mapped onto 4′-thionucleosides, and the corresponding analogues evaluated for their biological activities.

In 2014, Thottassery and colleagues identified 4′-thio-2′-deoxycytidine (T-dCyd) as a chemotherapeutic agent against leukaemia, with IC_{50} = 0.60 μM in CCRF-CEM cells.[103] Following this, in 2019, Haraguchi and co-workers reported the synthesis and biological evaluation of a small library of 4′-position modified analogues of T-DCyd for their antiviral and anticancer activity (Figure 18).[104]

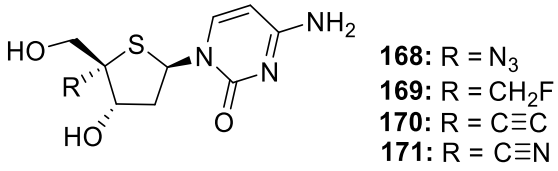
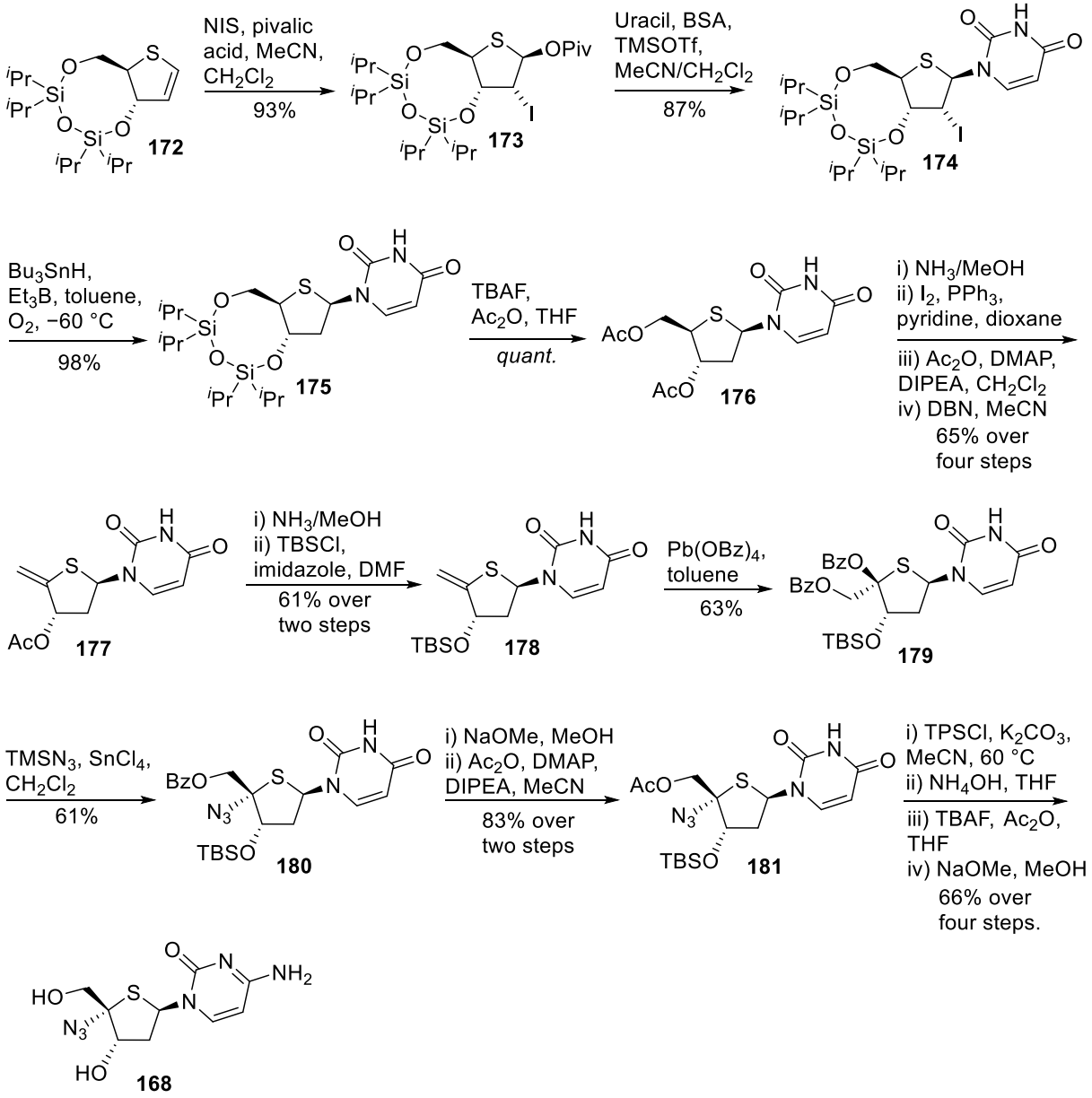


Figure 18. Structures of 2′-deoxy-4′-thiocytidine nucleosides **168** – **171**.

The synthesis started with thiofuranose **172** (Scheme 26), prepared in 12 steps from 2-deoxy-D-ribose using established procedures.[105] Treatment of **172** with *N*-iodosuccinamide (NIS) mediated electrophilic glycosidation with pivalic acid, furnished **173** as one diastereoisomer. Vorbrüggen-type glycosylation with uracil afforded the desired β-nucleoside **174** in 87% yield. Following this, the 2′-iodo moiety underwent $SnBu_3$ mediated radical reduction to obtain **175**. Removal of the TIPDS group, Appel iodination of the 5′-hydroxyl group, subsequent acetylation of the 3′-hydroxyl group and elimination of the 5′-iodo moiety furnished the 4′-methylene derivative **177** in 65% over four steps. Ammoniolysis of the acetate and substitution with a TBS group furnished **178** which was treated with $Pb(OBz)_4$ to furnish dibenzoate **179** in 63% yield from **178**. Inversion of C5′ to the desired D-ribo

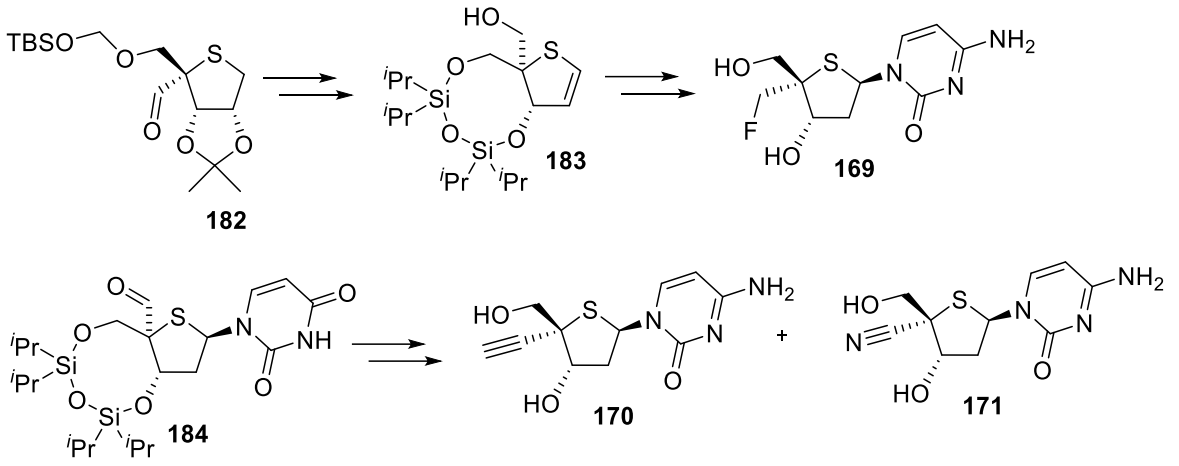
configuration and installation of the 4′-azido group was achieved *via* reaction of **179** with $TMSN_3$ and $SnCl_4$, which furnished **180** as the major product. The uracil nucleobase was then converted to cytosine using standard procedures and the protecting groups removed to afford the final product **168** in 7% yield over 18 steps.



Scheme 26. Synthesis of **168**.

2′-deoxy-4′-*C*-fluoromethyl-4′-thiocytidine **169** was prepared in 14 steps from known aldehyde **182** (Scheme 27).[106,107] The aldehyde was converted to the alcohol **183** which underwent DAST fluorination to install the 4′-α-fluoromethyl group, which was further developed into the final cytidine

derivative **169**. The 4′-α-alkynyl and nitrile analogues **170** and **171** were prepared from aldehyde **184**, employing late-stage insertion of the key functional group at the 4′-position following installation of the nucleobase.



Scheme 27. Key intermediates to access 2′-deoxy-4′-thiocytidine nucleosides **169**-**171**.

Of the four compounds synthesised **168** – **171**, **168** and **169** showed moderate neoplastic activity against B-cell leukaemia CCRF-5B cells (IC_{50} = 7.14 μM for **168**, IC_{50} = 3.19 μM for **169**) and in T-cell leukaemia Molt-4 cell lines (IC_{50} = 2.72 μM for **168**, IC_{50} = 2.24 μM for **169**). Similarly, **168** and **169** exhibited promising antiviral activities against varicella-zoster virus (VZV) (VZV OKA/TK^+ strain EC_{50} = 0.43 μM for **168**, EC_{50} = 2.38 μM for **39**; VZV/TK^- 07-1 strain EC_{50} = 0.76 μM for **168**, EC_{50} = 1.65 μM for **169**), with **168** proving particularly potent with sub micromolar EC_{50} values. Moreover, **168** and **169** had excellent inhibition of herpes simplex virus (HSV) (HSV-1 EC_{50} = 0.55 – 0.75 μM for **168**, EC_{50} = 0.39 – 1.13 μM for **169**; HSV-2 EC_{50} = 0.70 μM for **168**, EC_{50} = 0.71 μM for **169**). Unfortunately, **169** was found to be highly cytotoxic (CC_{50} = 0.57 μM in human embryonic lung cells), while **168** showed no toxicity (CC_{50} = >100 μM). Thus compound **168** has potential to be a promising antiviral therapeutic.

1.5. Summary

Nucleoside analogues make up a significant proportion of chemotherapeutics for viral infections and cancers. Synthetic trends appear to be moving away from the native nucleoside forms and towards

heteroatom-substituted varieties with functionalisation around the furanosyl ring. Such nucleoside analogues have shown improved resistance to hydrolytic metabolism as well as promising biological activities, which have often exceeded that of the native derivatives. Functionalisation at the 2′- and 4′-positions has been shown to improve antiviral and antineoplastic activities and direct selectivity for one virus over another as well as lowering host cell cytotoxicity.

There is significant chemical space to be explored for thionucleoside analogues due to the large scope for novel chemical modifications to the nucleoside scaffold. However, the development of such molecules will require adaptation of the synthetic methods for the 4′-oxo analogues as additional issues will need to be taken into consideration, such as changes in reactivities, electronegativity and conformational changes due to the large atomic radius of sulfur. Indeed, the synthesis of thionucleosides is known to be associated with difficulties with product separation and side reactions,[108] however, given their excellent potential as chemotherapeutic agents, it is a path worth exploring.

1.6. Work described in this thesis

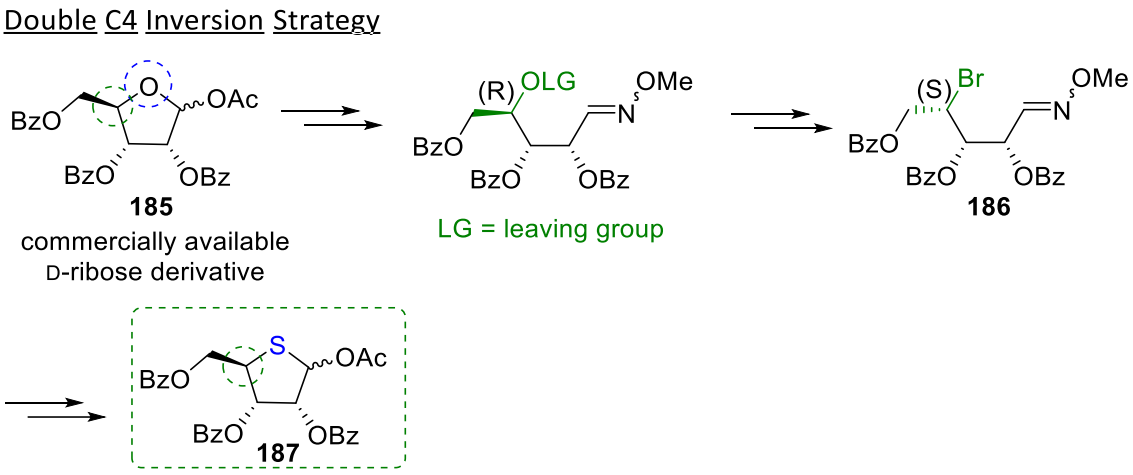
As discussed throughout this Chapter, nucleoside analogues make up a significant portion of the antiviral and anticancer drug market, and the synthesis of novel nucleoside analogues and their biological evaluation in various biological targets comprises a highly competitive and exciting area of research. Work in this thesis will take inspiration from nucleoside analogue drugs currently on the market, by following a mapping approach whereby chemical modifications seen in existing drugs will be incorporated into the design of novel thionucleoside analogues. Further, the thionucleoside analogues synthesised herein will be evaluated in antiviral and anticancer assays and their biological activities directly compared to the native 4′-oxo counterparts.

This thesis will involve the development of novel synthetic routes to thioribose and thionucleoside scaffolds, beginning in Chapter 2 with the scalable, high yielding synthesis of 1′-*O*-acetyl,2′,3′-*O*-benzoyl-1′-(4′-thio-D-ribo)furanose.

Chapter 2: Optimisation of large-scale synthesis of protected thioribose

2.1. Aims and rationale

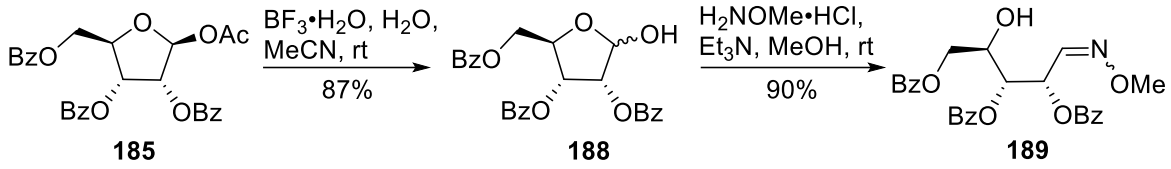
A scalable and reliable method for the synthesis of the key building block 1-β-*O*-acetyl-2,3,5-tri-*O*-benzoyl-1-(4-thio-D-ribofuranose) **187** was required to support the synthesis and chemical modification of thionucleoside analogues. The synthetic approach undertaken herein was based on procedures reported in the patent literature by Nakamura and colleagues (Fujifilm Inc)[109] which demonstrated the preparation of thioribose derivatives *via* a double inversion strategy whereby C4 undergoes stereochemical inversion *via* a bromination reaction, prior to S_N2 sulfur insertion and intramolecular ring closing to ensure the correct stereochemistry in the thioribose product **171** (Scheme 28).



Scheme 28. Synthetic strategy for the synthesis of **187**.

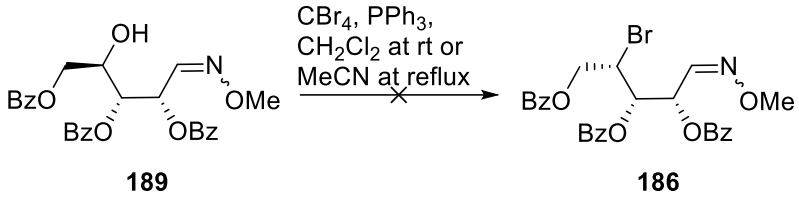
2.2. Synthesis of key 1-(4-thio) ribose derivative

Starting with **185**, the anomeric acetate was hydrolysed using wet $BF_3{\cdot}OEt_2$ as a Lewis acid to obtain **188** as a 1.1/1 α/β mixture. This reaction furnished **188** in excellent yields which was consistent on scale up to 50 g (93% yield) and 100 g (87 - 91%). Crude product hemiacetal **188** was treated with $H_2NOMe{\cdot}HCl$ and Et_3N which furnished the corresponding 1-oxime **189** as an isomeric mixture (3/1 ratio of isomers) (Scheme 29). NMR analysis of **189** indicated a significant downfield shift of imine proton H1 to δ_H 7.62 ppm and C1 to δ_C 145.1 ppm. In addition, the appearance of a singlet at δ_H 3.84 ppm for OCH_3 for the major geometric oxime isomer was observed.



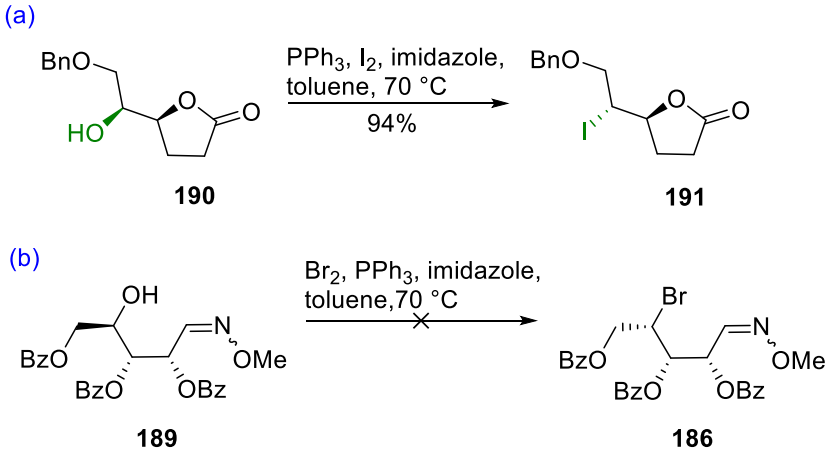
Scheme 29. Hydrolysis of **185** anomeric acetate to obtain **188** and conversion to the corresponding oxime **189**.

Appel halogenation conditions[110] were next trialled to convert the secondary 4-hydroxyl group to the corresponding alkyl bromide **186**, *via* treatment of **189** with PPh_3 and CBr_4 (Scheme 30). However, no reaction occurred and the starting material **189** was recovered.



Scheme 30. Attempted Appel halogenation of **189**.

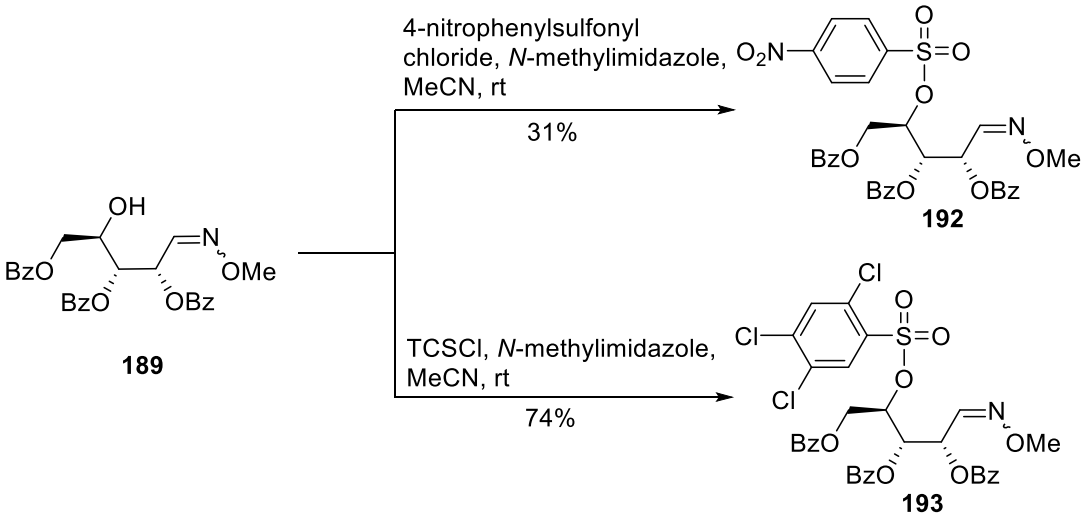
Thus, reaction of **189** with Br_2, PPh_3 and imidazole was investigated next, following similar conditions reported by Chavan *et al.* for iodination of a secondary alcohol on lactone **190** (Scheme 31).[111]



Scheme 31. a) Iodination of lactone **190** reported by Chavan et al; b) attempted bromination of **189** to prepare **186**.

However, no reaction was observed which may be due to the deactivating effect of the adjacent benzoate groups through electron withdrawal, reducing the nucleophilicity of the 4-OH. Thus, sulfonyl leaving groups (4-nitrophenylsulfonyl and 2,4,5-trichlorobenzenesulfonyl) were evaluated for their suitability as a transient 4-position leaving groups for subsequent bromination.

Reaction of crude product **189** with 4-nitrophenylsulfonyl chloride and *N*-methylimidazole furnished **192** in a low yield of 31% (Scheme 32). Attempts to purify **192** by crystallisation, precipitation or trituration failed to deliver purified **192** and thus column chromatography was necessary. NMR analysis of **192** showed H4 shifted downfield in the ^{1}H NMR spectrum to δ_H 5.52 ppm due to the electron withdrawing effects of the sulfonyl group and ESI-MS analysis confirmed the mass as 514.1494 corresponding to the [**192**+Na]$^{+}$ species. Due to the poor yield of **192** and requirement for column chromatography, preparation of **193** was next evaluated. Thus, crude product **189** was reacted with 2,4,5-trichlorobenzenesulfonyl chloride (TCSCl) and *N*-methylimidazole, which furnished **193** in 74% yield over three steps from **185**, starting on 5 g scale. Purification of **193** was achieved *via* trituration in room temperature Et_2O, which delivered **193** as an easy-to-handle white powder and negated the requirement for column chromatography.

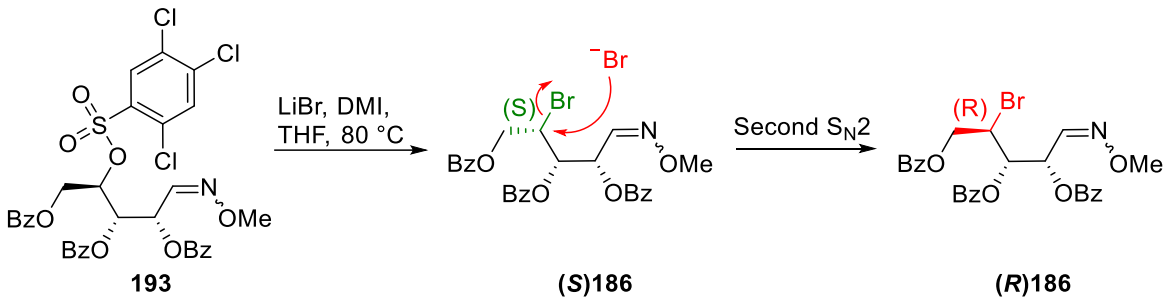


Scheme 32. Synthesis of **192** and **193**.

On scale up of the synthesis, starting with 50 g **185**, the yield for preparation of **193** was reduced to a moderate 53% over three steps from **185**. Intermediates **188** and **189** could be used crude product and purification of **193** *via* trituration was effective on large scale. Scale up to 100 g of **185** and use of crude product **188** and **189** furnished **193** in a reduced 46% yield over the three steps. At 100 g scale, trituration of crude product **193** in Et_2O only furnished 13% yield of **193** and a further 33% could be obtained by removal of the solvent from the mother liquor, applying it to a silica plug and eluting with 1/9 Et_2O/petroleum ether. The requirement for a silica plug to furnish moderate

quantities of **193** used large volumes of solvent (~25 L) and significantly increased the purification time, thus 50 g was selected as the optimum starting scale for this synthetic route. Analysis of the ^{1}H NMR data for **193** showed a significant downfield shift of H4 from δ_H 4.42 – 4.39 ppm to δ_H 5.53 ppm and disappearance of the 4-hydroxyl peak which was observed at δ_H 3.13 ppm for **189**. HRMS allowed further structural confirmation with a m/z of 734.0422 corresponding to the [**193**+H]$^{+}$ species.

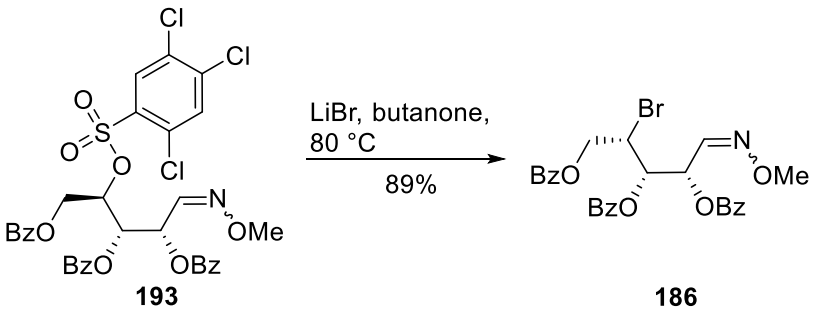
Substitution of the 4-position sulfonyl group with bromide with concomitant stereochemical inversion at C4 was achieved *via* treatment of **193** with LiBr (5 mol equivalents) in DMI and THF at 60 °C, which furnished **186** in 65% yield (Scheme 33). However, both the C4 (*S*) and (*R*) diastereoisomers were observable by ^{1}H NMR in a 3/1 (*S*)/(*R*) diastereomeric ratio (dr). One of the possible explanations for this outcome was that a second S_N2 reaction was occurring following the formation of (*S*)-**186**, whereby a second bromide ion attacks the C4-Br, resulting in a mixture of diastereoisomers (Scheme 33). Reaction time was thought to be a factor, as the bromination took 16 h to go to completion. However, NMR analysis after 2 h showed the diastereomeric mixture (10/1 (*S*)/(*R*)) also formed in shorter reaction periods before complete consumption of **193**. Further, reducing the equivalents of LiBr to 1.5 mol equivalents did not result in complete consumption of **193** after 24 h, nor prevent formation of a diastereomeric mixture.



Scheme 33. Synthesis of (*S*)- and (*R*)-**186**.

Choice of solvent is important in S_N2 reactions, for example, polar aprotic solvents increase the nucleophilicity of halide ions whilst polar protic solvents decrease the nucleophilicity of halide ions due to the formation of hydrogen bonds *in situ*.[112] Thus, alternative solvents for the reaction were evaluated. The use of acetone at reflux did not furnish **186**, despite good solubility of the LiBr in solution. This may have been due to the low boiling point of acetone not being sufficient to drive

reaction. Thus, butanone was trialled as solvent at reflux, which delivered **186** in 89% yield as the single (*S*)-diastereoisomer (Scheme 34).



Scheme 34. Improved synthesis of **186**.

Incorporation of bromine was confirmed by HRMS, with the distinctive Br isotope pattern observable and m/z 554.0802 corresponding to the [**186**+H]$^+$ species (Figure 19). Further, the ^{13}C NMR spectrum indicated an upfield shift of C4 to δ_C 47.8 ppm (from δ_C 79.6 ppm in **193**), which was consistent with literature values.[109]

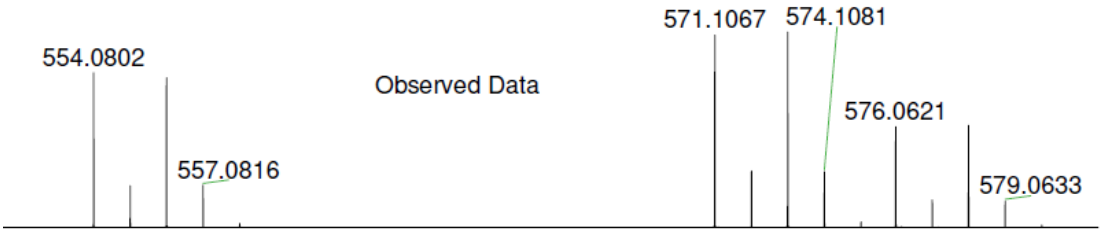
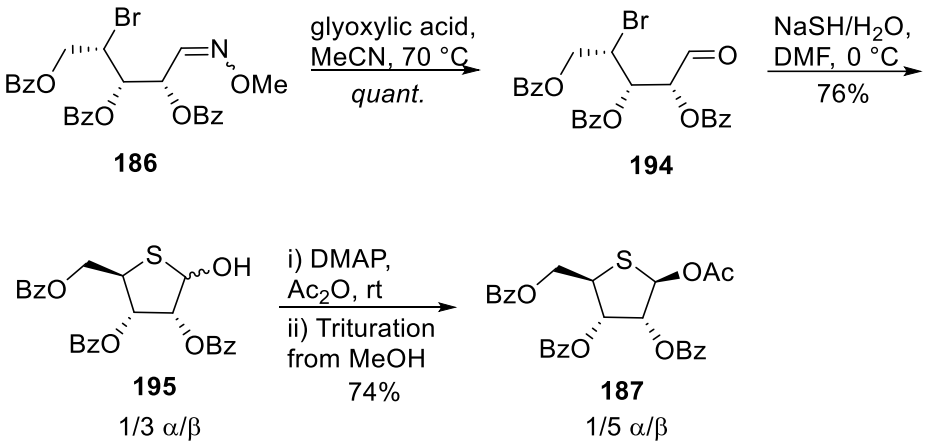


Figure 19. HRMS of **186** showing Br isotope pattern.

Treatment of **186** with glyoxylic acid at 70 °C hydrolysed the oxime to the corresponding aldehyde **194** (Scheme 35).[109] A downfield shift of H1 (from δ_H 7.50 ppm in **186** to δ_H 9.72 in **194**) and C1 (from δ_C 144.4 ppm in **186** to δ_C 194.7 ppm in **194**) confirmed the formation of an aldehyde and **194** was used immediately without further purification. Conversion to thiofuranose **195** was achieved *via* S_N2 reaction of NaSH with **194**, which afforded **195** as a mixture of anomers (3/1 β/α) in 76% yield on 11 g scale. The chemical shift of H1 and C1 in **195** shifted to the typical regions for an anomeric ribose CH (δ_H 5.51 ppm and δ_C 80.2 ppm) and matched literature values.[109] Upon reaction completion, indicated by TLC through full consumption of **194** (R_f 0.62, 2/8 Et_2O/petroleum ether) and formation of **195** (R_f 0.29, 2/8 Et_2O/petroleum ether), Ac_2O and DMAP were added to the reaction mixture, obtaining the crude product acetylated product **187** as a mixture of anomers (1/5 ratio α/β)

following workup. Crude product **187** was purified by trituration in MeOH which furnished the β-anomer in 74% yield, which was consistent on scale up to ~10 g.



Scheme 35. Synthesis of **187** from **186**.

2.3. Conclusions

Notably, the development of this synthetic route to **187** required no column chromatography, therefore, it was easily amenable to large scale and use of 50 g of **185** delivered 13 g of **187** in an overall yield of 29% over seven steps. Overall, this synthetic route was a significant improvement on similar syntheses reported in the literature; the yield was double that of comparable syntheses (11%[109] and 14%[113]), and no requirement for column chromatography was an excellent advancement which vastly simplified the process. The route may be carried out from start to finish in approximately 5 – 8 days which supported continual synthesis of large quantities of thionucleosides for further modification and development.

Chapter 3: Synthesis of *Ribo* and *Arabino* Configured Thionucleoside Analogues

3.1. Aims and rationale

Development of consistent, scalable and high yielding syntheses of 1′-(4′-thio-D-ribofuranosyl)uracil/cytosine **196** and **197** (Figure 20) from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-1-(4-thio-D-ribofuranose) was essential to deliver significant quantities of these key nucleosides. With multi-gram quantities of nucleosides **196** and **197**, further modification of the thionucleoside scaffold could be pursued, which would allow for late-stage diversification into numerous thionucleoside derivatives. Further, preparation of novel sulfinyl derivative **198** is pursued herein to evaluate whether this confers any chemotherapeutic activity in comparison to **197**.

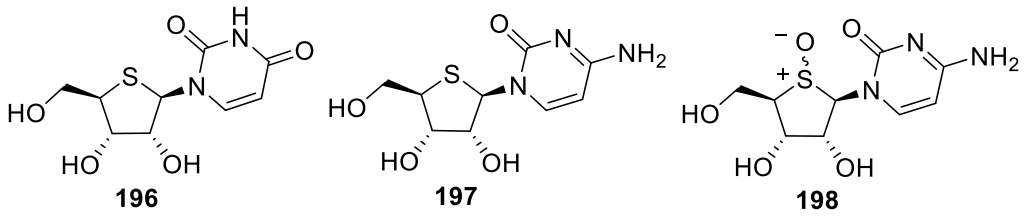


Figure 20. Structures of thionucleoside analogues **196**, **197** and **198**.

Cytarabine **23** is a potent chemotherapeutic agent used to treat leukemia (see Chapter 1, section 1.3.1. for details on its synthesis and biological activity). The thionucleoside analogue, 1′-(4′-thio-D-arabinofuranosyl)cytosine (Thiarabine) **199** (Figure 21) has excellent antitumour activity against various human tumour xenografts in mice. Indeed, Thiarabine was found to be a superior antitumour agent than anticancer drugs Gemcitabine **65** and Cytarabine **23**.[114] The synthesis of 1′-(4′-thio-D-arabinofuranosyl)uracil/cytosine and 1′-(4′-sulfinyl-D-arabinofuranosyl)cytosine from 1′-(4′-thio-D-ribofuranosyl)uracil is herein pursued to compare the thionucleoside analogues **199**, **200** and **201** against **23** in various cytotoxicity assays. It is of particular interest to examine whether oxidation to the novel sulfoxide **201** improves the anticancer activity.

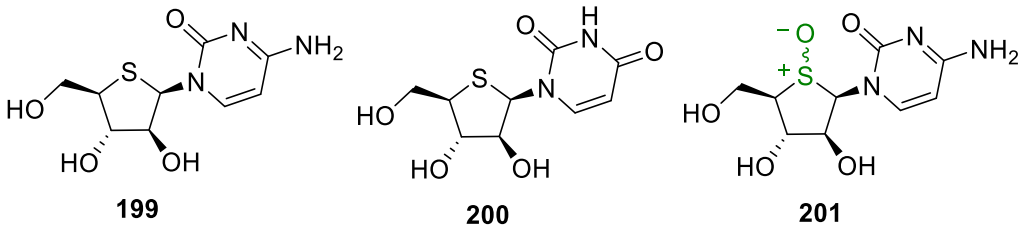
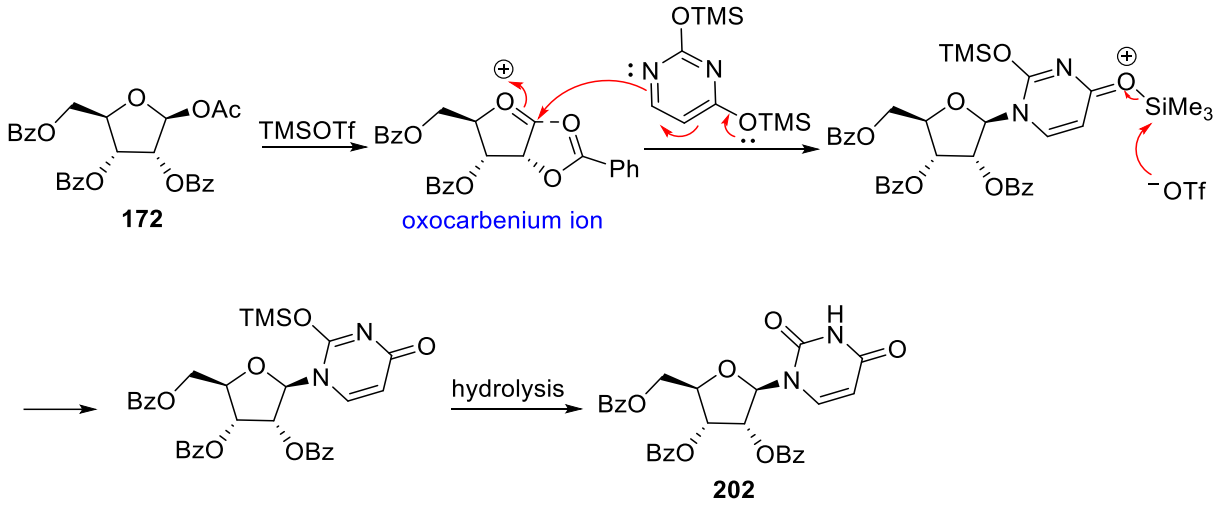


Figure 21. Structures of Thiarabine **100**, **200** and **201**.

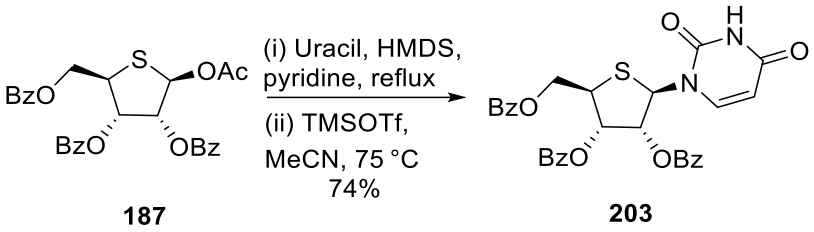
3.2. Synthesis of 1′-(4′-thiouridine) and 1′-(4′-thiocytidine)

To prepare 1′-(4′-thio-D-ribo/arabinofuranosyl) nucleosides, a method for installation of the nucleobase onto **187** was required. As discussed in Chapter 1, section 1.2.2., Vorbrüggen type glycosylation[7,8,10] is a useful means of preparing nucleoside analogues stereoselectively. Neighbouring group participation by a C2-*O*-ester group directs the stereochemistry of the product by hindrance of nucleophilic attack from beneath the ring (Scheme 36). Thus, nucleophilic attack by the nucleobase onto C1 occurs on the less hindered face of the ribofuranosyl ring, furnishing the desired β-nucleoside.



Scheme 36. Mechanism of Vorbrüggen reaction.

Vorbrüggen reactions are activated by Lewis acids such as $SnCl_4$ and TMSOTf. To avoid tin emulsions at workup, TMSOTf was selected as the Lewis acid for preparation of **203** (Scheme 37). This reaction required careful exclusion of moisture and optimisation of the reaction conditions and purification methods to maximise the yield, isolate the desired β-anomer and allow for scale up. The results of these efforts are shown in Table 4.



Scheme 37. Optimised Vorbrüggen synthesis of **203**.

Entry	T (ºC)	TMSOTf (eq)	Scale (g)	Yield (%)
1	rt	1	0.5	13 [a, c]
2	reflux	1	0.5	28 [a, c]
3	reflux	0.8	0.5	55 [a, c]
4	75	0.8	5.0	51 [b, c]
5	75	0.8	5.0	84 [b, d]
6	75	0.8	10	74 [b, d]

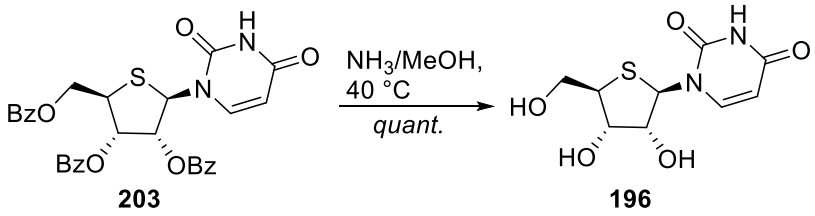
Table 4. Optimisation studies for the preparation of **203**; a) 1/10 α/β anomeric mixture; b) β-anomer only; c) column chromatography (EtOAc/hexanes); d) silica plug (3/7 EtOAc/petroleum ether) followed by trituration (1/2 boiling EtOAc/petroleum ether).

Reflux of uracil in HMDS and pyridine delivered the bis-*O*-silylated uracil intermediate in 3 h. Removal of excess HMDS and pyridine was best achieved under vacuum on a Schlenk line to exclude moisture. Removal of these reagents using a rotary evaporator caused the trimethylsilyl groups to hydrolyse, as evidenced by the precipitation of uracil as a white solid which was insoluble in organic solvent. Reaction of the silylated uracil with **187** and TMSOTf in MeCN at rt (Entry 1,Table 4) furnished **203** in 13% yield in a 1/10 α/β ratio. The reaction was trialled at reflux which furnished **203** in a slightly improved yield of 28% (Entry 2, Table 4). However, at this higher temperature, the reaction solution turned a dark brown colour and multiple side products were observable by TLC. Reaction at reflux, with a reduced equivalence (0.8 equivalents) of TMSOTf (Entry 3, Table 4) which improved the yield further to 55%. Subsequently, the lower temperatures were trialled with 0.8 equivalents of TMSOTf and it was found that reacting the reagents just below reflux, at 75 ° C, improved the yield significantly (Entry 4, Table 4), with the reaction mixture staying orange in colour.

Multiple rounds of column chromatography were required to separate **203** from the complex crude product mixture in adequate quantities as a 1/10 α/β mixture (Entries 1 – 4, Table 4). Anomeric separation *via* column chromatography proved difficult, delivering **203β** in varying yields of which the highest achieved was 51% (Entry 4, Table 4). Finally, the crude product solid, following passage through a silica plug, was triturated from 1/2 (*v/v*) EtOAc/petroleum ether solution which furnished the desired β-anomer in excellent yields of 74% which proved to be consistent on scale up from 5 g to 10 g (Entries 5 – 6, Table 4).

NMR analysis confirmed the correct structure for **203β**, notably with the presence of signals for the uracil C5 and C6. The uracil protons H6 and H5 appeared at δ_H 7.74 and 5.56 ppm respectively, with a large vicinal coupling to each other of 8.2 Hz. HSQC spectrum showed that these protons correlated to signals at δ_C 139.7 and 103.8 ppm. The desired stereochemistry at C1′ was confirmed by a large vicinal coupling $J_{H1'\text{-}H2'}$ = 6.8 Hz indicating a 1,2-*trans* relationship between H1′ and H2′. This improved synthetic method was recently published in Proven Synthetic Methods in Carbohydrate Chemistry: Volume 5.[115]

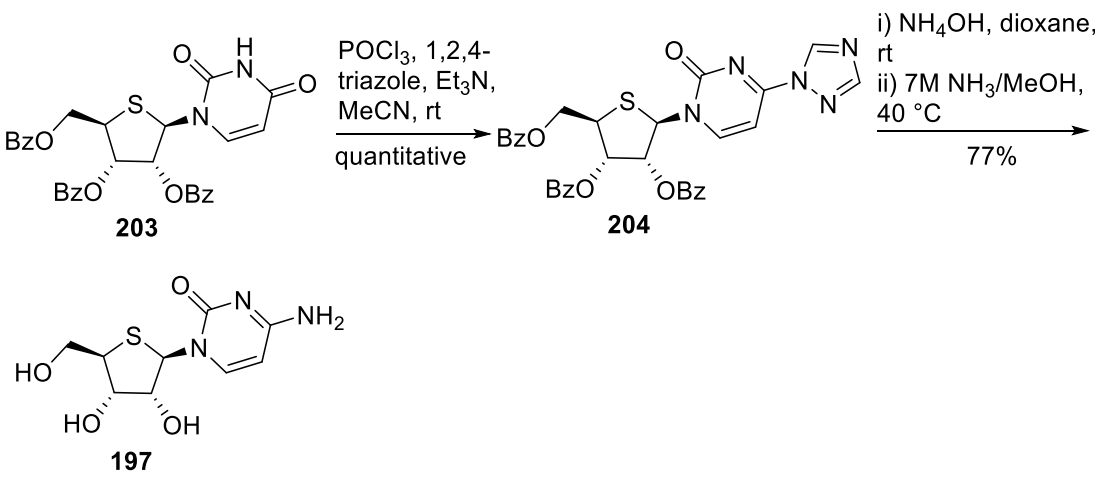
Global deprotection of **203** was achieved by stirring the nucleoside in 7M NH_3/MeOH which furnished **196** in quantitative yield which was consistent on scale-up to 8.0 g (Scheme 38). Purification *via* reverse phase column chromatography (H_2O) delivered **196** in high purity of 96%, as determined *via* analytical HPLC.



Scheme 38. Synthesis of **196**.

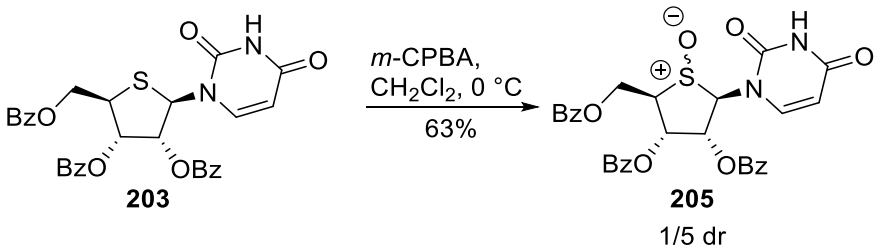
Synthesis of 1′-(4′-thio-D-ribofuranosyl)cytosine **197** was achieved by conversion of the uracil nucleobase in **203** to cytosine using standard conditions.[116] Treatment of **203** with $POCl_3$, 1,2,4-triazole and Et_3N furnished the C4-triazole intermediate **204** in quantitative yield (Scheme 39), marked by the appearance of triazole CH peaks at δ_C 154.2 and 143.4 ppm. Crude product **204** was

subsequently stirred in NH_4OH which substituted the triazole group with -NH_2 and then in 7M NH_3/MeOH at 40 °C to remove the benzoyl groups, which delivered **197** in 77% yield over the three steps. The preparation of **197** was confirmed by the marked downfield shift of C4 to δ_C 165.9 ppm and HRMS m/z of 260.0714 corresponding to the [**197**+H]$^+$ species. Purification *via* flash chromatography with MeOH/$CHCl_3$ and then *via* reverse phase flash chromatography with H_2O delivered **197** in 97% purity.



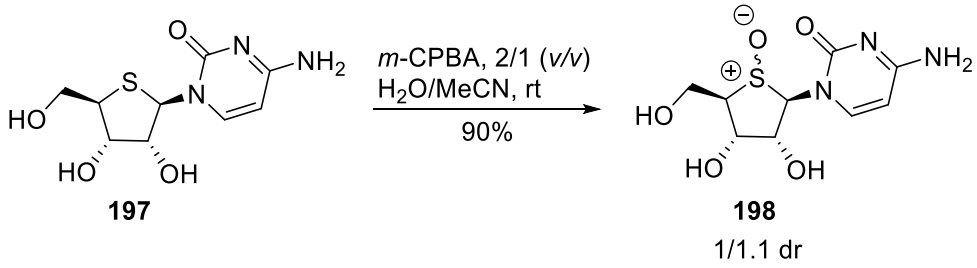
Scheme 39. Synthesis of **197** from **203**.

Oxidation to the corresponding sulfoxide was trialled *via* reaction of **203** with *m*-CPBA (Scheme 40) which furnished **205** in 63% yield as a 1/5 ratio of diastereoisomers. The installation of the polar sulfinyl group corresponded to a significant downfield shift of C1′ and C4′ from δ_C 79.7 and 46.2 ppm in **203** to δ_C 91.5 and 70.7 ppm respectively in **205** and HRMS displayed m/z 611.1092 corresponding to the [**205**+Na]$^+$ species. Attempted deprotection with 7M NH_3/MeOH resulted in the formation of an inseparable mixture of products, thus, sulfur oxidation was pursued on deprotected nucleoside **197**.



Scheme 40. Synthesis of **205**.

Reaction of **197** with *m*-CPBA in 2/1 (v/v) H_2O/MeCN solution at rt furnished the corresponding sulfoxide **198** in 90% yield in a 1/1.1 dr. An analytically pure (>95% purity) sample was obtained *via* preparative HPLC purification of a small quantity (20 mg) of **198**. The increased electron-withdrawing effects of the sulfinyl group resulted in the downfield shift of C1′ and C4′ from δ_C 65.1 and 51.7 ppm in **197** to δ_C 73.1 and 65.7 ppm respectively in **198**. HRMS displayed m/z of 274.0510 corresponding to the [**198**-H]⁻ species.



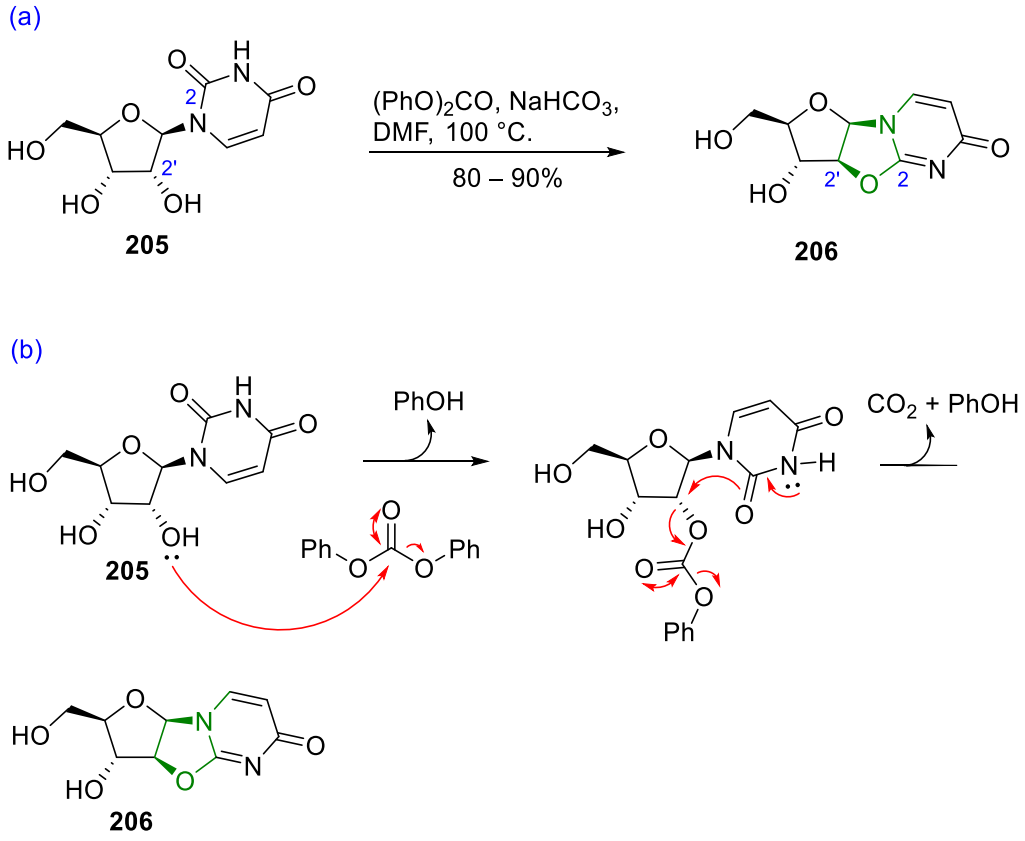
Scheme 41. Synthesis of **198**.

Overall, reliable, scalable and high yielding syntheses of **196**, **197** and **198** were developed. The optimisation of the Vorbrüggen reaction conditions and consequent purification for the preparation of **203β** enabled delivery of **196** in multi-gram quantities and vastly simplified purification of subsequent compounds. Nucleoside **196** was prepared in 74% yield over two steps from **187**. Nucleoside **197** was prepared in 57% yield over five steps from **187**. Sulfinyl derivative **198** was prepared in 51% overall yield from **187**. Further, the three nucleosides **196**, **197** and **198** were delivered in high purities >95%.

3.3. Synthesis of *arabino* configured analogues of 1′-(4′-thiouridine) and 1′-(4′-thiocytidine)

For the synthesis of 1′-(4′-thio-D-arabinofuranosyl)uracil **200** and cytosine **199**, inversion of the C2′ stereocentre was envisaged *via* an intramolecular cyclisation between the C2-carbonyl on the uracil nucleobase and the C2′-position on the furanosyl ring using standard conditions reported for the synthesis of 2,2′-anhydrouridine (Scheme 42).[117–120] Whilst the mechanism for this reaction is not formally known, no aqueous workup was required for this reaction, which removes the possibility of formation of a cyclic 2′,3′-carbonate and it is proposed that nucleophilic attack by the C2-uracil

carbonyl upon a 2′-carbonate leaving group results in the formation the **206**. As the reacting nucleoside is fully deprotected, in theory, each of the three available hydroxy groups may react with $(PhO)_2CO$. However, aforementioned procedures for the native ribose nucleoside unanimously report yields of 80 - 90% which suggests that only the 2′-positio in **205** can undergo attack by the uracil C2-carbonyl oxygen atom.

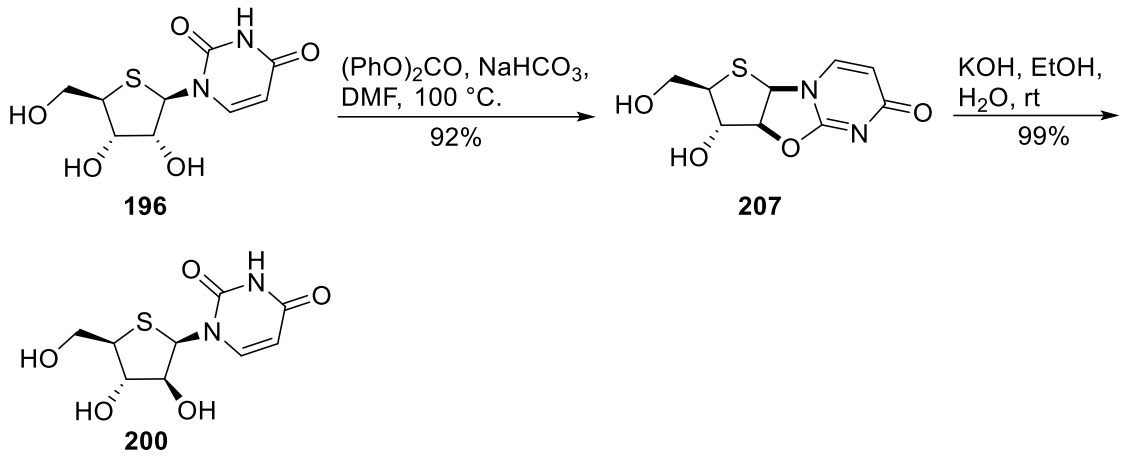


Scheme 42. (a) Standard conditions for the synthesis of 2,2′-anhdyrouridine **206** (b) proposed mechanism for the formation of **206**.

Treatment of **196** with $(PhO)_2CO$ and $NaHCO_3$ at 100 °C in DMF furnished the 2,2′-anhydro derivative **207** (Scheme 43). The reported work up procedures for the native analogue **206** could not be applied to the **207** due to differences in solubility.[121,122] Thus, the DMF was removed from the reaction mixture *in vacuo* and the crude product residue triturated from EtOAc which furnished **207** as a tan solid in excellent yield of 92%, which was consistent on scale-up from 200 mg to 1.5 g.

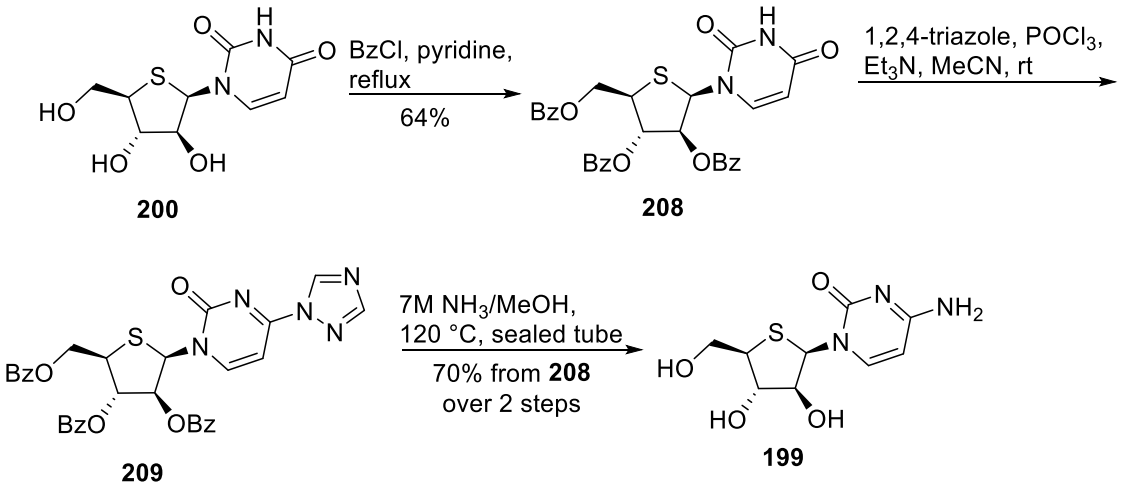
Ring opening of **207** was achieved by stirring with KOH in EtOH and H_2O, resulting in overall stereochemical inversion of the 2′-*O*-position to furnish the *arabino* derivative **200** in quantitative yield. The coupling of H2′ to H3′ for **200** ($J_{H2'-H3'}$ = 9.1 Hz), was significantly larger than that observed

for **196** ($J_{H2'\text{-}H3'}$ = 3.7 Hz), which confirmed inversion of C2′ stereochemistry. Purification of **200** *via* reverse phase flash column chromatography (H_2O) delivered **200** in 97% purity.



Scheme 43. Synthesis of **200**.

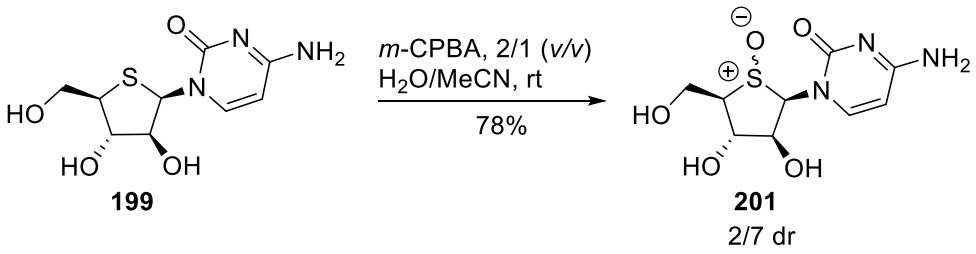
Preparation of the corresponding cytosine derivative **199** was next pursued. Global protection of **200** with benzoyl groups *via* reflux with BzCl in pyridine, afforded **208** in 64% yield. Conversion of the uracil nucleobase to cytosine was then achieved *via* reaction with 1,2,4-triazole, $POCl_3$ and Et_3N to furnish the C4-triazole intermediate **209**, followed by subsequent reaction of **209** with 7M NH_3/MeOH which furnished **199** in 70% yield from **200**. Purification *via* reverse phase flash column chromatography (H_2O) delivered **199** in 98% purity.



Scheme 44. Synthesis of **199**.

Conversion of uracil to cytosine was marked by the small upfield shift of C4 from δ_C 166.1 ppm in **200** to 165.8 ppm in **199**. HRMS further confirmed the preparation of **199** with *m/z* 260.0702 corresponding to the [**199**+H]$^+$ species.

Finally, reaction of **199** with *m*-CPBA in 2/1 (*v*/*v*) H_2O/MeCN solution at rt furnished the corresponding sulfoxide **201** in 78% yield in 7/2 dr at S (Scheme 45). An analytically pure (>95% purity) sample was obtained *via* crystallisation from a minimum of hot H_2O in 10% overall yield in a 5/2 dr.



Scheme 45. Synthesis of **201**.

The increased electron-withdrawing effects of the sulfinyl group caused a significant downfield shift of C1′ and C4′ from δ_C 59.8 and 49.1 ppm in **199** to δ_C 82.4 and 75.1 ppm in **201**. HRMS displayed m/z of 298.0469 corresponding to the [**201**+Na]$^+$ species. The dr of **201** was considerably different to that of sulfoxide **198** (1.1/1 dr at S; see Chapter 3, Section 3.2), which suggested that the *arabino* configuration at C2′ may direct the formation of one diastereoisomer over the other. It was postulated that due to increased steric bulk on the β-face, there was a preference for the sulfinyl oxygen to occupy the α-face. Attempted growth of a crystal of **201** to obtain a single diastereoisomer which may then have been analysed by X-ray crystallography were unsuccessful in numerous solvents (H_2O, H_2O/acetone, MeOH), and thus the stereochemistry of the major sulfinyl diastereoisomer was not identified.

3.4. Conclusions

Overall, **200**, **199** and **201** were successfully prepared from **196** *via* high yielding methods. The C2′ stereochemistry was inverted from the *ribo* configuration to the *arabino* configuration in two simple steps from **196** to deliver **200** and the uracil nucleobase subsequently

converted to cytosine to deliver **199**. The sulfinyl derivative **201** was then accessed *via* oxidation of **199**. Further, the three nucleosides **200**, **199** and **201** were delivered in high purities >95%.

Chapter 4: Studies towards the synthesis of 2′-α-flouro-1′-(4′-thio)uracil nucleoside analogues

4.1. Aims and rationale

2′-Deoxy-2′-fluoro cytidine (FdC) **210** (Figure 22) is a potent inhibitor of Crimean-Congo haemorrhagic fever virus (CCHFV) with nanomolar EC_{50} values against CCHFV (61 nM) and CCHFV/ZsG (31 nM) in Huh7 cells.[123] Moreover, it has shown excellent inhibitory activity against HCV with an EC_{90} value of 5.0 μM against HCV-replicon RNA-containing Huh-7 cells.[124,125]

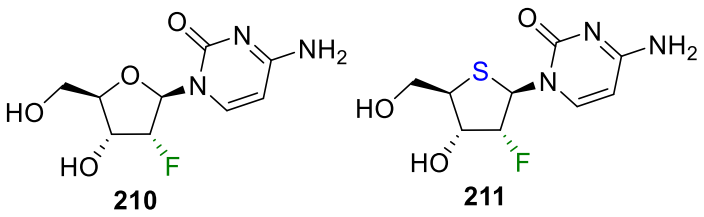
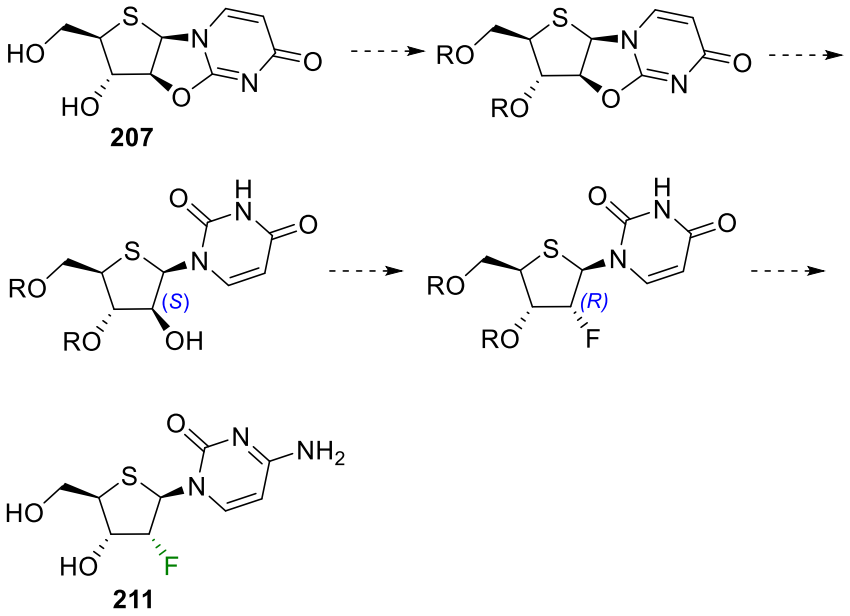


Figure 22. Structures of FdC **210** and the corresponding thionucleoside analogue **211**.

The thionucleoside analogue of FdC, **211**, has been synthesised by Takahashi and colleagues,[126] however, no antiviral activity has been reported to date.[126] Takahashi employed forcing conditions (HF, 125 °C in a steel bomb) to install the 2′-fluoro group. Thus, a novel and milder synthesis of **211** was pursued herein, with the aim to evaluate its antiviral activity and compare it to that of FdC. A DAST fluorination strategy was envisioned for the synthesis of **211**. To obtain the desired 2′-(*R*) stereochemistry in the product, stereochemical inversion of the C2′ hydroxyl group to the *arabino* configuration was required, and suitable protection of the 3′,5-hydroxy groups with base stable protecting groups would be necessary to avoid reaction with DAST at these positions (Scheme 46).

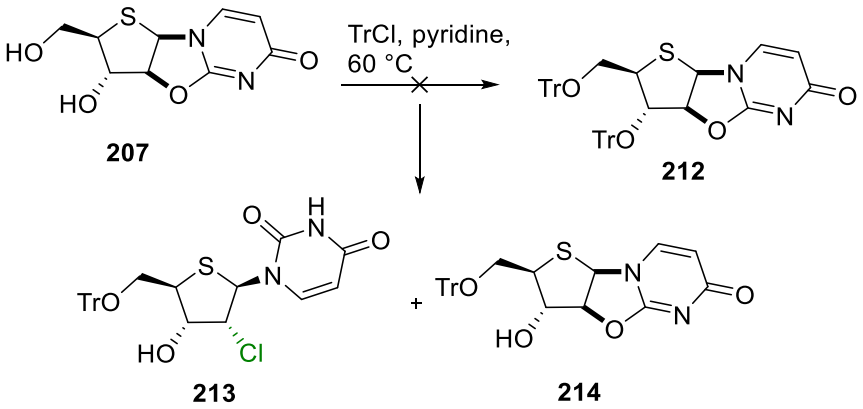


Scheme 46. Proposed strategy for the synthesis of **211**. R = suitable protecting group.

Aerschot and Herdewijn accessed **210** using trityl protecting groups for the 3′,5′-hydroxyl groups.[127,128] Interestingly, the Aerschot and Herdewijn reported that the 2′-chlorinated derivative was the major product (48%) of the tritylation reaction, which they propose is by nucleophilic attack of pyridine hydrochloride on the 2′-position of the 2,2′-anhydro bond. Thus, this protecting group strategy was applied to **207**, with the potential preparation of a 2′-chlorinated derivative considered a positive outcome as it may also be evaluated for antiviral and anticancer properties.

4.2. Synthesis

Reaction of **207** with TrCl in pyridine did not furnish the desired **212** and instead resulted in the formation of two products **213** and **214** (Scheme 47).

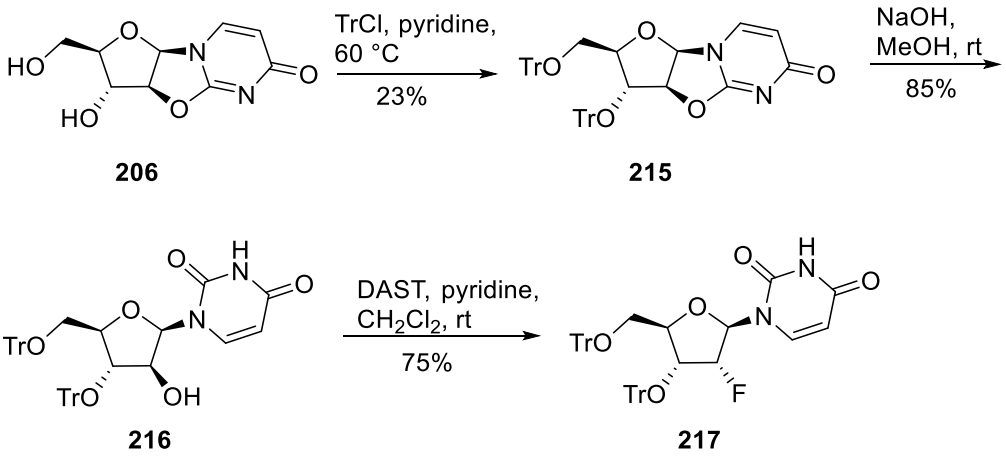


Scheme 47. Products **215** and **214** prepared from reaction of **207** with trityl chloride.

Nucleoside **213** was the major product, obtained in 34% yield. HRMS confirmed incorporation of chlorine with m/z 543.1127 corresponding to the [**213**+Na]$^+$ species. NMR analysis showed a significant downfield shift of the H2′ peak to δ_H 5.43 ppm due to the presence of the electron withdrawing Cl substituent, and a significant upfield shift of the C2′ peak to δ_C 66.2 ppm in the region typical for a C-Cl carbon. A small quantity of **214** (2.3% yield) was isolated, with one trityl group installed, which was expected to be on the less sterically hindered and more reactive primary 5′-hydroxyl group. The significant upfield shift of the H5′a and H5′b protons to δ_H 3.51 – 3.44 ppm supports the 5′-regiochemistry of the trityl group.

The addition of chloride to the 2′-position rather than at the 2-position in **217** may be accounted for as chloride is a softer nucleophile than hydroxide, which attacked at C2 in the synthesis of **200**. The 2-position is electron dense due to conjugation with the aromatic base and may therefore be a harder electrophile than the sp^3 hybridised 2′-position, and thus chloride attacked at C2′ to furnish the α-chloro nucleoside **213**.

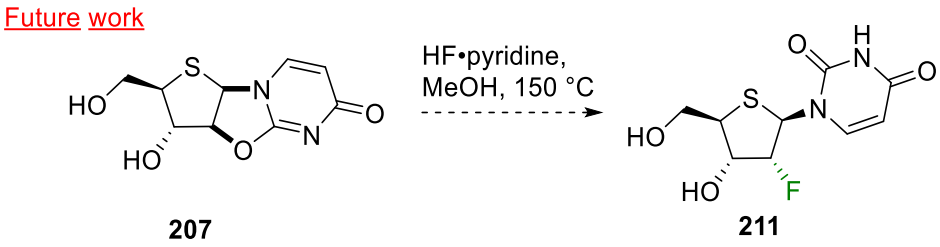
Repeated attempts to prepare **212** were unsuccessful, therefore, a control series of test reactions were run on the native 2,2′-anhydrouridine substrate **206**. Thus, **206** was treated with TrCl, obtaining exclusively **215** in 23% yield (Scheme 48), with recovery of starting material (61%). Contrary, to findings by Aerschot and Herdewijn, the 2′-chlorinated derivative was not prepared, perhaps due to carrying out the reaction at a lower temperature of 60 °C. Subsequent ring opening of **215** *via* treatment with NaOH provided **216** in 85% yield. Nucleoside **216** was then fluorinated at the 2′-position using DAST which furnished **217** in 75%. NMR analysis confirmed the incorporation of fluorine, with an ^{19}F NMR signal δ_F -200 ppm, and subsequent splitting of the anomeric H1′ ^{1}H NMR signal into a dd with $J_{H1'\text{-}F}$ = 14.5 Hz. Further, a smaller H2′-H3′ coupling was observed with $J_{H2'\text{-}H3'}$ = 3.1 Hz, which confirmed the inversion of the C2′ stereochemistry.



Scheme 48. Synthesis of **217**.

As the tritylation strategy to prepare a 2′-fluorinated derivative worked well on the native substrate **206**, it was concluded that the thionucleoside substrate **207** was the issue rather than the reaction conditions. The large size of the sulfur atom in the ring may have caused significant enough steric hindrance, such that installation of two trityl groups was highly unfavoured, even at higher temperatures. The requirement for the protecting groups to be base stable, due to the subsequent hydroxide-mediated opening of the 2,2′-anhydronucleoside to the *arabino* nucleoside and stable under DAST fluorination conditions, however, created necessity for the use of alkyl protecting groups.

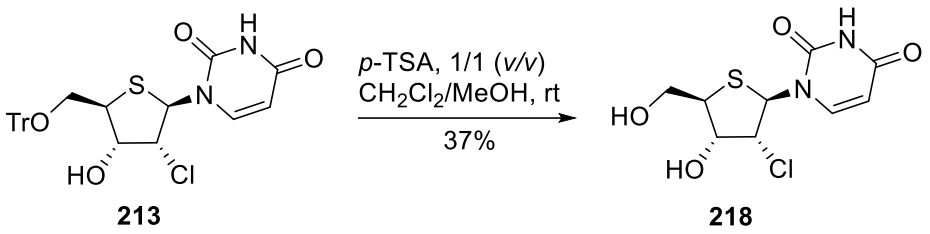
Future work for the synthesis of **211**, would involve the use of highly forcing conditions to install the 2′-fluoro group, as the milder protecting group strategy proved unsuccessful. Conditions reported by Takahashi *et al*,[126] could be trialled, whereby **211** was synthesised *via* reaction of **207** with HF·pyridine at 150 °C in a steel bomb (Scheme 49).



Scheme 49. Future work for the synthesis of **211**.

The 2′-chloro derivative **213** was deprotected *via* treatment with *p*-TSA in 1/1 (*v*/*v*) CH_2Cl_2/MeOH solution to obtain **218** in low yields of 37% due to incomplete deprotection (Scheme 50). Purification *via* reverse phase flash chromatography furnished **218** in 98% purity. HRMS

confirmed the presence of the chlorine with m/z 301.0025 corresponding to the [**218**+Na]$^+$ species (Figure 23).



Scheme 50. Synthesis of **218**.

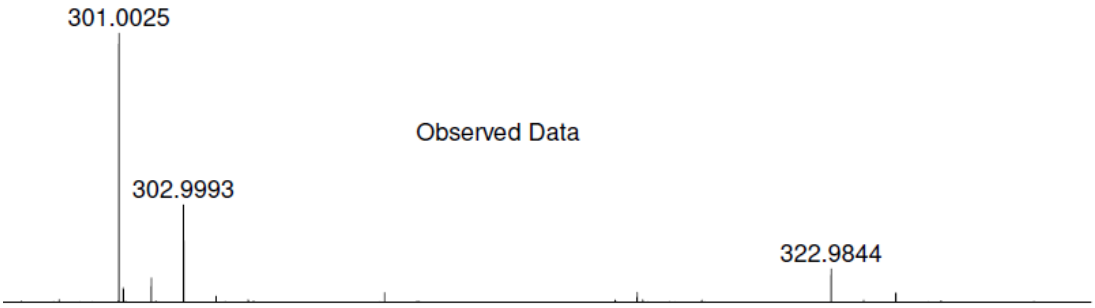


Figure 23. HRMS of **218** with observed chlorine isotope pattern.

The α-chloro configuration at C2′ was assigned *via* NMR analysis. Notably, the vicinal H1′-H2′ coupling was large, with $J_{H1'-H2'}$ = 7.8 Hz indicated a 1,2-*trans* coupling. Similarly, the vicinal H2′-H3′ coupling was small, with $J_{H2'-H3'}$ = 3.3 Hz, which indicated a 1,2-*cis* coupling. Further confirmation was sought *via* Nuclear Overhauser Effect (NOE) experiment analysis. Irradiation of H2′ (Figure 24) showed through space correlation H2′ to H1′, H3′ and H6. The through space correlation of H2′ to H3′ suggested that the two protons are both on the β-face, such that H2′,H3′ are in a 1,2-*cis* relationship. This was further confirmed by the absence of through space correlation of H2′ to H4′.

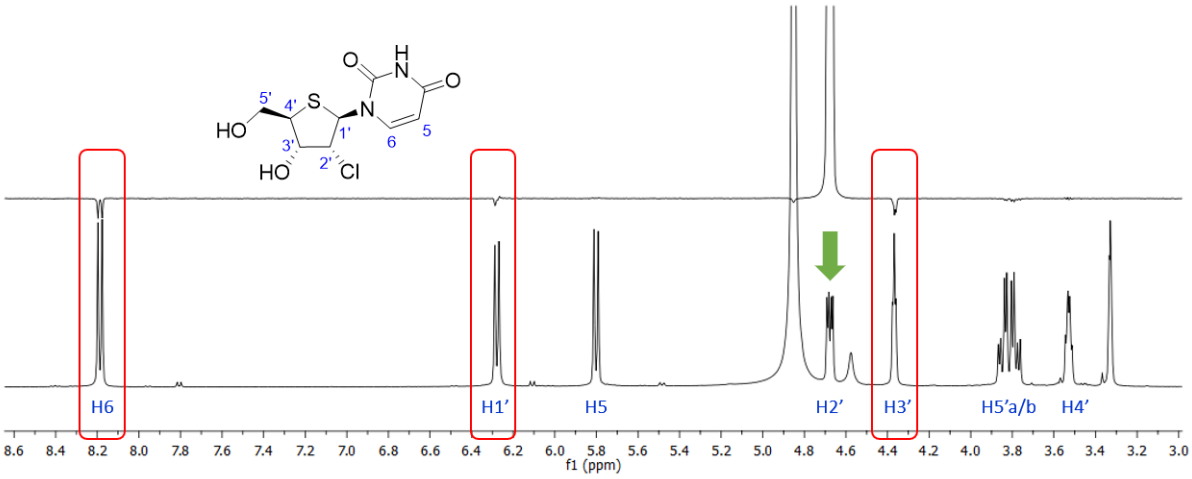


Figure 24. NOE of **218** with H2′ irradiated overlayed with the corresponding ^{1}H NMR (400 MHz, D_2O).

4.3. Conclusions and future work

Overall, attempts to synthesise **211** *via* a 3′,5′-*O*-tritylation and 2′-DAST fluorination strategy was unsuccessful. Control reactions on the native uridine counterparts indicated that the poor yields and formation of side products were due to inclusion of sulfur into the nucleoside scaffold. Nonetheless, the 2′-chlorinated thionucleoside **218** was prepared *via* serendipity and delivered in high purity >95%.

Chapter 5: Studies towards the synthesis of 4′-substituted 1′-(4′-thio) nucleosides

5.1. Aims and rationale

Syntheses of 4′-substituted nucleoside analogues are well documented in the literature, and these analogues are known to have interesting antiviral activities.[101,129–135] Access to 4′-position functionalised thionucleosides was envisioned *via* a late-stage functionalisation approach, such that multiple 4′-modified analogues **219** and **220** (Figure 25) could be synthesised.

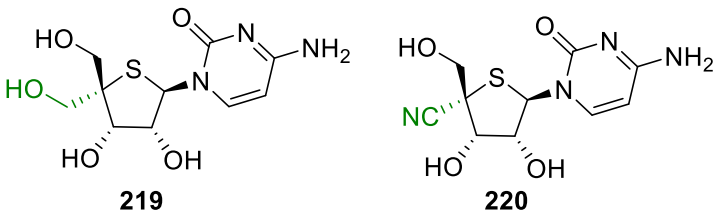
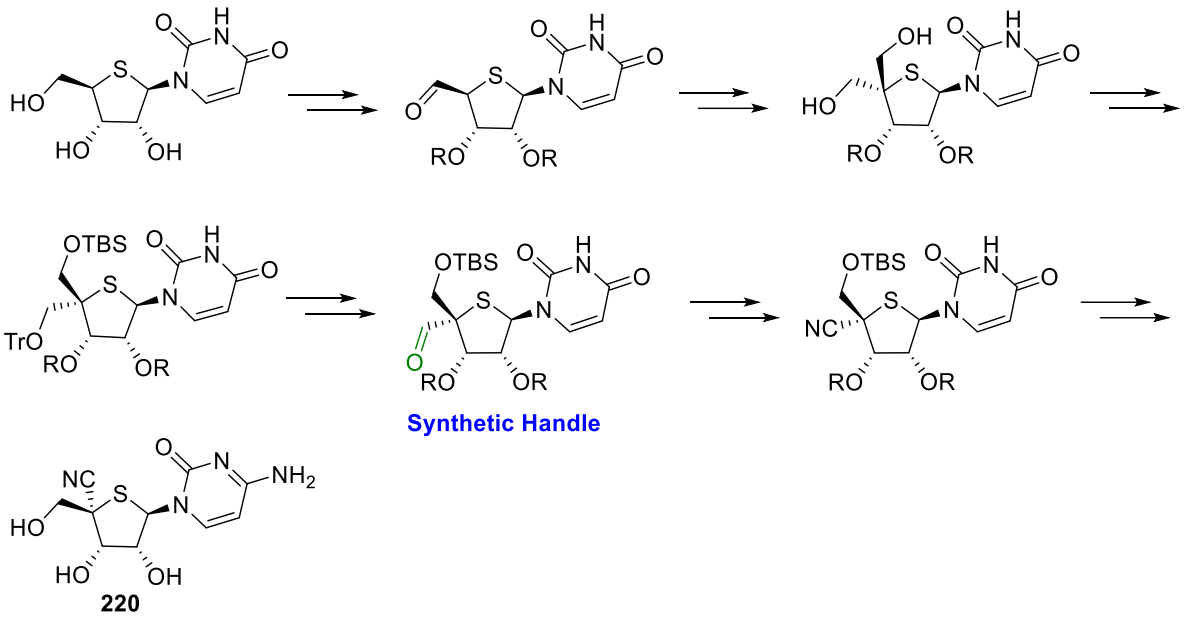


Figure 25. Proposed 4′-thionucleoside analogue target structures **219** and **220**.

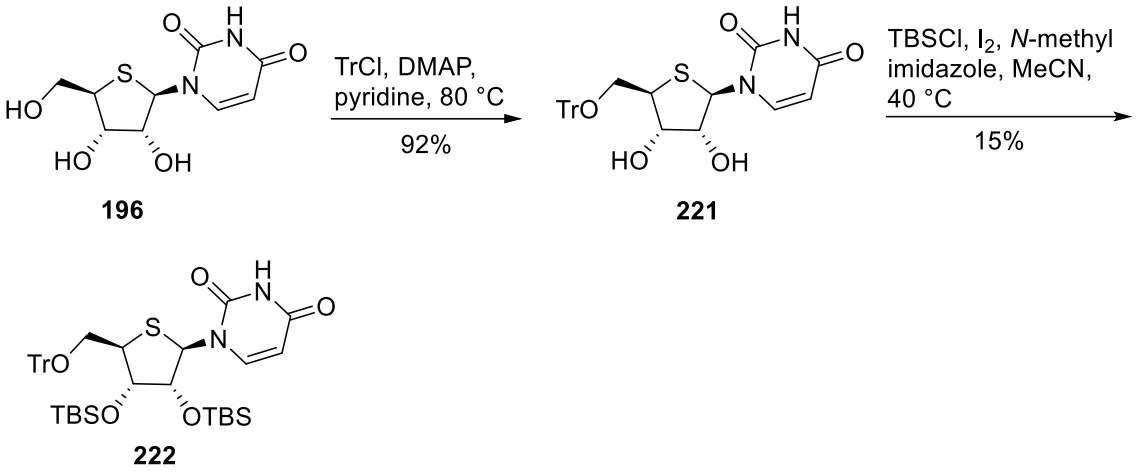
These analogues could be accessed through common synthetic intermediate, which would act as a synthetic handle for installation of various functionalities. In this chapter, similar synthetic methodology employed for the synthesis of 4'-modified analogues of native 4'-oxo nucleosides developed by Nomura and colleagues[101] was mapped onto 4'-thionucleoside analogues (Scheme 51).



Scheme 51. Proposed synthetic route to **220**.

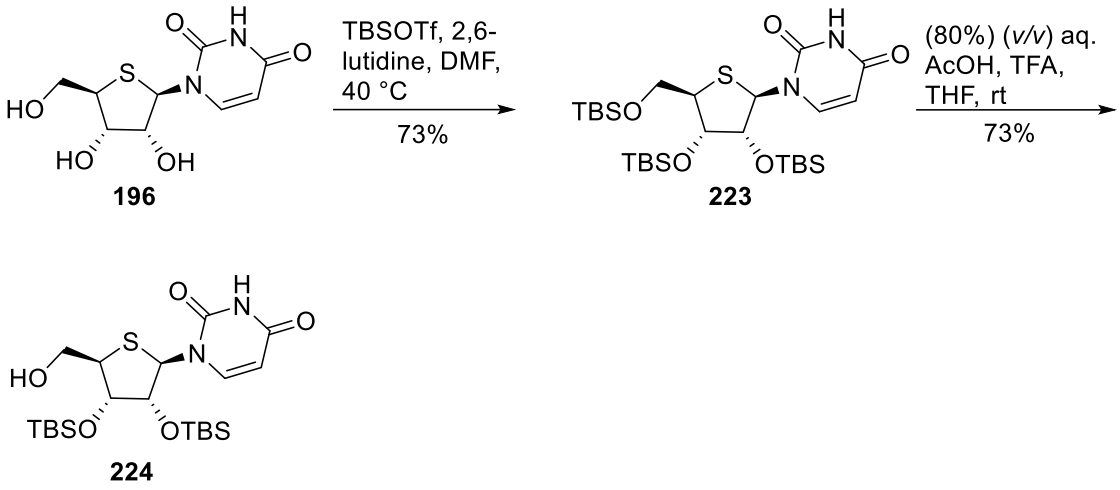
5.2. Synthesis of key 4′-*gem*-*bis*-diol 1′-(4′-thio) nucleoside intermediate

A series of protecting group manipulations were evaluated to prepare **222**. Starting with **196**, tritylation of the primary alcohol was achieved *via* reaction with TrCl, DMAP and pyridine at 80 °C to obtain **221** in 92% yield.[132] Nucleoside **221** was then heated to reflux with TBSCl and *N*-methylimidazole to install TBS groups at the 2′- and 3′- hydroxyl groups. However, this reaction did not go to completion, with very little conversion to **222** after 48 h (15% yield). Thus, iodine was employed to speed up the reaction,[130] however, it was found that the acid-labile 5′-trityl group was also removed during the reaction, which resulted in a complex mixture of various protected products. Further, the presence of the bulky trityl group appeared to hinder the installation of bulky TBS groups, and thus this protecting group strategy was deemed unsuitable.



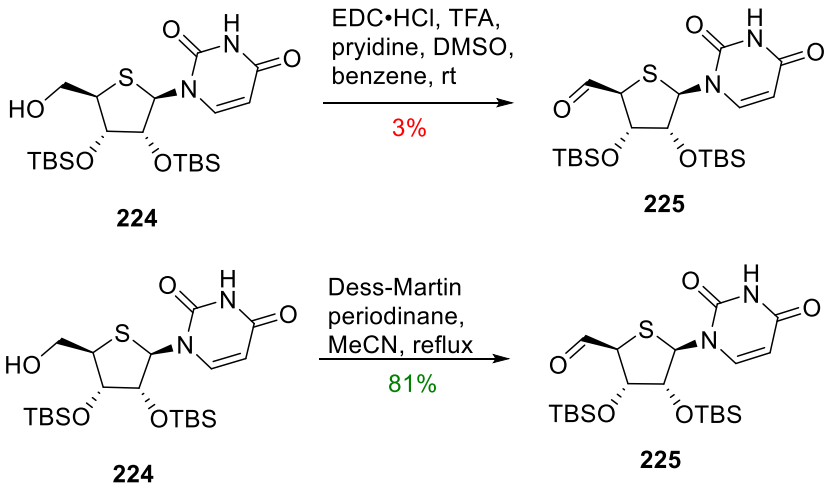
Scheme 52. Synthesis of **222**.

A more direct approach involved global protection of the 2′,3′ and 5′-*O*-positions with TBS groups, followed by selective removal of the primary 5′-TBS group (Scheme 53). The conditions reported by Bartoszewicz for silylation of nucleosides were followed,[130] which furnished **223** in 68% yield. However, on scale up from 50 mg to 700 mg a significant reduction in yield from 68% to 26% was observed as the reaction did not go to completion after 72 h. Heating the reaction to reflux or increasing the equivalents of reagents from 3.3 to 6 mol equivalents did not drive the reaction to completion. Thus, a stronger electrophile, TBSOTf, was employed, which improved the yield of **223** to 72% which was consistent on scale up to 4 g. Selective deprotection of the 5′-hydroxyl group was achieved *via* treatment of **223** with 80% AcOH and TFA which furnished **224** in 73% yield. This deprotection had excellent regioselectivity, and could be left stirring overnight with no reduction in yield.



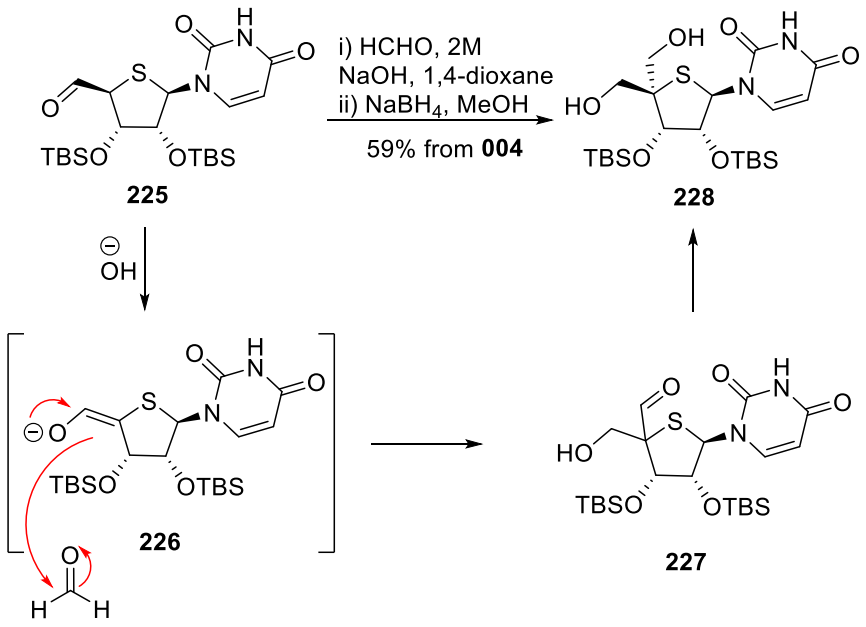
Scheme 53. Synthesis of **224**.

Following the synthetic strategy employed by Normura *et al.* on the native nucleoside substrate,[101] oxidation of the 5′-hydroxyl group was first attempted using a variation of the Pfitzner-Moffatt oxidation whereby EDC is used instead of DCC.[101] The EDC-derived urea which forms as a side product of the reaction is water soluble, and thus simplifies the purification process in comparision to the organic-soluble DCC urea which is troublesome to remove from the product without purification on silica gel. However, the reaction did not go to completion after 2 h, obtaining **225** in extremely low yields of 3% with revcovery of **224** (90%) (Scheme 54). Oxidation was more readily achieved using Dess-Martin periodinane. Reaction in CH_2Cl_2 did not occur at room temperature, and thus MeCN was used as solvent and the reaction heated to reflux which resulted in complete conversion to the desired 4′-formyl product **225** in 81% yield.



Scheme 54. Synthesis of **225**

Following this, a methylhydroxyl group was installed at the 4′-position *via* reaction of crude product **225** with 2M NaOH and HCHO to prepare the corresponding enolate **226** *in situ* which then reacted with HCHO to furnish **227** (Scheme 55). Upon scale up to 1.5 g, an insoluble gummy residue formed at workup which reduced the recovery of material. This was overcome by working up the crude product reaction mixture without evaporation of the reaction solvent 1,4-dioxane to aid solubility during the workup, and copious extractions of the aqueous layer with 1/9 (*v/v*) IPA/$CHCl_3$ solution. Intermediate **227** was highly unstable, and could not be purified on silica gel, thus, it was used crude product and immediately reacted with $NaBH_4$ in MeOH afforded the *gem*-diol **228** in a high yield of 59% over the three steps (from **224**).



Scheme 55. Synthesis of **228** *via* **227**.

Installation of the CH_2OH group was confirmed by NMR analysis, with the disappearance of the aldehyde H5′ peak at δ_H 9.64 ppm seen in **225** and two CH_2 peaks observable by HSQC-DEPT in the ^{13}C NMR of **228** at δ_C 64.5 and 63.2 ppm (Figure 26). Moreover, the disappearance of a H4′ peak in the ^1H NMR and change in the multiplicity of H3′ from an apparent t at δ_H 4.30 ppm in **225** to a doublet at δ_H 4.27 ppm in **228** further confirmed C4′ to be quarternary as H3′ only shows coupling to H2′ ($J_{H3'\text{-}H2'}$ = 3.0 Hz).

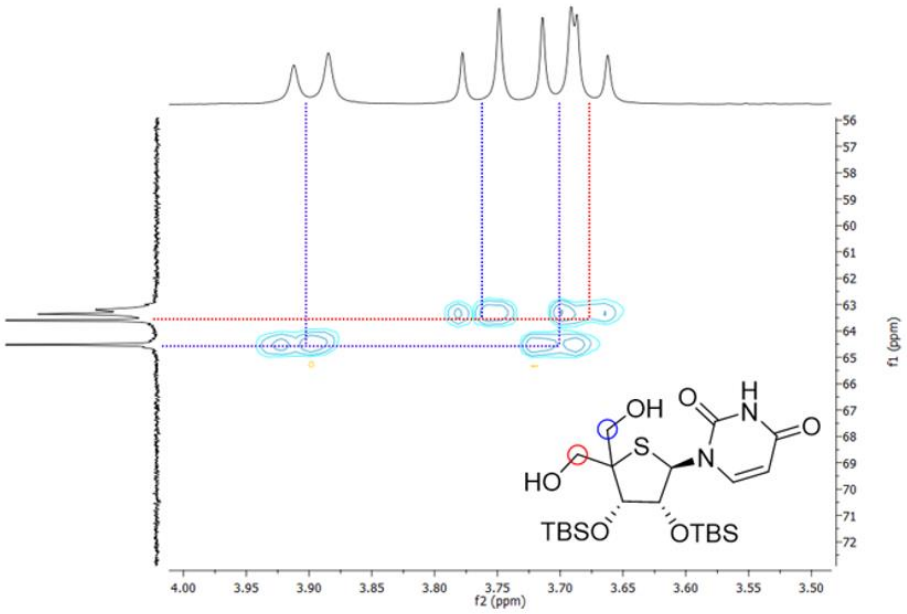
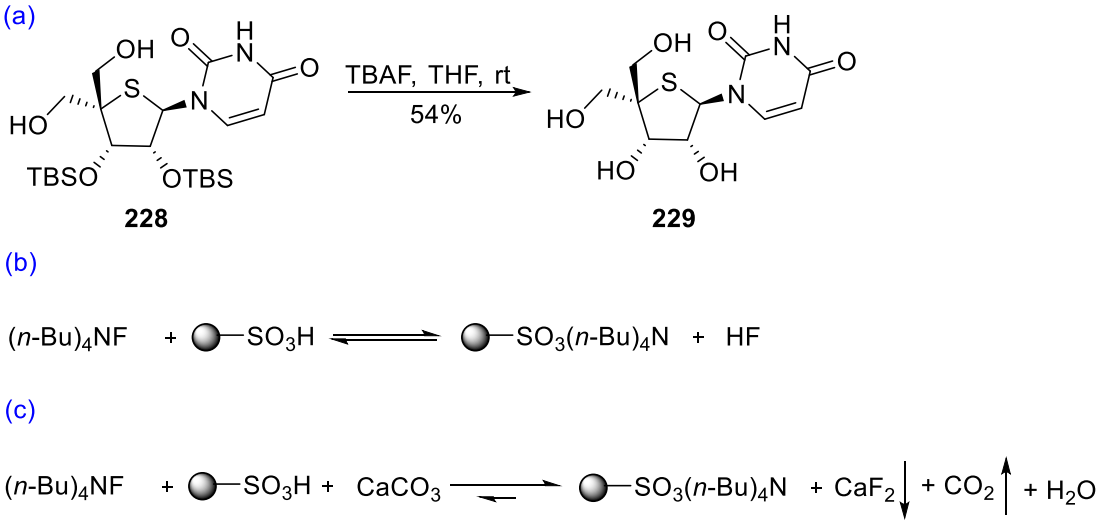


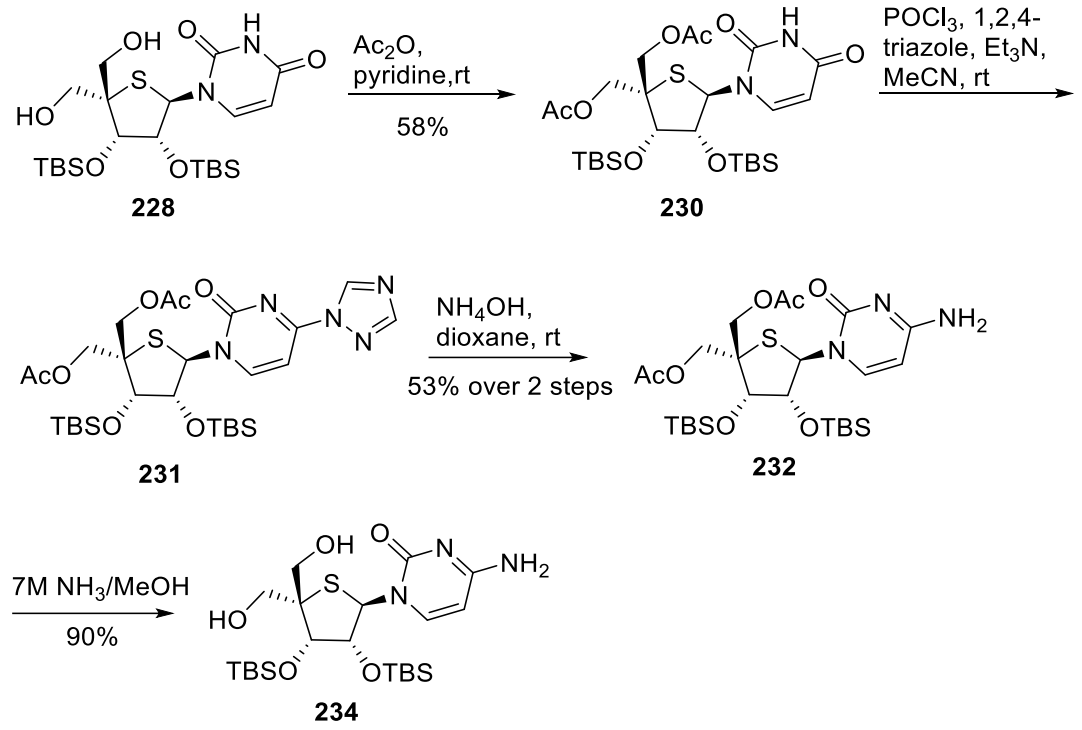
Figure 26. HSQC-DEPT of **228** with the C5′ and C6′ CH_2 signals highlighted. Red contours indicate CH or CH_3, blue contours indicate CH_2.

Deprotection of **228** using TBAF (Scheme 56, a), afforded **229** as an unknown *tert*-butylammonium salt, despite stirring a solution the nucleoside with amberlyst sulfonic acid resin. Kaburagi and Kishi developed a workup procedure to effectively remove TBAF salts from water-soluble compounds.[136] Use of $CaCO_3$ with sulfonic acid resin results in the formation of an insoluble CaF_2 precipitate, which drives the equilibrium to the right and therefore maximises TBAF removal from a sample (Scheme 56, b and c). Thus, the crude product **229** was treated with amberlyst sulfonic acid resin and $CaCO_3$, which successfully removed the TBAF salt from the product, furnishing **229** in 54% yield.



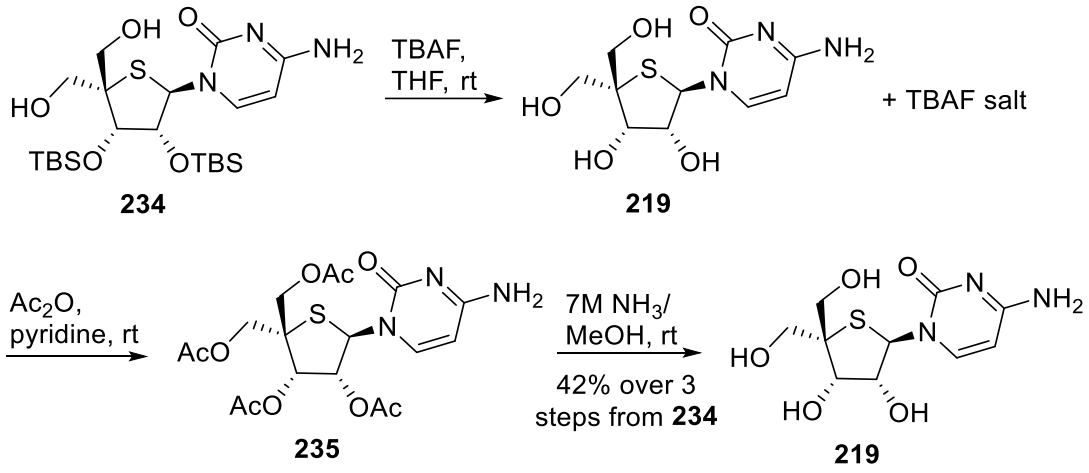
Scheme 56. a) Synthesis of **229**; b) Equilibrium of sulfonic acid ion exchange resin with TBAF; c) Equilibrium of sulfonic acid ion exchange resin with TBAF and $CaCO_3$.

To convert the uracil nucleobase to cytosine, **228** was first acetylated at the 5′- and 6′-hydroxyl groups to obtain **230** (Scheme 57) in 58% yield which was subsequently converted to the cytidine analogue **232** *via* C4-triazole **231** by reaction with $POCl_3$, 1,2,4-triazole and Et_3N, to furnish **231** followed by NH_4OH, which furnished **232** in 54% yield from **230**. Interestingly, the NH_4OH did not cleave the two acetyl groups, with the formation of one product observed by TLC analysis of the reaction, which indicated that the C4 position was significantly more electrophilic than the acetyl carbonyl positions. Thus, the acetyl groups were removed *via* treatment of **232** with 7M NH_3/MeOH solution which delivered **234** in excellent yield of 90%.



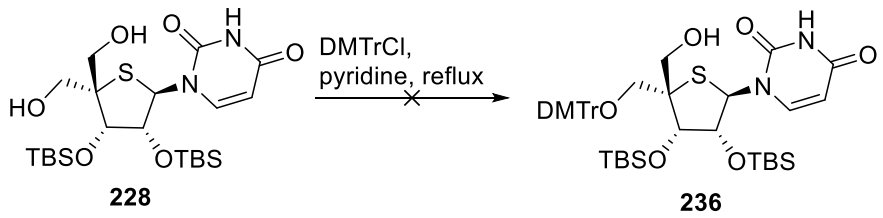
Scheme 57. Synthesis of **234**.

Finally, the TBS groups were removed using TBAF which furnished **219** (Scheme 58). Repeated treatment of the crude product **219** with Amberlyst sulfonic acid resin and $CaCO_3$ removed ~85% of the TBAF salts (by ^1H NMR integration) and attempts to remove the remaining salts *via* column chromatography or desalting columns were unsuccessful. Thus, **219** was acetylated *via* reaction with Ac_2O and pyridine to furnish **235** and the TBAF salts removed during aqueous workup. Subsequent deprotection using 7M NH_3/MeOH solution furnished **219** in 42% yield from **234**.



Scheme 58. Synthesis of **219**.

Nomura's synthesis of 4′-subsituted nucleoside analogues used dimethoxytrityl to regioselectively protect the 4′-α-methylhydroxy group.[101] Thus, the synthesis of **236** was pursued *via* treatment of **228** with dimethoxytrityl chloride (DMTrCl) (1 – 4 equivalents) in pyridine at reflux (Scheme 59), however, no reaction occurred and **228** was recovered. This suggested that **228** was very sterically hindered, due to the presence of two bulky TBS groups at the 2′- and 3′- positions, and the large sulfur atom in the ribofuranosyl ring. Attempted selective protection of either the α- or β- 4′-methylhydroxyl group with trityl (0.5 – 1 equivalents TBSCl, 0 °C) resulted in the formation of a complex mixture of inseparable products. Thus, these protecting groups were deemed unsuitable for regioselective protection of one 4′-methylhydroxy group.

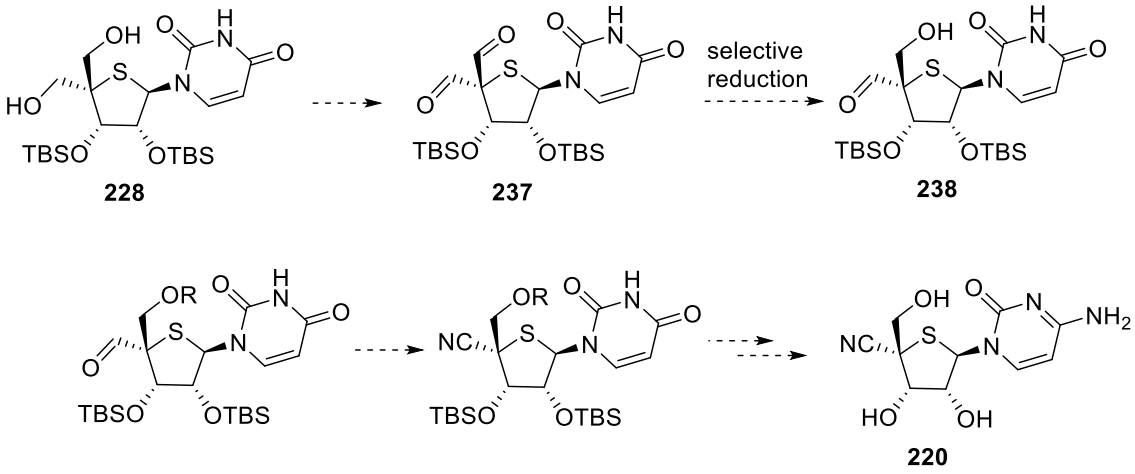


Scheme 59. Attempted synthesis of **236**.

5.3. Conclusions and future work

Overall, the key synthetic intermediate 4′-*gem*-*bis*-methylhydroxy 1′-(4′-)thio nucleoside analogue **229** was prepared *via* novel methodology in 31% yield over five steps, which may be used as a synthetic handle to synthesise corresponding 4′-subtituted analogues. The corresponding cytosine analogue **219** was easily prepared from intermediate **229** and delivered in excellent purity >95%. Further work is required to complete the synthesis, in particular development of a suitable method to prepare intermediate **238** the substrate for nucleophilic substitution to prepare **220**.

A method for the synthesis of **220** was not developed due to regioselectivity issues with selective protection of one of the 4′-methylhydroxyl groups. Future work could include trialling the use of monomethoxy trityl (MMTr) to selectively protect the more hindered 4′-methylhydroxyl group, as MMTr is less bulky than DMTr, but more so than a trityl group. Alternatively, oxidation of both primary alcohol groups in **228** and subsequent evaluation of a panel of bulky reducing agents could be pursued to explore selective reduction of the less-hindered aldehyde (Scheme 60).



Scheme 60. Future work for the synthesis of **220**.

Chapter 6: Synthesis of 1′-(4′-thio) nucleoside analogues of Sofosbuvir

6.1. Aims and rationale

Sofosbuvir **39** is a potent antiviral drug which is used for the treatment of HCV, and syntheses of its uracil **52** and cytosine **53** derivatives are well documented in the literature (as discussed in Chapter 1, section 1.3.3.).[49,51,137–139] However, to date there are no reported syntheses of thionucleoside analogues of sofosbuvir and its derivatives. Thus, there is novel chemical space to be explored in this area. In this section, synthetic efforts to prepare the thioribose analogue **239** and thionucleoside analogues **240** – **244** (Figure 27) are described.

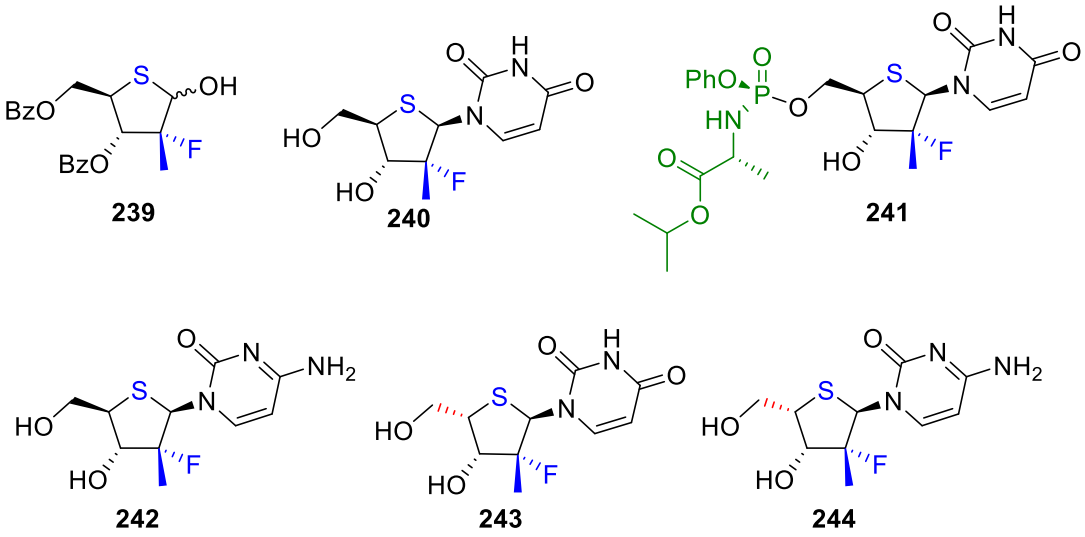


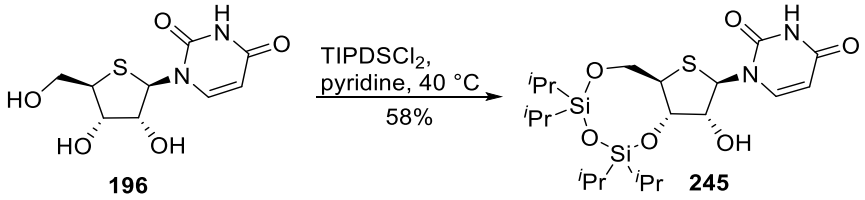
Figure 27. Proposed 2′-deoxy-2′-fluoro-2′-*C*-methyl synthetic targets **239** – **244**.

6.2. Studies towards a late-stage functionalisation approach to prepare 2′-fluoro-2′-*C*-methyl 1′-(4′-thio) nucleoside analogues

It was envisioned that with **196** in hand, a late-stage modification approach could be pursued *via* installation of the 2′-fluoro-2′-*C*-methyl group onto the thionucleoside scaffold. Clark and colleagues prepared 2′-deoxy-2′-fluoro-2′-*C*-methyl cytidine **53** *via* a 2′-ketone intermediate and subsequent 2′-methylation and 2′-fluorination.[49] Thus, this synthetic strategy was applied to **196**.

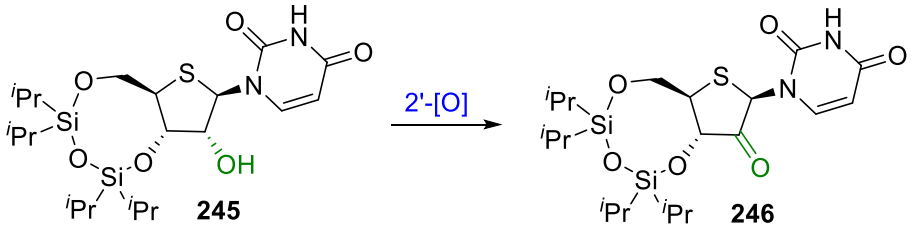
6.2.1. Synthetic studies towards late-stage installation of the 2′-fluoro-2′-*C*-methyl group

Starting from **196**, the nucleoside was 3′,5′-*O*-protected with a TIPDS group (Scheme 61). Reaction of **196** with $TIPDSCl_2$ in pyridine at rt afforded **245** in a moderate yield of 44% after 3 days.[140] The yield was improved to 58% with heating to 40 °C, which was consistent on scale-up from 250 mg to 1 g. Further heating above 40 °C did not improve the yield or reaction time. ^{1}H NMR analysis showed an OH δ_H 3.54 ppm coupling to H2′ ($J_{OH\text{-}H2'}$ = 8.7 Hz) which indicated the 2′-hydroxyl group was free while the 3′ and 5′ hydroxyl groups were protected.



Scheme 61. Synthesis of **245**.

Following this, various conditions for 2′-oxidation were evaluated and the results of these efforts are shown in Table 5.



Entry	Reagents	Solvent	Temperature	Result
1	Dess-Martin periodinane	CH_2Cl_2	rt	No reaction
2	Dess-Martin periodinane	MeCN	reflux	No reaction
3	TEMPO, BAIB	CH_2Cl_2	rt	No reaction
4	CrO_3, Ac_2O	Pyridine	rt	No reaction
5	TFAA, DMSO	THF	−78 °C	No reaction
6	TFAA, DMSO	THF	−15 °C – rt	No reaction

7	Ac_2O, DMSO	DMSO	rt	75% yield

Table 5. Reagents and conditions trialled for 2′-oxidation of **245** to prepare **246**.

Treatment of **245** with Dess-Martin periodinane (1.5 equiv.) in CH_2Cl_2 or MeCN at rt (Entry 1 and 2, Table 5)[141] did not result in any **246** formation, nor did TEMPO-mediated oxidation (Entry 2, Table 5) and the starting material **245** was recovered. Hansske *et al.* achieved 2′-oxidation of the native substrate using Cr(VI) *via* reaction of the nucleoside with CrO_3, Ac_2O and pyridine (Entry 4, Table 5),[142] however, no reaction occurred under these conditions. Applying Swern-type activated DMSO oxidation conditions (Table 5, Entries 5 – 6)[143] to **245** also proved unsuccessful. The TIPDS protecting group in **245** bears bulky *iso*propyl groups and sulfur has a larger atomic radius than that of oxygen, which may sterically hinder oxidation at the 2′-position. Albright-Goldman oxidation has been used to oxidise sterically hindered secondary alcohols, such as those in steroids *via* reaction of the alcohol with DMSO and acetic anhydride at rt,[144] thus, these conditions were trialled (Table 5, Entry 7), which successfully furnished **246** in 75% yield, which was consistent on scale-up to 2 g.

In the ^{1}H NMR spectrum, the loss of H2′ is evident as H1′ becomes a singlet at δ_H 5.84 ppm and H3′ becomes a doublet at δ_H 4.61 ppm, only coupling to H4′. Further, the characteristic C2′ ketone peak was observed at δ_C 201.1 ppm in the ^{13}C NMR spectrum (Figure 28).

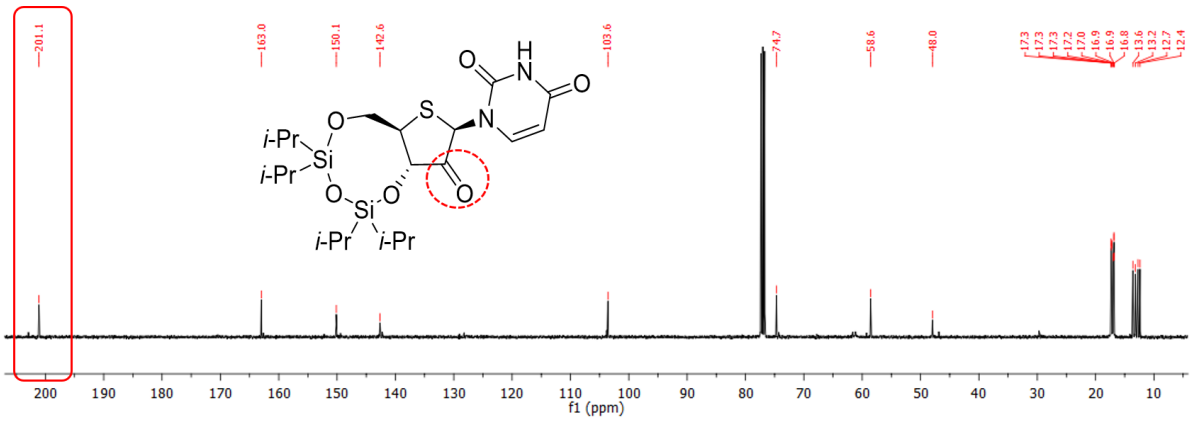
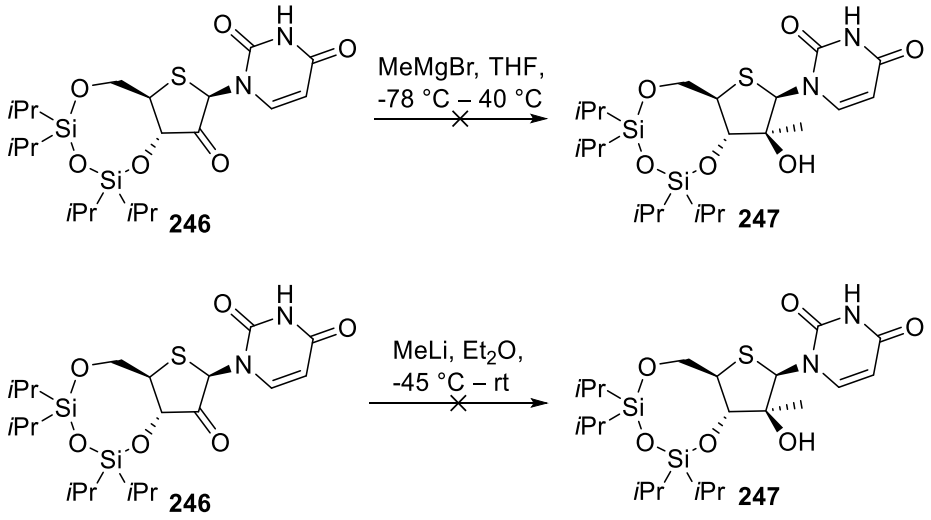


Figure 28. ^{13}C NMR ($CDCl_3$, 101 MHz) spectrum of **246**.

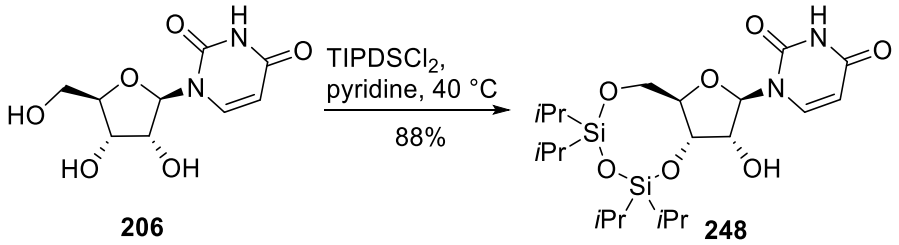
Stereoselective methylation of the 2′-ketone to prepare **247** was first attempted *via* reaction of **246** with MeMgBr (Scheme 62),[145] however, no reaction was observed over a range of temperatures (−78 °C, −45 °C, −10 °C, rt, 40 °C) using 1.5 equivalents of MeMgBr. Increasing the equivalents of MeMgBr to 5 equivalents did not drive the reaction, and long periods at rt or higher temperatures

resulted in decomposition of **246**. Next, a harder nucleophile, MeLi was trialled as a methylating agent.[46,146] Using 1.5 equivalents of MeLi over a range of temperatures (–45 °C, –10 °C, rt) did not result in any reaction, and **246** was recovered. Similarly, increasing the equivalents of MeLi to 2 did not drive reaction, and use of more than 3 equivalents resulted in decomposition at temperatures higher than -45 °C, as observed by consumption of the starting material and formation of a highly polar compound (R_f = 0, 5/95 MeOH/CH_2Cl_2).



Scheme 62. Attempted 2′-methylation of **246** using MeMgBr or MeLi.

A series of control reactions using uridine were pursued to compare the reactivity of a native nucleoside 1′-(4′-oxo) substrate to that of **246**. Uridine **206** was 3′,5′-protected with TIPDS[48] to obtain **248** in 88% yield.



Scheme 63. Synthesis of **248**.

Reaction of **248** under Albright Goldman oxidation conditions resulted in complete conversion of the starting material (R_f 0.30, 3/7 acetone/toluene) to a less polar product (R_f = 0.61, 3/7 acetone/toluene). However, no ketone peak was observed in the region of δ_C ~200 ppm in the ^{13}C

NMR spectrum of the product and H1′ and H3′ were observed to couple to H2′, which indicated no oxidation had occurred. Further analysis indicated the formation of a 2′-thioacetal product **249**. The methylene CH_2 of **249** was evident in the ^1H NMR spectrum (Figure 29), appearing as a singlet at δ_H 4.98 ppm and in the ^{13}C NMR spectrum at δ_C 74.2 ppm (see HSQC-DEPT in Figure 30). Moreover, the terminal thio-methyl CH_3 was evident as a singlet at δ_H 2.15 and at δ_C 12.9 ppm. HRMS analysis displayed a m/z peak at 545.2180, corresponding to the [**249**-H]$^-$ species.

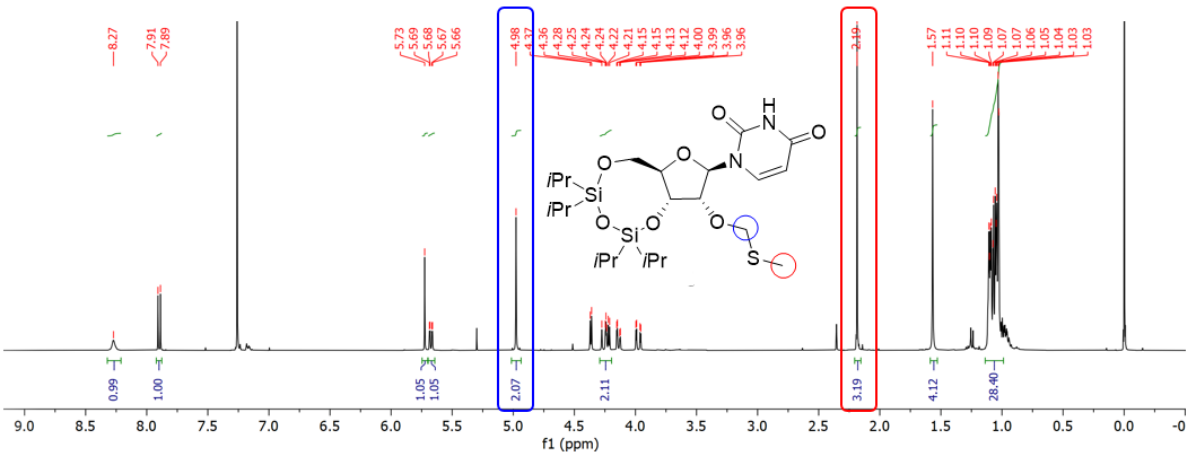


Figure 29. ^1H NMR ($CDCl_3$, 400 MHz) spectrum of **249**.

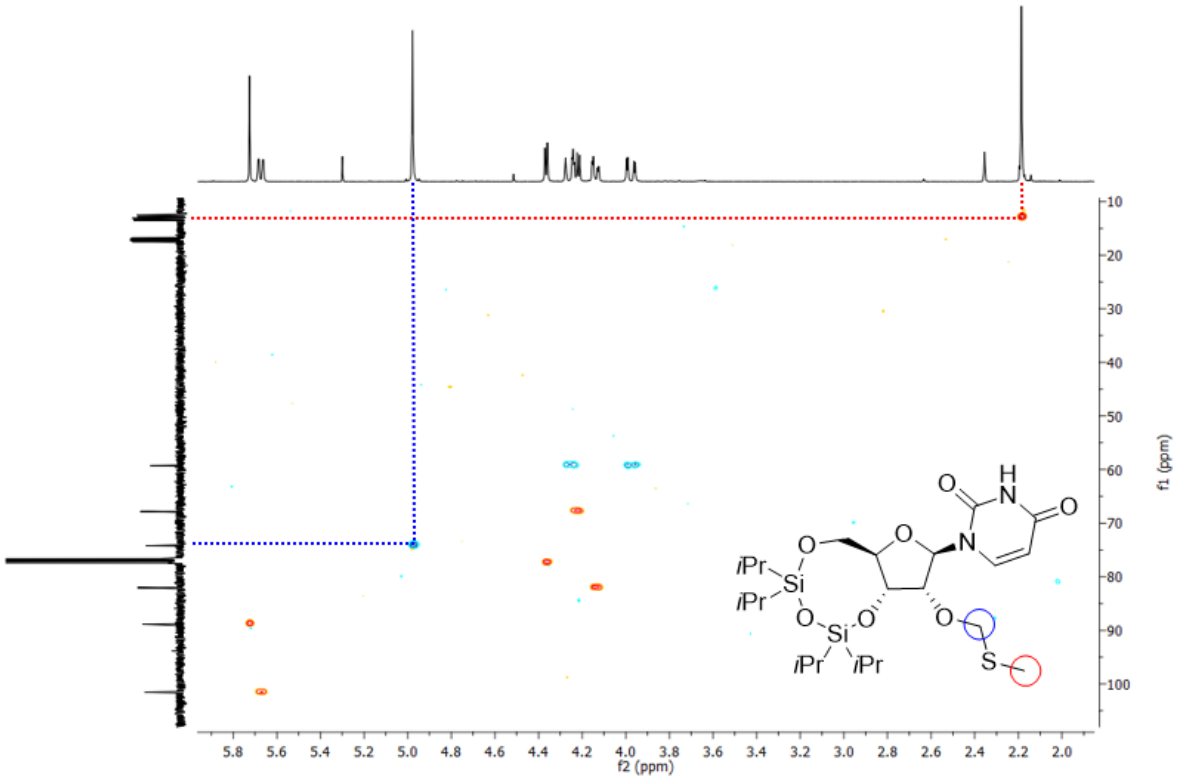
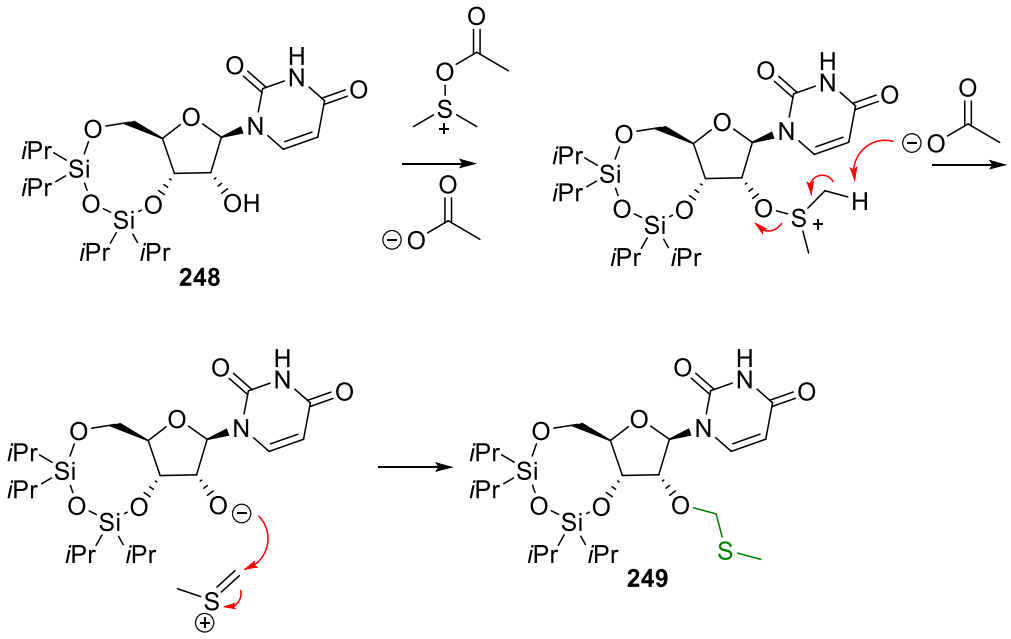


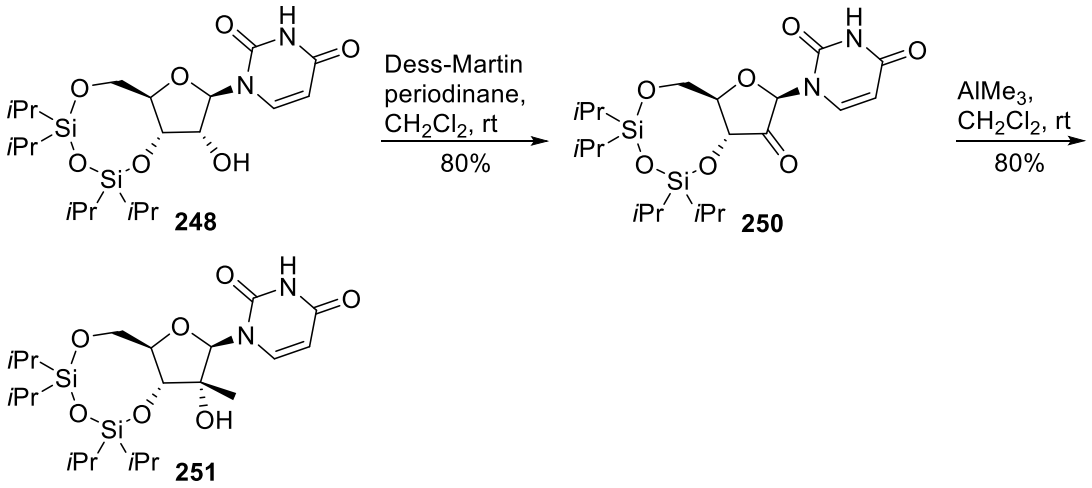
Figure 30. HSQC-DEPT correlation spectrum of **249**. Pale blue contours indicate CH_2, red contours indicate CH or CH_3.

Interestingly, carrying out the Albright-Goldman oxidation at rt on the thionucleoside substrate **245** did not furnish any thioacetal side product, with complete conversion of **245** to **246** observable by TLC, and no methylene CH_2 signals or methyl CH_3 signals observable by NMR analysis. It has been reported that increased steric hindrance tends to reduce the quantity of thiohemiacetal side product formed in Albright Goldman oxidations,[147] thus, the formation of **249** rather than the desired ketone may have been due to less steric hindrance for the native analogue **248** in comparison to **245** which bears the larger sulfur atom. A proposed mechanism for the formation of **249** is shown in Scheme 64, whereby a 1,2-rearrangement reaction occurs.



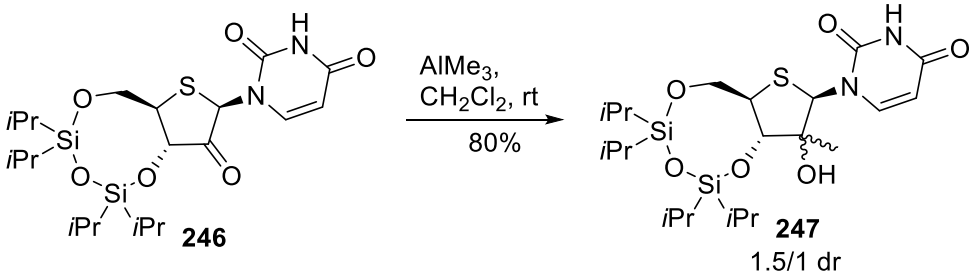
Scheme 64. Proposed mechanism for the formation of **249** *via* rearrangement under Albright Goldman conditions.

Reaction of **248** with Dess-Martin periodinane at rt delivered the desired ketone **250** in 80% yield (Scheme 65). A series of methylation reactions were trialled on **250**. Treatment of **250** with MeLi or MeMgBr was unsuccessful over a range of temperatures (-45° – 40 °C). Reaction at rt or higher temperatures for prolonged periods (>4h), or use of a large excess of methylating reagent led to decomposition of **250**. Trimethylaluminium ($AlMe_3$) has been reported to add the 2′-methyl stereoselectively to the α-face,[148–150] and indeed treatment of **250** $AlMe_3$ delivered exclusively the 2′-(*R*) diastereoisomer **251** in 80% yield.



Scheme 65. Synthesis of **251**.

With a methylation method established, focus was returned to the thionucleoside derivative **246**. Treatment of **246** with 5 equivalents of $AlMe_3$ (Scheme 66) furnished **247** as an inseparable mixture of diastereoisomers (1.5/1 ratio) in low yields of 25 – 30% with 26% recovery of **246**. A large excess of $AlMe_3$ (7 mol equivalents) was required to drive the reaction to completion, which improved the yield to a moderate 49%. However, due to the formation of an emulsion at workup, the yield could not be improved further. Moreover, scale-up from 500 mg to 1.5 g delivered **247** in significantly reduced yields of 15 – 20% due to incomplete reaction, and thus the reaction was not scaled-up.

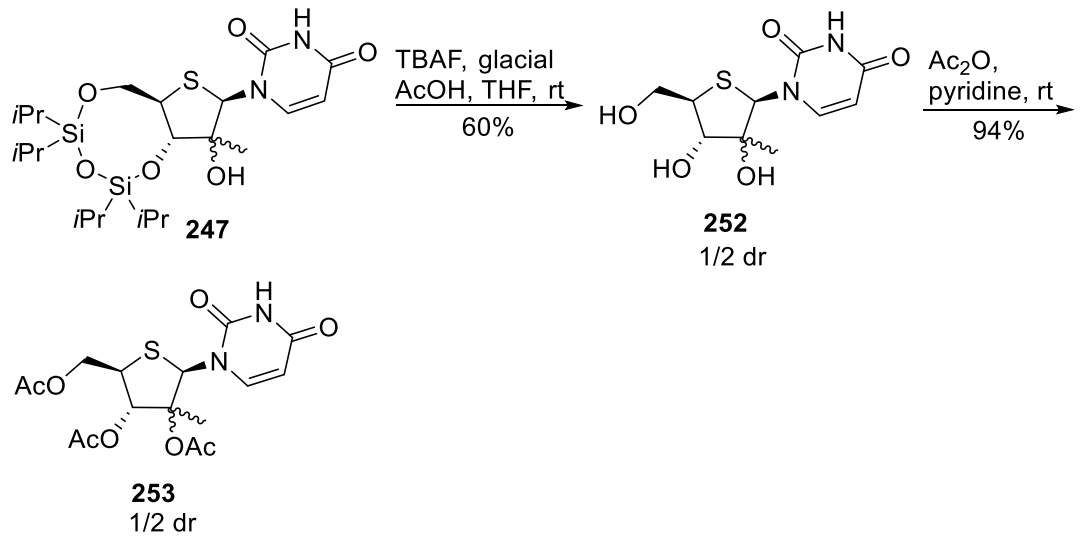


Scheme 66. Synthesis of **247**.

A possible explanation for the lack of stereoselectivity on the thionucleoside substrate **246** in comparison to **250** is that the increased steric bulk of the furanosyl sulfur atom directed the stereochemical outcome for **246**, whereas for **250**, the nucleobase as a β-substituent directed methyl addition to the α-face.

Attempts to separate the diastereomeric mixture proved unsuccessful due to similar R_f values for the starting material **246** (R_f = 0.47, 3/7 EtOAc/CH_2Cl_2) and the two diastereoisomers of **247** (R_f

= 0.40 and 0.37, 3/7 EtOAc/CH_2Cl_2). Thus, the TIPDS group was removed *via* treatment of **247** with TBAF to obtain **252** in 60% yield (Scheme 67). Following purification *via* column chromatography, **252** was furnished in a more favourable diastereomeric ratio (1/2). It was envisioned that one diastereoisomer could be isolated *via* fractional crystallisation or precipitation, however, no diastereomeric resolution was achieved. Thus **252** was globally acetylated with the aim to further trial diastereomeric resolution. Reaction of **252** with Ac_2O and pyridine prepared **253** in 94% yield. A small quantity (13%) of **253** was separated *via* column chromatography as a 1/4 mixture of diastereoisomers, however, attempted crystallisations did not furnish one diastereoisomer, which was likely due to the small sample size (22 mg).



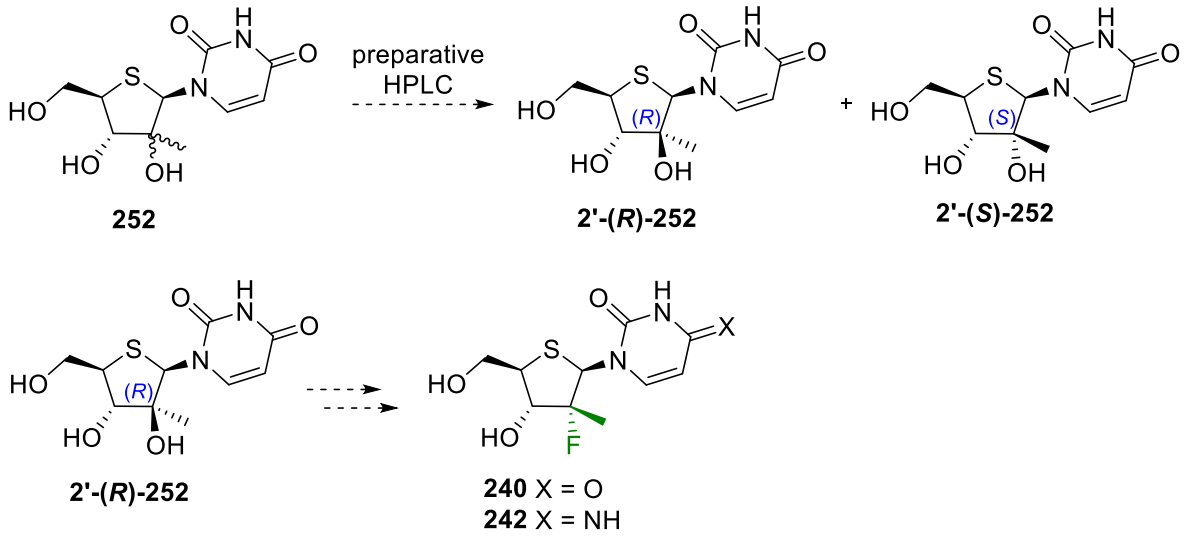
Scheme 67. Synthesis of **253**.

Clark's DAST fluorination of the native nucleoside substrate furnished three products; the desired fluorinated analogue, the 2'-methylene analogue and the C2'-epimerised analogue. Thus, DAST fluorination of **253** was not pursued as using a diastereomeric mixture would likely furnish an even more complicated mixture of products and isomers.

6.2.2. Conclusions and future work

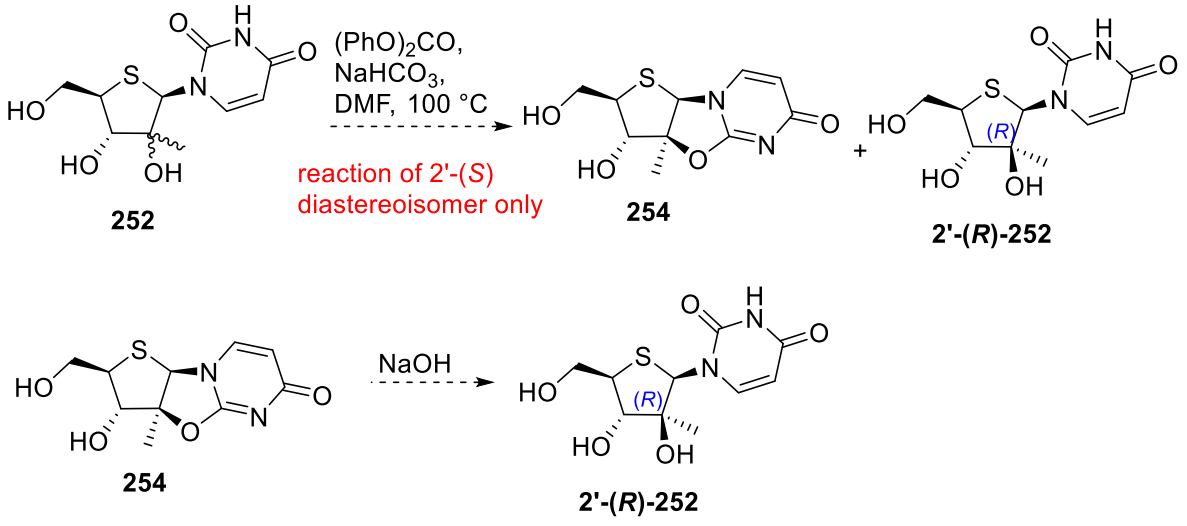
The late-stage functionalisation approach was deemed unsuitable for the synthesis of **240** or **242** due to low yields for the preparation of **245** and **247** and inability to resolve the diastereomeric mixtures of intermediates **247**, **252** and **253** for subsequent DAST fluorination.

Future work on this synthetic route could follow two different approaches. Firstly, a preparative HPLC purification method could be developed to isolate the desired 2′-(*R*) diastereoisomer of **252** and then further modifications could be undertaken (Scheme 68).



Scheme 68. Proposed strategy for synthesis of **240** and **242** *via* chiral resolution of **252** through preparative HPLC.

Secondly, application of conditions used to prepare **207** from **196** (Chapter 3, section 3.3.), could be trialled to resolve the **252** diastereomeric mixture, as only the 2′-(*S*) diastereoisomer should react to form **254**, and ring opening with hydroxide would result in stereochemical inversion at C2′ to furnish the desired 2′-(*R*) diastereoisomer (Scheme 69). However, the presence of the bulky 2′-*C*-methyl group may sterically hinder the formation of **254**.

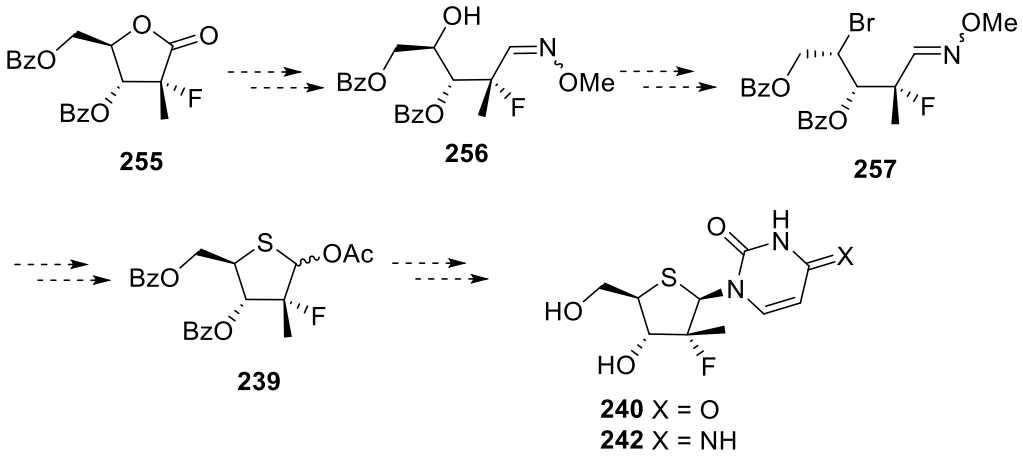


Scheme 69. Proposed strategy for synthesis of **240** and **242** *via* chiral resolution of **252** through preparation of 2,2′-anhydro intermediate **254**.

6.3. Synthesis of 2′-fluoro-2′-*C*-methyl 1′-(4′-thio) nucleosides *via* an oxime approach

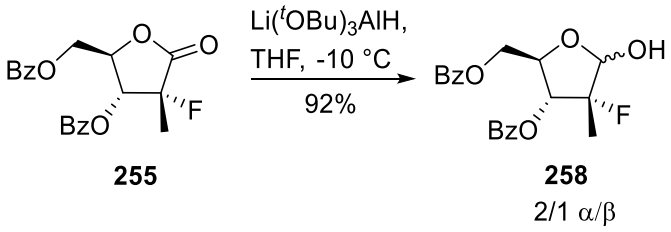
6.3.1. Synthesis of 2-fluoro-2-*C*-methyl 1-(4-thio)ribose

Thus, a new approach was followed, whereby the previously discussed and optimised conditions (Chapter 2) used to synthesise **187** were applied to the synthesis of thioribose derivative **239**, and a nucleobase installed to deliver **240** and **242** (Scheme 70). Commercially available ribonolactone **255**,[138] which has the 2-fluoro-2-*C*-methyl functionality pre-installed, was selected as the starting material for this synthesis.



Scheme 70. Suggested route to nucleosides **240** and **242**, starting from **255**.

Thus, lactone **255** was selectively reduced *via* treatment with Li(O^{t}Bu)$_3$AlH at -10 °C,[151] to obtain **258** as an anomeric mixture (2/1 ratio α/β) in 92% yield (Scheme 71). The yield was consistent on scale up from 200 mg to 20 g, however, the workup was adapted for large scale such that the quenched reaction mixture was filtered through glass wool rather than celite, which simplified the workup of the thick emulsion.



Scheme 71. Synthesis of **258**.

The reduction of the lactone was marked by the significant upfield shift of C1 from δ_C 169.1 ppm in **255** to δ_C 100.9 ppm in **258**. Further, the 2-F peak changed from a qd, ($J_{F\text{-}CH3}$ = 23.4 Hz, $J_{F\text{-}H3}$ = 17.9 Hz) in **255** to an apparent dp due to the similar F-H3 and F-CH_3 coupling of 22.7 Hz and additional F-H1 coupling of $J_{F\text{-}H1}$ = 9.7 Hz was observed (Figure 31).

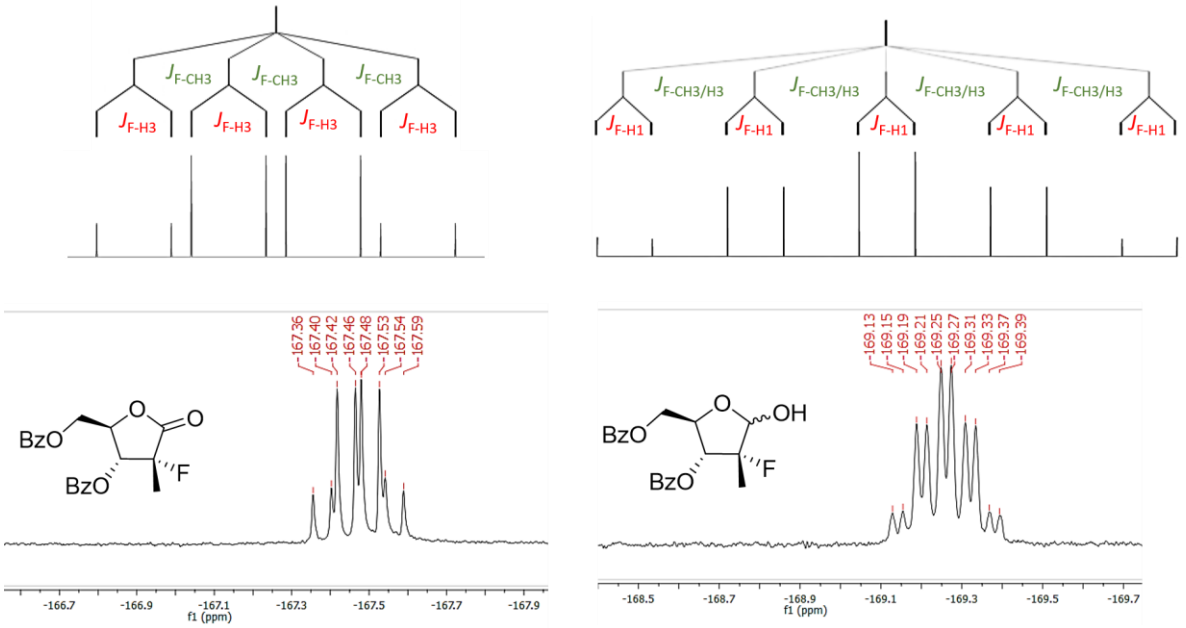
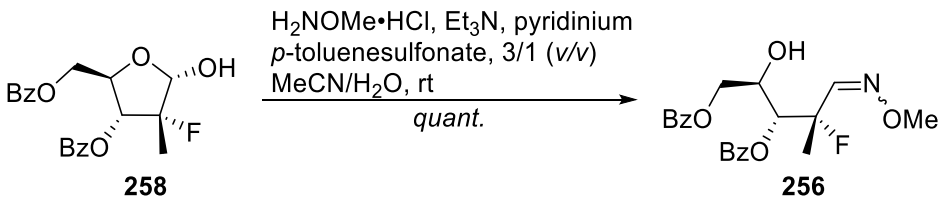


Figure 31. Tree diagram and ^{19}F NMR (377 MHz, $CDCl_3$) for **255** (left) and **258** (right).

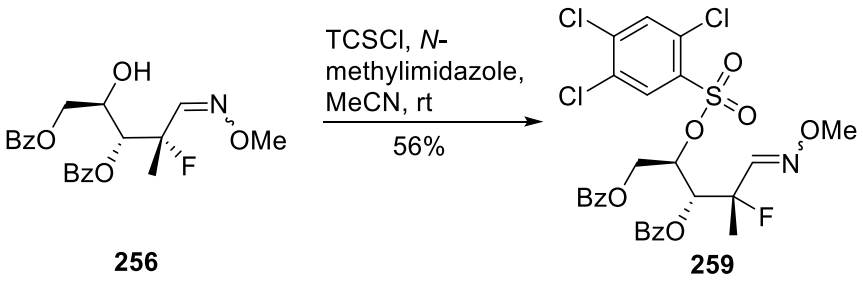
Following conditions used previously for the synthesis of **189**,[109] **258** was treated with $H_2NOMe{\cdot}HCl$ and Et_3N to prepare the corresponding oxime, however, no reaction occurred, with full recovery of the starting material **258**. The reduced reactivity of **258** may have been due to the presence of the 2-fluoro group deactivating the C1 carbonyl. In Nakamura's synthesis of a 2-*gem*-difluorinated oxime, pyridinium *p*-toluene sulfonate was added to the reaction to activate the C1 carbonyl group *via* protonation. Thus, these conditions were applied to **258** which furnished **256** in quantitative yield (Scheme 72).



Scheme 72. Synthesis of **256**.

The formation of the oxime was marked by the significant downfield shift of H1 from δ_H 5.34 ppm in **258** to δ_H 7.66 ppm in **256** and C1 from δ_C 100.9 ppm in **258** to δ_C 149.3 ppm in **256**. Further, the oxime OCH_3 was evident as a singlet at δ_H 3.88 ppm. Interestingly, **256** was obtained as a single isomer. Steric hindrance from the 2-methyl group and the ability of the 2-fluoro group to hydrogen bond with the incoming methoxy group, which would favour formation of the Z-oxime, could be a possible explanation for this result. However, the geometry of the oxime isomer was not determined.

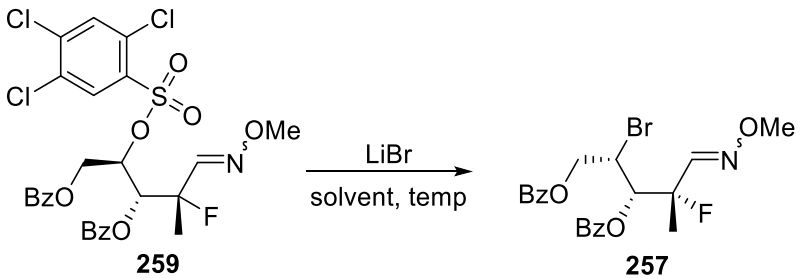
Substitution of the 4-hydroxyl group with a sulfonyl leaving group *via* treatment of oxime **256** with TCSCl and *N*-methylimidazole afforded **259** in a satisfactory yield of 86% on 500 mg scale. Scale-up to 10 g, and use of crude product **258** and **256** furnished **259** in 56% yield over three steps. The addition of the strong electron withdrawing sulfonyl group was evidenced by the significant downfield shift of H4 and C4 peaks from δ_H 4.48 and δ_C 69.2 ppm in **258** to δ_H 5.60 and δ_C 80.4 ppm in **259**.



256 **259**

Scheme 73. Synthesis of **259**.

Numerous conditions for the inversion of the C4 stereochemistry *via* S_N2 substitution of the 4-*O*-sulfonyl group with a halide were evaluated (Table 6).



259 **257**

Entry	Solvent	Temperature (°C)	257
1	Butanone	80	No reaction

2	DMF	130	12% yield
3	DMF	100	17% yield
4	DMF	80	53% yield
5	DMF	60	No reaction

Table 6. Optimisation studies for the synthesis of **257**.

Previously, the use of LiBr in butanone at 80 °C effectively produced bromide **186** in satisfactory yields (see Chapter 2, section 2.2.), however, no reaction was observed when **259** was reacted under these conditions (Table 6, Entry 1). Thus, the solvent was changed to DMF to allow for higher reaction temperatures, and indeed temperature had a significant effect on the reaction progression and yields. Heating to 130 °C or 100 °C (Table 6 , Entries 2 and 3 respectively) led to product formation, however, this competed with degradation of **259** and **257** *in situ*, and consequent low yields of **257**. No reaction occurred at 60 °C (Entry 5, Table 6). Thus, reaction at 80 °C in DMF was found to be optimal, which furnished **257** in a moderate yield of 53% (Table 6, Entry 4). Reaction in DMF was stereoselective and exclusively furnished the desired 4-(*S*) diastereoisomer, in comparison to a 3/1 dr for **186**. This may be due to the reduced reaction times for **259** in comparison to **193**, or perhaps steric hindrance by the 2-methyl group blocking a second S_N2 reaction of bromide onto 4-(*S*) configured **257**. In the ^{13}C NMR, C4 shifted upfield from δ_C 80.4 ppm in **259** to the region typical for a carbon bonded to a halide δ_C 46.3 ppm in **257**. Moreover, the inversion of the C4 stereochemistry was marked by the small H3-H4 coupling of $J_{H3\text{-}H4}$ = 2.3 Hz, which indicated a 1,2-*cis* relationship. Further, HRMS, displayed m/z 446.0586, corresponding to the [**257**+H]$^+$ species, and the distinctive Br isotope (^{79}Br and ^{81}Br) pattern was observed (Figure 32).

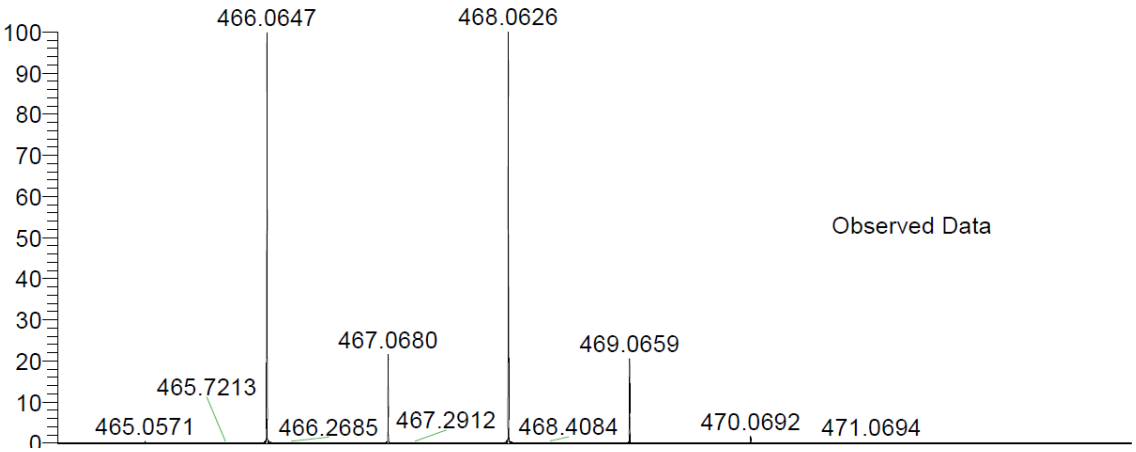
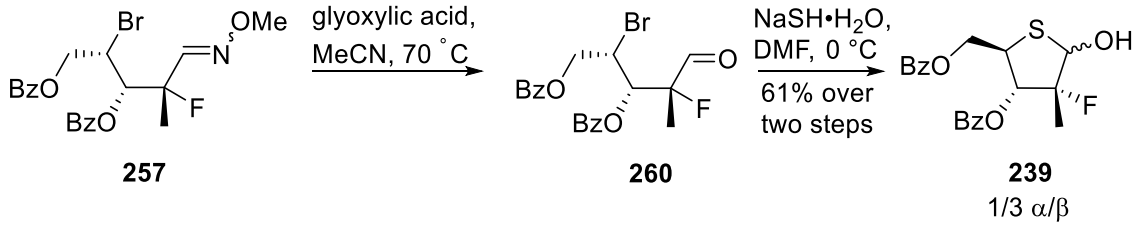


Figure 32. HRMS of **257** with observed Br isotope pattern.

Hydrolysis of the oxime group to obtain the aldehyde intermediate **260** was achieved *via* reaction of **257** in glyoxylic acid at 70 °C, which was immediately followed by S_N2 sulfur insertion with NaSH·H_2O to afford **239** in a 1/3 α/β ratio in 61% yield over the two steps (Scheme 74). The inversion of the C4 stereocentre from (*S*)- to (*R*)- was marked by the significant difference in H3-H4 coupling. In **257**, H3 and H4 are on the same face, in a 1,2-*cis* relationship with a small coupling of $J_{H3\text{-}H4}$ = 2.3 Hz, whilst in **239** H3 and H4 are on opposite faces, in a 1,2-*trans* relationship with a large coupling of $J_{H3\text{-}H4}$ = 9.1 Hz. The intramolecular ring-closing was confirmed by the upfield shift from that of H1 and C1 in oxime **257** at δ_H 7.92 – 7.84 and δ_C 147.6 ppm to thiohemiacetal in **239** at δ_H 5.16 and δ_C 81.3 ppm.



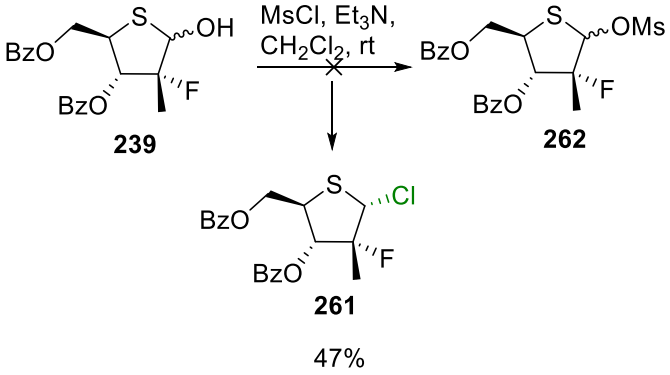
Scheme 74. Synthesis of **239** from **257**.

Overall, novel 2-fluoro-2-*C*-methyl 1-(4-thio)ribose derivative **239** was furnished in six steps in 18% overall yield from commercially available ribonolactone **255**. The bromination step reduced the overall yield significantly, due to competing decomposition of **259** and formation of the desired bromide **257**. The synthetic route was scaled to 20 g, which allowed for the preparation of multi-gram

quantities of **239** which would act as a key synthetic intermediate in the preparation of 1′-(4′-thio) nucleoside analogues of **39**.

6.3.2. Synthesis of 2′-fluoro-2′-*C*-methyl 1′-(4′-thio) nucleosides

With the key synthetic intermediate **239** in hand, the installation of an anomeric mesylate was trialled *via* treatment of **239** with MsCl and Et_3N at rt (Scheme 75). Formation of a product was observed by TLC analysis (R_f product = 0.71, R_f **239** = 0.44, 1/2 Et_2O/petroleum ether), however, no characteristic mesyl CH_3 was evident in the ^1H and ^{13}C NMR spectra. The product obtained was one anomer and NOE analysis showed through space correlation from H1 to H4 and 2-CH_3 protons, which indicated H1 was on the β-face, and thus the anomeric substituent was on the α-face. Furthermore, C1 shifted upfield from δ_C 81.3 ppm in **239** to δ_C 68.0 ppm in the product, which suggested installation of a chloride.[152] HRMS confirmed formation of the anomeric chloride **261**, with m/z of 431.0488 corresponding to the [**261**+Na]$^+$ species (Figure 33).



Scheme 75. Attempted synthesis of mesylate **262** and resulting formation of **261**.

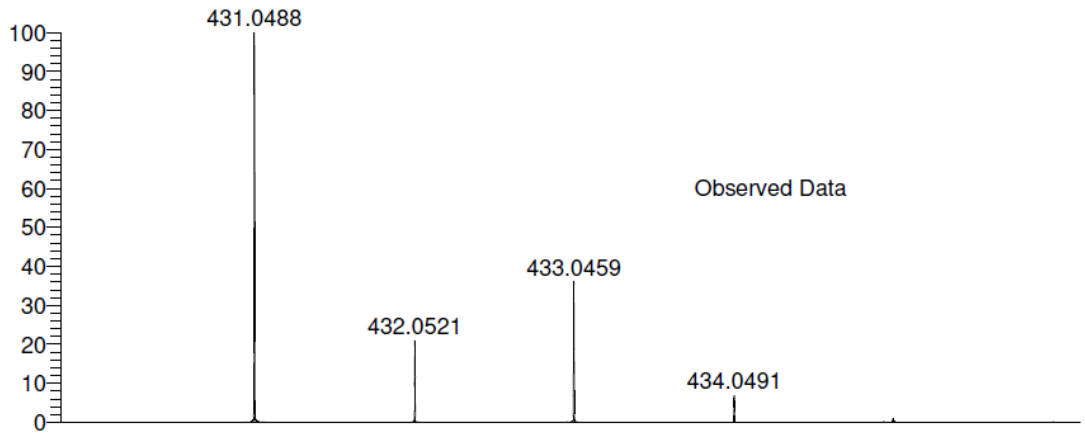
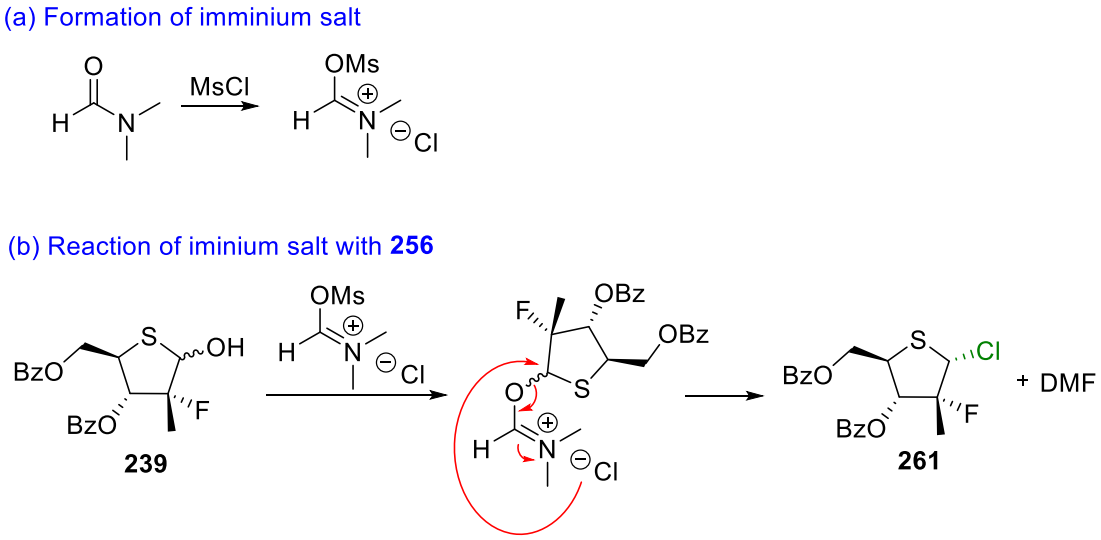


Figure 33. HRMS of **261** with observed Cl isotope pattern.

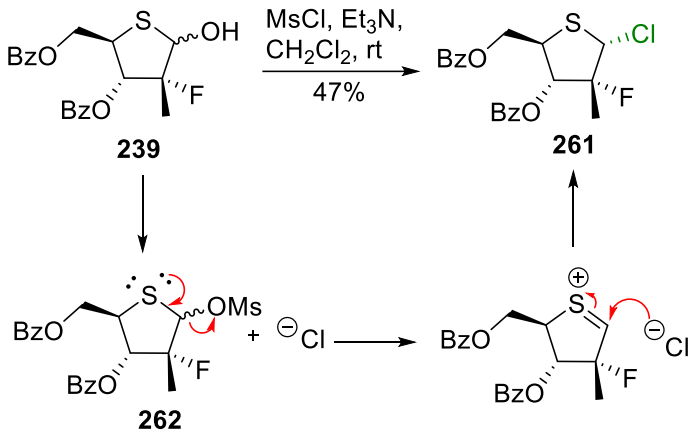
It has been reported that chloro glycosides can be prepared through reaction of a sugar alcohol with MsCl and DMF.[153] This is proposed to occur *via* the formation of an iminium salt by reaction of DMF with MsCl. It is possible that residual DMF from the preparation of **239** has served to catalytically drive the formation of **261** in this way (Scheme 76).



Scheme 76. A) Formation of iminium salt from reaction of MsCl with DMF; b) Formation of chloroglycoside **261** by reaction of **239** with the iminium salt.

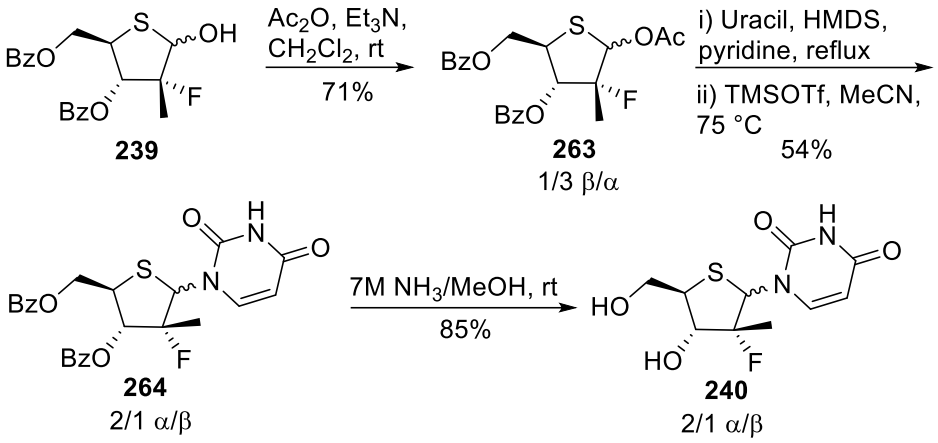
It was also postulated that the anomeric mesylate formed *in situ* and was substituted by the chloride ion. The stereochemical outcome of the reaction, whereby only one anomer is formed, suggests that the mesylate would have to leave first, to form the corresponding sulfenium ion, which then undergoes nucleophilic attack by chloride at the less hindered face of the furanosyl ring (Scheme

77). However, it is worth noting that mesylate intermediate **262** was not observed in the ^{1}H NMR spectrum of the reaction mixture.



Scheme 77. Alternative proposed mechanism for the formation of **261**.

Due to issues with preparation of the anomeric mesylate, the acetyl glycoside was prepared, obtaining **263** in 71% yield in a 1/3 β/α ratio (Scheme 78). The uracil nucleobase was installed under the optimised Vorbrüggen conditions discussed in Chapter 3, section 3.2., which delivered **264** in 54% yield. Nucleoside **264** was obtained in a 2/1 α/β ratio, which was characterised by the large 1,2-*trans* H1′-F coupling of $J_{H1'\text{-}F}$ = 22.5 Hz for the major anomer, and the smaller 1,2-*cis* H1′-F coupling of $J_{H1'\text{-}F}$ = 14.2 Hz for the minor anomer. The characteristic uracil 5-Ar-CH and 6-Ar-CH peaks were observed at δ_H 8.09 and 5.85 ppm and at δ_C 165.8 and 152.1 ppm for **264β**. Deprotection of the benzoate groups *via* treatment of **264** with neat 7M NH_3/MeOH solution furnished **240** in 85% yield as a 2/1 α/β mixture. A preparative HPLC method was developed to separate and isolate **240α** and **240β** in purity of >95%. Analytically pure samples of **240α** and **240β** were obtained in overall yields of 26% and 15% respectfully from **264**.



Scheme 78. Synthesis of **240**.

The anomeric assignments of **240α** and **240β** were confirmed *via* NOE analysis, with irradiation of H1′. For the more polar anomer **240α** (retention time = 25.7 minutes), through space correlation was evident from H1′ to H3′ (Figure 34), which indicated that H1′ was on the β-face and concomitantly, the nucleobase was on the α-face. Further, no through space correlation of H1′ to H4′ was evident, as H4′ was on the opposite face.

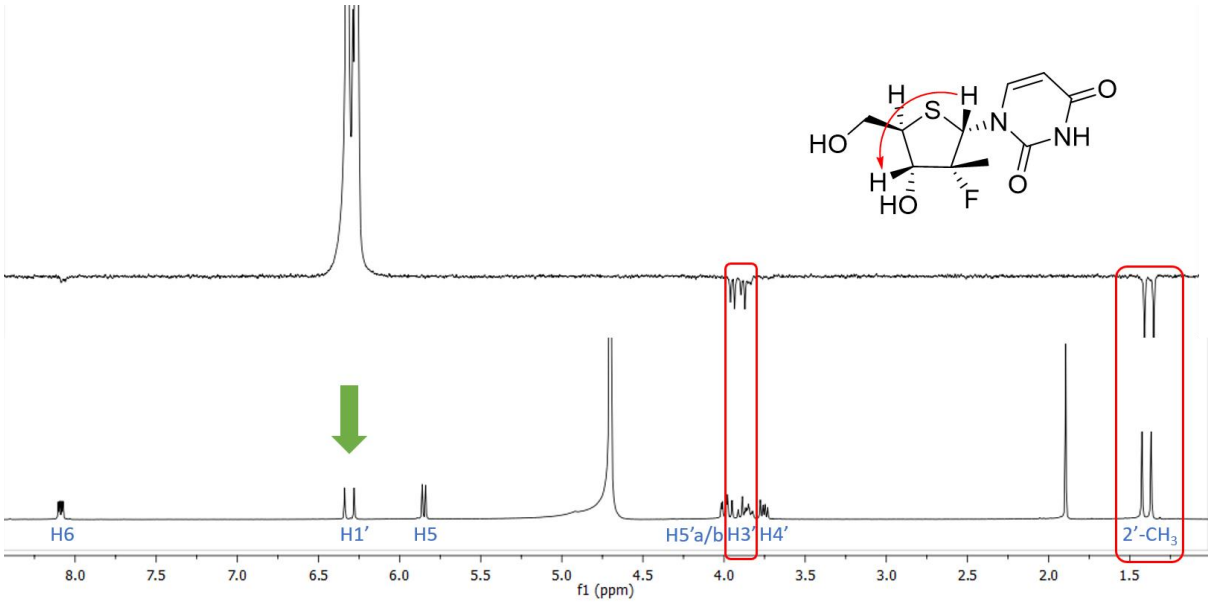


Figure 34. NOE of **240α** where H1′ is irradiated and corresponding ^{1}H NMR spectrum (D_2O, 400 MHz).

For the less polar anomer (retention time = 26.7 minutes), through space correlation was evident from H1′ to H4′ (Figure 35), which indicated that H1′ was on the α-face and consequently the nucleobase was on the β-face. Further, no through space correlation of H1′ to H3′ was evident, as H3′ was on the opposite face.

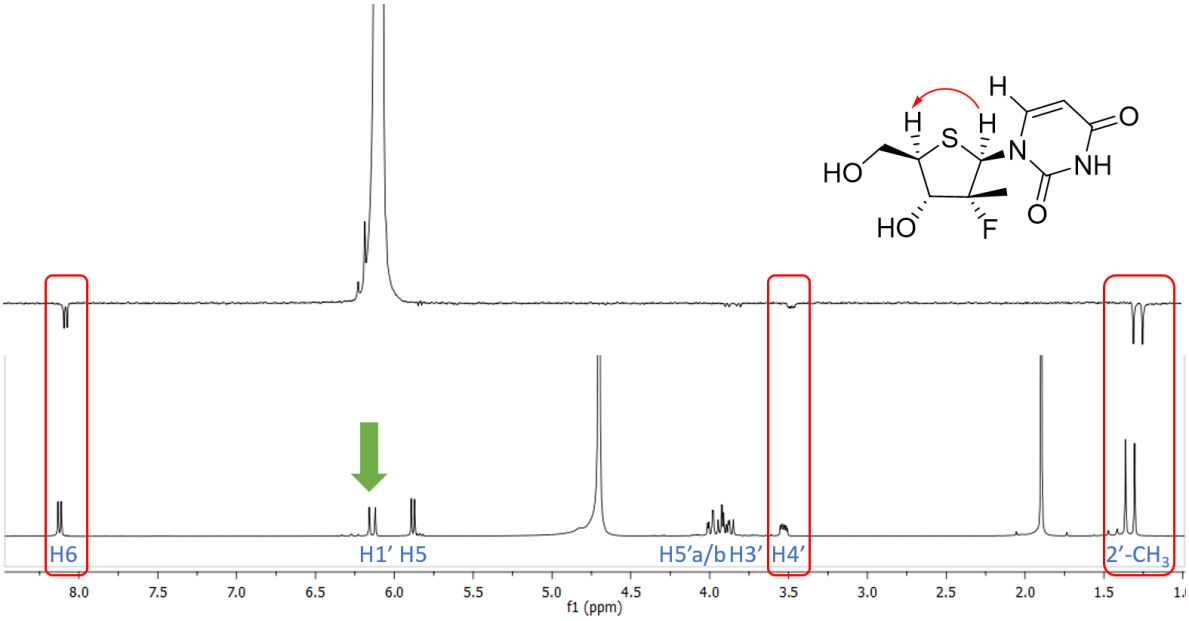
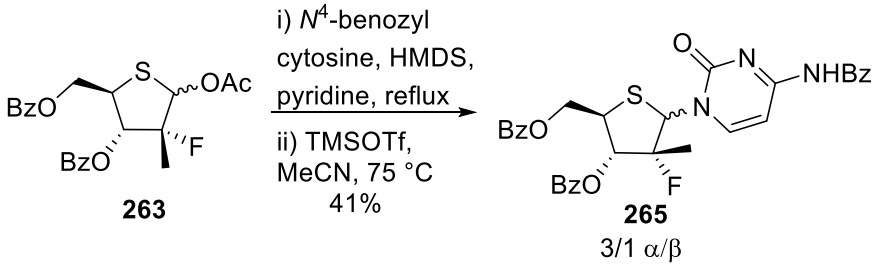


Figure 35. NOE of **240β** where H1′ is irradiated and corresponding ^{1}H NMR spectrum (D_2O, 400 MHz).

It was observed that for **264** and **240**, a H1′ coupling constant of ~15 Hz or less corresponded to the β-anomer, whilst a H1′ coupling constant of ~22 Hz or greater corresponded to the α-anomer. Indeed, **240β** had $J_{H1'\text{-}F}$ = 15.2 Hz, whilst **240α** had $J_{H1'\text{-}F}$ = 23.2 Hz.

The corresponding cytosine analogue **265**, was prepared from **263** under the same Vorbrüggen conditions, using N^4-benzoyl cytosine as the nucleobase (Scheme 79). Nucleoside **265** was obtained in a low yield of 41% with 36% recovery of the starting material **263**. The anomeric ratio of **265** (3/1 α/β) was less favourable in comparison to **264** (2/1 α/β), which may be due to increased steric hindrance between the 2′-*C*-methyl group and the bulkier the N^4-benzoylated cytosine nucleobase.

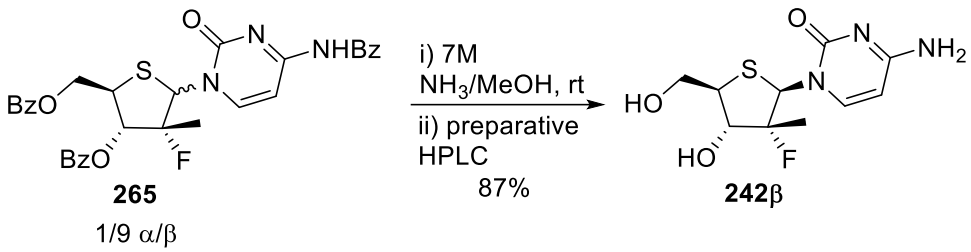


Scheme 79. Synthesis of **265**.

However, this anomeric ratio allowed for fractional precipitation of small quantities of the α- and β-anomers following column chromatography. Pure β-anomer was isolated in 4% overall yield and the α-anomer was isolated in 3% overall yield. The anomers were identified based on the coupling

constant for H1′. For **265β** has $J_{H1'\text{-}F}$ = 13.8 Hz, whilst **265α** has larger coupling of $J_{H1'\text{-}F}$ = 22.4 Hz. Installation of the nucleobase was evident by NMR, notably the N^4-benzoyl C=O peak at δ_C 162.2 ppm, and the cytosine 5-Ar-CH and 6-Ar-CH signals at δ_H 8.45 and 7.78 – 7.34, δ_C 145.4 and 97.7 ppm for **265β**.

Deprotection of the benzoate groups *via* treatment of **265** (1/9 α/β) with neat 7M NH_3/MeOH solution furnished **242β** in quantitative yield (Scheme 80). An analytically pure (>95% purity) sample was obtained *via* preparative HPLC which delivered **242β** in an excellent overall yield of 87% and a small quantity of **242α** in 10% yield.



Scheme 80. Synthesis of **242β**.

The C1′ stereochemistry of each isolated anomer was confirmed by NOE analysis. In **242α**, H1′ was irradiated and through space correlation was evident between H1′ and H3′ (Figure 36). As H3′ is on the β-face, this implied that H1′ was also on the β-face and consequently the cytosine nucleobase was on the α-face, such that H1′ is in a 1,2-*cis* relationship with 2′-F.

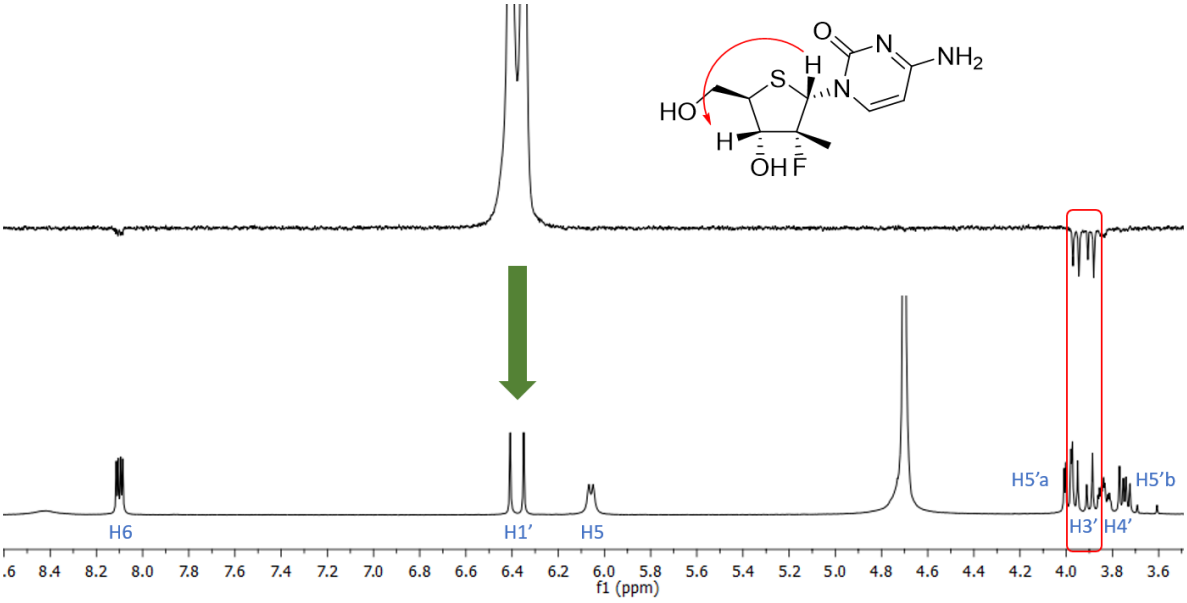
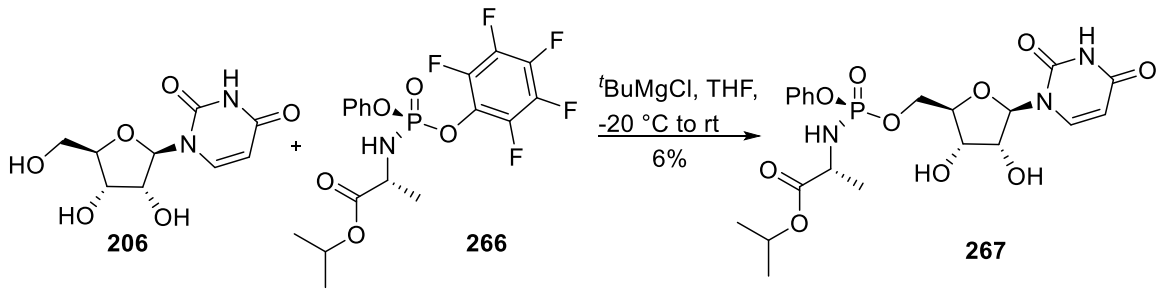


Figure 36. NOE of **242α** where H1′ is irradiated and corresponding ^{1}H NMR spectrum (D_2O, 400 MHz).

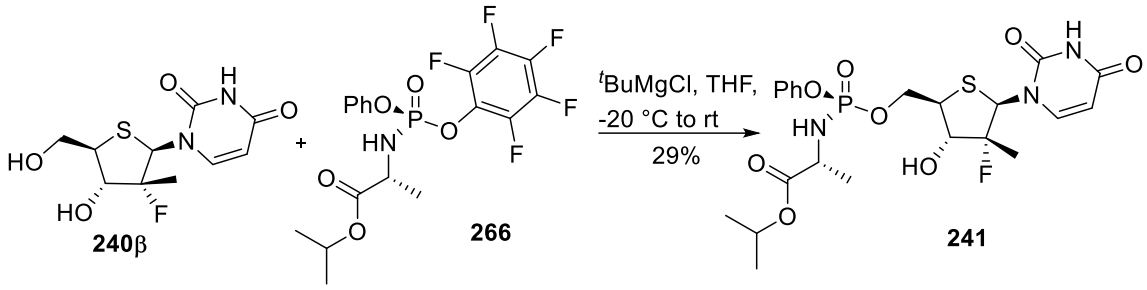
Preparation of a phosphoramidate prodrug was first trialled on a basic nucleoside substrate, uridine **206**. Treatment of **206** with ^{t}BuMgCl which acts as a strong base followed by addition of **266** in THF at −20 °C furnished the desired phosphoramidate product **267** in low yields of 6% (Scheme 81). Uridine was not soluble in THF and this hindered the reaction progression greatly, with a significant quantity of **206** still evident by TLC after 5 days. The installation of the phosphoramidate group was confirmed by the presence of a signal in the ^{31}P NMR at δ_P 3.16 ppm and by the D-alanine C2″ CH and CH_3 signals at δ_H 3.98 – 3.86 and 1.32 ppm respectively. Only one diastereoisomer was observed in the NMR spectra due to the use of chiral phosphorus reagent **266**.



Scheme 81. Synthesis of **267**.

Synthesis of the corresponding phosphoramidate prodrug of **240β** was then carried out under the same conditions, which furnished **241** in low yields of 29% (Scheme 82). Solubility of **240β** in THF was poor, and thus significant quantities of **240β** was evident by TLC after 2 weeks (R_f **240β** =

0.33 1/1 acetone/toluene). The reaction mixture was applied directly to silica gel and the solvent removed to avoid material loss during an aqueous workup, which allowed for excellent recovery of unreacted, water-soluble **240β** (63%). Following purification *via* flash column chromatography, an analytically pure sample of **241** was obtained in 18% overall yield *via* preparative HPLC which delivered **241** in >99% purity. The installation of the phosphoramidate group was confirmed by the presence of a signal in the ^{31}P NMR at δ_P 3.81 ppm and by the D-alanine C2″ CH and CH_3 signals at δ_H 3.99 – 3.86 and 1.34 ppm respectively and δ_C 50.3 and 20.7 ppm respectively.



Scheme 82. Synthesis of **241**.

To conclude, uracil and cytosine nucleobases were successfully installed on the acetyl glycoside **263**, and deprotected to deliver the desired target molecules **240** and **242**. Preparative HPLC methods were developed to separate the α/β anomers of **240** and **242** and to purify the nucleosides to >95%. Lastly, the phosphoramidate prodrug **241** was prepared from **240** in one step and a preparative HPLC method developed to purify to >95%. Overall, the novel synthetic targets **240**, **242** and **241** were successfully prepared.

6.3.3. Conclusions

Novel synthetic targets **239** and **240** – **242** were successfully prepared starting from commercially available ribonolactone **255**. The key 1′-(4′-thio) hemiacetal intermediate **239** was delivered in a moderate yield of 18% over six steps, due to the low yielding bromination step, in comparison to the synthesis of **187** described in Chapter 1. Glycosylation of uracil and cytosine with **239** was achieved *via* TMSOTf activated Vorbrüggen reaction. Preparative HPLC was necessary to separate the α/β anomers of 1′-(4′-thio) nucleoside analogues **240** and **242** due to lack of β-selectivity in the glycosylation step. The 1′-(4′-thio) nucleoside analogue **241** of the antiviral drug Sofobuvir was

successfully prepared and delivered in high purity of >99%. These 1′-(4′-thio) nucleoside analogues **240** – **242** require evaluation in antiviral assays, as described in Chapter 8.

6.4. Studies towards the synthesis of L-lyxo 2′-fluoro-2′-*C*-methyl-1′-(4′-thio) nucleoside analogues

6.4.1. Aims and rationale

The C4′-epimers of naturally configured D-nucleosides are L-nucleosides, where the stereochemistry at C4′ is (*S*) such that the 5′-CH_2OH group is on the α-face. There is growing interest in the synthesis of L-nucleosides and the evaluation of their potential antiviral activity.[154–156] For example, 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU) **268** (Figure 37) has potent antiviral activities against both hepatitis B virus (IC_{50} 0.1 μM in primary hepatocytes from chronically hepatitis B infected ducklings) and Epstein-Barr virus (IC_{50} 5.0 μM in H1 cells).[157] Thus, the synthesis of the L-thionucleosides **243** and **244** was pursued, with the aim to evaluate their antiviral activities and compare to thionucleoside analogues **240** and **242** described in Chapter 6, section 6.3.2.

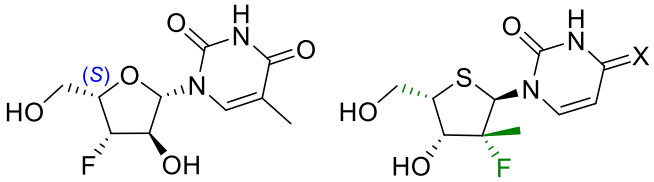
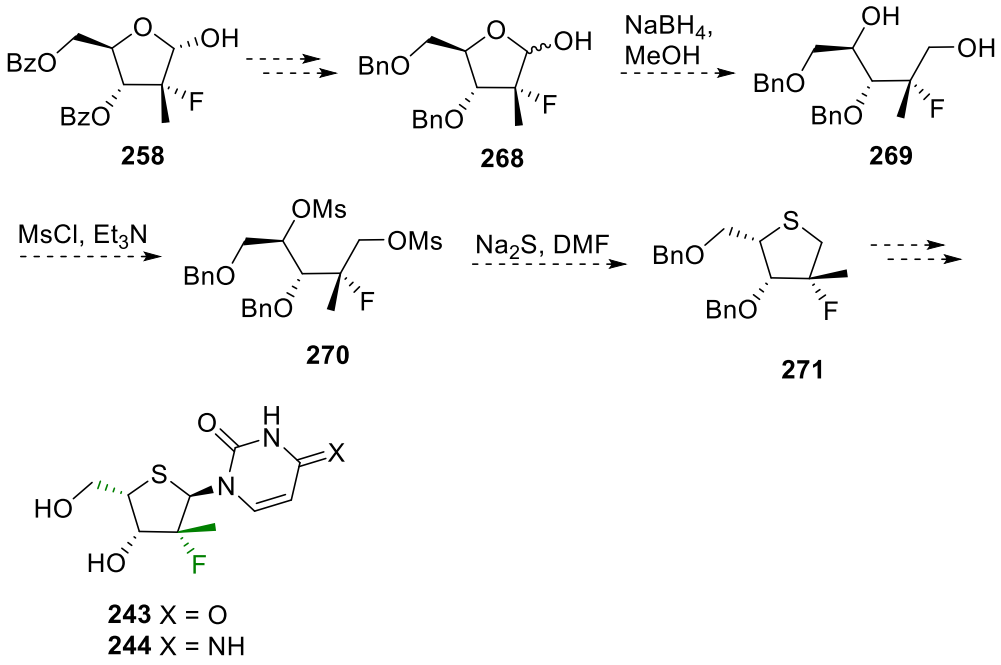


Figure 37. Structure of L-FMAU **268** and proposed synthetic targets **243** and **244**.

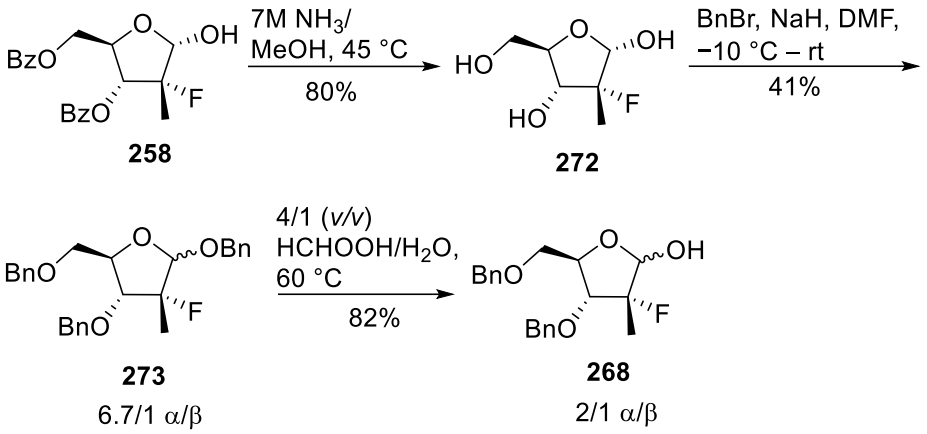
Following similar methodology to that reported by Codée and colleauges,[158] it was envisioned that the L-ribothionucleosides **243** and **244** could be synthesised *via* diol **269** (Scheme 83).



Scheme 83. Proposed strategy for the preparation of **243** and **244** *via* diol **269**.

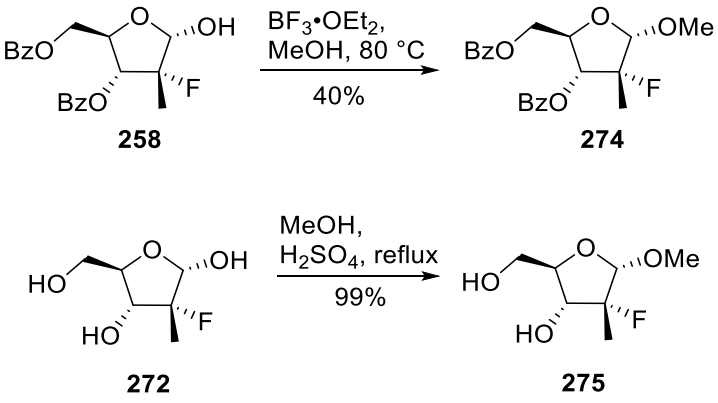
6.4.2. Synthesis of L-lyxo 2′-fluoro-2′-*C*-methyl-1′-(4′-sulfinyl) furanosyl derivative

Double reduction of the lactone to the corresponding diol required substitution of the existing benzoyl protecting groups with benzyl groups prior to reaction with $NaBH_4$. Hemiacetal **258** was globally deprotected *via* reaction with 7M NH_3/MeOH solution at 45 °C or with 1M NaOMe/MeOH. However, treatment with ammonia afforded **272** (Scheme 84) in higher yields of 80%, compared to 57% with NaOMe and thus was selected as the preferred deprotection method. Preparation of the benzyl glycoside **273** was pursued to evaluate whether the anomeric benzyl group could be selectively cleaved *via* acid hydrolysis to obtain **268** in two steps, which would eliminate the requirement to install an anomeric methoxy group. Global benzylation was achieved *via* reaction of **272** with BnBr and NaH in DMF at rt, obtaining **273** in a low yield of 41%. Reaction of **273** with aqueous formic acid at 60 °C furnished **268** as an anomeric mixture (2/1 α/β) in 82% yield.



Scheme 84. Synthesis of **268**.

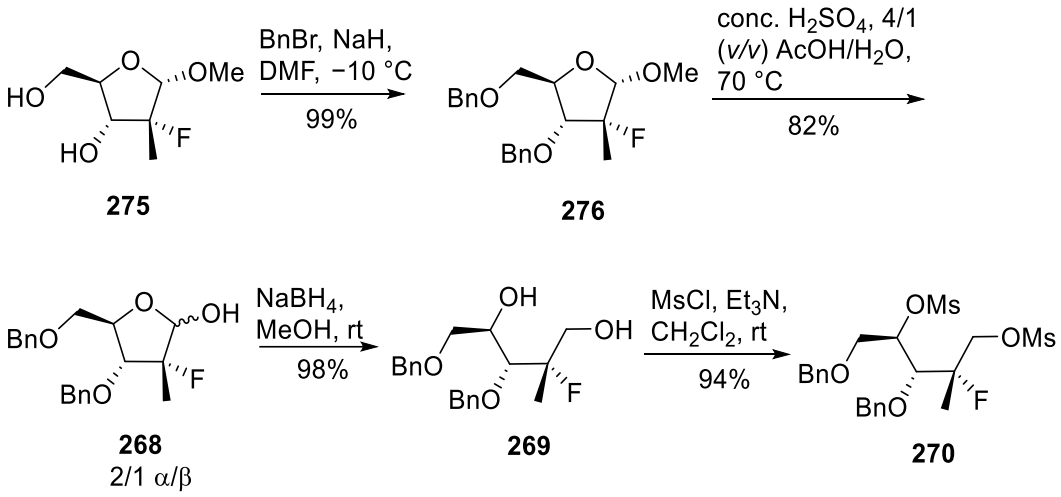
Due to the low-yielding benzylation step to prepare **273**, use of a methyl glycoside was evaluated. Hemiacetal **258** was reacted with $BF_3{\cdot}OEt_2$ in MeOH to furnish **274** in a low yield of 40% (Scheme 85) following conditions reported by Vedula *et* al.[159] Due to the low yield for methyl glycoside formation using $BF_3{\cdot}OEt_2$, an alternative method reported by Madern *et al.* on a similar substrate was followed.[158] The methyl glycoside **275** was prepared *via* reflux of **272** with conc. H_2SO_4 in MeOH to afford **275** in quantitative yield. The α-stereochemistry was retained, which was indicated by the small H1-F coupling of $J_{H1\text{-}F}$ = 9.8 Hz in comparison to the large 1,2-*trans* H3-F coupling of $J_{H3\text{-}F}$ = 23.8 Hz.



Scheme 85. Syntheses of methyl glycoside **274** and **275**.

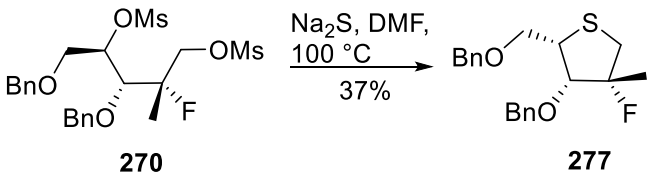
Subsequent 3,5-*O*-benzylation of **275** was achieved by reaction with BnBr and NaH (Scheme 86), which afforded **276** in quantitative yield. Hydrolysis of the anomeric methoxy group was achieved *via* reaction of **276** with conc. H_2SO_4 and aqueous AcOH at 70 °C, which afforded **268** in 82% yield (Scheme 86). Double reduction of **268** *via* treatment with 3 mol equivalents of $NaBH_4$ furnished diol

269 in excellent yield of 98% which was consistent on scale up from 300 mg to 2 g. The structure of **269** was confirmed *via* ^{1}H NMR, with HSQC-DEPT showing a total of four CH_2 peaks which correspond to the two benzyl CH_2 moieties, C5 and the reduced C1 position. The two hydroxyl groups at the 1- and 4- positions were evident in the ^{1}H NMR at δ_H 2.68 and δ_H 2.44 ppm. Double mesylation of the 1- and 4-hydroxyl groups was effected *via* reaction of **269** with MsCl and Et_3N, which delivered **270** in 94% yield.



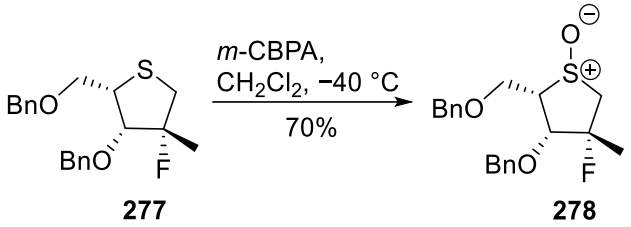
Scheme 86. Synthesis of **270**.

Sulfur incorporation *via* S_N2 reaction at the 4-position and subsequent intramolecular ring closing onto the 1-position was achieved *via* treatment of **270** with Na_2S at 100 °C to afford **277** in low yield of 37% (Scheme 87). Significant decomposition occurred, which may have been due to the high reaction temperatures, however, reaction at lower temperatures (60 °C) did not go to completion, and long reaction periods resulted in decomposition of **270**. A significant upfield shift of the H1 protons (δ_H 4.34 – 4.17 ppm in **270** to δ_H 3.07 and 2.67 ppm in **277**) and smaller upfield shift of H4 protons (δ_H 5.18 ppm in **270** to δ_H 4.76 – 4.62 ppm in **277**) was observed due to the removal of the electron withdrawing mesylate groups.



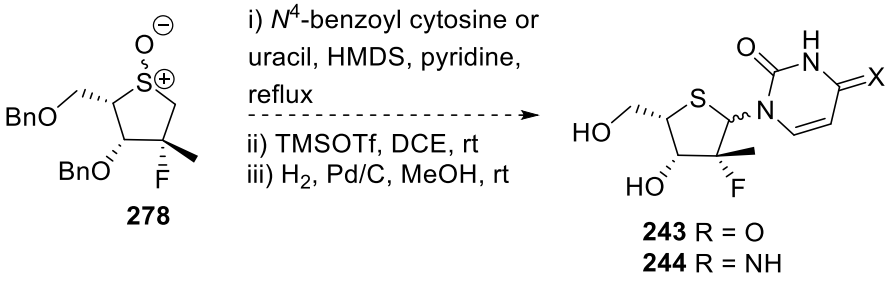
Scheme 87. Synthesis of **277**.

Oxidation to the corresponding sulfoxide **278** was trialled on small scale (~130 mg) *via* treatment of **277** with *m*-CPBA at -40 °C, which afforded **278** in 70% yield (Scheme 88). Interestingly, **278** was obtained as a single diastereoisomer at sulfur, which may be due to steric hindrance by the bulky 2-methyl substituent. It was observed that the H1a and H1b proton peaks shifted downfield to from δ_H 3.07 and 2.67 ppm in **270** to δ_H 3.77 – 3.63 and 2.79 ppm in **278**. Similarly, C1 and C4 shifted downfield from δ_C 36.2 and 45.4 ppm respectively in **270** to δ_C 59.9 and 69.7 ppm in **278**, which is consistent with the inclusion of an adjacent sulfinyl group.



Scheme 88. Synthesis of **278**.

Future work on this synthesis should include the growth of crystals of **278** to identify the sulfinyl diastereoisomer. Further, adequate quantities of **278** should be prepared to pursue installation of the cytosine and uracil nucleobases using Pummerer-type thioglycosylation and ultimately prepare **243** and **244** which should then be fully deprotected and purified to >95% (Scheme 89).



Scheme 89. Proposed future work to prepare **243** and **244**.

6.4.3. Conclusions and future work

To conclude, novel key synthetic intermediate **278** was prepared in 15% yield over eight steps from commercially available ribonolactone **255**. Further optimisation of the sulfur insertion and intramolecular ring closing to prepare **277** is required in order to improve the low yield of 37%.

Moreover, further work is required to complete the synthesis of **243** and **244**, in particular, glycosylation of uracil and cytosine with **278** needs to be developed.

Chapter 7: Towards the development of a novel synthetic route to 2′-*C*-methyl-1′-(4′-thio-D-ribofuranosyl) cytosine

7.1. Aims and rationale

2′-*C*-methylcytidine **29** (Figure 38) is an antiviral drug used to treat flaviviridae infections including HCV. Further, it has demonstrated excellent antiviral activity against ZKV and DFV as described in further detail in Chapter 1, section 1.3.2.

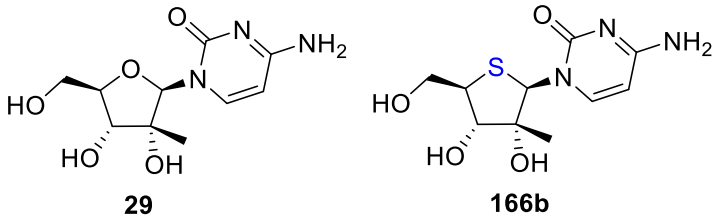
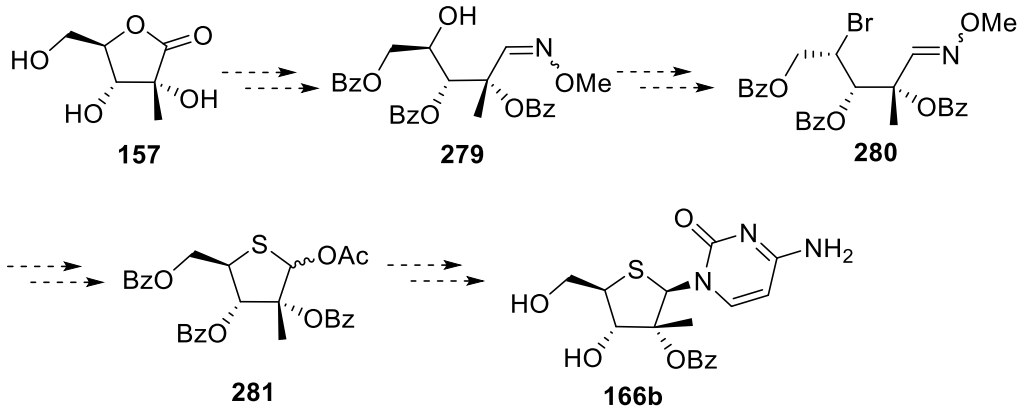


Figure 38. Structures of **29** and **166b**.

2′-*C*-methyl-1′-(4′-thio-D-ribofuranosyl)cytosine **166b** has been synthesised by Dukhan and colleagues (see Chapter 1, section 1.4.4.).[100] There is no alternative synthetic strategy to prepare **166b** reported in the literature. A novel synthesis of **166b** is herein pursued with the aim to develop scalable and high yielding synthetic methodology, and to evaluate the potential antiviral and anticancer activity of **166b.**

7.2. Synthetic study 1: towards the synthesis of 2′-*C*-methyl-1′-(4′-thio) ribose derivative *via* an oxime intermediate

Following issues with the late-stage functionalisation of thionucleosides discussed in Chapter 6, section 6.2, the development of a synthetic route to 2′-*C*-methyl-1′-(4′-thio-D-ribofuranosyl) uracil and cytosine started from commercially available 2-*C*-methyl-D-ribono-1,4-lactone **157**, with the 2-*C*-methyl moiety already installed. It was proposed that synthetic methodology developed Chapter 2 could be applied to the synthesis of the 2-*C*-methyl-1-(4-thio-D-ribofuranose) intermediate.



Scheme 90. Suggested route to **166b**, starting from commercially available lactone **157**.

The 2,3,5-hydroxyl groups of **157** were protected with acetyl (**282**), benzoyl (**283**) and benzyl (**284**) groups (Figure 39) and the protected lactones evaluated for reduction to the corresponding hemiacetals.

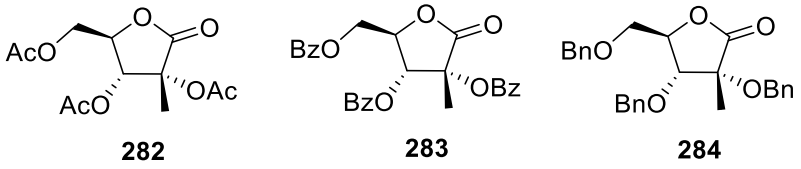
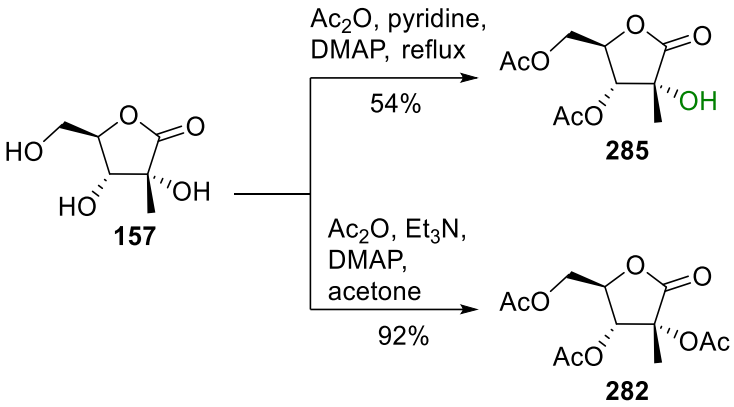


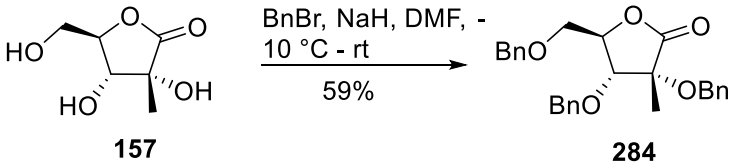
Figure 39. Structures of protected ribonolactone derivatives **282**, **283** and **284**.

The per-benzoylated lactone **283** was purchased from Carbosynth. The acetylated lactone **282** was prepared *via* treatment of **157** with Ac_2O, Et_3N and DMAP in acetone to obtain **282** in 92% yield (Scheme 91). Interestingly, the use of pyridine in place of Et_3N as a base prepared the 3,5-acetylated product **285** in 54% yield. Heating the reaction to reflux and the addition of 1 equivalent of DMAP did not drive acetylation of the tertiary alcohol at the 2-position, due to the reduced acidity of the hydroxyl proton ($pK_a \approx 18$) and thus the requirement for a base with a higher pK_aH than 18 (pK_aH pyridine = 5.2, pK_aH Et_3N = 10.8).



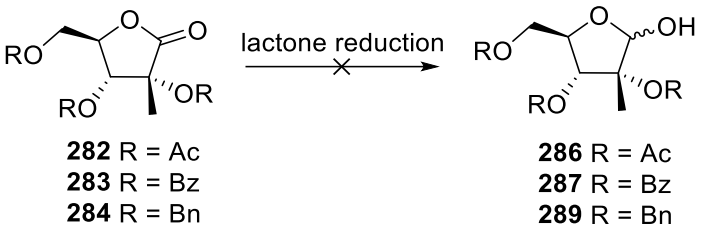
Scheme 91. Acetylation of **157**.

Treatment of **157** with BnBr and NaH afforded **284** in 59% yield, which was consistent on scale-up from 200 mg to 1.5 g (Scheme 92). However, upon scale-up to 5 g, the yield dropped significantly to 19% with a reaction time of 4 days at rt. Thus, a catalytic quantity (1 mol %) of TBAI was added to assess its potential to improve the yield due to the increased reactivity of BnI generated *in situ*, however, no improvement in yield was observed. Moreover, use of a large excess of NaH and BnBr (6.0 equivalents each) did not improve reaction yields, nor did heating the reaction to 50 – 80 °C. Thus, multiple 1.5 g scale benzylation reactions were carried out in parallel to prepare significant quantities of **284**.



Scheme 92. Synthesis of **284**.

The reduction of the three lactone derivatives **282 – 284**, was evaluated using a panel of reducing agents. Use of Li(^{t}OBu)AlH, Li(Et)$_3$BH, L-selectride and DIBAL-H with lactones **282** and **283** led to deprotection of the ester groups over prolonged reaction times, and no reduction to the corresponding hemiacetals **286** or **287** was observed. Moreover, treatment of benzyl protected lactone **284** with Li(^{t}OBu)AlH, Li(Et)$_3$BH, L-selectride or DIBAL-H did not deliver **289** This may be due to steric hindrance by the 2-methyl substituent blocking reaction with the bulky reducing agents.

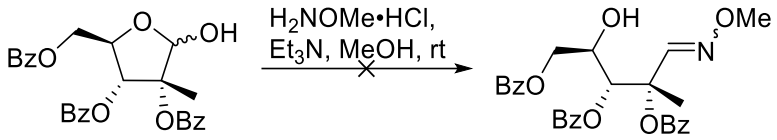


282 R = Ac
283 R = Bz
284 R = Bn

286 R = Ac
287 R = Bz
289 R = Bn

Scheme 93. Studies towards the reduction of lactones **282** – **283** to the corresponding hemiacetals.

A method for reduction of the lactone was therefore not developed, and a quantity of hemiacetal **290** was purchased from Carbosynth to evaluate the feasibility of the next steps in the proposed synthetic route. Treatment of **290** with $H_2NOMe{\cdot}HCl$ and Et_3N in MeOH afforded one new product in 40% yield (Scheme 94), with 50% recovery of the starting material after 4 days. NMR analysis confirmed the formation of the 1-oxime and the characteristic 2-OCH_3 NMR signal was observed at δ_H 7.49 and δ_C 62.1 ppm and the oxime H1/C1 peak observed downfield δ_H 3.79 and δ_C 151.6 ppm. However, it was observed that hydrolysis of one benzoate group had occurred, as only two benzoyl carbonyl peaks were evident in the ^{13}C NMR at δ_C 167.1 165.5 ppm. This deprotection was likely a result of prolonged reaction times in basic medium.

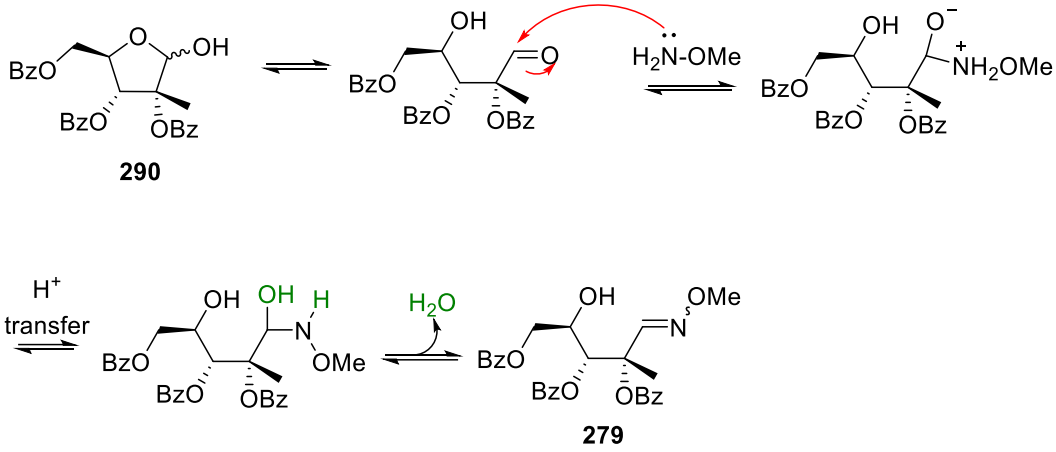


290 **279**

Scheme 94. Attempted synthesis of **279**.

The last mechanistic step in oxime formation is the loss of a molecule of water (Scheme 95), and thus 4Å molecular sieves were added to the reaction to drive the equilibrium to the right and

ensure no water was present to facilitate the benzoate hydrolysis, however, the desired oxime product did not form and the benzoyl deprotection still occurred.

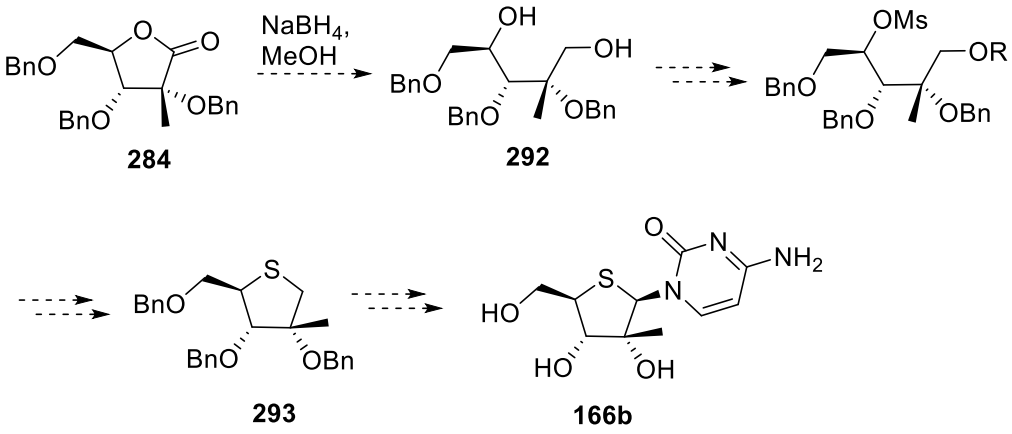


Scheme 95. Mechanism for the formation of oxime **279.**

Thus, due to issues with both the lactone reduction and oxime formation steps, this synthetic strategy was deemed unsuitable for the synthesis of 2′-*C*-methyl-1′(4′-thio-D-ribofuranosyl) uracil and cytosine.

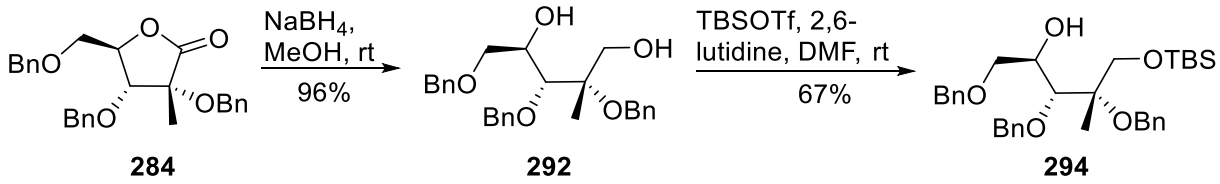
7.3. Synthetic study 2: towards the synthesis of 2′-*C*-methyl-1′-(4′-thio) nucleosides *via* 2-*C*-methyl-pentane-1,4-diol intermediate

As a means of overcoming the issue of selectively reducing lactones **282** – **284**, it was proposed that the lactone **284** could be reduced twice to the diol **292** using synthetic methodology similar to that described by Codée[158] and in section 2.6.1. (installation of a good leaving group at C4, S_N2 halogenation and sulfur insertion) be applied at the lower oxidation level.



Scheme 96. Proposed synthetic route to **166b** *via* diol **292**.

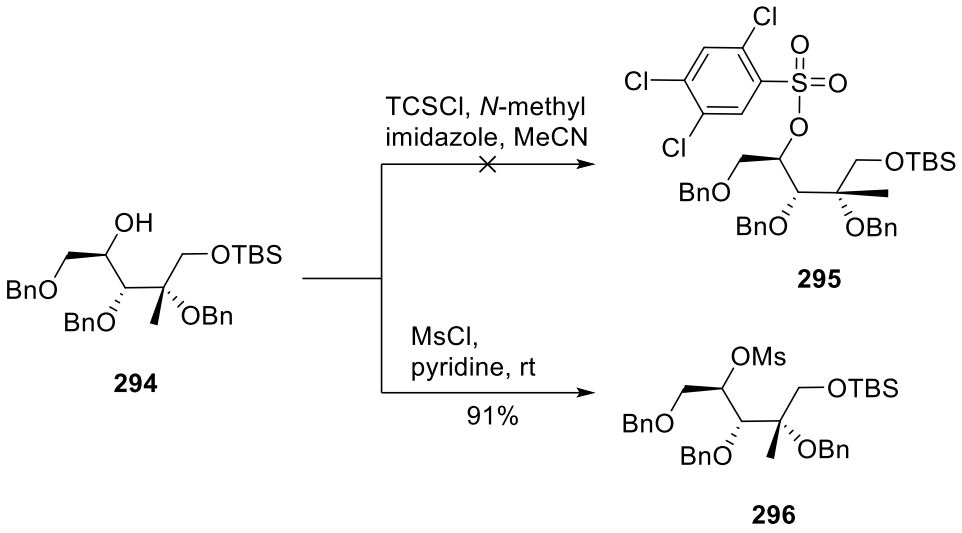
Thus **284** was treated with 2.2 equivalents of $NaBH_4$, which afforded **292** in quantitative yield (Scheme 97). The significant upfield shift of C1 from that typical of a lactone from δ_C 173.4 ppm in **284** to δ_C 71.5 ppm in **292** and the appearance of a new CH_2 proton peak at δ_H 3.79 – 3.52 ppm was a key marker of the transformation of the lactone to CH_2OH. Further, the change in mass was confirmed by HRMS with m/z 437.2341 corresponding to the [**292**+H]$^+$ species.



Scheme 97. Synthesis of **294**.

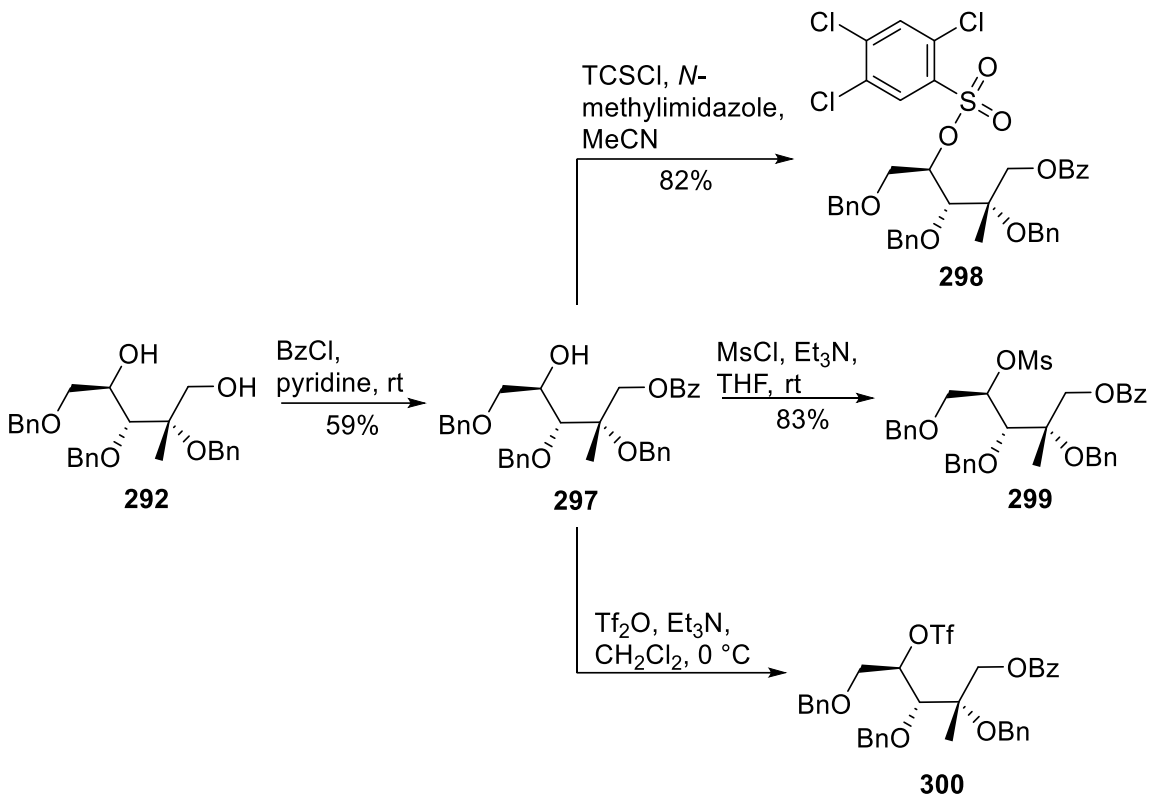
Protection of the 1-primary alcohol with a TBS group was first attempted *via* treatment of **292** with TBSCl and *N*-methylimidazole, however, the reaction did not go to completion. Thus, a stronger electrophile, TBSOTf was used with 2,6-lutidine which afforded **294** in a satisfactory yield of 67%. The increase in mass was observed *via* HRMS with m/z 551.3203 corresponding to the [**294**+H]$^+$ species.

The 4-hydroxyl group was then substituted with a good leaving group. Reaction with TCSCl and *N*-methylimidazole did not furnish **295**, with complete recovery of the starting material **294** (Scheme 98). Heating to reflux did not drive the reaction and it was postulated that steric hindrance from the bulky 2-*C*-methyl group was the cause of this poor reactivity. Thus, **294** was treated with MsCl in pyridine at rt, which afforded **296** in excellent yield of 91%.



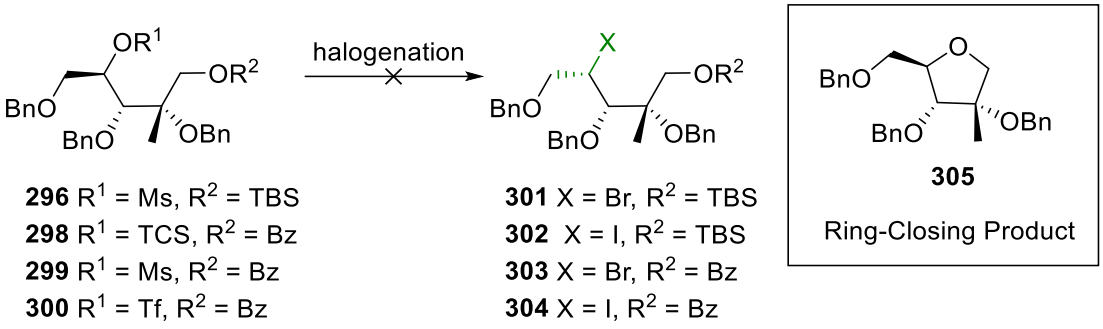
Scheme 98. Attempted installation of sulfonyl leaving groups onto **294**.

The 1-*O*-benzoyl analogue **297** was also synthesised. Treatment of **292** with BzCl in pyridine afforded **297** in 59% yield (Scheme 99). Sulfonyl leaving groups (TCS, Ms, Tf) were installed at the C4 hydroxyl moiety. The TCS moiety was installed *via* treatment of **297** with TCSCl, *N*-methylimidazole in MeCN at rt, which afforded **298** in 82% yield. No reaction was observed following treatment of **297** with MsCl in pyridine, however use of stronger base Et_3N afforded **299** in 83% yield. Lastly, reaction of **297** with Tf_2O in 1/5 pyridine/CH_2Cl_2 afforded triflate **300** which was used immediately without purification, due to its instability and susceptibility to degradation.



Scheme 99. Synthesis of **298** – **300** (yield not determined for **300**).

Stereochemical inversion of C4 *via* S_N2 halogenation was evaluated using various reagents and conditions (Table 7).



Entry	R^1	R^2	Reagent	Solvent	Temp. (°C)	Observation
1	Ms	TBS	LiBr	butanone	80	Formation of **305**
2	Ms	TBS	TBAI	butanone	80	Formation of **305**
3	Ms	TBS	KI	acetone	rt	Formation of **305**
4	TCS	Bz	LiBr	butanone	80	Decomposition

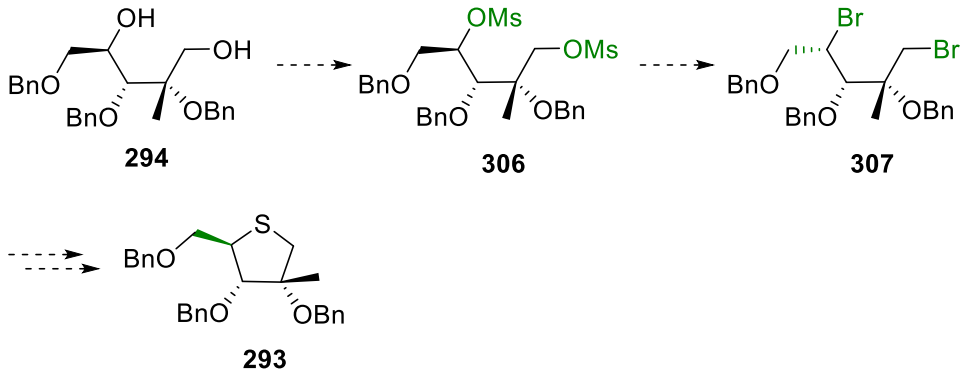
5	TCS	Bz	LiBr	butanone	60	No reaction
6	TCS	Bz	LiBr	butanone	rt	No reaction
7	TCS	Bz	KI	acetone	rt	No reaction
8	TCS	Bz	NaI	acetone	rt	No reaction
9	Ms	Bz	LiBr	butanone	60	No reaction
10	Tf	Bz	NaI	acetone	rt	Undesired products[a]

Table 7. Studies towards S_N2 halogenation at C4. [a] side products not identified.

Reaction of **296** with halide salts (Entries 1 – 3, Table 7) resulted in cleavage of the TBS group and subsequent intramolecular cyclisation to **305**, which was likely due to liberation or HI or HBr *in situ*. Due to the instability of the TBS group to halogenation conditions, the 1-*O*-benzoyl derivatives **298**, **299** and **300** were evaluated. Reaction of **298** with LiBr at 80 °C (Entry 4, Table 7) resulted in rapid decomposition of the starting material, however, no reaction occurred at rt or 60 °C (Entries 5 and 6, Table 7). Similarly, use of KI or NaI in acetone did not deliver the corresponding halide (Entries 7 and 8, Table 7). Reaction of **299** with LiBr in butanone at 60 °C (Entry 9, Table 7) did not result in product formation. Thus, the highly electrophilic triflate analogue **300** was treated with NaI in acetone at rt (Table 7, Entry 13), however, no product formation was indicated by HRMS, and the formation of multiple unidentified side products was observed by TLC and NMR analysis.

Further work is required to develop a double inversion strategy at C4. It is proposed that, following similar synthetic methodology to that reported by Codée and colleagues,[158] 1,4-di-*O* mesylate **306** could be prepared and subsequent 1,4-di-*O* halogenation to **307** could be trialled. Subsequent sulfur installation and intramolecular ring closing could then be achieved *via* reaction of

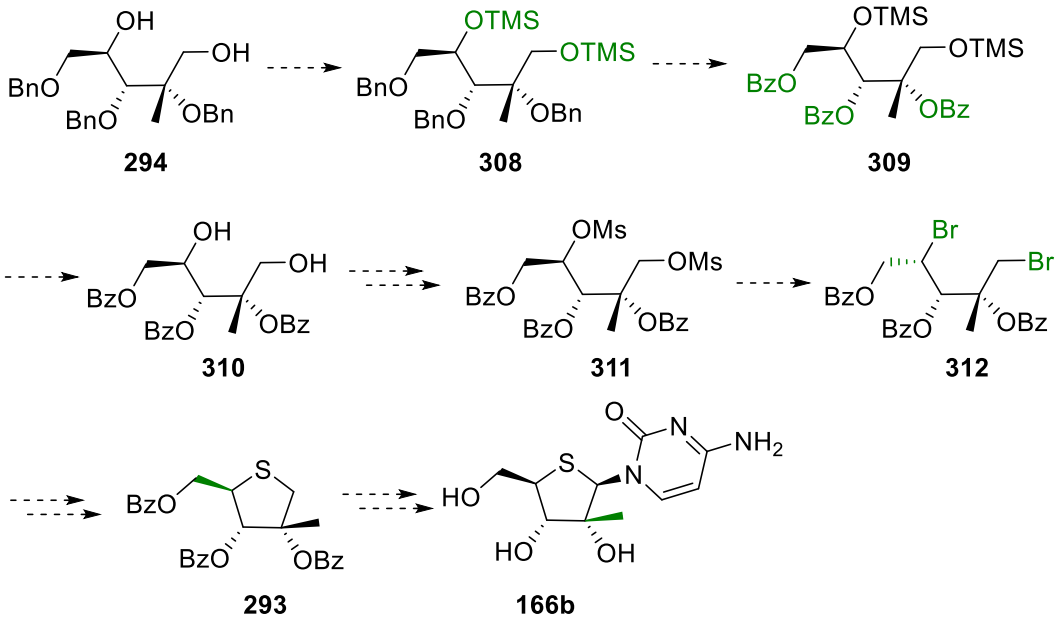
307 with Na_2S to obtain **293** (Scheme 100). However, due to previous reactivity issues with S_N2 halogenation of the secondary C4-mesyl group, similar issues with this approach may arise.



Scheme 100. Proposed alternative strategy for double inversion of the C4 stereocentre and subsequent preparation of **293**.

7.4. Conclusions and future work

Overall, attempts to develop a novel synthesis of **166b** *via* adaptation of Nakamura′s oxime strategy[109] or *via* diol **294** were unsuccessful. No improvement on Dukhan′s synthesis of **166b** was realised due to poor reactivities of the 2-*C*-methyl intermediates and consequent low yields. The 2-methyl substituent may have caused significant steric hindrance, which was clearly evident in attempted reductions of the lactones **282** – **284** with multiple bulky reducing agents. Reduced reactivity at C4 was observed in the synthetic approach *via* pentane 1,4-diol derivatives, as S_N2 halogenation at C4 failed to deliver the desired halides from the corresponding 4-sulfonyl derivatives **298** – **300**. This reduced reactivity may be due to inductive deactivation of the 4-position by adjacent electron donating benzyl groups, leading to reduced electrophilicity of C4. Thus, future work could include a protecting group alteration, such that the benzyl groups are substituted for ester groups to activate C4 for the halogenation step (Scheme 101). This would add three synthetic steps such that the overall route would be nine steps to key intermediate **293** and 12 steps to the deprotected nucleoside **166b** from ribonolactone **157**, which would be a moderate improvement on Dukhan′s 14 step synthesis. However, should the proposed synthesis deliver **166b** in a higher yield (8.6% overall yield for Dukhan′s synthesis), it could prove to be a worthwhile alternative synthetic strategy.



Scheme 101. Proposed alternative protecting group strategy to synthesise **166b**.

Chapter 8: Synthesis of 2′-deoxy-2′,2′-*gem*-difluoro-1′-(4′-thio-D-ribofuranosyl)cytosine analogues

8.1. Aims and rationale

The synthesis of 2′-deoxy-2′,2′-*gem*-difluoro-1′-(4′-thio-D-ribofuranosyl)cytosine **156** was reported in 1996/1997 by Yoshimura *et al.*, who employed DAST fluorination of a 2-keto thioriboside derivative to install the 2-gemdifluoro moiety (as described in Chapter 1, section 1.4.4.).[97,98,160] The anticancer activity of **156** was evaluated in T-cell leukemia CCRF-HSB-2 cells and human solid tumour KB cells where it had little activity. Gemcitabine **65** (Figure 40) was originally intended as an antiviral drug, and was recently found to be active against SARS-CoV (EC_{50} = 5.0 μM, see Chapter 1, section 1.3.4. for further detail). Thus, the synthesis of **156** was pursued with the aim to evaluate potential antiviral and anticancer activities. Further, synthesis of the novel sulfoxide derivative **313** was pursued, with the aim to compare its chemotherapeutic activity to that of **65** and **156**.

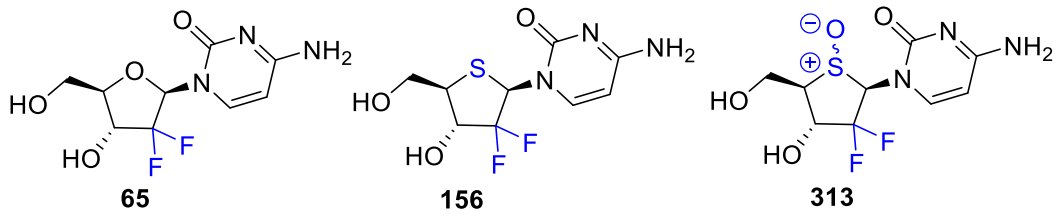
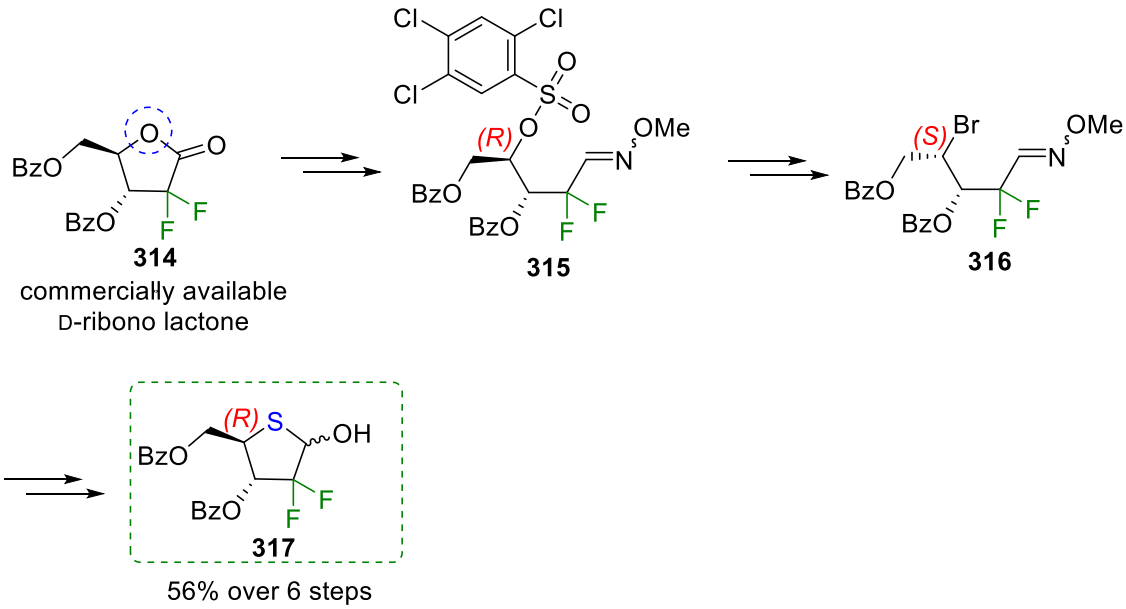


Figure 40. Structures of **65**, **156** and **313**.

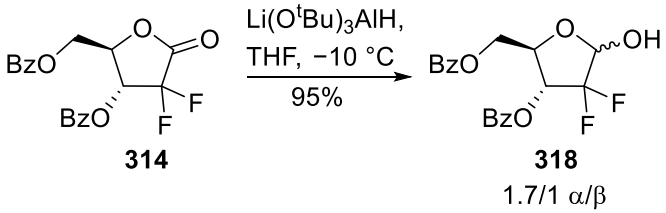
8.2. Synthesis of 2-deoxy-2-*gem*-difluoro-1-(4-thio) ribose derivative

Procedures established for preparation of **187** (see Chapter 2) and described by Nakamura and colleagues, Fujifilm Inc,[109] were adopted in the synthesis of the difluorinated thioribose intermediate 2-deoxy-2-difluoro-1-(4-thio-D-ribofuranose) **317** (Scheme 102).



Scheme 102. Synthetic route to **317**.

Lactone **314** was reduced with $Li(O^tBu)_3AlH$ at −10 °C, which afforded hemiacetal **318** in a 1.7/1 α/β ratio (determined by comparison of the NMR data to literature values)[109,145] in quantitative yield of 95%. The high yield proved consistent on scale-up from 200 mg to 20 g. Upon quenching of the reaction with saturated aqueous NH_4Cl, an insoluble emulsion formed, which impeded workup at scales >10 g. This was overcome by filtration of the emulsion through a pad of glass wool layered on top of the celite filter aid which simplified the workup process. Further, **318** could be used crude product for the next step, which eliminated the requirement for column chromatography.



Scheme 103. Synthesis of **318**.

In the ^{13}C NMR spectrum, a significant upfield shift of C1 from δ_C 162.5 in **314** to δ_C 121.6 ppm in **318** was evident, which is consistent with lactone reduction. Indeed, the C1 signal was evident in HSQC-DEPT correlation plot, which further confirmed the change from a quaternary carbon to a CH. The ^{19}F NMR spectrum clearly showed the changes in multiplicity from a dd in **314** to a ddd in

318 (for α-anomer J = 252.0 Hz, J = 16.3 Hz, J = 6.7 Hz; for β-anomer J = 242.1 Hz, J = 10.1 Hz, J = 6.2 Hz) due to the additional H1 coupling to fluorine (Figure 41).

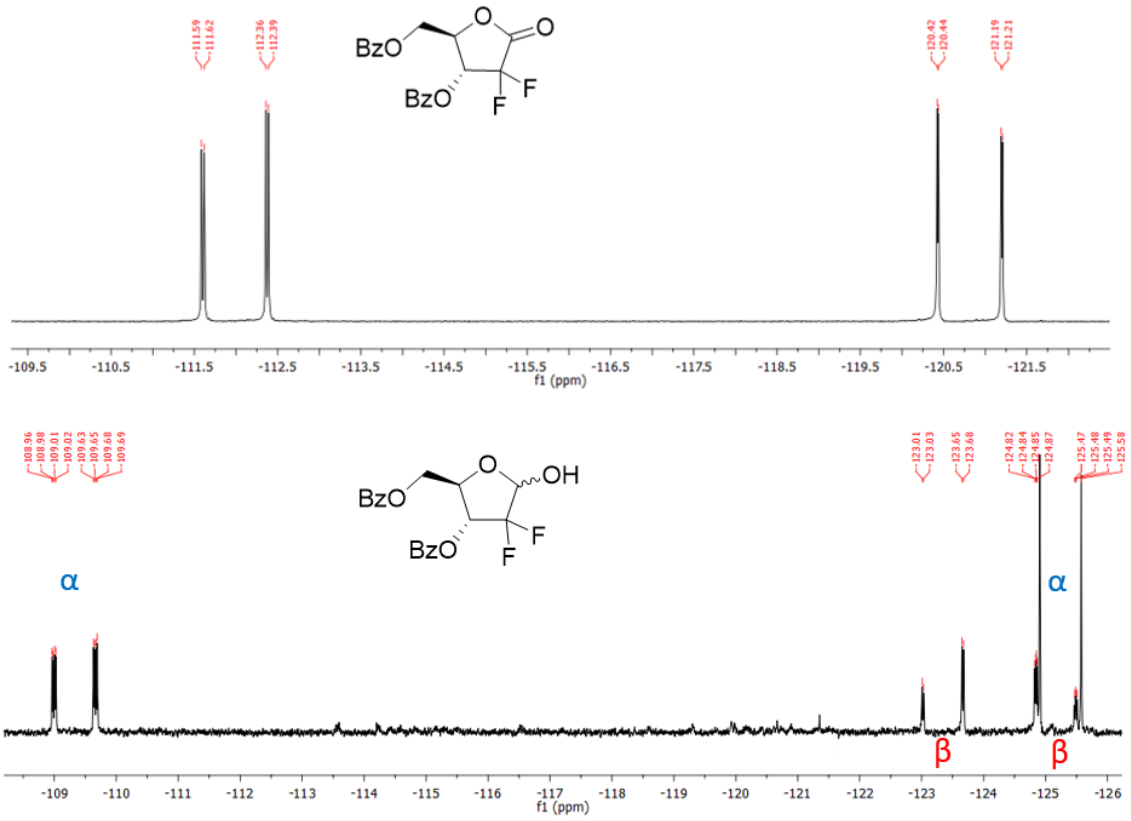
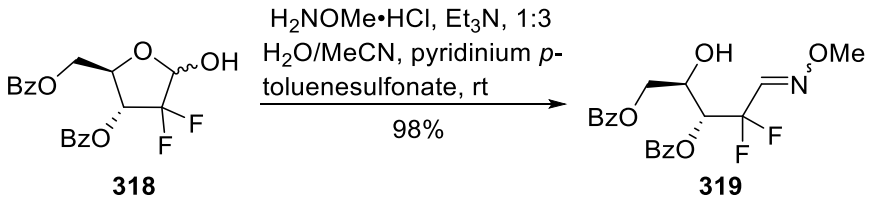


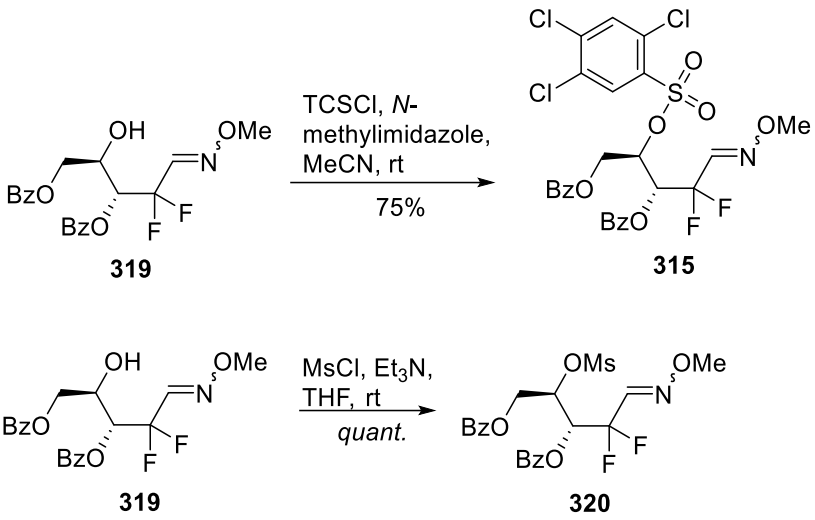
Figure 41. ^{19}F NMR spectrum ($CDCl_3$, 376 MHz) of **314** (top) and **318** (bottom).

Conversion of **318** to the corresponding oxime **319** was achieved *via* reaction with $H_2NOMe{\cdot}HCl$ and Et_3N in the presence of pyridinium *p*-toluene sulfonate (Scheme 104). The oxime **319** was obtained in a 10/1 isomer ratio. It may be hypothesised that the *Z*-alkene was favoured in this reaction, due to the ability of the 2-fluorine atoms to hydrogen bond with the incoming OCH_3 group and direct *cis* stereochemistry at the oxime.



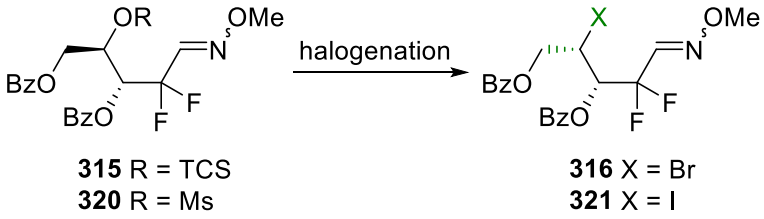
Scheme 104. Synthesis of **319**.

Trichlorobenzene sulfonyl and mesyl leaving groups were installed at the 4-hydroxyl group. Reaction of **319** with TCSCl and *N*-methylimidazole afforded **315** in 75% yield (Scheme 105), which was consistent on scale-up from 500 mg to 20 g. Reaction of **319** with MsCl and Et_3N afforded **320** in quantitative yield.



Scheme 105. Synthesis of **315** and **320**.

Sulfonate esters **315** and **320** were next evaluated for halogenation and inversion of stereochemistry at C4 (Scheme 106 and Table 8).



Scheme 106. S_N2 C4 halogenation reaction.

Entry	R	Reagent	Solvent	Temp. (°C)	Result
1	Ms	KI	acetone	rt	No reaction
2	Ms	LiBr	butanone	60 °C	No reaction
3	TCS	LiBr	butanone	80 °C	97% yield **316**

Table 8. Studies for preparation of **316** or **321**.

Treatment of mesylate **320** with KI in acetone at rt did not result in any formation of **321**, and **320** was recovered (Entry 1, Table 8). Thus, **320** was treated with LiBr in butanone at 80 °C (Entry 2,

Table 8), however, no reaction occurred and **320** was recovered. Reaction of **315** with LiBr in butanone at 80 °C effected the desired S_N2 substitution of the *O*-TCS group to afford **316** in quantitative yield of 97% (Entry 3, Table 8). The excellent yield was consistent on scale-up to 20.0 g, and bromide **316** could be used crude product in the next step. The presence of the C4 bromide was confirmed by HRMS, displaying m/z 487.0668, corresponding to the [**316**+NH_4]$^+$ species (Figure 42).

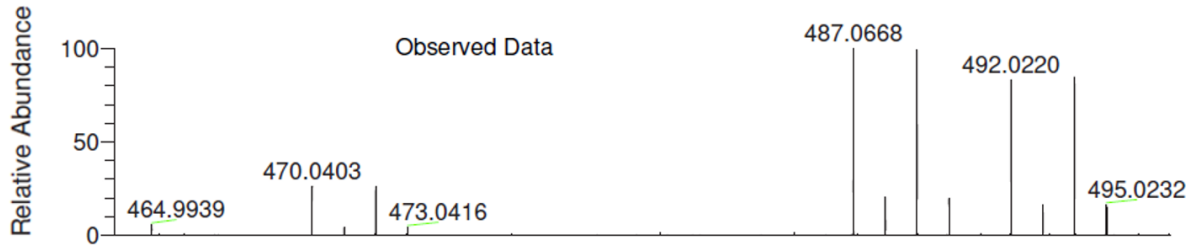
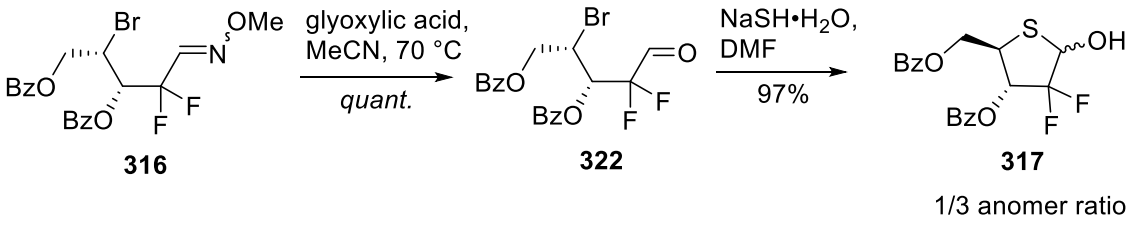


Figure 42. HRMS of **316** with the observed bromine isotope pattern.

In the ^{1}H NMR spectrum of **316**, the H3-H4 coupling constant only increases slightly from $J_{H3\text{-}H4}$ = 2.6 Hz in **315** to $J_{H4\text{-}H3}$ = 2.8 Hz in **316**, however, this may be accounted for by the significant affect the large bromide group may have on the conformation of **316**. Confirmation of the double inversion of C4 stereochemistry is discussed later in section 8.3.

Acidic hydrolysis of **316** using glyoxylic acid at 70 °C afforded aldehyde **322** in quantitative yield (Scheme 107). The characteristic aldehyde peak was evident as an apparent triplet at δ_H 9.70 ppm with equivalent coupling of $J_{H1\text{-}F}$ = 3.0 Hz to both vicinal fluorine atoms. Crude product **322** was immediately reacted with NaSH·H_2O in DMF which afforded the thiohemiacetal **317** as a mixture of anomers (1/3 ratio) in quantitative yield of 97%. HRMS displayed m/z of 395.0764 corresponding to the [**317**+H]$^+$ species, which confirmed the loss of bromide and incorporation of sulfur.

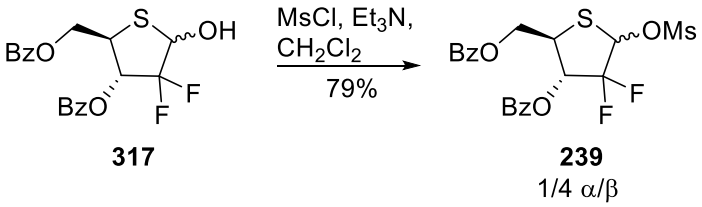


Scheme 107. Synthesis of **317**.

Overall, **317** was synthesised in 56% yield over six steps from commercially available lactone **314**. The excellent yields were consistent on scale-up to 20 g, which allowed for preparation of multi-gram quantities of the key intermediate **317**. Further, this synthesis was column chromatography free, and thus highly efficient.

8.3. Synthesis of 2′-deoxy-2′,2′-gem-difluoro-1′-(4′-thio-D-ribofuranosyl)cytosine and 2′-deoxy-2′,2′-gem-difluoro-1′-(4′-sulfinyl-D-ribofuranosyl)cytosine

Following the preparation of key intermediate **317**, the mesyl glycoside was synthesised *via* treatment of **317** with MsCl and Et_3N at rt which afforded **323** in 79% yield as a 1/4 α/β mixture of anomers (Scheme 108).



Scheme 108. Synthesis of **323**.

The β-anomer was separated by crystallisation from hot Et_2O and recrystallised by diffusion using CH_2Cl_2/hexane to afford crystals of **323β** which could be analysed by X-ray crystallography (Figure 43).

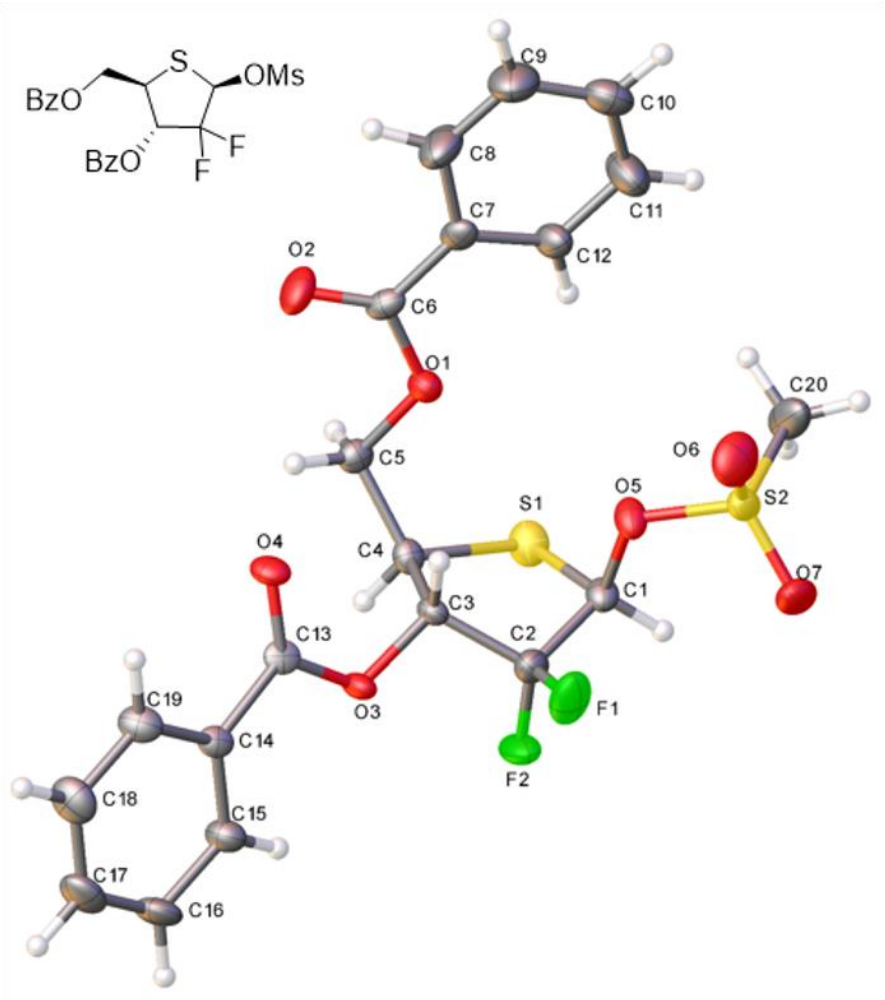
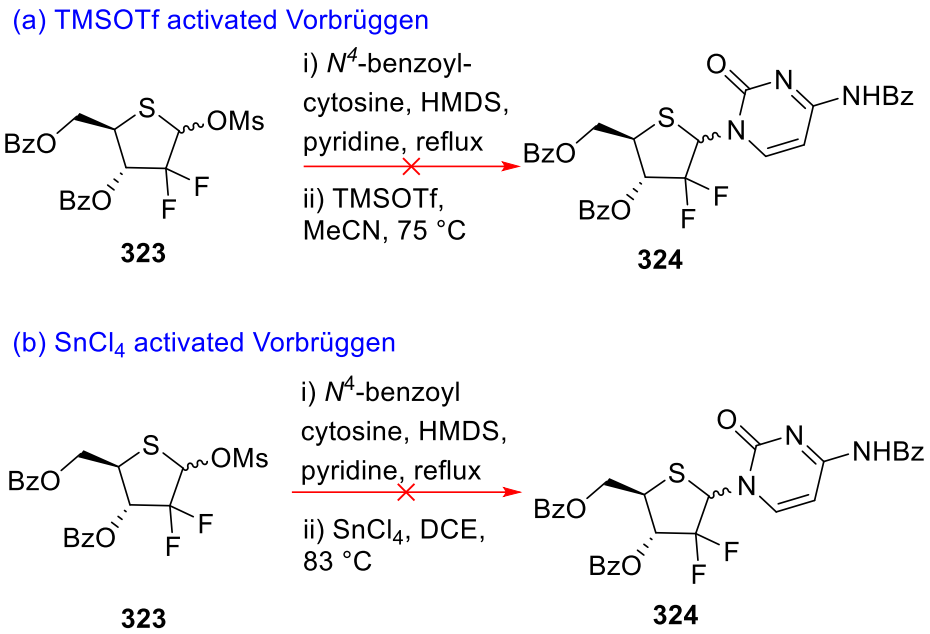


Figure 43. X-ray crystal structure of **323**. Slight positional disorder in one phenyl ring of **323** was modelled by splitting C7 and C10-C12 over two overlapping orientations with occupancies refined to approximately 0.7:0.3. EADP constraints were applied to the closely overlapping carbon atoms C7/C7A and C10/C10A, and the ring geometry and U_{ij} tensors were restrained with SADI, ISOR and/or RIGU cards where appropriate to maintain sensible geometries.

The crystal structure of **323β** confirmed the double inversion of the stereochemistry at C4, with the structure clearly adopting a D-*ribo* configuration. The anomeric mesylate was observed above the plane of the thiofuranose ring, consistent with 1′-β-D-*ribo* stereochemistry.

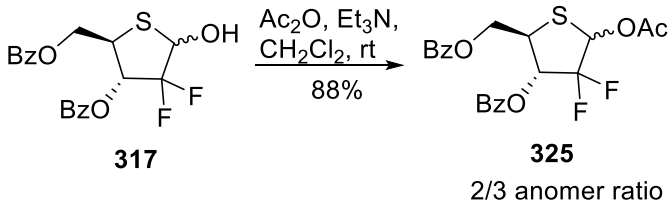
Glycosylation of silylated N^4-benozyl cytosine was trialled initially with the anomeric mixture of **323** to evaluate suitable conditions. Reaction of N^4-benozyl cytosine with HMDS and pyridine at reflux prepared the corresponding 2-*O*,4-*N* silylated cytosine derivative, which was subsequently treated with **323** and TMSOTf at 75 °C. Formation of **324** was not observed and prolonged reaction times (>30 h) resulted in decomposition of the starting material **323**. Thus, a stronger Lewis acid $SnCl_4$,[161] was trialled, however, **324** was not prepared and decomposition of **323** occurred after less

than 24 h. Moreover, reaction at lower temperatures (rt, 50 °C) did not furnish **324** and decomposition was evident by TLC analysis.



Scheme 109. Attempted synthesis of **324** from **323** *via* a) TMSOTf or b) $SnCl_4$ activated Vorbrüggen.

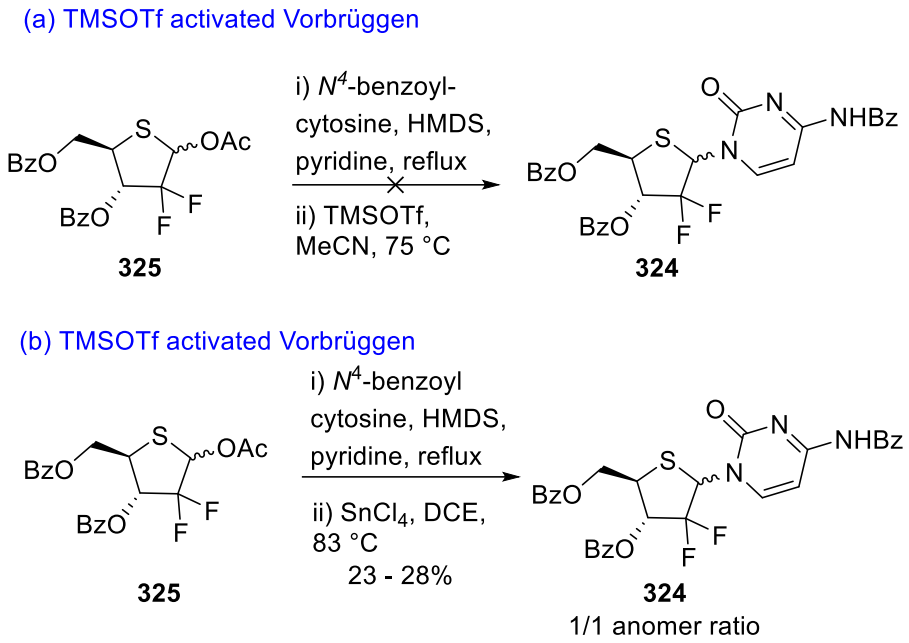
It was postulated that liberation of the strong acid, methanesulfonic acid, could be responsible for the significant decomposition in these reactions. Thus, **233** was acetylated *via* treatment with Ac_2O and Et_3N at rt (Scheme 110), which furnished **325** in 88% yield as a 2/3 anomeric mixture.



Scheme 110. Synthesis of **325**.

Acetyl glycoside **325** and TMSOTf were added to the 2-*O*,4-*N* silylated cytosine derivative and the mixture heated to 75 °C, however, no reaction occurred and **325** was recovered (Scheme 111). However, no decomposition was evident by TLC, thus, stronger Lewis acid $SnCl_4$ was trialled as an activator for the glycosylation with **325**, which furnished **324** as a 1/1 anomeric mixture, in low yields of 23 – 28% with 25% recovered yield of unreacted **325**. Quenching of the reaction with saturated aqueous $NaHCO_3$ resulted in the formation of an insoluble emulsion, which reduced the recovery of material (Crude product yield = 75%). Following column chromatography, small quantities of pure

324α (5%) and **324β** (3.3%) were isolated by fractional precipitation from EtOH or EtOAc, however, significant quantities could not be purified in this way.



Scheme 111. Attempted synthesis of **324** from **325** *via* a) TMSOTf or b) $SnCl_4$ activated Vorbrüggen.

NMR analysis of **324α/β** confirmed the installation of the N^4-benzoyl cytosine nucleobase, with the cytosine 5-Ar-CH and 6-Ar-CH signals evident at δ_H 8.31 and δ_C 146.1 ppm and at δ_H 7.56 – 7.44 and δ_C 132.9 ppm in the β-anomer. HRMS displayed m/z of 592.1344 corresponding to the [**324**+H]$^+$ species. The α/β anomers were determined retrospectively *via* deprotection to the free nucleoside **156**, NOE analysis and comparison to literature values.[98] Interestingly, in **324β** the two fluorine signals overlap to form an apparent broad dt such that individual fluorine peaks cannot be discerned (Figure 44), with similar coupling constants for each fluorine to H1′ and H3′.

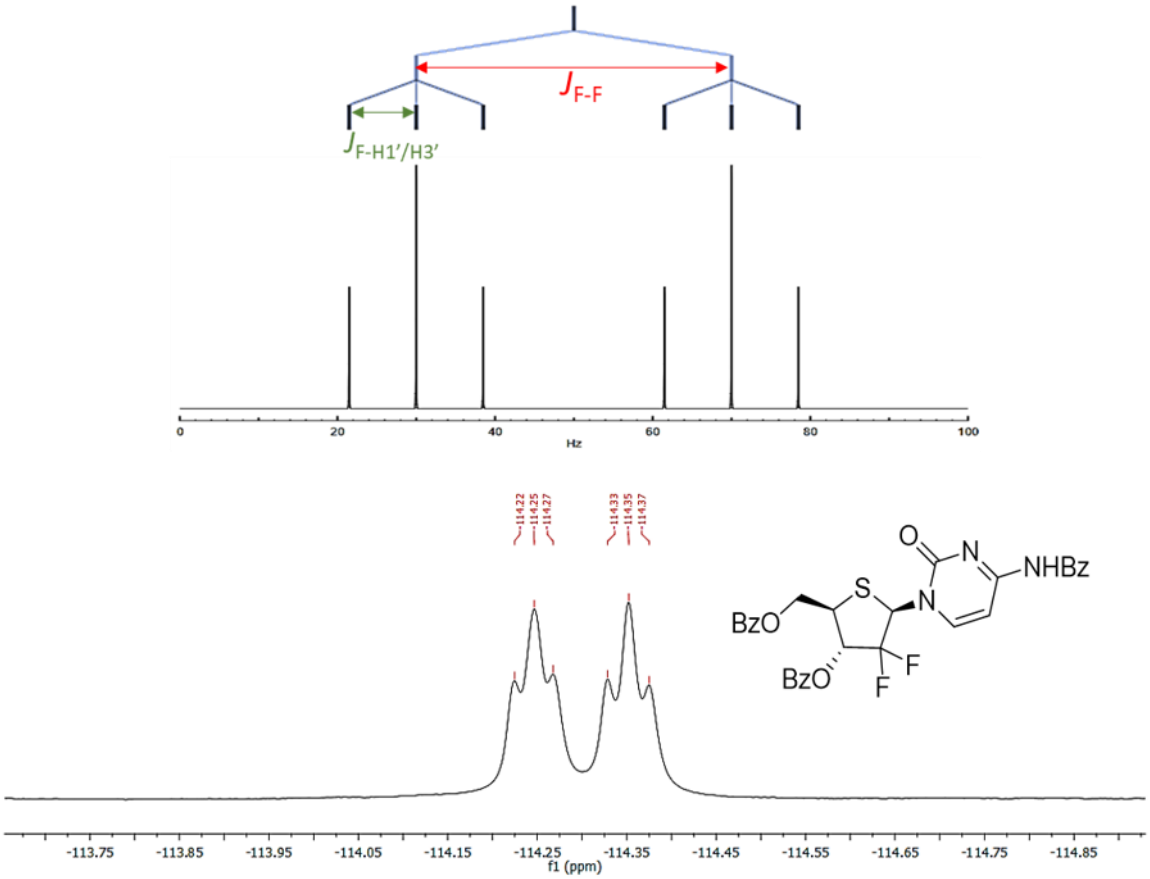


Figure 44. Tree diagram showing splitting of the 2-flourine peaks for **324β** and ^{19}F NMR ($CDCl_3$, 376 Hz) of **324β**.

This suggests that the two fluorine atoms in **324β** are nearly equivalent rather than diastereotopic as one may expect. Guschlbauer and Jankowski reported that increasing electronegativity of the 2′-subtituent of the ribofuranose ring increases the contribution of North (C3′-endo, C2′-exo) conformations of the ribose ring,[162] and this conformation may rationalise the interesting coupling of the two 2′-flourine atoms for **324β** (Figure 45); the coupling differences of each fluorine to H1′ and H3′ are so small that they are not discernible in the ^{19}F NMR spectrum.

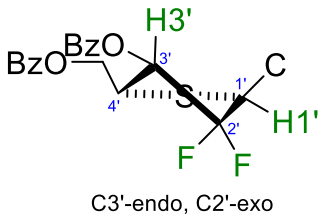


Figure 45. C3′-endo, C2′-exo conformation of **324β**. C = *N*^{4}-benzoyl cytosine.

In contrast, for **324α** the two fluorine signals were distinct, and separated considerably in the ^{19}F NMR spectrum (Figure 46). One fluorine peak corresponded to a broad doublet, such that $J_{F\text{-}H1'/H3'}$

was too small to be discerned at 400 MHz. The other fluorine signal split into an apparent broad dt with $J_{F\text{-}H1'/H3'}$ = 10.7 Hz.

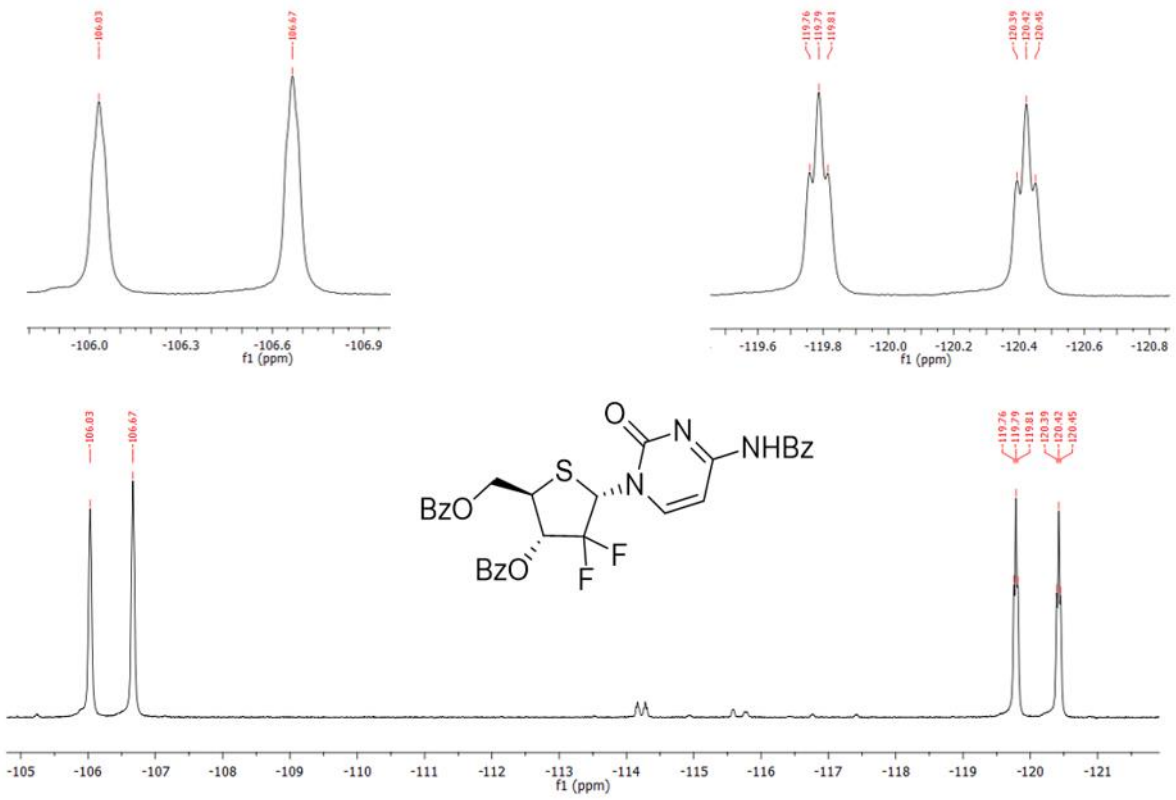


Figure 46. ^{19}F NMR ($CDCl_3$, 376 Hz) of **324*α***.

If **324*α*** also assumes the C3′-endo, C2′-exo conformation due to the presence of the electronegative 2′-flourine substituents, it may be rationalised that the fluorine highlighted in green in the figure below, experiences similar coupling to H1′ and H3′.

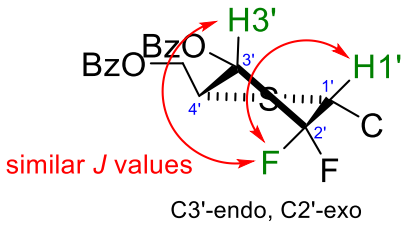
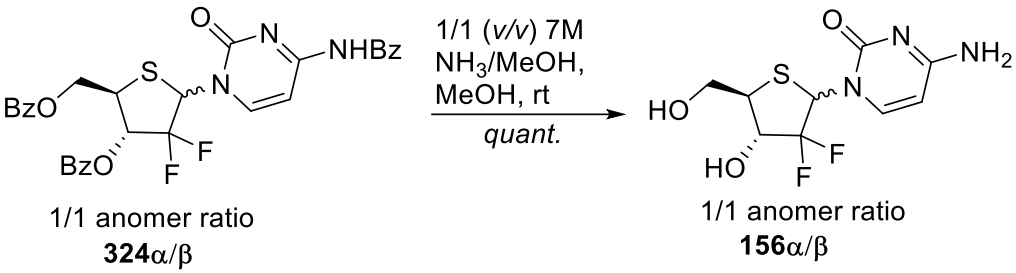


Figure 47. C3′-endo, C2′-exo conformation of **324*α***. C = *N*^{4}-benzoyl cytosine.

Deprotection of **324** was achieved *via* treatment with 7M NH_3/MeOH at rt, which afforded **156*α*/β** in quantitative yield (Scheme 112). Moreover, the small quantities of pure **324*α*** and **324β** were also deprotected using these conditions.



Scheme 112. Synthesis of **156α/β**.

Attempted crystallisation/precipitation of pure α- or β- anomers from the anomeric mixture proved unsuccessful and thus the anomers were separated *via* preparative HPLC which furnished **156α** in >95% purity in excellent yield of 44%. The β-anomer, **156β**, was isolated in 87% purity, and thus required two rounds of preparative HPLC to obtain **156β** in >99% purity, in satisfactory yield of 20%. Following separation of the two anomers, the stereochemistry of the 1′-position was assigned *via* NOE by irradiation of H1′ in **156α** (Figure 48). Through space correlation was observed from H1′ to H3′, which indicated H1′ was on the β-face and consequently, the nucleobase was on the α-face. This was further confirmed by the absence of through space correlation for H1′ to H4′, as would be expected for the α-nucleoside.

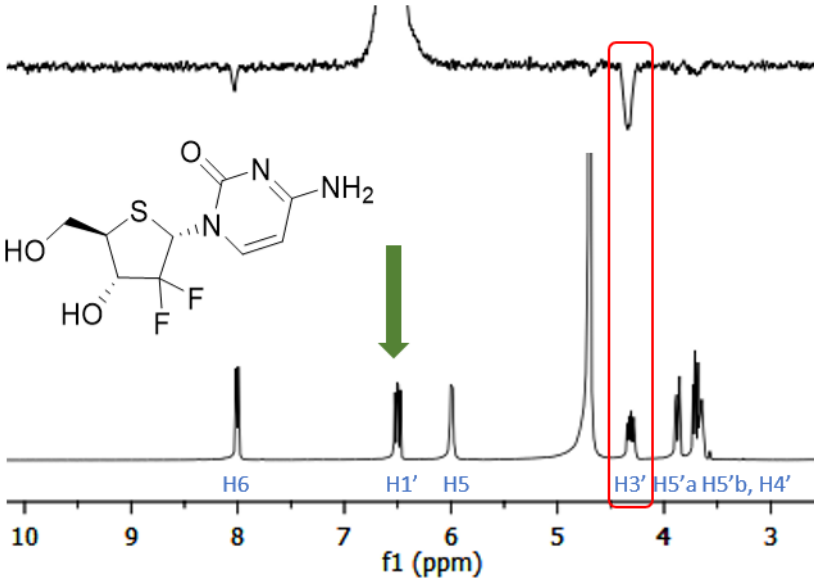
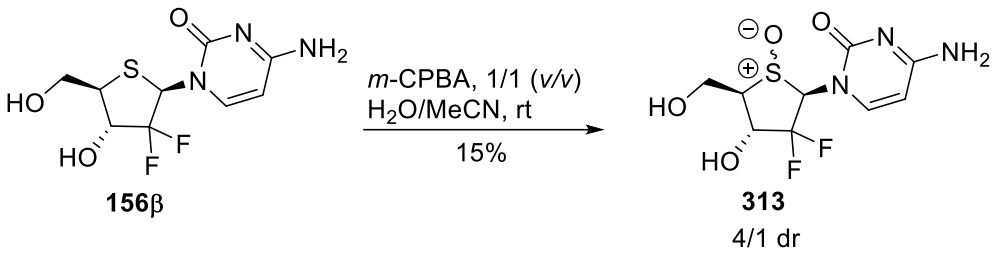


Figure 48. NOE of **156α** with H1′ irradiated overlayed with the corresponding ^{1}H NMR (400 MHz, D_2O).

A small quantity of purified **156β** (20 mg) was then oxidised to the corresponding sulfoxide **313** *via* reaction with *m*-CPBA in 1/1 (*v*/*v*) H_2O/MeCN (Scheme 113). Sulfoxide **313** was obtained in

a 4/1 dr in 15% yield after purification *via* preparative HPLC. The reaction did not go to completion after prolonged reaction times (18 h), which accounted for the low yield.



Scheme 113. Synthesis of **313**.

The sulfoxide **313** was considerably more polar than **156β** with R_f = 0.57 for **156β** and R_f = 0.41 for **313** (1/9 H_2O/MeCN), and indeed the oxidation was marked by a significant downfield shift of C1′ and C4′ from δ_C 59.7 – 59.1 and 46.3 ppm respectively in **156β** to δ_C 84.6 and 72.8 ppm respectively in **313**. HRMS displayed m/z of 296.0516 corresponding to the [**313**+H]$^+$ species.

Overall, methods for cytosine glycosylation with **323** or **325** were evaluated and $SnCl_4$ mediated Vorbrüggen reaction with the acetyl glycoside **325** deemed the most suitable. However, formation of an insoluble tin emulsion led to low yields for the glycosylation step. Nucleoside analogue **156** was successfully prepared and a preparative HPLC method developed to separate the α/β anomers, delivering **156** in high purity. Subsequent oxidation furnished novel nucleoside analogue **313** which was also purified to high standard *via* preparative HPLC.

8.4. Conclusions

A scalable and high-yielding synthesis of the 2-deoxy,2-*gem*-difluoro thiohemiacetal **317** was developed which allowed for preparation of multigram quantities **317**. The overall yield of 56% over six steps from the commercially available lactone **314** was double that for the preparation of **187**. The higher yield may have been due through electron withdrawal by the difluoro moiety causing improved reactivity for steps including installation of a sulfonyl leaving group, bromination and sulfur incorporation. Further, the 1′-(4′-thio) nucleoside targets **156** and **313** were prepared *via* $SnCl_4$ activated Vorbrüggen glycosylation and preparative HPLC methods developed and optimised to deliver **156β** and **313** in high purity of >99%. These analogues were evaluated in cytotoxicity assays, as described in Chapter 9.

Chapter 9: Cytotoxicity assays of nucleoside analogue panel

A panel of 1′-(4′-thio) nucleoside analogues (Figure 49) were evaluated in cytotoxicity assays against human pancreatic cancer (PANC-1) and human primary glioblastoma (U87-MG) cells and their CC_{50} values compared to AraC **23** and Gemcitabine **65**.

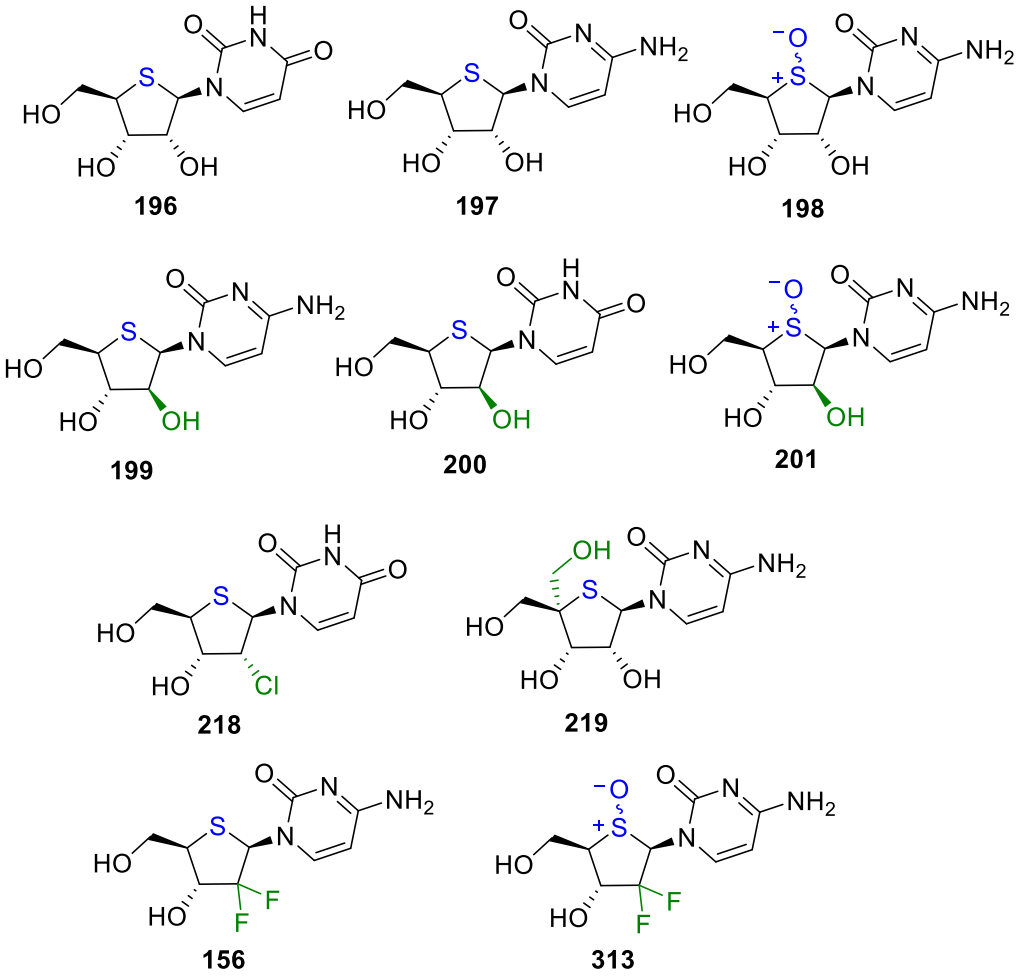


Figure 49. Structures of 1′-(4′-thio) nucleoside analogues evaluated in cytotoxicity assays.

Of the 1′-(4′-thio) nucleosides evaluated, only **199** had any measurable cytotoxicity (Table 9), with a low CC_{50} value of 0.59 μM in U87-MG cells, and a moderate CC_{50} value of 12 μM in PANC-1 cells (Entry 3, Table 9). However, the native analogue **23**, and gemcitabine **65** had superior CC_{50} values in both cell lines (Entry 1 and 2, Table 9).

Entry	Compound	CC_{50} (μM) U87-MG	CC_{50} (μM) PANC-1

1	**23**	0.19	0.43
2	**65**	0.01	0.21
3	**199**	0.59	12.0

Table 9. Cytotoxicity data for **23**, **65** and **199** in U87-MG and PANC-1 cells.

Further work is required to determine the IC_{50} values in various antiviral assays for the panel of 1′-(4′-thio) nucleoside analogues synthesised (Figure 50). The antiviral assay data could be compared to that of the corresponding native nucleosides (where applicable) for the purpose of establishing structure-activity relationships (SAR) based on modifications to the nucleoside scaffold. Indeed, should any compounds display promising antiviral activity, further chemical development of the active compounds could be pursued with the aim to improve the IC_{50} values further for use as a potential chemotherapeutic agent for the treatment of viral disease.

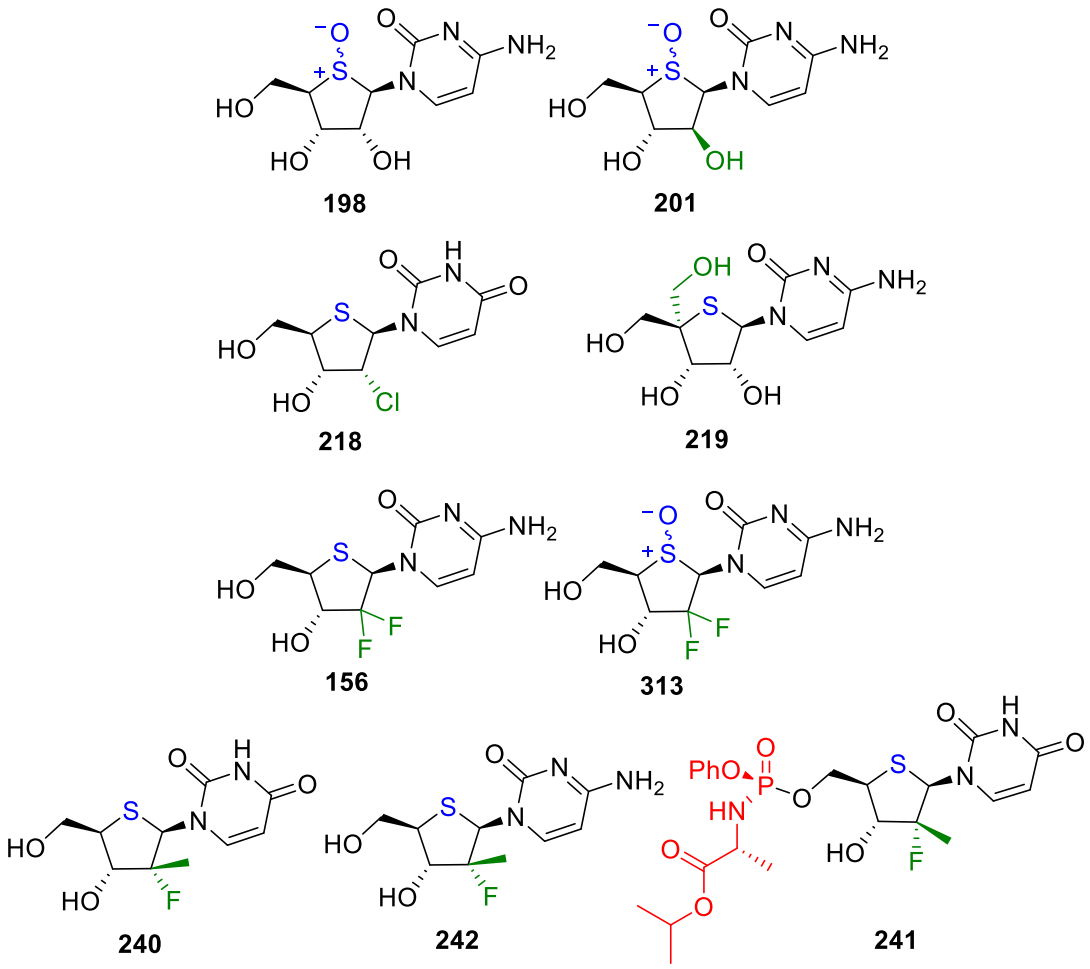


Figure 50. Structures of 1′-(4′-thio) nucleoside analogues which require evaluation in antiviral assays.

Chapter 10: Experimental

10.1 Materials and Methods

^{1}H NMR spectra were recorded on a Bruker Advance 400 (400 MHz) instrument using deuterochloroform (or other indicated solvent) as reference. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); dt (doublet of triplets); ddt (doublet of doublet of triplets); dqd (doublet of quartet of doublets); ddq (doublet of doublet of quartets); sp (septet) or m (multiplet). The multiplicity of each signal may be described as app. (apparent); ov. (overlapping); br. (broad). The number of protons (n) for a given resonance is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. ^{1}H NMR resonances were assigned with the aid of gDQCOSY. ^{13}C NMR spectra were recorded on a Bruker Advance 400 (100 MHz) instrument using the PENDANT sequence and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm relative to TMS where δ (TMS) = 0.00 ppm. ^{13}C NMR resonances were assigned with the aid of gHSQCAD. ^{19}F NMR were recorded on a Bruker Advance 400 (376 MHz) instrument. ^{31}P NMR were recorded on a Bruker Advance 400 (161 MHz) instrument. NMR data were analysed using Mestrenova software. Analytical thin layer chromatography (TLC) was carried out on pre-coated 0.25 mm ICN Biomedicals GmbH 60 F254 silica gel plates. Visualisation was by absorption of UV light or thermal development after dipping in 5% H_2SO_4 in MeOH. Optical activities were recorded on automatic Rudolph Autopol I or Bellingham and Stanley ADP430 polarimeters (concentration in g/100 mL). HRMS (ESI, NSI) were obtained on Agilent 6530 Q-TOF, LQT Orbitrap XL1 or Waters (Xevo, G2-XS TOF or G2-S ASAP) Micromass LCT spectrometers using a methanol mobile phase in positive/negative ionisation modes as appropriate. Manual column chromatography was carried out on silica gel (Sigma Aldrich 40–63 μm) under a positive pressure of compressed air. Automatic flash chromatography was carried out on silica gel (Reveleris® X2 system) under a positive pressure of compressed N_2. Dry CH_2Cl_2 and DMF was acquired from an Innovative Technology solvent purification system. Anhydrous MeOH, dioxane, EtOH, Et_2O, DMF, acetone was dried over

4 Å molecular sieves. Chemicals were purchased from Acros Organics UK, Aldrich UK, Alfa Aesar UK, Carbosynth, Fisher Scientific, Tokyo Chemical Industry. All solvents and reagents were purified and dried where necessary, by standard techniques. Where appropriate and if not stated otherwise, all non-aqueous reactions were performed under an inert atmosphere of nitrogen, using a vacuum manifold with nitrogen passed through 4 Å molecular sieves and self-indicating silica gel. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to n-hexane and petroleum ether to the fraction boiling between 40 and 60 °C. Volumes of less than 0.2 mL were measured and dispensed *via* automatic micropipette or Luer-lock micro-syringe. All reactions requiring heating were conducted using heating blocks atop stirrer hotplates with temperature controlled by an external probe. Reactions requiring lower temperatures were cooled using the following bath compositions: 0 °C (ice/water); -10 °C (acetone/ice). Reactions requiring lower temperature conditions or low temperatures for periods over 3 h were maintained using a Huber chiller unit and an acetone bath. An Agilent preparative HPLC system equipped with 1260, variable wavelength detector, auto sampler and 1260 series preparative fraction collector were used. The data was collected and processed using Agilent "Chemstation" 1260 series software. The UV detection wavelength was 254 nm.

10.2 ×-Ray Crystallography

All data were collected on a Bruker D8 Quest ECO diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystals were mounted on Mitegen micromounts in NVH immersion oil, and all collections were carried out at 150 K using an Oxford cryostream. Data collections were carried out using φ and ω scans, with collections and data reductions carried out in the Bruker APEX-3 2016 suite of programs. Multi-scan absorption corrections were applied for all datasets using SADABS 2016. The data were solved with the intrinsic phasing routine in SHELXT, and all data were refined on F^2 with full-matrix least squares procedures in SHELXL,[163] operating within the OLEX-2 GUI.[164] All non-hydrogen atoms were refined with anisotropic displacement parameters. Carbon-bound hydrogen atoms were placed in riding positions and refined with isotropic displacement parameters equal to 1.2 or 1.5 times the isotropic equivalent of their carrier atom.

10.3 Cytotoxicity Assays

Cell culture: PANC-1 (ATCC, Catalog# CRL-1469) cells were cultured at 37 °C with 5% CO_2 in DMEM (Corning, Catalog# 10-013CV), supplemented with 10% heat-inactivated FBS (Corning, Catalog# 35016CV) and 1X Non-essential amino acids (0.1 mmol each amino acids) (Corning, Catalog# 25025CI) and 1X Penicillin-Streptomycin Solution (Penicillin (100 IU) and Streptomycin (100 μg/mL) (Corning, Catalog# 30002CI). U87-MG (ATCC, Catalog# HTB-14) cells were cultured at 37 °C with 5% CO_2 in EMEM (Lonza, Catalog# 12-611F), supplemented with 10% heat-inactivated FBS (Corning, Catalog# 35016CV) and 1X Non-essential amino acids (0.1 mmol each amino acids) (Corning, Catalog# 25025CI) and 1X Corning™ Penicillin-Streptomycin Solution (Penicillin (100 IU) and Streptomycin (100 μg/mL) (Corning, Catalog# 30002CI). Testing compound stock solution: 40 mM (first experiment) and 5 mM (second experiment), respectively, were in DMSO. Compound DMSO stock solutions were diluted for 20-fold in culture medium, followed by 8 points of 3-fold serial dilutions in medium with 5% DMSO. The tenth point contained no compounds, only medium with 5% DMSO served as DMSO control. Reference compound Gemcitabine was 1 mmol in H_2O. The top concentration for PANC-1 was 5 μmol and for U87-MG was 0.185 μmol. Cytarabine was 2 mmol in H_2O. The top concentration was 10 μmol for both cell lines. The control for these two compounds were cells treated with medium only. The cells were seeded in a density of 4000 cells/well/100 μL for PANC-1 cells and 5000 cells/well/100 ul for U87-MG cells on 96-well white plates and incubated at 37C with 5% CO2 for 24 h. On day two, 10 μL of the serial diluted compounds were added onto the plate with cells, either in duplicate or triplicate. The top concentration of the testing compound was 200 uM (first experiment) and 25 μmol (second experiment). The final DMSO concentration in the assay for all wells was 0.5%. The cells were incubated with the compounds for three days at 37 °C with 5% CO_2. Cell *via*bilities were then determined using CellTiter-Glo, 2.0 (Promega, Catalog# G9243), which quantitated the amount of ATP present, which indicated the presence of metabolically active cells. Briefly, 100 μL of the CellTiter-Glo, 2.0 reagent was added to each well and the luminescent signal was recorded for 0.5 s/well on an EnSpire plate reader. The luminescent signals from 4 wells containing only medium were used as background which was subtracted from all other testing wells. The wells treated with only 0.5% DMSO were DMSO control,

was set as 100% of cell *via*bility. All the wells treated with cells will be as % of the Control. Data analysis was performed using GraphPad Prism software.

Assignment of proton and carbon atoms for NMR analysis follow the generic ring numbering systems shown in Figure 51. Symbol † denotes novel compounds; * denotes compound reported in the literature but not characterised.

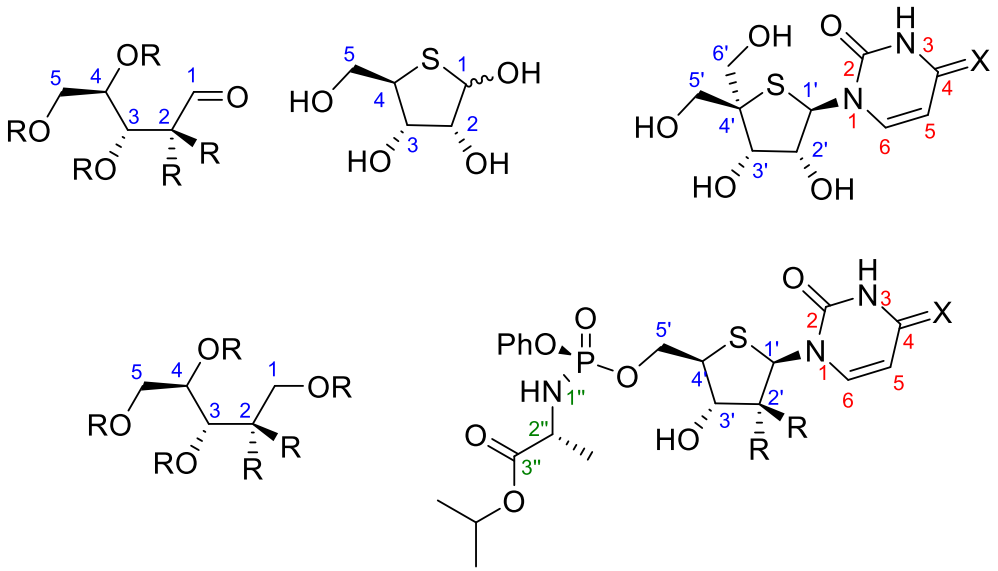
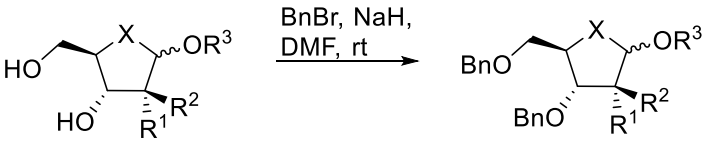


Figure 51. Numbering convention for compounds synthesised.

10.4 General Procedures

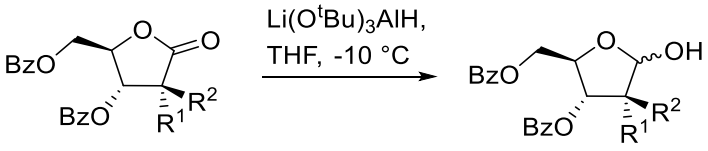
A) Benzylation



Alcohol (1.0 equiv.) was dissolved in DMF (0.25 M) and the solution cooled to -10 °C. A 60% dispersion of NaH (1.0 equiv.) was added and the solution stirred for 30 minutes. BnBr (1.5 equiv.) was added and the solution stirred for 1 h. Additions of NaH (1.0 equiv.) and BnBr (1.5 equiv.) were repeated for each alcohol group and the solution stirred vigorously at rt until TLC analysis indicated the reaction was complete. The reaction was quenched with MeOH and the solvent reduced *in vacuo* to <10 mL. The crude product was diluted with EtOAc and washed with H_2O (1 ×) and brine (1 ×).

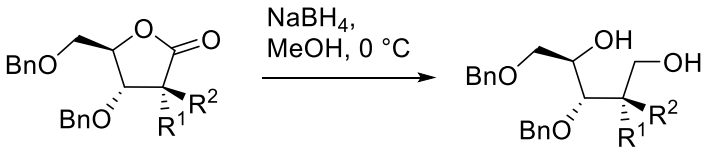
The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* and to obtain the crude product compound.

B) Lactone reduction to hemiacetal



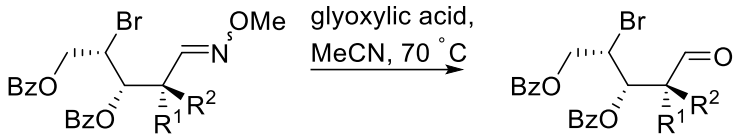
Ribonolactone (1.0 equiv.) was dissolved in THF (0.5 M) and the solution cooled to −10 °C. Li(O^{t}Bu)AlH (1.5 equiv.) was added at −10 °C and the solution gradually warmed to 0 °C. After TLC analysis indicated the reaction was compete, the reaction was quenched with saturated aqueous NH_4Cl solution until pH = 7, filtered through a glass wool packed sintered funnel and the organic solvents removed from the mother liquor *in vacuo*. The aqueous was extracted with EtOAc (3 ×) and the combined organic layers were washed with H_2O (2 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and dried *in vacuo* to obtain the crude product compound.

C) Lactone reduction to 1,4-diol



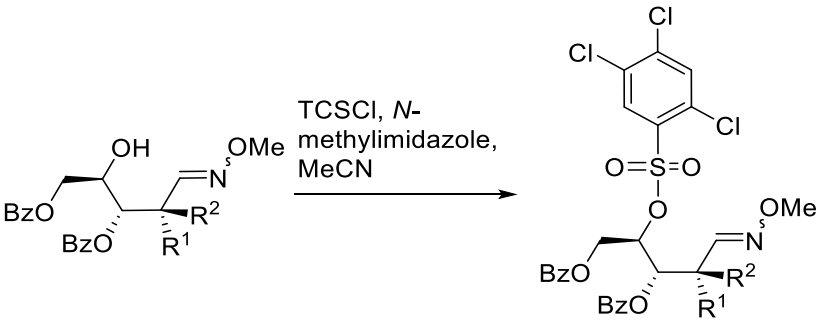
Benzyl protected ribolactone (1.0 equiv.) was dissolved in MeOH (0.15 M), and the solution cooled to 0 °C. Sodium borohydride (2.2 equiv.) was added in small portions over a period of 30 minutes at 0 °C. The reaction was then stirred at room temperature until TLC analysis indicated the reaction was complete. The solvent was removed *in vacuo* and the residue dissolved in EtOAc, washed with water (2 ×) and brine (1 ×), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to obtain the crude product compound.

D) Acidic hydrolysis of oxime



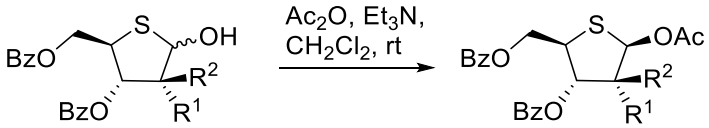
Oxime (1.0 equiv.) was dissolved in MeCN (0.5 M), glyoxylic acid (7.0 equiv.) added, and the solution heated to 70 °C overnight. The reaction was cooled to rt, poured onto H_2O and extracted with EtOAc (2 ×). The combined organic phases were washed with H_2O (5 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and dried *in vacuo* to obtain a mixture of the crude product aldehyde and the hydrate of the aldehyde.

E) Installation of 4-*O*-TCS group



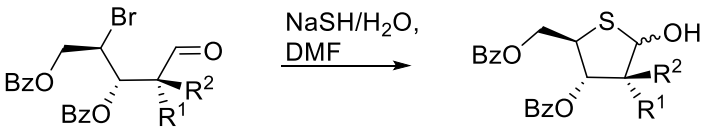
Oxime (1.0 equiv.), was dissolved in MeCN (0.38 M) and 2,4,5-trichlorobenzenesulfonyl chloride (1.1 equiv.) and *N*-methyl imidazole (1.1 equiv.) added. The solution stirred vigorously at rt until TLC analysis indicated the reaction was complete, and H_2O (10 mL) was added, and the solvent removed *in vacuo*. The residue was diluted in EtOAc/H_2O, the organic separated and the aqueous layer extracted with EtOAc (2 ×). The combined organic phases were washed with H_2O (3 ×) and brine (1 ×), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product compound.

F) Preparation of acetyl riboside



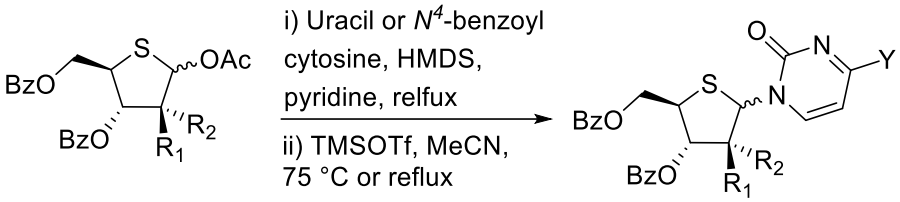
Thiohemiacetal (1.0 equiv.) was dissolved in CH_2Cl_2 (0.2 M) and Ac_2O (1.5 equiv.) and Et_3N (1.5 equiv.) added. The solution was stirred at rt until TLC analysis indicated the reaction was complete, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution (1 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and dried in vacuo to obtain the crude product compound.

G) Sulfur insertion and ring Closing



Aldehyde (1.0 equiv.) was dissolved in DMF (0.8 M) and cooled to 0 °C. $NaSH{\cdot}H_2O$ (1.3 equiv.) was dissolved in a minimum volume of H_2O, added to the solution and the solution stirred at 0 °C until TLC analysis indicated the reaction was complete. The solution was diluted with EtOAc, washed with H_2O (2 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and dried in vacuo to obtain the crude product compound.

H) TMSOTf activated Vorbrüggen



Uracil or N^4-benzoylcytosine (1.4 equiv.) was suspended in pyridine (1.7 M) and the flask charged with hexamethyldisilazane (9.9 equiv.) and the mixture heated to reflux for 3 h. The solution was cooled over ice, dried *in vacuo* and flushed with N_2 to obtain crude product silylated uracil/N^4-benzoyl cytosine as a colourless oil. Ribofuranoside (1.0 equiv.) was dissolved in MeCN (0.1 M) and the solution transferred under N_2 to the flask containing silylated nucleobase, and the resultant suspension cooled to 0 °C. TMSOTf (0.78 – 1.0 equiv.) was added dropwise and the solution stirred at 0 °C for a further 10 minutes before heating and stirring vigorously until the reaction was observed to be complete by TLC. The reaction was cooled to 0 °C and quenched with Et_3N until pH = 7 and stirred at 0 °C for 10 minutes. The solvent was reduced to <10 mL *in vacuo* and the residue diluted in EtOAc.

The organic phase was washed with saturated aqueous $NaHCO_3$ solution (3 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to obtain the crude product compound.

I) $SnCl_4$ activated Vorbrüggen

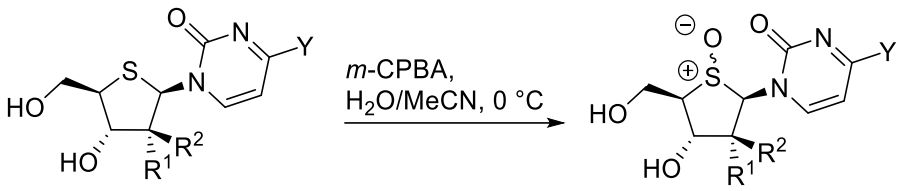
Uracil or N^4-benzoylcytosine (1.4 equiv.) was suspended in pyridine (1.7 M) and the flask charged with hexamethyldisilazane (9.9 equiv.) and the mixture heated to reflux for 3 h. The solution was cooled over ice, dried *in vacuo* and flushed with N_2, to obtain crude product silylated uracil/N^4-benzoyl cytosine as a colourless oil. Ribofuranoside derivative (1.0 equiv.) was dissolved in DCE (0.1 M) and the solution transferred under N_2 to the flask containing silylated nucleobase, and the resultant suspension cooled to 0 °C. $SnCl_4$ (7.0 equiv.) was added dropwise over 5 minutes and the solution stirred at 0 °C for 10 minutes before heating to reflux. Once TLC analysis indicated reaction was complete, the reaction solution was cooled to rt and poured onto an ice-cold solution of saturated aqueous $NaHCO_3$, the suspension stirred for 30 minutes, filtered through celite, and the organic solvents removed *in vacuo*. The aqueous was extracted with CH_2Cl_2 (4 ×) and the combined organic layers washed with saturated $NaHCO_3$ solution (1 ×), H_2O (1 ×) and brine (1 ×), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was purified on silica gel *via* automated flash chromatography to obtain an anomeric mixture of the nucleoside product.

J) Uracil to cytosine conversion

Nucleoside (1.0 equiv.) was suspended in MeCN (0.1 M) and the solution cooled to 0 °C. Et_3N (23 equiv.), 1,2,4-triazole (23 equiv.) and $POCl_3$ (2.4 equiv.) were added and the solution stirred for a further 10 minutes at 0 °C before warming to rt, stirring vigorously. Once TLC analysis indicated the reaction was complete, the solution was poured into an ice-cold saturated aqueous $NaHCO_3$ solution and diluted with EtOAc. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (2 ×) and brine (1 ×), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product which was dissolved in 1,4-dioxane (0.25 M) and the solution charged with an equal volume of 25% (*w/v*) NH_4OH solution. The solution was stirred at rt in a sealed flask overnight. The solvents were removed *in vacuo* and the crude product suspended in MeOH (0.25 M) and 7M NH_3/MeOH solution (3.0 equiv. per ester group) and stirred at 40 °C in a sealed flask until

TLC analysis indicated reaction was complete. The solvent was removed *in vacuo* to obtain the crude product compound.

K) *m*-CPBA mediated oxidation to sulfoxide



Nucleoside (1.0 equiv.) was dissolved in 2/1 (*v*/*v*) H_2O/MeCN solution and the solution cooled to < 5 °C over ice. *m*-CPBA (1.1 equiv.) was added, and the solution stirred over ice for 10 minutes. and then stirred at rt until TLC analysis indicated reaction was complete. The solvents were removed *in vacuo* and the crude product residue partitioned between H_2O/CH_2Cl_2. The aqueous layer was separated and washed with CH_2Cl_2 (2 ×), the solvent removed *in vacuo* and the crude product purified on octadecyl modified silica gel *via* automated flash chromatography.

L) Purity Quantification *via* Analytical HPLC – Method 1

Purity was quantified *via* analytical HPLC as follows: a 4.6 × 100 mm column packed 4 µ particle size with Poroshell 5 EC-C18 was employed to load the sample. A linear gradient system was optimised to quantify the purity of nucleoside samples where the initial mobile phase ratio was a mixture of mobile phase A (H_2O) and mobile phase B (MeOH) in a 0/100. The ratio of mobile phase B was increased from 0/100 to 100/0 over 10 minutes and the gradient held at 100/0 for 3 minutes before returning to initial conditions over 0.1 minutes. The flow rate was 2 mL/minute. A solution of nucleoside in H_2O (0.5 mg/mL) was prepared and 10 μL injected into the preparative HPLC system.

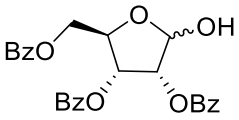
M) Purity Quantification *via* Analytical HPLC – Method 2

Purity was quantified *via* analytical HPLC as follows: a 250 x 10.5 mm column packed 5 µ particle size with Zorbax C18-A was employed to load the sample. A linear gradient system was optimised to quantify the purity of nucleoside samples where the initial mobile phase ratio was a mixture of mobile phase A (H_2O) and mobile phase B (MeOH) in a 0/100 and held at 0/100 for 5 minutes. The ratio of mobile phase B was increased from 0/100 to 100/0 over 5 minutes and the gradient held at 100/0 for

3 minutes before returning to initial conditions over 0.1 minutes. The flow rate was 10 mL/minute. The UV detection wavelength was 254 nm. A solution of nucleoside in H_2O (1 mg/mL) was prepared and 10 μL injected into the preparative HPLC system.

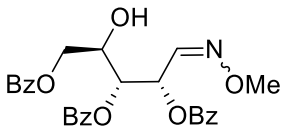
10.5. 1,4-Thio-D-ribose configured analogues

2,3,5-Tri-*O*-benzoyl-1′-α,β-D-ribofuranose **188**



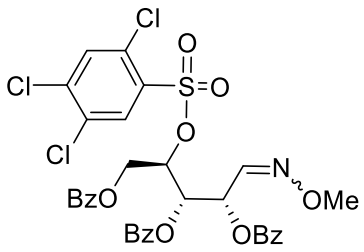
1-β-*O*-acetyl-2,3,5-tri-*O*-benzoyl-1-D-ribofuranose (100 g, 198 mmol, 1.0 equiv.) was dissolved in MeCN (2.0 L) and H_2O added (10 mL) and the solution cooled to 0 °C. $BF_3{\cdot}OEt_2$ (51 mL, 416 mmol, 1.6 equiv.) was added over 20 minutes and the solution stirred for a further 10 minutes at 0 °C before warming to rt and stirring vigorously for 2.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (1.2 L) and stirred for 5 minutes. The organic layer was separated and the organic solvent removed *in vacuo* and the crude product diluted in EtOAc (500 mL). The aqueous layer was extracted with EtOAc (6 × 300 mL) and the organic layers combined and washed with saturated aqueous $NaHCO_3$ solution (3 × 1 L) and brine (2 × 1 L), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain crude product **188** as a white foam (83.0 g, 179 mmol, 91%) which was used without further purification. R_f 0.22 (5/15, EtOAc/petroleum ether); 1.0/1.1 ratio anomers; **major anomer:** ^{1}H NMR (400 MHz, $CHCl_3$) δ 8.10 – 7.98 (m, 6H, Ar-H), 7.57 – 7.51 (m, 3H, Ar-H), 7.43 – 7.33 (m, 6H, Ar-H), 5.91 (dd, $J_{H3\text{-}H4}$ = 6.4 Hz, $J_{H3\text{-}H2}$ = 4.8 Hz, 1H, H3), 5.69 (dd, $J_{H2\text{-}H3}$ = 4.9 Hz, $J_{H2\text{-}H1}$ = 1.1 Hz, 1H, H2), 5.64 (dd, $J_{H1\text{-}OH}$ = 3.6 Hz, $J_{H1\text{-}H2}$ = 1.1 Hz, 1H, H1), 4.74 (dd, $J_{H5a\text{-}H5b}$ = 11.4 Hz, $J_{H5a\text{-}H4}$ = 3.4 Hz, 1H, H5a), 4.72 – 4.69 (m, 1H, H4), 4.63 (dd, $J_{H5b\text{-}H5a}$ = 11.0 Hz, $J_{H5b\text{-}H4}$ = 5.2 Hz, 1H, H5b), 3.81 (br s, 1H, OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6 (C=O, Bz), 165.5 (C=O, Bz), 165.4 (C=O, Bz), 133.5 (C_q, Ar-C), 133.4 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.84 (CH, Ar-C), 129.79 (CH, Ar-C), 129.77 (CH, Ar-C), 128.63 (CH, Ar-C), 128.58 (CH, Ar-C), 128.51 (CH, Ar-C), 128.50 (CH, Ar-C), 128.45 (CH, Ar-C), 128.4 (CH, Ar-C), 100.5 (CH, C1), 79.4 (CH, C4), 76.2 (CH, C2), 72.4 (CH, C3), 65.2 (CH_2, C5); ESI HRMS *m*/*z* found: $(M+H)^+$ 463.1401 $C_{26}H_{22}N_2O_8$, requires $(M+H)^+$ 463.1387. NMR data is consistent with literature values.[109]

(2*R*,3*R*,4*S*)-2,3,5-Tri-*O*-benzoyl-4-hydroxy-1-(methoxyimino)pentane (*E*/*Z*) **189**



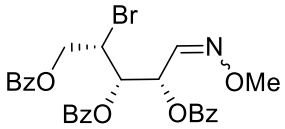
To a solution of **188** (79.7 g, 172 mmol, 1.0 equiv.) in MeOH (115 mL) was added $H_2NOMe{\cdot}HCl$ (21.5 g, 268 mmol, 1.6 equiv.) and the solution cooled to 0 °C. Et_3N (36 mL, 258 mmol, 1.5 equiv.) was added and the solution stirred for a further 15 minutes at 0 °C before warming to rt. After 21 h vigorous stirring, the solvent was removed *in vacuo* and the residue partitioned between EtOAc (1 L) and H_2O (1.5 L). The organic layer was separated and the aqueous extracted with EtOAc (2 × 500 mL). The organic layers were combined and washed with H_2O (1 L) and brine (1 L), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product **189** as a white foamy syrup (84.9 g, ~172 mmol, *quant.*) which was used without further purification. R_f 0.45 (1/9, acetone/toluene); 3/1 ratio isomers; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.98 (m, 6H, Ar-H), 7.62 (d, $J_{H1\text{-}H2}$ = 6.9 Hz, 1H, H1), 7.58 – 7.52 (m, 3H, Ar-H), 7.43 – 7.37 (m, 6H, Ar-H), 6.17 (dd, $J_{H2\text{-}H1}$ = 6.9 Hz, $J_{H2\text{-}H3}$ = 3.2 Hz, 1H, H2), 5.83 (dd $J_{H3\text{-}H4}$ = 8.2 Hz, $J_{H3\text{-}H2}$ = 3.2 Hz, 1H, H3), 4.71 – 4.63 (m, 1H, H5a), 4.46 – 4.43 (m, 1H, H5b), 4.42 – 4.39 (m, 1H, H4), 3.84 (s, 3H, OCH_3), 3.13 (d, $J_{OH\text{-}H4}$ = 5.8 Hz, 1H, 4-OH); ^{13}C NMR (101 MHz, $CDCl_3$) 166.9 (C=O, Bz), 165.3 (C=O, Bz), 165.1 (C=O, Bz), 145.1 (CH=N, C1), 133.6 (C_q, Ar-C), 133.6 (C_q, Ar-C), 133.4 (C_q, Ar-C), 133.3 (CH, Ar-C), 129.9 (CH, Ar-C), 129.83 (CH, Ar-C), 129.81 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 73.3 (CH, C3), 71.1 (CH, C2), 69.0 (CH, C4), 65.8 (CH_2, C5), 62.3 (OCH_3); ESI HRMS *m/z* found: $(M+Na)^+$ 514.1494 $C_{26}H_{26}NO_8$, requires $(M+Na)^+$ 514.1472. NMR data is consistent with literature values.[109]

(2*R*,3*R*,4*S*)-2,3,5-Tri-*O*-benzoyl-4-*O*-(2′,4′,5′-trichlorophenylsulfonyl)-1-(methoxyimino)pentane (*E*/*Z*) **193** †



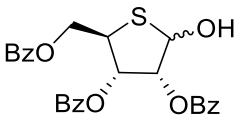
Compound **193** was prepared according to general procedure E using **189** (17.4 g, 33.4 mmol, 1.0 equiv.), 2,4,5-trichlorobenzenesulfonyl chloride (10.9 g, 38.9 mmol, 1.1 equiv.), *N*-methyl imidazole (3.1 mL, 38.9 mmol, 1.1 equiv.) and MeCN (93 mL). Reaction time = 5 h, as indicated by TLC (R_f = 0.26 for **189**, R_f = 0.57 for **193** in 1/1 Et_2O/petroleum ether). Purification: the crude product foam was triturated from ice-cold Et_2O (200 mL), the white solid collected by suction filtration and the filtrate washed with ice-cold Et_2O (100 mL) to obtain **193** as a white amorphous solid (13.5 g). The solvent in the mother liquor was removed *in vacuo* and triturated a second time from ice-cold Et_2O (50 mL), the white solid collected by suction filtration and the filtrate washed with ice-cold Et_2O (20 mL) to obtain a further quantity of **193** (3.50 g); (17.0 g total, 23.1 mmol, 69%). R_f 0.57 (1/1, Et_2O/petroleum ether); 5.7/1.0 ratio isomers; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.89 (m, 8H, Ar-H), 7.62 – 7.55 (m, 3H, Ar-H), 7.47 – 7.41 (m, 6H, Ar-H), 7.48 – 7.39 (m, 6H, Ar-H) 7.44 (d, $J_{H1\text{-}H2}$ = 6.2 Hz, 1H, H1), 6.03 (dd, $J_{H2\text{-}H1}$ = 6.2 Hz, $J_{H2\text{-}H3}$ = 5.2 Hz, 1H, H2), 5.98 (dd, $J_{H3\text{-}H2}$ = 5.2 Hz, $J_{H3\text{-}H4}$ = 3.8 Hz, 1H, H3), 5.53 (ddd, $J_{H4\text{-}H5b}$ = 7.4 Hz, $J_{H4\text{-}H3}$ = 3.8 Hz, $J_{H4\text{-}H5a}$ = 2.9 Hz, 1H, H4), 4.85 (dd, $J_{H5a\text{-}H5b}$ = 12.7 Hz, $J_{H5a\text{-}H4}$ = 2.9 Hz, 1H, H5a), 4.67 (dd, $J_{H5b\text{-}H5a}$ = 12.7 Hz, $J_{H5b\text{-}H4}$ = 7.3 Hz, 1H, H5b), 3.85 (s, 1H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7 (C=O, Bz), 164.8 (C=O, Bz), 164.8 (C=O, Bz), 143.9 (CH=N, C1), 139.0 (C_q, Ar-C), 133.9 (C_q, Ar-C), 134.4 (C_q, Ar-C), 133.7 (C_q, Ar-C), 133.5 (C_q, Ar-C), 133.4 (C_q, Ar-C), 132.0 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.7 (CH, Ar-C), 129.6 (CH, Ar-C), 128.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.64 (CH, Ar-C), 128.6 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 79.6 (CH, C4), 71.6 (CH, C3), 69.7 (CH, C2), 62.4 (OCH_3), 62.3 (CH_2, C5); ESI HRMS *m*/*z* found: $(M+H)^+$ 734.0422 $C_{33}H_{26}{}^{35}Cl_3NO_{10}S$, requires $(M+H)^+$ 734.0421.

(2*S*,3*R*,4*S*)-2,3,5-Tri-*O*-benzoyl-4-bromo-1-(methoxyimino)pentane (*E*/*Z*) **186**



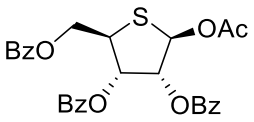
To a solution of **193** (8.60 g, 12.7 mmol, 1.0 equiv.) in 2-butanone (49 mL) was added LiBr (5.52 g, 63.5 mmol, 5.0 equiv.) and the solution stirred at 80 °C. After 18 h, the solution was cooled to rt and the solvent removed *in vacuo*. The residue was partitioned between EtOAc (250 mL) and H_2O (200 mL), the organic layer separated, and the aqueous layer extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with H_2O (200 mL) and brine (200 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow oil which was purified on silica gel *via* automated flash chromatography (0 – 32% Et_2O/petroleum ether) to obtain **186** as a white foamy syrup (6.29 g, 11.4 mmol, 89%). R_f 0.36 (1/4, Et_2O/petroleum ether); 3.3/1.0 ratio isomers; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.04 (m, 6H, Ar-H) 7.62 – 7.57 (m, 3H, Ar-H), 7.48 – 7.43 (m, 6H, Ar-H), 7.50 (d, 1H, $J_{H1\text{-}H2}$ = 3.3 Hz, H1), 6.03 (dd, 1H, $J_{H2\text{-}H3}$ = 6.5 Hz, $J_{H2\text{-}H1}$ = 3.3 Hz, H2), 6.00 (dd, 1H, $J_{H3\text{-}H2}$ = 6.5 Hz, $J_{H3\text{-}H4}$ = 3.0 Hz, H3), 4.81 – 4.75 (m, 1H, H5a), 4.70 (ddd, $J_{H4\text{-}H5}$ = 7.2 Hz, $J_{H4\text{-}H5}$ = 6.0 Hz, $J_{H4\text{-}H3}$ = 2.9 Hz, 1H, H4), 4.61 – 4.55 (1H, m, H5b), 3.70 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7 (C=O, Bz), 165.1 (C=O, Bz), 164.7 (C=O, Bz), 144.4 (CH=N, C1), 133.8 (C_q, Ar-C), 133.7 (CH, C1′), 133.5 (C_q, Ar-C), 133.4 (CH, Ar-C), 133.3 (CH, Ar-C), 130.2 (CH, Ar-C), 130.1 (CH, Ar-C), 130.0 (CH, Ar-C), 129.9 (CH, Ar-C), 129.9 (CH, Ar-C), 129.9 (CH, Ar-C), 129.3 (CH, Ar-C), 129.3 (CH, Ar-C), 129.0 (CH, Ar-C), 129.0 (CH, Ar-C), 128.9 (CH, Ar-C), 128.9 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 71.4 (CH, C2), 70.2 (CH, C3), 64.8 (CH_2, C5), 62.2 (OCH_3), 47.8 (C-Br, C4); ESI HRMS *m/z* found: $(M+H)^+$ 554.0808 $C_{27}H_{24}{}^{79}BrNO_7$, requires $(M+H)^+$ 554.0809. ^{1}H NMR data is consistent with literature values.[109]

2,3,5-Tri-*O*-benzoyl-1-α,β-(4-thio-D-ribofuranose) **195**



Thiohemiacetal **194** was prepared according to general procedure D using **186** (10.4 g, 18.8 mmol, 1.0 equiv.), glyoxylic acid (7.3 mL, 131 mmol, 7.0 equiv.) and MeCN (38 mL). Reaction time = 18 h. Obtained a mixture of the crude product **194** and the hydrate of aldehyde **194** (9.06 g, 17.2 mmol, 92%) which was used immediately without further purification. Hemiacetal **195** was prepared according to general procedure G using **194** (1.02 g, 1.94 mmol, 1.0 equiv.), $NaSH{\cdot}H_2O$ (187 mg, 2.46 mmol, 1.3 equiv.) and DMF (2.4 mL). Reaction time = 30 minutes. Purification: the crude product orange syrup was purified on silica gel *via* automated flash chromatography (0 – 30% EtOAc/petroleum ether) to obtain **195** as a white foam (0.703 g, 1.47 mmol, 76%). R_f 0.29 (1/1, Et_2O/petroleum ether); 1/3 ratio anomers; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.01 (m, 2H, Ar-H), 8.01 – 7.93 (m, 2H, Ar-H), 7.93 – 7.86 (m, 2H, Ar-H), 7.61 – 7.28 (m, 9H, Ar-H), 6.05 (dd, $J_{H3\text{-}H4}$ = 8.1 Hz, $J_{H3\text{-}H2}$ = 3.6 Hz, 1H, H3), 5.90 (dd, $J_{H2\text{-}H3}$ = 3.6 Hz, $J_{H2\text{-}H1}$ = 2.1 Hz, 1H, H2), 5.51 (d, $J_{H1\text{-}H2}$ = 2.1 Hz, 1H, H1), 4.74 (dd, $J_{H5a\text{-}H5b}$ = 11.4 Hz, $J_{H5a\text{-}H4}$ = 6.4 Hz, 1H, H5a), 4.61 (dd, $J_{H5b\text{-}H5a}$ = 11.4 Hz, $J_{H5b\text{-}H4}$ = 6.1 Hz, 1H, H5b), 4.23 (app. dt, $J_{H4\text{-}H3}$ = 8.0 Hz, $J_{H4\text{-}H5a/b}$ 6.1 Hz, 1H, H4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1 (C=O, Bz), 165.4 (C=O, Bz), 165.4 (C=O, Bz), 133.6 (C_q, Ar-C), 133.4 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.8 (CH, Ar-C), 129.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 80.2 (CH, C1), 79.3 (CH, C2), 75.6 (CH, C3), 65.8 (CH_2, C5), 46.3 (CH, C4); ESI HRMS *m/z* found: $(M+Na)^+$ 501.1001 $C_{26}H_{22}O_7S$, requires $(M+Na)^+$ 501.0984. ^{1}H NMR data is consistent with literature values.[109]

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-1-β-(4-thio-D-ribofuranose) **187**

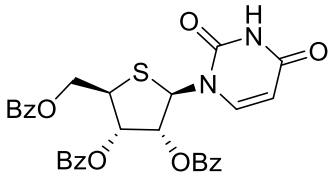


Ac_2O (0.20 mL, 2.14 mmol, 1.5 equiv.) was added to a solution of the crude product anomeric mixture of **195** (0.683 g, 1.43 mmol, 1.0 equiv.) in pyridine (7.2 mL) and the solution stirred at rt for 30

minutes. The solution was poured onto 1M aqueous HCl solution (100 mL) and diluted with EtOAc (100 mL). The organic layer was separated and washed with 1M aqueous HCl solution (100 mL), saturated aqueous $NaHCO_3$ solution (3 × 100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered and dried *in vacuo* to obtain the crude product as a yellow foam which was triturated from ice-cold MeOH (20 mL), the precipitate collected by suction filtration and the filtrate washed with ice-cold MeOH (10 mL) to obtain **187** as a white solid (372 mg, 0.715 mmol, 50% from **193**). R_f 0.67 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 8.00 (m, 2H, Bz), 7.97 (m, 2H, Ar-H), 8.00 – 7.92 (m, 2H, Ar-H), 7.66 – 7.54 (m, 1H, Ar-H), 7.54 – 7.42 (m, 4H, Ar-H), 7.38 – 7.28 (m, 4H, Ar-H), 6.06 (d, $J_{H1\text{-}H2}$ = 1.7 Hz, 1H, H1), 5.99 (dd, $J_{H2\text{-}H3}$ = 3.6 Hz, $J_{H2\text{-}H1}$ = 1.7 Hz, 1H, H2), 5.91 (dd, $J_{H3\text{-}H4}$ = 8.6 Hz, $J_{H3\text{-}H2}$ = 3.6 Hz, 1H, H3), 4.73 (dd, $J_{H5a\text{-}H5b}$ = 11.5 Hz, $J_{H5a\text{-}H4}$ = 6.0 Hz, 1H, H5a), 4.54 (dd, $J_{H5b\text{-}H5a}$ = 11.5 Hz, $J_{H5b\text{-}H4}$ = 6.2 Hz, 1H, H5b), 4.25 (app. dt, $J_{H4\text{-}3}$ = 8.6 Hz, $J_{H4\text{-}H5a/b}$ = 6.1 Hz, 1H, H4), 2.12 (s, 3H, Ac-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4 (C=O, Ac), 166.0 (C=O, Bz), 165.4 (C=O, Bz), 165.0 (C=O, Bz), 133.7 (C_q, Ar-C), 133.5 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.8 (CH, Ar-C), 129.7 (CH, Ar-C, 129.4 (CH, Ar-C), 129.0 (CH, Ar-C0, 128.8 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 79.7 (CH, C1), 76.8 (CH, C2), 75.1 (CH, C3), 65.2 (CH_2, C5), 46.2 (CH, C4), 20.9 (Ac-CH_3); ESI HRMS *m/z* found: $(M+Na)^+$ 543.1079 $C_{28}H_{24}O_8S$, requires $(M+Na)^+$ 543.1084. ^{1}H NMR data is consistent with literature values.[109]

10.6. 1′-(4′-Thio-D-*ribo*) nucleoside analogues

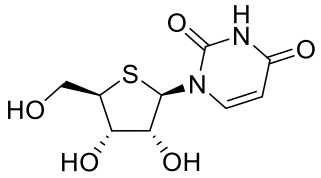
2′,3′,5′-Tri-*O*-benzoyl,1′-β-(4′-thio-D-ribofuranosyl)uracil **203** †



Nucleoside **203** was prepared according to general procedure H using uracil (2.90 g, 26.0 mmol, 1.4 equiv.), pyridine (19 mL), hexamethyldisilazane (40 mL, 190 mmol, 9.9 equiv.), **187** (10.0 g, 19.2 mmol, 1.0 equiv.), MeCN (200 mL) and TMSOTf (2.7 mL, 15.0 mmol, 0.78 equiv.). Reaction time = 72 h. Purification: the crude product orange-brown syrup was passed through a silica gel plug (3/7 EtOAc/petroleum ether), the solvents were removed *in vacuo* and the crude product triturated

from a boiling solution of petroleum ether (120 mL) and EtOAc (50 mL), the solid collected by suction filtration and the filtrate washed with rt petroleum ether (50 mL) to furnish **203** as a white amorphous solid (8.12 g, 14.2 mmol, 74%). R_f 0.80 (1/1, EtOAc/hexane); mp 213 – 215 °C; $[\alpha]_D^{27}$ –80.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 – 8.11 (m, 2H, Ar-H), 8.08 – 8.02 (m, 2H, Ar-H), 7.97 – 7.90 (m, 2H, Ar-H), 7.74 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 7.61 – 7.44 (m, 7H, Ar-H), 7.43 – 7.35 (m, 2H, Ar-H), 6.69 (d, $J_{H1'\text{-}H2'}$ = 6.8 Hz, 1H, H1′), 5.99 (dd, $J_{H2\text{-}H3}$ = 3.6 Hz, $J_{H2\text{-}H1}$ = 1.7 Hz, 1H, H2), 5.91 (dd, $J_{H3\text{-}H4}$ = 8.6 Hz, $J_{H3\text{-}H2}$ = 3.6 Hz, 1H, H3), 5.56 (dd, $J_{H5\text{-}H6}$ = 8.2 Hz, $J_{H5\text{-}NH}$ = 1.2 Hz, H5), 4.85 (dd, $J_{H5'a\text{-}H5'b}$ = 12.0 Hz, $J_{H5'a\text{-}H4'}$ = 5.6 Hz, 1H, H5′a), 4.71 (dd, $J_{H5'b\text{-}H5'a}$ = 12.0 Hz, $J_{H5'b\text{-}H4'}$ = 4.7 Hz, 1H, H5′b), 4.06 (m, 1H, H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1 (C=O, Bz), 165.4 (C=O, Bz), 165.2 (C=O, Bz), 162.1 (C=O, C4), 150.4 (C=O, C2), 133.7 (C_q, Ar-C), 133.5 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.8 (CH, Ar-C), 129.7 (CH, Ar-C), 129.4 (CH, Ar-C), 129.0 (CH, Ar-C0, 128.8 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 79.7 (CH, C1), 76.8 (CH, C2), 75.1 (CH, C3), 65.2 (CH_2, C5), 46.2 (CH, C4), 20.9 (Ac-CH_3); ESI HRMS *m/z* found: $(M+Na)^+$ 543.1079 $C_{28}H_{24}O_8S$, requires $(M+Na)^+$ 543.1084; Anal. Calcd for $C_{30}H_{24}N_2O_8S$: C, 62.93; H, 4.23; N, 4.89; S, 5.60, Found C, 63.18; H, 4.30; N. 5.09; S, 5.61.

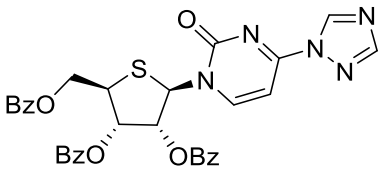
1′-β-(4′-Thio-D-ribofuranosyl)uracil **196**



To a suspension of **203** (8.12 g, 14.2 mmol, 1.0 equiv.) in MeOH (95 mL) and cooled to 0 °C was added 7M NH_3/MeOH solution (18 mL, 128 mmol, 9.0 equiv.). The solution was warmed to 40 °C and stirred for 72 h. The solvent was removed *in vacuo* to give an orange solid which triturated from CH_2Cl_2 (150 mL), filtered through a sintered funnel and the filtrate washed with CH_2Cl_2 (30 mL) and acetone (15 mL) to obtain a beige solid which was then purified on octadecyl modified silica gel *via* automated flash chromatography (H_2O) to afford **196** as a white foam (3.55 g, 13.6 mmol, 96%). R_f 0.28 (15/85, MeOH/CH_2Cl_2); $[\alpha]_D^{24.1}$ +16.0 (*c* 1.0, H_2O);[165,166] ^{1}H NMR (400 MHz, D_2O) δ 8.18 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H H6), 5.95 (d, $J_{H1'\text{-}H2'}$ = 5.7 Hz, 1H, H1′), 5.90 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.32

(dd, $J_{H2'\text{-}H1'}$ = 5.7 Hz, , $J_{H2'\text{-}H3'}$ = 3.7 Hz, 1H, H2′), 4.19 (app. t, $J_{H3'\text{-}H2',H3'\text{-}H4'}$ = 4.1 Hz, 1H, H3′), 3.87 (dd, $J_{H5'a\text{-}H5'b}$ = 12.0 Hz, $J_{H5'a\text{-}H4'}$ = 5.3 Hz, 1H, H5′a), 3.82 (dd, $J_{H5'b\text{-}H5'a}$ = 12.0 Hz, $J_{H5'b\text{-}H4'}$ = 5.5 Hz, 1H), 3.45 – 3.39 (m, 1H, H4′); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1H, N-H), 8.01 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 5.91 (d, $J_{H1'\text{-}H2'}$ = 7.4 Hz, 1H, H1′), 5.70 (d, $J_{H5\text{-}H6}$ = 8.0 Hz, 1H, H5), 4.28 – 4.08 (m, 1H, H2′), 4.04 (s, 1H, H3′), 3.90 – 3.69 (m, 2H, H4′ and H5a′), 3.19 (dd, $J_{H5'b\text{-}H5'a}$ = 11.8 Hz, $J_{H5'b\text{-}H4'}$ = 5.2 Hz, 1H, H5′b); ^{13}C NMR (101 MHz, D_2O) δ 166.1 (C=O, C4), 152.3 (C=O, C2), 143.0 (CH, C6), 102.4 (CH, C5), 77.4 (CH, C2′), 73.4 (C3′), 64.3 (CH, C1′), 62.4 (CH_2, C5′), 52.2 (CH, C4′); ESI HRMS *m/z* found: $(M+H)^+$ 261.0530 $C_9H_{12}N_2O_5S$, requires $(M+H)^+$ 261.0540. NMR data is consistent with literature values (in DMSO-D_6).[167]

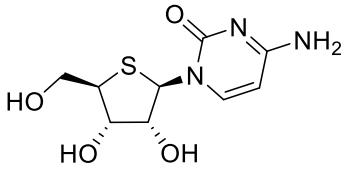
2′,3′,5′-Tri-*O*-benzoyl-4-*C*-(1,2,4-triazole)-1′-β-(4′-thio-D-ribofuranosyl)uracil **204** †



A suspension of **208** (2.00 g, 3.49 mmol, 1.0 equiv.) in MeCN (35 mL) was cooled to 0 °C. Et_3N (11 mL, 80.3 mmol, 23 equiv.), 1,2,4-triazole (5.44 g, 78.5 mmol, 23 equiv.) and $POCl_3$ (0.80 mL, 8.52 mmol, 2.4 equiv.) were added and the solution stirred for a further 10 minutes at 0 °C before warming to rt, stirring vigorously. After 3 h, the solution was poured into an ice-cold saturated aqueous $NaHCO_3$ solution (150 mL) and diluted with EtOAc (150 mL). The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (2 × 100 mL) and brine (150 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product **204** as a yellow foam (2.29 g, ~3.49 mmol, *quant.*), which was used immediately in the next step without further purification. R_f 0.31 (1/1, EtOAc/petroleum ether); $[\alpha]_D^{24.2}$ –78.4 (c 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H, triazole CH), 8.51 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6), 8.21 – 8.10 (m, 3H, 2 × Ar-H and triazole CH), 8.06 – 8.02 (m, 2H, Ar-H), 8.00 – 7.91 (m, 2H, Ar-H), 7.70 – 7.36 (m, 9H, Ar-H), 6.92 (d, $J_{H5\text{-}H6}$ = 7.3 Hz, 1H, H5), 6.85 (d, $J_{H1'\text{-}H2'}$ = 6.5 Hz, 1H, H1′), 6.06 (dd, $J_{H2'\text{-}H1'}$ = 6.5 Hz, $J_{H2'\text{-}H3'}$ = 4.0 Hz, 1H, H2′), 5.95 (app. t, $J_{H3'\text{-}H2'/H4'}$ = 3.9 Hz, 1H, H3′), 4.86 (dd, $J_{H5'a\text{-}H5'b}$ = 12.0 Hz, $J_{H5'a\text{-}H4'}$ = 5.5 Hz, 1H, H5′a), 4.72 (dd, $J_{H5'b\text{-}H5'a}$ = 12.0 Hz, $J_{H5'b\text{-}H4'}$ = 5.0 Hz, 1H, H5′b), 4.21 – 4.14 (m,

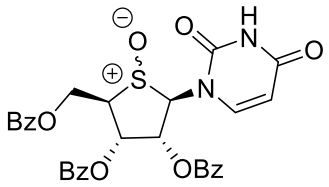
1H, H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1 (C=O, Bz), 165.4 (C=O, Bz), 165.2 (C=O, Bz), 159.1 (C-N, C4), 154.8 (C=O, C2), 154.2 (triazole CH), 147.1 (CH, C6), 143.4 (triazole CH), 133.9 (C_q, Ar-C), 133.8 (C_q, Ar-C), 130.1 (C_q, Ar-C), 130.0 (CH, Ar-C), 129.8 (CH, Ar-C), 129.1 (CH, Ar-C), 128.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.3 (CH, Ar-C), 96.1 (CH, C5), 76.7 (CH, C2′), 74.4 (CH, C3′), 64.3 (CH_2, C5′), 63.9 (CH, C1′), 48.2 (CH, C4′); ESI HRMS *m/z* found: $(M+H)^+$ 624.1567 $C_{32}H_{25}N_5O_7S$, requires $(M+H)^+$ 624.1547.

1′-β-(4′-Thio-D-ribofuranosyl)cytosine **197**



Crude product **204** (2.29 g, 3.49 mmol, 1.0 equiv.) was dissolved in 1,4-dioxane (15 mL) and the solution charged with 25% (*w*/*v*) aqueous NH_4OH solution (15 mL, 107 mmol, 30 equiv.) and stirred at rt in a sealed flask overnight. The solvents were removed *in vacuo* and the crude product suspended in MeOH (16 mL) and 7M NH_3 in MeOH solution (4.5 mL, 31.4 mmol, 9.0 equiv.) added and stirred at 40 °C in a sealed flask for 24 h. The solvent was removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 30%, MeOH/$CHCl_3$) to obtain **197** as a yellow foam (0.698 g, 2.69 mmol, 77%). R_f 0.27 (1/9, H_2O/MeCN); ^{1}H NMR (400 MHz, D_2O) δ 8.18 (d, J_{H6-H5} = 7.6 Hz, 1H, H6), 6.07 (d, J_{H5-H6} = 7.6 Hz, 1H, H5), 5.97 (d, $J_{H1'-H2'}$ = 5.2 Hz, 1H, H1′), 4.39 – 4.26 (m, 1H, H3′), 4.26 – 4.10 (m, 1H, H2′), 3.92 (dd, $J_{H5'a-H5'b}$ = 12.0 Hz, $J_{H5'a-H4'}$ = 5.1 Hz, 1H, H5′a), 3.84 (dd, $J_{H5'b-H5'a}$ = 12.0 Hz, $J_{H5'b-H4'}$ = 5.6 Hz, 1H, H5b), 3.63 – 3.44 (m, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 165.9 (C-NH_2, C4), 158.2 (C=O, C2), 142.8 (CH, C6), 96.4 (CH, C5), 77.6 (CH, C3′), 73.1 (CH, C2′), 65.0 (CH, C1′), 62.2 (CH_2, C5′), 51.7 (CH, C4′); ESI HRMS *m/z* found: $(M+H)^+$ 260.0714 $C_{13}H_{13}N_3O_4S$, requires 260.0700. NMR data is consistent with literature values.[168]

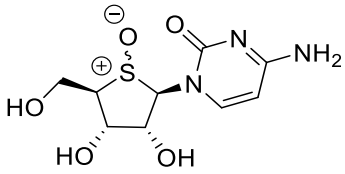
2′,3′,5′-Tri-*O*-benzoyl-1′-β-(4′-sulfinyl-D-ribofuranosyl)uracil **205** †



A solution of **203** (245 mg, 0.428 mmol, 1.0 equiv.) in CH_2Cl_2 (4.3 mL) was cooled to –40 °C and *m*-CPBA (148 mg, 0.856 mmol, 2.0 equiv.) added. The suspension was stirred at –40 °C for 1 h. The reaction was quenched *via* addition of saturated aqueous $Na_2S_2O_3$ solution until pH = 7 and the solution extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a white foam (5/1 ratio of diastereoisomers) which was purified on silica gel *via* automated flash chromatography (20 – 66% EtOAc/petroleum ether) to obtain the major diastereoisomer of **205** as a white solid (131 mg, 0.223 mmol, 52%) and the minor diastereoisomer of **205** as a white solid (28.0 mg, 47.6 μmol mmol, 11%). **Major diastereoisomer:** R_f 0.07 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{25.7}$ +13.8 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 8.10 – 8.02 (m, 4H, Ar-H), 7.96 – 7.91 (m, 2H, Ar-H), 7.61 – 7.52 (m, 3H), 7.46 – 7.36 (m, 6H), 7.35 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 6.21 (dd, $J_{H3'\text{-}H4'}$ = 7.2 Hz, $J_{H3'\text{-}H2'}$ = 4.8 Hz, 1H, H3′), 6.08 (t, $J_{H2'\text{-}H1'/H3'}$ = 4.8 Hz, 1H, H2′), 5.81 (d, $J_{H5\text{-}H6}$ = 8.0 Hz, 1H, H5), 5.05 (d, $J_{H1'\text{-}H2'}$ = 4.8 Hz, H1′) 5.03 (dd, $J_{H5'a\text{-}H5'b}$ = 13.2 Hz, $J_{H5'a\text{-}H4'}$ = 5.3 Hz, 1H, H5′a), 4.96 (dd, $J_{H5'b\text{-}H5'a}$ = 12.2 Hz, $J_{H5'b\text{-}H4'}$ = 7.4 Hz, 1H, H5′b), 4.18 – 4.06 (m 1H, H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 165.7 (C=O, Bz), 165.0 (C=O, Bz), 162.7 (C=O, C4), 150.5 (C=O, C2), 144.1 (CH, C6), 134.1 (C_q, Ar-C), 133.9 (C_q, Ar-C), 133.5 (C_q, Ar-C), 130.0 (CH, Ar-C), 129.9 (CH, Ar-C), 129.0 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.2 (CH, Ar-C), 103.9 (CH, C5), 91.5 (CH, C1′), 75.0 (CH, C2′), 72.5 (CH, C3′), 70.7 (CH, C4′), 60.4 (CH_2, C5′); **minor diastereoisomer:** R_f 0.23 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{25.8}$ –37.5 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.03 (m, 4H, Ar-H), 7.99 – 7.90 (m, 2H, Ar-H), 7.59 – 7.50 (m, 3H, Ar-H), 7.45 – 7.36 (m, 7H, H6 and Ar-H), 6.22 (dd, $J_{H3'\text{-}H4'}$ = 7.2 Hz, $J_{H3'\text{-}H2'}$ = 4.8 Hz, 1H, H3′), 6.09 (app. t, $J_{H2'\text{-}H1'/H3'}$ = 4.8 Hz, 1H, H2′), 5.82 (d, $J_{H5\text{-}H6}$ = 7.8 Hz, 1H, H5), 5.19 (d, $J_{H1'\text{-}H2'}$ = 4.8 Hz, 1H, H1′), 5.04 (dd, $J_{H5'a\text{-}H5'b}$ = 10.8 Hz, $J_{H5'a\text{-}H5'b}$ = 4.7 Hz, 1H, H5′a), 4.97 (dd, $J_{H5'a\text{-}H5'b}$ = 10.3 Hz, $J_{H5'a\text{-}H5'b}$ = 5.3 Hz, 1H, H5′b), 4.22 – 4.11 (m, 1H, H4′); ^{13}C NMR (101

MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 165.7, (C=O, Bz) 165.1 (C=O, Bz), 163.1 (C=O, C4), 150.6 (C=O, C2), 144.4 (CH, C6), 134.1 (C_q, Ar-C), 133.9 (C_q, Ar-C), 133.5 (C_q, Ar-C), 130.0 (CH, Ar-C), 129.9 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.2 (CH, Ar-C), 103.8 (CH, C5), 91.2 (CH, C1′), 75.1 (CH, C2′), 72.5 (CH, C3′), 70.8 (CH, C4′), 60.4 (CH_2, C5′); NSI HRMS *m/z* found: $(M+Na)^+$ 611.1092 $C_{30}H_{24}N_2O_9S$, requires $(M+Na)^+$ 611.1095.

1′-β-(4′-Sulfinyl(*S*/*R*)-D-ribofuranosyl)cytosine **198** †



Nucleoside **198** was prepared according to general procedure K using **197** (45.0 mg, 0.174 mmol, 1.0 equiv.), *m*-CPBA (43.0 mg, 0.191 mmol, 1.1 equiv.) and 2/1 (*v*/*v*) H_2O/MeCN solution (0.87 mL). Reaction time = 18 h. Purification: the crude product white solid was purified on octadecyl modified silica gel *via* flash chromatography (0 – 100% MeCN/H_2O) to obtain **198** as a white solid (43.0 mg, 0.156 mmol, 90%) and 20.0 mg separated and purified by preparative HPLC (Table 15).

Time (minutes)	**%A (H_2O)**	**%B (MeOH)**
0.0	100	0
10.0	100	0
12.0	0	100
15.0	0	100
15.1	100	0

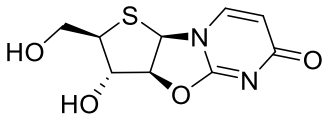
Table 10. Preparative HPLC linear gradient system for purification of **198**. A 250 x 21.2 mm column packed 5 µ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 20 mL/minute. A solution of **198** in H_2O (100 mg/mL) was prepared and 50 μL injected into the prep HPLC system.

Retention time = 4.4 minutes. **198** was obtained as a white solid, a mixture of diastereoisomers (7.1 mg, 26.5 μmol). The purity was determined using method 0. R_f 0.57 (1/4, H_2O/MeCN); 1.1/1 diastereoisomer ratio; **major diastereoisomer:** ^{1}H NMR (400 MHz, D_2O) δ 7.60 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6), 6.00 (ov. d, $J_{H5\text{-}H6}$ = 7.3 Hz, 1H, H5), 5.77 (d, $J_{H1'\text{-}H2'}$ = 9.2 Hz, 1H, H1′), 4.78 (dd, $J_{H2'\text{-}H1'}$ =

9.1 Hz, $J_{H2'\text{-}H3'}$ = 4.9 Hz, 1H, H2′), 4.30 (app. t, $J_{H3'\text{-}H2'/H4'}$ = 4.7 Hz, 1H, H3′), 4.02 (dd, $J_{H5'a\text{-}H5'b}$ = 12.2 Hz, $J_{H5'a\text{-}H4'}$ = 5.2 Hz, 1H, H5′a), 3.99 – 3.95 (ov. m, 1H, H5′b), 3.57 (app. dt, $J_{H4'\text{-}H5'b}$ = 9.5 Hz, $J_{H4'\text{-}H5'a/H3'}$ = 4.9 Hz, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 166.1 (C-NH_2, C4), 157.8 (C=O, C2), 146.4 (CH, C6), 96.4 (CH, C5), 73.1 (CH, C1′), 71.5 (CH, C2′), 70.4 (CH, C3′), 65.7 (CH, C4′), 56.3 (CH_2, C5′); **minor diastereoisomer:** ^{1}H NMR (400 MHz, D_2O) δ 7.55 (d, $J_{H6\text{-}H5}$ = 7.6 Hz, 1H, H6), 6.01 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 5.00 (d, $J_{H1'\text{-}H2'}$ = 8.1 Hz, 1H), 4.68 – 4.64 (ov. m, 1H, H2′), 4.41 (app. t, $J_{H3'\text{-}H2'/H4'}$ = 3.5 Hz, H3′), 3.99 – 3.94 (ov. m, 2H, H5′a and H5′b), 3.39 – 3.30 (m, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 166.8 (C-NH_2, C4), 157.2 (C=O, C2), 142.8 (CH, C6), 123.1, 96.7 (CH, C5), 91.5 (CH, C1′), 74.8 (CH, C4′), 73.3 (CH, C2′), 70.4 (CH, C3′), 58.2 (CH_2, C5′); NSI HRMS *m/z* found: $(M\text{-}H)^-$ 274.0510 $C_9H_{12}O_5N_3S$, requires 274.0498.

10.7. 1′-(4′-Thio-D-*arabino*) nucleoside analogues

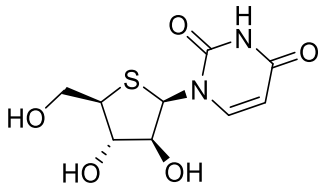
2′,2-Anhydro-1′-β-(4′-thio-D-ribofuranosyl)uracil **207**



A solution of **196** (1.00 g, 3.84 mmol, 1.0 equiv.), $(PhO)_2CO$ (0.910 g, 4.23 mmol, 1.1 equiv.) and $NaHCO_3$ (32.0 mg, 0.384 mmol, 0.10 equiv.) in DMF (1.5 mL) was stirred vigorously at 100 °C for 18 h. The solvent was removed *in vacuo* and the residue triturated from EtOAc (100 mL) and the solid collected by suction filtration, and the filtrate washed with rt EtOAc (20 mL) to afford **207** as a tan solid (0.910 g, 3.76 mmol, 98%). R_f 0.35 (1/4, MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O) δ 7.73 (d, $J_{H6\text{-}H5}$ = 7.5 Hz, 1H, H6), 6.21 (d, $J_{H1'\text{-}H2'}$ = 7.7 Hz, 1H, H1′), 6.08 (d, $J_{H5\text{-}H6}$ = 7.4 Hz, 1H, H5), 5.46 (d, $J_{H2'\text{-}H1'}$ = 7.7 Hz, 1H, H2′), 4.74 (app. s, 1H, H3′), 3.69 – 3.32 (m, 3H, H5′a, H5′b, H4′); ^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6), 6.24 (d, $J_{H1'\text{-}H2'}$ = 7.6 Hz, 1H, H1′), 6.08 (d, $J_{H5\text{-}H6}$ = 7.4 Hz, 1H, H5), 5.46 (dd, $J_{H2'\text{-}H1'}$ = 7.5 Hz, $J_{H2'\text{-}H3'}$ = 0.8 Hz, 1H, H2′), 4.81 – 4.74 (m, 1H, H3′), 3.58 – 3.50 (m, 2H, H4′ and H5′a), 3.43 (dd, $J_{H5'b\text{-}H5'a}$ = 9.5 Hz, $J_{H5'b\text{-}H4'}$ = 4.2 Hz, 1H, H5′b); ^{13}C NMR (101 MHz, D_2O) δ 175.4 (C-O, C2), 160.1 (C=O, C4), 138.9 (CH, C6), 109.0 (CH, C5), 92.1 (CH, C2′), 80.5 (CH, C3′), 70.3 (CH, C1′), 62.3 (CH_2, C5′), 59.1 (CH, C4′); ESI HRMS *m/z* found:

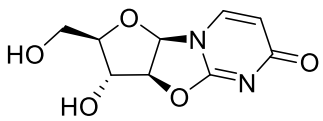
$(M+H)^+$ 243.0442 $C_9H_{10}N_2O_4S$, requires $(M+H)^+$ 243.0434. NMR data is consistent with literature values (in MeOD).[169]

1′-β-(4′-Thio-D-arabinofuranosyl)uracil **200**



A suspension of **207** (0.500 g, 2.06 mmol, 1.0 equiv.) and KOH (116 mg, 2.06 mmol 1.0 equiv.) in a 9/1 (*v*/*v*) solution of EtOH/H_2O (10 mL) was stirred vigorously at rt for 18 h. Amberlyst 15(H^+) ion exchange resin was added, the solution stirred for 10 minutes and filtered, washing with H_2O (15 mL) and freeze dried to afford **200** as an orange solid (482 mg, 1.85 mmol, 90%). R_f 0.36 (1/4, MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O) δ 8.28 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 6.10 (d, $J_{H1'\text{-}H2'}$ = 6.5 Hz, 1H, H1′), 5.84 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.29 (dd, $J_{H2'\text{-}H3'}$ = 9.1 Hz, $J_{H2'\text{-}H1'}$ = 6.5 Hz, 1H, H2′), 4.03 – 3.81 (m, 3H, H5′a, H5′b, H3′), 3.29 (ddd, $J_{H4'\text{-}H3'}$ = 8.7 Hz, $J_{H4'\text{-}H5'a}$ 5.2 Hz, $J_{H4'\text{-}H5'b}$ = 3.8 Hz, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 166.1 (C=O, C4), 152.4 (C=O, C2), 144.2 (CH, C6), 101.3 (CH, C5), 77.1 (CH, C2′), 74.1 (CH, C3′), 60.7 (CH_2, C5′), 59.3 (CH, C1′), 48.8 (CH, C4′); ESI HRMS *m/z* found: $(M+H)^+$ 261.0545 $C_9H_{12}N_2O_5S$, requires $(M+H)^+$ 261.0540. NMR data is consistent with literature values.[170]

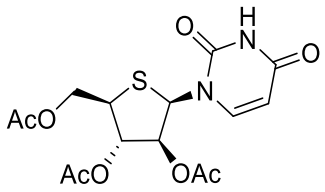
2,2′-Anhydrouridine **206**



Uridine (10.0 g, 41.0 mmol, 1.0 equiv.) was dissolved in DMF (36 mL) and $(PhO)_2CO$ (9.65 g, 45.0 mmol, 1.1 equiv.) and $NaHCO_3$ (98.0 mg, 4.10 mmol, 0.10 equiv.) added. The solution was heated to 100 °C and stirred overnight. The solution was cooled to rt, resulting in the formation of a white precipitate which was suspended in MeCN (50 mL) and the precipitate collected *via* Buchner filtration, washing with CH_2Cl_2 (50 mL) to obtain **206** as a white solid (6.52 g, 22.8 mmol, 56%). R_f 0.49 (1/4, MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6),

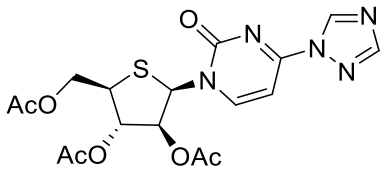
6.30 (d, $J_{H1'\text{-}H2'}$ = 5.7 Hz, 1H, H1′), 5.89 (d, $J_{OH\text{-}H3'}$ = 8.2 Hz, 1H, C3′-OH), 5.84 (d, $J_{H5\text{-}H6}$ = 7.4 Hz, 1H, H5), 5.19 (app. d, $J_{H2'\text{-}H3'}$ = 5.7 Hz, 1H, H2′), 4.98 (app. br t, $J_{OH\text{-}H5'a,\ OH\text{-}H5'b}$ = 5.0 Hz, 1H, C5′-OH), 4.38 (app. br s, 1H, H3′), 4.13 – 4.03 (m, 1H, H4′), 3.27 (dd, $J_{H5'a\text{-}H5'b}$ = 10.7 Hz, $J_{H5'a\text{-}H4'}$ 5.7 Hz, 1H, H5′a), 3.19 (dd, $J_{H5'b\text{-}H5'a}$ = 11.3 Hz, $J_{H5'b\text{-}H4'}$ = 5.6 Hz, 1H, H5′b); ^{13}C NMR (101 MHz, DMSO) δ 172.6 (C=O, C4), 160.4 (C-O, C2), 137.6 (CH, C6), 109.0 (CH, C5), 90.6 (CH, C1′), 89.7 (CH, C4′), 89.3 (CH, C2′), 75.3 (CH, C3′), 61.3 (CH_2, C5′); ESI HRMS *m/z* found: $(M+K)^+$ 265.0234 $C_9H_{10}N_2O_5$, requires $(M+K)^+$ 265.0221. ^{1}H NMR data is consistent with literature values.[122]

2′,3′,5′-Tri-*O*-acetyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **208** †



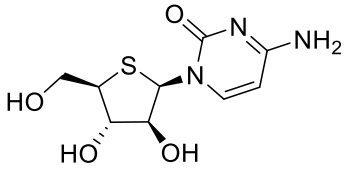
Ac_2O (3.5 mL, 11.1 mmol, 6.0 equiv.) was added to a solution of **200** (481 mg, 1.84 mmol, 1.0 equiv.) in pyridine (12 mL), and the solution stirred vigorously at rt for 22 h. The solution was poured on 1M aqueous HCl solution (70 mL) and diluted with EtOAc (70 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (3 × 70 mL) and brine (70 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as an orange foam which was purified on silica gel *via* automated flash chromatography (50 – 65% EtOAc/hexane) to obtain **208** as a white foam (0.527 g, 1.36 mmol, 74%). R_f 0.62 (EtOAc); $[\alpha]_D^{25.8}$ +41.2 (*c* 0.7, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H, N-H), 7.96 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 6.52 (d, $J_{H1'\text{-}H2'}$ = 5.4 Hz, 1H, H1′), 5.78 (dd, $J_{H5\text{-}H6}$ = 8.2 Hz, $J_{H5\text{-}NH}$ = 2.1 Hz, 1H, H5), 5.58 – 5.51 (m, 1H, H2′), 5.37 (dd, $J_{H3\text{-}H2'}$ = 4.8 Hz, $J_{H3'\text{-}H4'}$ = 4.0, 1H, H3′), 4.40 (dd, $J_{H5'a\text{-}H5'b}$ = 11.6 Hz, $J_{H5'a\text{-}H4'}$ = 7.1 Hz, 1H, H5′a), 4.35 (dd, $J_{H5'b\text{-}H5'a}$ = 11.6 Hz, $J_{H5'b\text{-}H4'}$ = 6.6 Hz, 1H, H5′b), 3.65 (app. td, $J_{H4'\text{-}H5'a/b}$ = 6.9 Hz, $J_{H4'\text{-}H3'}$ = 4.0 Hz, 1H, H4′), 2.14 (s, 3H, Ac-CH_3), 2.13 (s, 3H, Ac-CH_3), 2.05 (s, 3H, Ac-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4 (C=O, Ac), 169.4 (C=O, Ac), 168.6 (C=O, Ac), 162.6 (C=O, C4), 150.6 (C=O, C2), 141.5 (CH, C6), 102.1 (CH, C5), 75.6 (CH, C3′), 75.6 (CH, C2′), 63.7 (CH_2, C5′), 60.5 (CH, C1′), 48.5 (CH, C4′), 20.8 (Ac-CH_3), 20.7 (Ac-CH_3), 20.6 (Ac-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 387.0873 $C_{15}H_{18}N_2O_8S$, requires $(M+H)^+$ 387.0857.

2′,3′,5′-*O*-Tri-*O*-acetyl-4-*C*-(1,2,4-triazole)-1′-β-(4′-thio-D-arabinofuranosyl)uracil **209** †



A suspension of **059** (360 mg, 0.299 mmol, 1.0 equiv.) in MeCN (9.3 mL) was cooled to 0 °C. Et_3N (3.0 mL, 21.4 mmol, 23 equiv.), 1,2,4-triazole (1.44 g, 20.9 mmol, 23 equiv.) and $POCl_3$ (0.21 mL, 2.27 mmol, 2.4 equiv.) were added and the solution stirred for a further 5 minutes at 0 °C before warming to rt with vigorous stirring. After 3 h, the solution was poured onto ice-cold saturated aqueous $NaHCO_3$ solution (100 mL) and diluted with EtOAc (100 mL). The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (2 × 75 mL) and brine (75 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product **209** as a yellow foam (376 mg, 0.299 mmol, *quant.*), which was used crude product in the next step without further purification. R_f 0.31 (1/1, EtOAc/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H, triazole CH), 8.71 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6), 8.14 (s, 1H, triazole-CH), 7.11 (d, $J_{H5\text{-}H6}$ = 7.3 Hz, 1H, H5), 6.73 (d, $J_{H1'\text{-}H2'}$ = 5.4 Hz, 1H, H1′), 5.70 (app. t, $J_{H2'\text{-}H1'/H3'}$ = 5.4 Hz, 1H, H2′), 5.45 – 5.32 (m, 1H, H3′), 4.49 – 4.33 (m, 2H, H5′a and H5′b), 3.80 – 3.61 (m, 1H, H4′), 2.16 (s, 3H, Ac-CH_3), 2.13 (s, 3H, Ac-CH_3), 2.01 (s, 3H, Ac-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5 (uracil C-N, C4), 159.3 (uracil C=O, C2), 154.2 (triazole-CH), 148.7 (CH, C6), 143.5 (triazole-CH), 94.7 (CH, C5), 75.1 (CH, C3′), 75.1 (CH, C2′) 63.5 (CH_2, C5′), 62.3 (CH, C1′), 48.3 (CH, C4′), 20.8 (Ac-CH_3), 20.8 (Ac-CH_3), 20.6 (Ac-CH_3); ESI HRMS *m/z* found $(M+H)^+$ 438.1099 $C_{17}H_{19}N_5O_7S$ requires $(M+H)^+$ 438.1078.

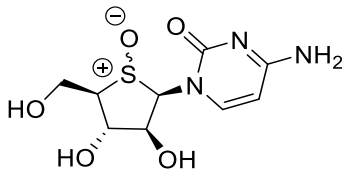
1′-β-(4′-Thio-D-arabinofuranosyl)cytosine **199** †



A solution of **209** (460 mg, 1.05 mmol, 1.0 equiv.) in neat 7M NH_3/MeOH solution (3.5 mL, 24.5 mmol, 23 equiv.) was heated to 120 °C in a sealed tube for 24 h. The solvents were removed *in vacuo* and the crude product purified on octadecyl modified silica gel *via* automated flash chromatography

(0/100, 10/90, 100/0 MeCN/H_2O) to obtain **199** as a white solid (190 mg, 0.733 mmol, 70%, 98% purity by HPLC). R_f 0.09 (1/4, MeOH/EtOAc); ^{1}H NMR (400 MHz, D_2O) δ 8.25 (d, $J_{H6\text{-}H5}$ = 7.6 Hz, 1H, H6), 6.26 (d, $J_{H1'\text{-}H2'}$ = 6.5 Hz, 1H, H1′), 6.06 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 4.35 (app. dd, $J_{H2'\text{-}H3'}$ = 8.7 Hz, $J_{H2'\text{-}H1'}$ = 6.5 Hz, 1H, H2′), 4.13 – 3.95 (m, 2H, H3′ and H5′a), 3.90 (dd, $J_{H5'b\text{-}H5'a}$ = 12.1 Hz, $J_{H5'b\text{-}H4'}$ = 5.6 Hz, 1H, H5′b), 3.44 – 3.23 (m, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 165.8 (cytosine C-NH_2, C4), 158.5 (cytosine C=O, C2), 144.1 (CH, C6), 95.6 (CH, C5), 77.2 (CH, C2′), 74.6 (CH, C3′), 61.1 (CH_2, C5′), 59.8 (CH, C1′), 49.1 (CH, C4′); ESI HRMS *m/z* found: $(M+H)^+$ 260.0702 $C_9H_{13}N_3O_4S$, requires $(M+H)^+$ 260.0700. NMR data is consistent with literature values.[171]

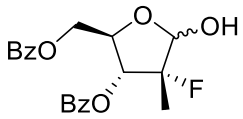
1′-(4′-Sulfinyl(*S*/*R*)-D-arabinofuranosyl)cytosine **201**



Nucleoside **201** was prepared according to general procedure K using **199** (116 mg, 0.447 mmol, 1.0 equiv.), *m*-CPBA (121 mg, 0.492 mmol, 1.1 equiv.) and 2/1 (*v*/*v*) H_2O/MeCN solution (2.2 mL). Reaction time = 20 h. Purification: the crude product white solid was purified on octadecyl modified silica gel *via* flash chromatography (0 – 100% MeCN/H_2O) to obtain **201** as a white solid (2.5/1 diastereoisomer ratio, 95.6 mg, 0.347 mmol, 78%) and an analytically pure sample (9/1 diastereoisomer ratio, >95% purity as determined by method 0) obtained *via* precipitation from a minimum of hot H_2O to obtain a quantity of **201** as a white solid (12 mg, 43.6 μmol, 10%). R_f 0.41 (1/4, H_2O/MeCN); **major diastereoisomer:** ^{1}H NMR (400 MHz, D_2O) δ 7.57 (d, $J_{H6\text{-}H5}$ = 7.4 Hz, 1H, H6), 5.99 (d, $J_{H5\text{-}H6}$ = 7.4 Hz, 1H, H5), 5.03 (d, $J_{H1'\text{-}H2'}$ = 8.3 Hz, 1H, H1′), 4.77 – 4.73 (ov. m, 1H, H2′), 4.25 (dd, $J_{H3'\text{-}H2'}$ = 8.1 Hz, $J_{H3'\text{-}H4\text{-}}$ = 4.6 Hz, 1H, H3′) 4.19 (ov. dd, $J_{H5'a\text{-}H5'b}$ = 12.8 Hz, $J_{H5'a\text{-}H4'}$ = 4.6 Hz, 1H, H5′a), 4.05 (dd, $J_{H5'b\text{-}H5a}$ = 11.9 Hz, $J_{H5'b\text{-}H4'}$ = 10.1 Hz, 1H, H5′b), 3.05 (app. td, $J_{H4'\text{-}H5'b}$ = 9.4 Hz, $J_{H4'\text{-}H5'a/H3'}$ = 4.6 Hz, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 166.8 (C-NH_2, C4), 157.8 (C=O, C2), 146.6 (CH, C6), 96.2 (CH, C5), 82.4 (CH, C1′), 76.9 (CH, C3′), 75.5 (CH, C2′), 75.1 (CH, C4′), 59.1 (CH_2, C5′); NSI HRMS *m/z* found: $(M+Na)^+$ 298.0469 $C_9H_{13}N_3O_5S$, requires $(M+Na)^+$ 298.0468.

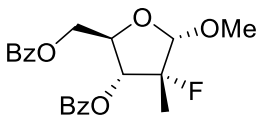
10.8. 2′-Deoxy-2′-*C*-methyl-2′-fluoro-1′-(4′-thio-D-*ribo*) nucleoside analogues

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methyl-1-α,β-D-ribofuranose **258**



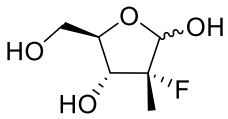
Hemiacetal **258** was prepared according to general procedure B using 2-deoxy-2-fluoro-2-*C*-methyl-3,5-di-*O*-benzoyl-D-ribono-1,4-lactone (20.0 g, 53.7 mmol, 1.0 equiv.), Li(O^{t}Bu)AlH (16.4 g, 64.4 mmol, 1.2 equiv.) and THF (270 mL). Reaction time = 30 minutes. Obtained the crude product as a colourless syrup (20.4 g, 53.4 mmol, *quant.*) which was used in the next step without further purification. Purification (for analytical sample): the crude product syrup was purified on silica gel *via* automated flash chromatography (10 – 20% Et_2O/petroleum ether) to obtain **258** as a colourless syrup (18.5 g, 49.4 mmol, 92% purified yield). R_f 0.15 (1/4, Et_2O/petroleum ether); 2.5/1.0 ratio α/β; **α-anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.07 (m, 2H, Ar-H), 8.03 – 7.99 (m, 2H, Ar-H), 7.63 – 7.58 (m, 1H, Ar-H), 7.56 – 7.43 (m, 2H), 7.41 – 7.33 (m, 3H, Ar-H), 5.65 (dd, $J_{H3\text{-}F}$ = 23.3 Hz, $J_{H3\text{-}H4}$ = 7.5 Hz, 1H, H3), 5.34 (d, $J_{H1\text{-}F}$ = 9.7 Hz, 1H, H1), 4.74 – 4.44 (m, 3H, H4, H5a and H5b), 3.47 (br s, 1H, 1-OH), 1.66 – 1.47 (d, $J_{CH3\text{-}F}$ = 22.6 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5 (C=O, Bz), 165.9 (C=O, Bz), 133.8 (C_q, Ar-C), 133.7 (C_q, Ar-C), 133.2 (C_q, Ar-C), 133.1 (CH, Ar-C), 130.0 (CH, Ar-C), 129.73 (CH, Ar-C), 129.67 (CH, Ar-C), 128.9 (CH, Ar-C), 128.6 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 100.9 (d, $J_{C1\text{-}F}$ = 33.0 Hz, CH, C1), 100.0 (d, $J_{C2\text{-}F}$ = 181.1 Hz, C_q, C2), 78.3 (CH, C4), 74.7 (d, $J_{C3\text{-}F}$ = 14.7 Hz, CH, C3), 65.0 (CH_2, C5), 16.7 (2-CH_3), 16.5 ($J_{CH3\text{-}F}$ = 30.3 Hz, 2-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -169.27 (app. dp, $J_{F\text{-}H3/CH3}$ = 22.7 Hz, $J_{F\text{-}H1}$ = 9.6 Hz); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -169.27; ESI HRMS *m/z* found: $(M+Na)^+$ 397.1076 $C_{20}H_{19}FO_6$, requires $(M+Na)^+$ 397.1058. ^{1}H NMR data is consistent with literature values.[138]

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose **274**



A solution of **258** (3.10 g, 8.28 mmol, 1.0 equiv.) in MeOH (17 mL) was cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3.1 mL, 24.8 mmol, 3.0 equiv.) was added dropwise and the solution heated to 80 °C for 3.5 h. The solution as cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$ until pH = 7. The solvent was removed *in vacuo* and the residue diluted in EtOAc (300 mL), the organic layer was separated and washed with H_2O (300 mL) and brine (300 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a colourless syrup which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **274** as a colourless syrup (1.28 g, 3.30 mmol, 40%). R_f 0.66 (1/1, Et_2O/petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.06 (m, 2H, Ar-H), 8.06 – 7.96 (m, 2H, Ar-H), 7.68 – 7.41 (m, 4H, Ar-H), 7.41 – 7.30 (m, 2H, Ar-H), 5.64 (dd, $J_{H3\text{-}F}$ = 23.8 Hz, $J_{H3\text{-}H4}$ = 7.7 Hz, 1H, H3), 4.86 (d, $J_{H1\text{-}F}$ = 9.8 Hz, 1H, H1), 4.65 (dd, $J_{H5a\text{-}H5b}$ = 11.6 Hz, $J_{H5a\text{-}H4}$ = 4.0 Hz, 1H, H5a), 4.62 – 4.53 (m, 1H, H4), 4.46 (dd, $J_{H5b\text{-}H5a}$ = 11.6 Hz, $J_{H5b\text{-}H4}$ = 4.8 Hz, 1H, H5b), 3.38 (s, 3H, 1-OCH_3), 1.52 (d, $J_{CH3\text{-}F}$ = 22.7 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2 (C=O, Bz), 165.8 (C=O, Bz), 133.7 (C_q, Ar-C), 133.1 (C_q, Ar-C), 130.0 (CH, Ar-C), 129.8 (CH, Ar-C), 129.7 (CH, Ar-C), 129.0 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 106.8 (d, $J_{C1\text{-}F}$ = 31.9 Hz, CH, C1), 99.7 (d, $J_{C2\text{-}F}$ = 181.5 Hz, C_q, C2), 78.0 (CH, C4), 75.0 (d, $J_{C3\text{-}F}$ = 14.7 Hz, C3), 64.7 (CH_2, C5), 55.3 (1-OCH_3), 16.6 (d, $J_{CH3\text{-}F}$ = 23.7 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -168.75 – -169.22 (m); ^{19}F {^1H} NMR (376 MHz, $CDCl_3$) δ -169.01; ESI HRMS *m/z* found: $(M+H)^+$ 389.1408 $C_{21}H_{21}FO_6$, requires $(M+H)^+$ 389.1395. NMR data is consistent with literature values.[138]

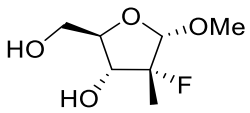
2-Deoxy-2-fluoro-2-*C*-methyl-1-α,β-D-ribofuranose **272**



To a solution of **258** (10.8 g, 28.9 mmol, 1.0 equiv.) in MeOH (96 mL) was added 7M NH_3/MeOH solution (41 mL, 289 mmol, 10 equiv.). The solution was heated to 45 °C. After 18 h, the solvent was removed *in vacuo* and the residue triturated from ice-cold EtOAc (200 mL) and filtered through a sintered funnel and the filtrate washed with ice-cold EtOAc (100 mL) to obtain **272** as a white amorphous solid (3.82 g, 23.0 mmol, 80%). R_f 0.11 (5/95, MeOH/CH_2Cl_2); 12.5/1.0 ratio α/β mixture; **α-anomer:** ^1H NMR (400 MHz, MeOD) δ 4.97 (d, $J_{H1\text{-}F}$ = 6.0 Hz, 1H, H1), 3.96 (dd, $J_{H5a\text{-}H5b}$ = 11.9

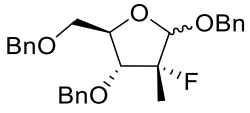
Hz, J_{H5a-H4} = 2.8 Hz, 1H, H5a), 3.82 – 3.70 (m, 2H, H3, H4), 3.65 (dd, $J_{H5b-H5a}$ = 11.8 Hz, J_{H5b-H4} = 5.0 Hz, 1H, H5b), 1.35 (d, J_{CH3-F} = 22.7 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, MeOD) δ 94.5 (d, J_{C2-F} = 176.5, CH, C2), 94.4 (d, J_{C1-F} = 30.7 Hz, CH, C1), 70.1 (d, J_{C3-F} = 16.5 Hz, CH, C3), 68.0 (CH, C4), 63.2 (CH_2, C5), 17.1 (d, J_{CH3-F} = 24 Hz, 2-CH_3); ^{19}F NMR (376 MHz, MeOD) δ -172.74 (app. pd J_{F-CH3} = 22.6 Hz, J_{F-H3} = 11.9 Hz); ^{19}F {^{1}H} NMR (376 MHz, MeOD) δ -172.70; ESI HRMS *m/z* found: $(M+Na)^+$ 189.0536 $C_6H_{11}FO_4$, requires $(M+Na)^+$ 189.0533. NMR data is consistent with literature values.[172]

1-α-*O*-Methyl-2-deoxy-2-fluoro-2-*C*-methyl-D-ribofuranose **275**



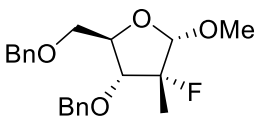
To a solution of **274** (1.28 g, 3.30 mmol, 1.0 equiv.) in MeOH (11 mL) was added 7M NH_3/MeOH solution (5.6 mL, 39.6 mmol, 12 equiv.). The solution was heated to 45 °C and after 20 h, the solvent was removed *in vacuo* and the residue triturated from ice-cold CH_2Cl_2 (50 mL) and filtered through a sintered funnel and the filtrate washed with ice-cold CH_2Cl_2 (10 mL) to obtain **275** as a white amorphous solid (0.590 g, 0.327 mmol, 99%). R_f 0.33 (5/95, MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD) δ 4.72 (d, J_{H1-F} = 10.6 Hz, 1H, H1), 3.97 – 3.85 (m, 1H, H4), 3.71 (dd, J_{H3-F} = 24.9 Hz, J_{H3-H4} = 8.2 Hz, 1H, H3), 3.69 (dd, $J_{H5a-H5b}$ = 11.9 Hz, J_{H5a-H4} = 2.9 Hz, 1H, H5a), 3.54 (dd, $J_{H5b-H5a}$ = 11.9 Hz, J_{H5b-H4} = 6.3 Hz, 1H, H5b), 3.38 (s, 1H, 1-OCH_3), 1.38 (d, J_{CH3-F} = 22.6 Hz, 1H, 2-CH_3); ^{13}C NMR (101 MHz, MeOD) δ 106.5 (d, J_{C1-F} = 32.7 Hz, CH, C1), 99.5 (d, J_{C2-F} = 176.2 Hz, CH, C2), 82.6 (CH, C4), 73.6 (d, J_{C3-F} = 17.0 Hz, CH, C3), 63.2 (CH, C5), 54.1 (1-OCH_3), 14.8 (d, J_{CH3-F} = 24.2 Hz, 2-CH_3); ^{19}F NMR (376 MHz, MeOD) δ -173.32 (app. dp, $J_{F-H3/CH3}$ = 23.1 Hz, J_{F-H1} = 10.6 Hz, 1F, C2-F); ^{19}F {^{1}H} NMR (376 MHz, MeOD) δ -173.30; ESI HRMS *m/z* found: $(M+Na)^+$ 203.0693 $C_7H_{13}FO_4$, requires $(M+Na)^+$ 203.069. This compound was reported in the literature but not characterised.[138]

1,3,5-Tri-*O*-benzyl-2-deoxy-2-fluoro-2-*C*-methyl-1-α,β-D-ribofuranose **273** †



A solution of **272** (300 mg, 1.81 mmol, 1.0 equiv.) in DMF (7.2 mL) was cooled to 0 °C. A 60% dispersion of NaH (290 mg, 7.24 mmol, 4.0 equiv.) was added and the solution stirred for 40 minutes. BnBr (1.3 mL, 10.8 mmol, 6.0 equiv.) was added and the solution stirred for 3 days at rt. The reaction was quenched with a few drops of ice-water and the solvent removed *in vacuo*. The crude product was diluted in EtOAc (70 mL) and washed with H_2O (70 mL) and brine (70 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 15% Et_2O/petroleum ether) to obtain **273** as a colourless oil (324 mg, 0.742 mmol, 41%) and **268** as a colourless oil (6.7/1.0 α/β, 208 mg, 0.600 mmol, 33%). R_f 0.53 (1/4, Et_2O/petroleum ether); 2.27/1.0 ratio α/β; **α-anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.17 (m, 15H, Ar-H), 4.90 (d, J_{H1-F} = 10.6 Hz, 1H, H1), 4.73 (d, J_{gem} = 11.9 Hz, 1H, Bn-CH_2), 4.69 (d, J_{gem} = 11.9 Hz, 1H, Bn-CH_2), 4.63 (d, J_{gem} = 11.9 Hz, 1H, Bn-CH_2), 4.56 (d, J_{gem} = 12.1 Hz, 1H, Bn-CH_2), 4.51 (d, J_{gem} = 12.1 Hz, 1H, Bn-CH_2), 4.46 (d, J_{gem} = 11.9 Hz, 1H, Bn-CH_2), 4.29 (ddd, J_{H4-H3} = 7.7 Hz, J_{H4-H5b} = 5.3 Hz, J_{H4-H5a} = 3.9 Hz, 1H, H4), 3.95 (dd, J_{H3-F} = 23.6 Hz, J_{H3-H4} = 7.7 Hz, 1H, H3), 3.59 (dd, $J_{H5a-H5b}$ = 10.6 Hz, J_{H5a-H4} = 3.9 Hz, 1H, H5a), 3.52 (dd, $J_{H5b-H5a}$ = 10.6 Hz, J_{H5b-H4} = 5.3 Hz, 1H, H5b), 1.47 (d, J_{CH3-F} = 22.5 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1 (C_q, Ar-C), 137.8 (C_q, Ar-C), 137.4 (C_q, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 128.1 (CH, Ar-C), 128.0 (CH, Ar-C), 128.0 (CH, Ar-C), 127.8 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 127.6 (CH, Ar-C), 104.7 (d, J_{C1-F} = 32.7 Hz, CH, C1), 99.9 (d, J_{C2-F} = 179.6 Hz, C_q, C2) , 81.3 (d, J_{C3-F} = 15.4 Hz, CH, C3) , 80.4 (CH, C4), 73.8 (CH_2, Bn), 73.3 (CH_2, Bn), 71.0 (CH_2, C5), 69.1 (CH_2, Bn), 17.1 (d, J_{CH3-F} = 24.2 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -169.85 (app. pd, $J_{F-CH3/H3}$ = 22.6 Hz, J_{F-H1} = 10.6 Hz); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -169.86; ESI HRMS *m/z* found: $(M+Na)^+$ 459.1962, $C_{27}H_{29}FO_4$, requires $(M+Na)^+$ 459.1948.

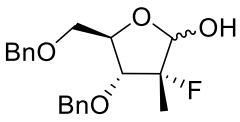
3,5-Di-*O*-benzyl-2-deoxy-2-fluoro-2-*C*-methyl-1-*O*-methyl-1-α-D-ribofuranose **276** †



Methyl glycoside **276** was prepared according to general procedure A using **268** (2.99 g, 16.6 mmol, 1.0 equiv.), NaH (60% dispersion in mineral oil, 1.66 g, 41.5 mmol, 2.5 equiv.), BnBr (5.9 mL,

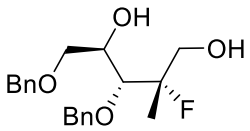
49.8 mmol, 3.0 equiv.) and DMF (37 mL) at rt. Reaction time = 48 h. Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to afford **276** as a colourless oil (5.95 g, 16.5 mmol, *quant.*). R_f 0.89 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ **–**37.4 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.09 (m, 10H, Ar-H), 4.73 – 4.63 (m, 3H, H1 and Bn-CH_2), 4.31 – 4.15 (m , 1H, H4), 3.86 (dd, J_{H3-F} = 23.5 Hz, J_{H3-H4} = 7.6 Hz, 1H, H3), 3.55 (dd, $J_{H5a-H5b}$ = 10.6 Hz, J_{H5a-H4} = 4.0 Hz, 1H, H5a), 3.48 (dd, $J_{H5b-H5a}$ = 10.6 Hz, J_{H5b-H4} = 5.4 Hz, 1H, H5b), 3.33 (s, 3H, 1-OCH_3), 1.42 (d, J_{CH3-F} = 22.5 Hz, 1H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2 (C_q, Ar-C), 137.8 (C_q, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 128.2 (CH, Ar-C), 128.0 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 127.7 (CH, Ar-C), 106.8 (d, J_{C1-F} = 32.5 Hz, CH_2, C1), 99.7 (d, J_{C2-F} = 179.3 Hz, C2), 81.2 (d, J_{C3-F} = 15.9 Hz, C3), 80.2 (d, J_{C4-F} = 0.98 Hz, C4), 73.7 (CH_2, Bn), 73.3 (CH_2, Bn), 71.0 (C5, CH_2), 55.1 (1-OCH_3), 17.0 (d, J_{CH3-F} = 24 Hz, 2-CH_3), ^{19}F NMR (376 MHz, $CDCl_3$) δ -169.79 (app. pd, $J_{F-CH3/H3}$ = 22.7, J_{F-H1} = 10.5 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 361.1836 $C_{21}H_{25}FO_4$, requires $(M+H)^+$ 361.1810.

3,5-Di-*O*-benzyl-2-deoxy-2-fluoro-2-*C*-methyl-1-α,β-D-ribofuranose **268** †



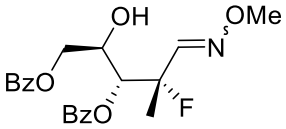
To a solution of 4/1 (*v*/*v*) AcOH/H_2O (37 mL) was added **200** (2.41 g, 6.69 mmol, 1.0 equiv.) and conc. H_2SO_4 (0.96 mL, 18.0 mmol, 2.7 equiv.). The solution was heated to 70 °C for 18 h, cooled to rt and slowly poured onto saturated aqueous $NaHCO_3$ solution (1 L) over 10 minutes with stirring. The aqueous was extracted with EtOAc (4 × 150 mL) and the combined organic phases washed with saturated aqueous $NaHCO_3$ solution (2 × 200 mL) and brine (200 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain **268** as a yellow solid (1.89 g, 5.46 mmol, 82%) which was used without further purification. R_f 0.19 (1/4, Et_2O/petroleum ether); 2/1 anomeric ratio; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.04 (m, 10H, Ar-H), 5.03 (ov. d, $J_{H1\text{-}F}$ = 7.8 Hz, 1H, H1), 4.73 (d, J_{gem} = 12.1 Hz, 1H, Bn-CH_2), 4.55 (d, J_{gem} = 8.4 Hz, 1H, Bn-CH_2), 4.52 (d, J_{gem} = 8.3 Hz, 1H, Bn-CH_2), 4.43 (d, J_{gem} = 11.7 Hz, 1H, Bn-CH_2), 4.06 (dd, $J_{H3\text{-}F}$ = 22.9 Hz, $J_{H3\text{-}H4}$ = 7.0 Hz, 1H, H3), 3.61 (dd, $J_{H5a\text{-}H5b}$ = 10.4 Hz, $J_{H5a\text{-}H4}$ = 2.6 Hz, 1H, H5a), 3.44 (d, $J_{OH\text{-}H1}$ = 7.4 Hz, 1H, 1-OH), 3.29 (dd, $J_{H5b\text{-}H5a}$ = 10.4 Hz, $J_{H5b\text{-}H4}$ = 2.5 Hz, 1H, H5b), 1.50 (d, $J_{CH3\text{-}F}$ = 22.2 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.6 (C_q, Ar-C), 137.0 (C_q, Ar-C), 128.6 (CH, Ar-C), 128.6 (CH, CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 128.2 (CH, Ar-C), 128.0 (CH, Ar-C), 127.8 (CH, Ar-C), 101.2 (d, $J_{C1\text{-}F}$ = 32.4 Hz, CH, C1), 100.3 (d, $J_{C2\text{-}F}$ = 182.0 Hz, C_q, C2), 81.0 (CH, C4), 79.3 (d, $J_{C3\text{-}F}$ = 15.4 Hz, CH C3), 73.9 (CH_2, Bn), 73.6 (CH_2, Bn), 69.1 (CH_2, C5), 17.2 (d, $J_{CH3\text{-}F}$ = 24.5 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -169.97 (app. pd, $J_{F\text{-}CH3/H3}$ = 22.3 Hz, $J_{F\text{-}H1}$ = 8.1 Hz); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -169.98; NSI HRMS *m/z* found: $(M+Na)^+$ 369.1466 $C_{20}H_{23}O_4F$, requires $(M+Na)^+$ 369.1473.

(2*S*,3*R*,4*R*)-3,5-Di-*O*-benzyl-2-fluoro-2-*C*-methyl-1,4-pentanediol **269** †



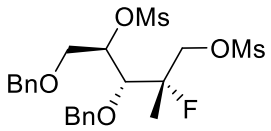
A solution of **268** (1.89 g, 5.46 mmol, 1.0 equiv.) in MeOH (27 mL) was cooled to 0 °C and $NaBH_4$ (0.620 g, 16.4 mmol, 3.0 equiv.) was added in small portions over 30 minutes. The reaction was then stirred for 3 h at room temperature, after which the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (150mL), washed with water (2 × 100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to obtain the crude product as a colourless oil which was purified on silica gel *via* automated flash chromatography (20 – 100% Et_2O/petroleum ether) to obtain **269** as a colourless oil (1.86 g, 5.34 mmol, 98%). R_f 0.13 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.7}$ −16.1 (*c* 0.8, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.20 (m, 10H, Ar-H), 4.72 (d, J_{gem} = 11.1 Hz, 1H, Bn-CH_2), 4.62 (d, J_{gem} = 11.0 Hz, 1H, Bn-CH_2), 4.60 – 4.53 (m, 2H, Bn-CH_2), 4.08 (app. td, $J_{H4\text{-}H5a/b}$ = 6.2 Hz, $J_{H4\text{-}H3}$ = 3.2 Hz, 1H, H4), 3.88 – 3.63 (m, 5H, H3, H5a and H5b, H1a and H1b), 2.68 (br s, 1H, OH), 2.44 (br s, 1H, OH), 1.42 (d, $J_{CH3\text{-}F}$ = 23.2 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.8 (C_q, Ar-C), 137.8 (C_q, Ar-C), 128.8 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.0 (CH, Ar-C), 128.0 (CH, Ar-C), 127.9 (CH, Ar-C), 127.9 (CH, Ar-C), 127.7 (CH, Ar-C), 127.7 (CH, Ar-C), 98.5 (d, $J_{C2\text{-}F}$ = 172.4 Hz, C_q, C2), 80.1 (d, $J_{C3\text{-}F}$ = 23.4 Hz, CH, C3), 75.0 (d, $J_{C5\text{-}F}$ = 1.6 Hz, C5), 73.5 (CH_2, Bn), 71.2 (d, $J_{CH2\text{-}F}$ = 2.5 Hz, CH_2, Bn), 70.5 (d, $J_{C4\text{-}F}$ = 2.9 Hz, CH, C4), 66.5 (d, $J_{C1\text{-}F}$ = 24.3 Hz, CH_2, C1) 18.9 (d, $J_{CH3\text{-}F}$ = 22.7 Hz, 2-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -159.13 – -161.77 (m); ^{19}F {^1H} NMR (377 MHz, $CDCl_3$) δ -160.14; ESI HRMS *m/z* found: $(M+H)^+$ 349.1846 $C_{20}H_{25}FO_4$, requires $(M+H)^+$ 349.1810.

(2*R*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-2-fluoro-4-hydroxy-1-(methoxyimino)-2-*C*-methyl-pentane (*E*/*Z*) **256**[†]



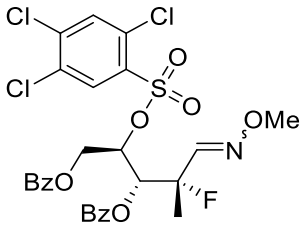
To a 1/3 (*v*/*v*) H_2O/MeCN solution (470 mL) was added **258** (20.0 g, 53.4 mmol, 1.0 equiv.), $H_2NOMe{\cdot}HCl$ (7.62 g, 91.2 mmol, 1.5 equiv.), Et_3N (13 mL, 91.2 mmol, 1.5 equiv.) and pyridinium *p*-toluenesulfonate (9.93 g, 39.5 mmol, 0.65 equiv.). The solution was stirred at rt for 4 days. The solvent was removed *in vacuo* and the residue partitioned between EtOAc (900 mL) and saturated aqueous $NaHCO_3$ (850 mL). The organic layer was separated and washed with H_2O (850 mL) and brine (850 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product **256** as a colourless syrup (21.4 g, 53.0 mmol, *quant.*) which was used without further purification. R_f 0.74 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.6}$ +64.4 (*c* 1.3, CH_2Cl_2) for isomeric mixture; 13/1 isomer ratio; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.04 (m, 2H, Ar-H), 8.04 – 7.94 (m, 2H, Ar-H), 7.66 (d, $J_{H1\text{-}F}$ = 10.2 Hz, 1H, H1), 7.64 – 7.51 (m, 2H, Ar-H), 7.50 – 7.37 (m, 4H, Ar-H), 5.61 (dd, $J_{H3\text{-}F}$ = 21.9 Hz, $J_{H3\text{-}H4}$ = 6.4 Hz, 1H, H3), 4.66 (dd, $J_{H5a\text{-}H5b}$ = 11.8 Hz, $J_{H5a\text{-}H4}$ = 2.4 Hz, 1H, H5a), 4.48 (app td, $J_{H4\text{-}H3/H5b}$ = 6.2 Hz, $J_{H4\text{-}H5a}$ = 2.5 Hz, 1H, H4), 4.39 (dd, $J_{H5b\text{-}H5a}$ = 11.8 Hz, $J_{H5b\text{-}H4}$ = 6.1 Hz, 1H, H5b), 3.88 (s, 3H, OCH_3), 2.83 (d, $J_{OH\text{-}H4}$ = 6.1 Hz, 1H, 4-OH), 1.62 (d, $J_{CH3\text{-}F}$ = 23.1 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9 (C=O, Bz), 165.5 (C=O, Bz), 149.3 (d, $J_{C1\text{-}F}$ = 31.7 Hz, CH=N, C1), 133.7 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.0 (CH, Ar-C), 129.8 (CH, Ar-C), 129.5 (CH, Ar-C), 128.9 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 94.8 (d, $J_{C2\text{-}F}$ = 175.5 Hz, C_q, C2), 76.5 (CH, C3), 69.2 (CH, C4), 66.2 (CH_2, C5), 62.3 (OCH_3), 21.5 (d, $J_{CH3\text{-}F}$ = 23.5 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -157.74 (app. pd, $J_{F\text{-}CH3/H3}$ = 22.7 Hz, pd, $J_{F\text{-}H1}$ = 10.0 Hz); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -157.73; ESI HRMS *m/z* found: $(M+H)^+$ 404.1515 $C_{10}H_{16}IO_4$ requires $(M+H)^+$ 404.1509.

(2*S*,3*R*,4*R*)-3,5-Di-*O*-benzyl-2-fluoro-1,4-di-*O*-mesyl-2-*C*-methyl -pentane **270** †



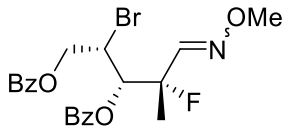
A solution of **269** (1.33 g, 3.81 mmol, 1.0 equiv.) in CH_2Cl_2 (19 mL) was cooled to 0 °C. MsCl (0.89 mL, 11.4 mmol, 3.0 equiv.) and Et_3N (1.6 mL, 11.4 mmol, 3.0 equiv.) were added over 5 minutes and the solution stirred at rt for 24 h. The solvent was removed *in vacuo* and the residue partitioned between EtOAc (120 mL) and saturated aqueous $NaHCO_3$ solution (120 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (120 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow oil which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **270** as a yellow oil (1.80 g, 3.57 mmol, 94%). R_f 0.24 (1/2, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ –16.5 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 10H, Ar-H), 5.18 (app. dt, $J_{H4\text{-}H5a/b}$ = 7.2 Hz, $J_{H4\text{-}H3}$ = 3.0 Hz, 1H, H4), 4.81 (d, J_{gem} = 10.8 Hz, 1H, Bn-CH_2), 4.60 (d, J_{gem} = 10.8 Hz, 1H, Bn-CH_2), 4.57 (s, 2H, Bn-CH_2), 4.34 – 4.17 (m, 2H, H1a and H1b), 4.02 (dd, $J_{H3\text{-}F}$ = 9.3 Hz, $J_{H3\text{-}H4}$ = 2.7 Hz, 1H, H3), 3.83 – 3.81 (ov. m, 2H, H5a and H5b), 3.03 (s, 3H, Ms-CH_3), 3.02 (s, 3H, Ms-CH_3), 1.42 (d, $J_{CH3\text{-}F}$ = 22.4 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.3 (C_q, Ar-C), 136.6 (C_q, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 128.1 (CH, Ar-C), 127.9 (CH, Ar-C), 95.4 (d, $J_{C2\text{-}F}$ = 179.5 Hz, C_q, C2), 81.1 (CH, C4), 78.3 (d, $J_{C3\text{-}F}$ = 27.1 Hz, CH, C3), 74.5 (CH_2, Bn), 73.5 (CH_2, Bn), 71.1 (d, $J_{C1\text{-}F}$ = 5.7 Hz, CH_2, C1), 69.1 (CH_2, C5), 38.8 (Ms-CH_3), 37.7 (Ms-CH_3), 18.0 (d, $J_{CH3\text{-}F}$ = 22.6 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -158.9 – -159.22 (m); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -159.1; ESI HRMS *m/z* found: $(M+H)^+$ 505.1380 $C_{22}H_{29}FO_8S_2$, requires $(M+H)^+$ 505.1361.

(2*R*,3*R*,4*S*)-3,5-Di-*O*-benzoate-4-*O*-(2′,4′,5′-trichlorophenylsulfonyl)-2-fluoro-1-(methoxyimino)-2-*C*-methyl-pentane (*E*/*Z*) **259** †



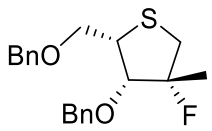
To a solution of **256** (21.3 g, 52.8 mmol, 1.0 equiv.) in MeCN (140 mL) was added 2,4,5-trichlorobenzenesulfonyl chloride (16.3 g, 58.1 mmol, 1.1 equiv.) and *N*-methyl imidazole (4.6 mL, 58.1 mmol, 1.1 equiv.). The solution was stirred at rt for 5 h and poured onto H_2O (900 mL) and extracted with EtOAc (3 × 400 mL). The organic layers were combined and washed with water (2 × 900 mL) brine (900 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a colourless syrup which was purified on silica gel *via* column chromatography (0 – 30% Et_2O/petroleum ether) to obtain **259** as a white foam (21.1 g, 34.1 mmol, 56%). R_f 0.72 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ +147.7 (*c* 1.5, CH_2Cl_2); 8/1 isomer ratio; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.04 (m, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 7.92 – 7.84 (m, 2H, Ar-H and H1), 7.69 – 7.38 (m, 6H, Ar-H), 7.33 (s, 1H, Ar-H), 5.94 (dd, $J_{H3\text{-}F}$ = 23.4 Hz, $J_{H3\text{-}H4}$ = 2.5 Hz, 1H, H3), 5.60 (dt, $J_{H4\text{-}H5a/b}$ = 8.8 Hz, $J_{H4\text{-}H3}$ = 2.3 Hz, 1H, H4), 4.86 (dd, $J_{H5a\text{-}H5b}$ = 12.9 Hz, $J_{H5a=H4}$ = 1.8 Hz, 1H, H5a), 4.64 (d, $J_{H5b\text{-}H5a}$ = 12.9 Hz, $J_{H5b\text{-}H4}$ = 8.8 Hz, 1H, H5b), 3.94 (s, 3H, OCH_3), 1.61 (d, $J_{CH3\text{-}F}$ = 22.8 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 164.8 (C=O, Bz), 147.6 (d, $J_{C1\text{-}F}$ = 30.6, CH=N, C1), 138.9 (Ar-C), 134.6 (C_q, Ar-C), 134.0 (C_q, Ar-C), 133.5 (C_q, Ar-C), 133.3 (CH, Ar-C), 131.9 (CH, Ar-C), 131.7 (CH, Ar-C), 131.6 (CH, Ar-C), 130.1 (CH, Ar-C), 129.6 (CH, Ar-C), 129.0 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 94.4 (d, $J_{C2\text{-}F}$ = 176.4 Hz, C_q, C2), 80.4 (CH, C4), 74.7 (d, $J_{C3\text{-}F}$ = 21.9 Hz, CH, C3), 62.8 (d, $J_{C5\text{-}F}$ = 9.2 Hz, CH_2, C5), 62.6 (OCH_3), 21.6 (d, $J_{CH3\text{-}F}$ = 24.1 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -156.26 (app. pd, $J_{F\text{-}CH3/H3}$ = 22.9 Hz, $J_{F\text{-}H1}$ = 9.5 Hz); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -156.26; ESI HRMS *m*/*z* found: $(M+H)^+$ 646.0271 $C_{27}H_{23}{}^{35}Cl_3FNO_8S$, requires $(M+H)^+$ 646.0267.

(2*S*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-2-bromo-2-fluoro-1-(methoxyimino)-2-*C*-methyl-pentane (*E*/*Z*) **257** †



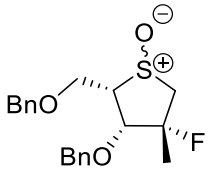
To a solution of **259** (1.50 g, 2.32 mmol, 1.0 equiv.) in DMF (16 mL) was added LiBr (1.00 g, 11.6 mmol, 5.0 equiv.) and the solution stirred at 80 °C. After 2 h, the solution was cooled to rt and the solvent removed *in vacuo*. The residue was poured onto ice-water (200 mL) and extracted with CH_2Cl_2 (6 × 60 mL) and the combined organic phases washed with brine (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers dried over anhydrous $MgSO_4$, filtered and the solution treated with activated charcoal, filtered through Celite and the solvent removed *in vacuo* to obtain the crude product as a brown oil which was purified on silica gel *via* automated flash chromatography (0 – 15% Et_2O/petroleum ether) to obtain **257** as a colourless oil (0.575 g, 1.24 mmol, 53%). R_f 0.78 (1/2, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ +17.0 (*c* 1.4, CH_2Cl_2) for single isomer; one isomer, geometry not determined; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.11 (m, 2H, Ar-H), 8.10 – 8.01 (m, 2H, Ar-H), 7.66 – 7.39 (m, 7H, Ar-H and H1), 5.90 (dd, $J_{H3\text{-}F}$ = 16.9 Hz, $J_{H3\text{-}H4}$ = 2.5 Hz, 1H, H3), 4.76 – 4.62 (m, 2H, H4 and H5a), 4.46 (dd, $J_{H5b\text{-}H5a}$ = 10.8 Hz, $J_{H5'b\text{-}H4'}$ = 6.8 Hz, 1H, H5′b), 3.75 (s, 3H, OCH_3), 1.67 (d, $J_{CH3\text{-}F}$ = 22.3 Hz, 3H, 2′-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7 (C=O, Bz), 165.2 (C=O, Bz), 148.2 (d, *J* = 29.9 Hz, CH=N, C1), 133.9 (C_q, Ar-C), 133.4 (C_q, Ar-C), 130.2 (CH, Ar-C), 129.9 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 94.7 (d, $J_{C2\text{-}F}$ = 175.0 Hz, C_q, C2), 72.4 (d, $J_{C3\text{-}F}$ = 25.2 Hz, CH, C3), 65.3 (CH_2, C5), 62.2 (OCH_3), 46.3 (d, $J_{C4\text{-}F}$ = 3.5 Hz, C-Br, C4), 20.8 (d, $J_{CH3\text{-}F}$ = 23.4 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -152.26 – -152.58 (m); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -152.42; FTMS + APCI HRMS *m/z* found: $(M+H)^+$ 446.0586 $C_{21}H_{21}{}^{79}Br_1F_1N_1O_5$, requires $(M+H)^+$ 466.0660.

(2*S*,3*R*,4*S*)-3,5-Di-*O*-benzyl-2-fluoro-2-*C*-methyl-1-(4-thio)cyclopentane **277** †



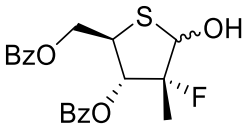
To a solution of **270** (0.500 g, 0.991 mmol, 1.0 equiv.) in DMF (3.3 mL) was added Na_2S (93.0 mg, 1.19 mmol, 1.2 equiv.) and the solution heated to 100 °C for 35 minutes. The solution was cooled to rt, poured onto H_2O and the aqueous extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic layers washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **277** as a yellow oil (127 mg, 0.367 mmol, 37%). R_f 0.65 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ –9.3 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 6.99 (m, 10H, Ar-H), 4.60 (d, J_{gem} = 11.7 Hz, 1H, Bn-CH_2), 4.55 (d, J_{gem} = 11.7 Hz, 1H, Bn-CH_2), 4.45 (d, J_{gem} = 11.8 Hz, 1H, Bn-CH_2), 4.39 (d, J_{gem} = 11.8 Hz, 1H, Bn-CH_2), 3.92 – 3.80 (m, 1H, H5a), 3.71 – 3.59 (m, 1H, H3), 3.55 – 3.42 (m, 2H, H5b and H4), 3.07 (dd, J_{H1a-F} = 17.3 Hz, $J_{H1a-H1b}$ = 11.4 Hz, 1H, H1a), 2.67 (dd, J_{H1b-F} = 20.1 Hz, $J_{H1b-H1a}$ = 11.5 Hz, 1H, H1b), 1.39 (d, J_{CH3-F} = 21.6 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2 (C_q, Ar-C), 137.6 (C_q, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.2 (CH, Ar-C), 128.0 (CH, Ar-C), 127.9 (CH, Ar-C), 127.7 (CH, Ar-C), 102.1 (d, J_{C2-F} = 186.4 Hz, C_q, C2), 83.5 (d, J_{C3-F} = 17.4 Hz, CH, C3), 73.5 (d, J_{CH2-F} = 1.7 Hz, CH_2, Bn), 73.4 (CH_2, Bn), 71.1 (d, J_{C5-F} = 5.1 Hz, CH_2, C5), 45.4 (d, J_{C4-F} = 1.4 Hz, CH, C4), 36.2 (d, J_{C1-F} = 24.9 Hz, CH_2, C1), 21.9 (d, J_{CH3-F} = 25.6 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -155.68 – -156.07 (m); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -155.86; ESI HRMS *m/z* found: $(M+H)^+$ 347.1491 $C_{20}H_{23}FO_2S$, requires $(M+H)^+$ 347.1476.

(2*S*,3*R*,4*S*)-3,5-Di-*O*-benzyl-2-fluoro-2-*C*-methyl-1-(4-sulfinyl)cyclopentane **278** †



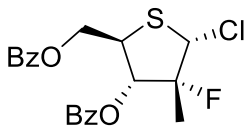
A solution of **277** (127 mg, 0.339 mmol, 1.0 equiv.) in CH_2Cl_2 (3.4 mL) was cooled to –40 °C and a suspension of *m*-CPBA (88.0 mg, 0.509 mmol, 1.5 equiv.) in CH_2Cl_2 (1.0 mL) added. The mixture was stirred at –40 °C for 1.5 h. The reaction was quenched *via* addition of saturated aqueous $Na_2S_2O_3$ (0.50 mL), poured into CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a white residue which was purified on silica gel *via* automated flash chromatography (0 – 100% EtOAc/petroleum ether) to obtain **278** as a colourless oil (92.0 mg, 0.236 mmol, 70%). R_f 0.47 (EtOAc); $[\alpha]_D^{22.8}$ –22.1 (*c* 2.1, CH_2Cl_2) for single diastereoisomer; one diastereoisomer, geometry not determined; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 10H, Ar-H), 4.70 (d, J_{gem} = 11.5 Hz, 1H, Bn-CH_2), 4.60 (d, J_{gem} = 11.6 Hz, 1H, Bn-CH_2), 4.54 (s, 2H, Bn-CH_2), 4.32 (dd, $J_{H3\text{-}F}$ = 14.5 Hz, $J_{H3\text{-}H4}$ 5.8 Hz, 1H, H3), 3.89 (dd, $J_{H5a\text{-}H5b}$ = 10.6 Hz, $J_{H5a\text{-}H4}$ 5.9 Hz, 1H, H5a), 3.77 – 3.63 (m, 2H, H5b and H1a), 3.47 – 3.38 (m, 1H, H4), 2.79 (dd, $J_{H1b\text{-}F}$ = 16.7 Hz, $J_{H1a\text{-}H1b}$ = 14.6 Hz, 1H, CH_2 H1b), 1.66 (d, $J_{CH3\text{-}F}$ = 22.6 Hz, 1H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5 (C_q, Ar-C), 137.0 (C_q, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.3 (CH, Ar-C), 128.2 (CH, Ar-C), 127.9 (CH, Ar-C), 127.8 (CH, Ar-C), 102.0 (d, $J_{C2\text{-}F}$ = 188.8 Hz, C_q, C2), 82.8 (d, $J_{C3\text{-}F}$ = 17.4 Hz, CH, C3), 74.2 (CH_2, Bn), 73.5 (CH_2, Bn), 69.7 (CH, C4), 64.6 (app. d, $J_{C5\text{-}F}$ = 4.3 Hz, CH_2, C5), 59.9 (d, $J_{C1\text{-}F}$ = 21.5 Hz, CH_2, C1), 23.4 (d, $J_{CH3\text{-}F}$ = 24.7 Hz, 2-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -148.39 – -148.89 (m); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -148.63; ESI HRMS *m/z* found: $(M+H)^+$ 365.1415, $C_{19}H_{24}O_5S$, requires 363.1425.

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methyl-1-α,β-(4-thio-D-ribofuranose) **239** †



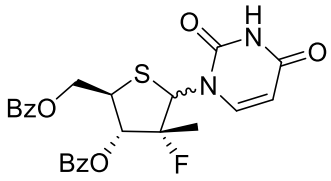
Aldehyde **260** was prepared according to general procedure D using **257** (5.63 g, 12.1 mmol, 1.0 equiv.), MeCN (24 mL) and glyoxylic acid (4.7 mL, 84.7 mmol, 7.0 equiv.) to obtain aldehyde **260** as an orange oil (4.44 g, ~12.1 mmol, *quant.*) which was used without further purification. R_f 0.23 (1/2, Et_2O/petroleum ether); ESI HRMS *m/z* found: $(M+Na)^+$ 459.0252 $C_{20}H_{18}{}^{79}BrFO_5$, requires $(M+Na)^+$ 459.0225. Thiohemiacetal **239** was prepared according to general procedure G using **260** (4.44 g, ~12.1 mmol, 1.00 equiv.), DMF (24 mL) and $NaSH{\cdot}H_2O$ (1.16 g, 15.7 mmol, 1.3 equiv.) to obtain the crude product as a brown syrup which was purified on silica gel *via* automated flash chromatography (0 – 30% Et_2O/petroleum ether) to obtain **239** as a colourless syrup, an anomeric mixture (2.89 g, 7.40 mmol, 61%) and recovered starting material **257** (1.03 g, 2.36 mmol, 20% recovery). R_f 0.35 (1/2, Et_2O/petroleum ether). R_f 0.35 (1/2, Et_2O/petroleum ether); 2.9/1 anomer ratio; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.04 (m, 2H, Ar-H), 7.91 – 7.85 (m, 2H, Ar-H), 7.59 (t, J_{vic} = 7.5 Hz, 1H, Ar-H), 7.49 – 7.42 (m, 3H, Ar-H), 7.31 – 7.22 (m, 2H, Ar-H), 5.80 (dd, J_{H3-F} = 26.7 Hz, J_{H3-H4} = 9.1 Hz, 1H, H3), 5.16 (d, J_{H1-F} = 7.5 Hz, 1H, H1), 4.64 (dd, $J_{H5a-H5b}$ = 11.5 Hz, J_{H5a-H4} = 6.1 Hz, 1H, H5a), 4.51 (dd, $J_{H5b-H5a}$ = 11.3 Hz, J_{H5b-H4} 5.8 Hz, 1H, H5b), 4.15 – 4.05 (m, 1H, H4), 1.54 (d, J_{CH3-F} = 22.6 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 165.7 (C=O, Bz), 133.7 (C_q, Ar-C), 133.1 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.6 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.3 (CH, Ar-C), 128.2 (CH, Ar-C), 102.8 (d, J_{C2-F} = 183.8 Hz, C_q, C2), 81.3 (d, J_{C1-F} = 33.4 Hz, CH, C1), 77.2 (d, J_{C3-F} = 17.0 Hz, CH, C3), 65.5 (CH_2, C5), 45.1, 17.6 (d, J_{CH3-F} = 23.6 Hz, CH_3, 2-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -163.70 (dqd, J_{F-H3} = 26.9 Hz, J_{F-CH3} = 22.5 Hz, J_{F-H1} = 7.5 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 391.0996 $C_{20}H_{19}FO_5S$, requires $(M+H)^+$ 391.1010.

3,5-Di-*O*-benzoyl-1-chloro-2-deoxy-2-fluoro-2-*C*-methyl-1-α-(4-thio-D-ribofuranosyl)uracil **261** †



A solution of **239** (1.18 g, 3.01 mmol, 1.0 equiv.) in CH_2Cl_2 (14 mL) was cooled to 0 °C. Et_3N (0.50 mL, 4.51 mmol, 1.5 equiv.) and MsCl (0.35 mL, 4.51 mmol, 1.5 equiv.) added. The solution stirred at rt for 2 days when complete consumption of the starting material was observed by TLC (new R_f 0.71, 1/2 Et_2O/petroleum ether). The solution was poured onto saturated aqueous $NaHCO_3$ solution (100 mL), the organic layer separated, and the aqueous layer extracted with CH_2Cl_2 (150 mL). The combined organic phases were washed with H_2O (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 30% Et_2O/petroleum ether) to obtain **261** as a colourless syrup (409 mg, 1.00 mmol, 47%). R_f 0.71 (1/2, Et_2O/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.01 (m, 2H, Ar-H), 7.97 – 7.89 (m, 2H, Ar-H), 7.65 – 7.55 (m, 1H, Ar-H), 7.53 – 7.40 (m, 3H, Ar-H), 7.33 – 7.24 (m, 2H, Ar-H), 5.97 (dd, $J_{H3\text{-}F}$ = 26.1 Hz, $J_{H3\text{-}H4}$ = 9.0 Hz, 1H, H3), 5.36 (d, $J_{H1\text{-}F}$ = 9.1 Hz, 1H, H1), 4.67 (dd, $J_{H5a\text{-}H5b}$ = 11.6 Hz, $J_{H5a\text{-}H4}$ = 5.8 Hz, 1H, H5a), 4.53 (dd, $J_{H5b\text{-}H5a}$ = 11.6 Hz, $J_{H5b\text{-}H4}$ = 5.9 Hz, 1H, H5b), 4.16 (app. dt, $J_{H4\text{-}H3}$ = 9.0 Hz, $J_{H4\text{-}H5a/b}$ = 5.9 Hz, 1H, H4), 1.65 (d, $J_{CH3\text{-}F}$ = 22.4 Hz, 1H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 165.4 (C=O, Bz), 133.8 (C_q, Ar-C), 133.1 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.7 (CH, Ar-C), 129.4 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.3 (CH, Ar-C), 104.1 (d, $J_{C2\text{-}F}$ = 187.3 Hz, C_q, C2), 76.3 (d, $J_{C3\text{-}F}$ = 16.6 Hz, CH, C3), 68.0 (d, $J_{C1\text{-}F}$ = 32.2 Hz, C-Cl, C1), 64.7 (CH_2, C5), 46.1 (d, $J_{C4\text{-}F}$ = 3.2 Hz, CH, C4), 19.1 (d, $J_{CH3\text{-}F}$ = 24.6 Hz, 2-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -155.67 (dqd, $J_{F\text{-}H3}$ = 26.0 Hz, $J_{F\text{-}CH3}$ = 22.5 Hz, $J_{F\text{-}H1}$ = 9.1 Hz); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -155.67; NSI HRMS *m/z* found: $(M+Na)^+$ 431.0488 $C_{20}H_{18}{}^{35}ClFO_4S$, requires $(M+Na)^+$ 431.0491.

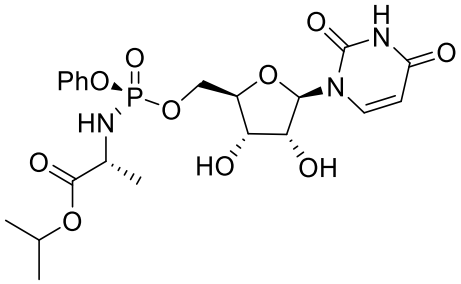
3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-α,β-(4′-thio-D-ribofuranosyl)uracil **264** †



Nucleoside **264** was prepared according to general procedure H using uracil (175 mg, 1.56 mmol, 1.4 equiv.), pyridine (0.90 mL), hexamethyldisilazane (2.4 mL, 11.5 mmol, 9.9 equiv.) **263** (0.500 g, 1.16 mmol, 1.0 equiv.), MeCN (12 mL) and TMSOTf (0.16 mL, 0.905 mmol, 0.78 equiv.). Reaction time = 72 h. Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 15% acetone/toluene) to obtain **264** as a yellow syrup (302 mg, 0.623 mmol, 54%). R_f 0.16 (1/9, acetone/toluene); 2/1 α/β anomer ratio; **α-anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H, NH), 8.13 – 8.01 (ov. m, 3H, Ar-H), 7.95 – 7.90 (ov. m, 1H, Ar-H), 7.62 – 7.56 (ov. m, 1H, Ar-H), 7.54 – 7.29 (ov. m, 4H, Ar-H), 7.20 – 7.14 (ov. m, 1H, Ar-H), 6.52 (d, $J_{H1'-F}$ = 22.5 Hz, 1H, H1′), 5.83 (d, J_{H5-H6} = 8.2 Hz, 1H, H5), 5.60 (dd, $J_{H3'-F}$ = 25.4 Hz, $J_{H3'-H4'}$ = 9.9 Hz, 1H, H3′), 4.69 (dd, $J_{H5'a-H5'b}$ = 11.3 Hz, $J_{H5'a-H4'}$ = 4.9 Hz, 1H), 4.50 – 4.43 (ov. m, 1H, H5′b) 4.45 – 4.37 (ov. m, 1H, H4′), 1.45 (d, J_{CH3-F} = 22.3 Hz, 3H, 2′-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 165.3 (C=O, Bz), 163.1 (C=O, C4), 151.4 (C=O, C2), 142.6 (d, *J* = 7.4 Hz, CH, C6), 134.0 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.7 (CH, Ar-C), 129.6 (CH, Ar-C), 128.7 (CH, Ar-C), 128.7 (CH, Ar-C), 128.4 (CH, Ar-C), 102.7 (CH, C5), 101.0 (d, $J_{C2'-F}$ = 192.5 Hz, C_q, C2′), 76.7 (d, $J_{C3'-F}$ = 14.7 Hz, CH, C3′), 64.3 (CH_2, C5′), 60.1 (d, $J_{C1'-F}$ = 16.0 Hz, CH, C1′), 46.7 (d, $J_{C4'-F}$ = 3.1 Hz, CH, C4′), 19.0 (d, J_{CH3-F} = 25.3 Hz, 2′-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -169.73 – -170.12 (m); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -169.94; **β-anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H, NH), 8.13 – 8.01 (ov. m, 2H, Ar-H), 7.97 – 7.85 (ov. m, 2H, Ar-H), 7.62 – 7.56 (ov. m, 1H, Ar-H), 7.54 – 7.29 (ov. m, 4H, Ar-H), 7.20 – 7.14 (m, 1H, Ar-H), 6.33 (d, $J_{H1'-F}$ = 14.2 Hz, 1H, H1′), 5.60 (dd, $J_{H3'-F}$ = 25.4 Hz, $J_{H3'-H4'}$ = 9.9 Hz, 1H, H3′), 5.48 (d, J_{H5-H6} = 8.2 Hz, 1H, H5), 4.90 (dd, $J_{H5'a-H5'b}$ = 12.6 Hz, $J_{H5'a-H4'}$ = 3.5 Hz, 1H, H5′), 4.49 – 4.40 (ov. m, 1H, H5′b), 4.22 – 4.06 (m, 1H, H4′), 1.38 (d, J_{CH3-F} = 22.1 Hz, 1H, 2′-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 165.5 (C=O, Bz), 162.8 (C=O, C4), 151.0 (C=O, C2), 140.1 (CH, C6), 134.1 (C_q, Ar-C), 133.7 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.3 (CH, Ar-C), 129.1 (CH, Ar-C), 128.7 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 103.6 (CH,

C5), 102.5 (d, $J_{C2'\text{-}F}$ = 188.5 Hz, C_q, C2′), 75.3 (d, $J_{C3'\text{-}F}$ = 18.1 Hz, CH, C3′), 63.9 (d, $J_{C1'\text{-}F}$ = 38.2 Hz, CH, C1′), 61.7 (CH_2, C5′), 45.9 (d, $J_{C4'\text{-}F}$ = 3.0 Hz, CH, C4′), 17.5 (d, $J_{CH3\text{-}F}$ = 24.1 Hz, 2′-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -155.10 – -155.76 (m); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -155.33; ESI HRMS *m/z* found: $(M+H)^+$ 485.1177 $C_{24}H_{22}O_6N_2FS$, requires 485.1104.

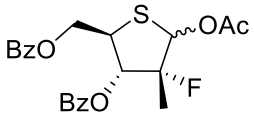
P(*S*)-*N*-(Uridin-5′-*O*-yl)(phenoxy)phosphoryl-L-alanine-1′′-methylethyl ester **267** †



Uridine (0.500 g, 2.08 mmol, 1.0 equiv.) was suspended in THF (8.0 mL) and the suspension cooled to –20 °C. *Tert*-butylmagnesium chloride (2.3 mL, 2.29 mmol, 1.1 equiv.) was added dropwise over 15 minutes and the suspension stirred at –20 °C for a further 30 minutes. N-[(S)-(2,3,4,5,6-pentafluorophenoxy)phenoxyphosphinyl]-L-alanine 1-methylethyl ester (1.13 g, 2.50 mmol, 1.2 equiv.) was added and the suspension stirred at rt. After 6 days, the suspension was cooled to -10 °C and quenched with saturated aqueous NH_4Cl solution until pH = 7. The THF was removed *in vacuo* and the crude product reside diluted in CH_2Cl_2 (80 mL) and washed with 15% (*v*/*v*) saturated aqueous NH_4Cl solution/H_2O (45 mL), 6% (*v*/*v*) saturated aqueous $NaHCO_3$ solution/H_2O (45 mL), and 5% (*v*/*v*) brine/H_2O solution (45 mL). The organic layer was dried over $MgSO_4$, filtered through celite and the solvent removed *in vacuo* to obtain the crude product as a white solid which was purified on silica gel *via* automated flash chromatography (15 – 100% EtOAc/*n*-heptane) to obtain **267** as a white solid (60.0 mg, 0.117 mmol, 6%). R_f 0.11 (EtOAc); $[\alpha]_D^{22.6}$ –0.5 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.24 (s, 1H, NH), 7.54 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 7.36 – 7.27 (m, 2H, Ar-H), 7.22 – 7.17 (m, 2H, Ar-H), 7.13 (t, J_{vic} = 7.3 Hz, 1H, Ar-H), 5.84 (d, $J_{H1'\text{-}H2'}$ = 2.5 Hz, 1H, H1′), 5.64 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 5.03 – 4.92 (m, 1H, ^{i}Pr -CH), 4.62 (dd, $J_{NH\text{-}P}$ = 23.2 Hz, $J_{NH\text{-}H2''}$ = 13.0 Hz, 1H, NH), 4.44 – 4.28 (m, 2H, H5′a and H5′b), 4.28 – 4.24 (m, 1H, H3′), 4.23 – 4.18 (m, 1H, H4′), 4.18 – 4.14 (m, 1H, H2′), 3.98 – 3.86 (m, 1H, H2′′), 1.32 (d, $J_{CH3\text{-}P}$ = 7.0 Hz, 3H, 2′′-CH_3), 1.22 – 1.16 (m, 6H, 2 × ^{i}Pr-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2 (d, $J_{C3''\text{-}P}$ = 6.4 Hz, alanine C=O, C3′′),

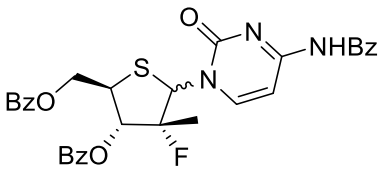
164.0 (C=O, C4), 151.2 (C=O, C2), 150.5 (d, $J_{C\text{-}P}$ = 6.7 Hz, C_q, Ar-C), 140.4 (CH, C6), 129.9 (CH, Ar-C), 125.2 (CH, Ar-C), 120.0 (CH, Ar-C), 120.0 (CH, Ar-C), 102.6 (CH, C5), 89.8 (CH, C1′), 82.5 (d, $J_{C4'\text{-}P}$ = 6.8 Hz, CH, C4′), 74.5 (CH, C2′), 69.7 (CH, C3′), 69.4 (CH, ^{i}Pr), 65.92 (d, $J_{C5'\text{-}P}$ = 4.7 Hz, CH_2, C5′) 50.3 (CH, C2′′), 21.7 (CH_3, ^{i}Pr), 21.6 (CH_3, ^{i}Pr), 20.8 (d, J = 5.8 Hz, 2′′-CH_3); ^{31}P {^{1}H} NMR (162 MHz, $CDCl_3$) δ 3.16 (s, 1P); NSI HRMS *m/z* found: $(M+NH_4)^+$ 531.1848 $C_{21}H_{28}N_3O_{10}P$, requires 531.1851.

1-*O*-Acetyl-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methyl-1-α,β-(4-thio-D-ribofuranose) **263** †



Acetyl glycoside **263** was prepared according to General Procedure E using **239** (1.40 g, 3.59 mmol, 1.0 equiv.), Ac_2O (0.41 mL, 4.30 mmol, 1.2 equiv.), Et_3N (0.60mL, 4.30 mmol, 1.2 equiv.) and CH_2Cl_2 (14 mL). Reaction time = 5 h. Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 30% Et_2O/petroleum ether) to obtain **263** as a yellow foam (1.11 g, 2.56 mmol, 71%). R_f 0.50 (1/2, Et_2O/petroleum ether); 1/3 anomer ratio; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 – 8.06 (m, 2H, Ar-H), 7.91 – 7.87 (m, 2H, Ar-H), 7.60 (t, J_{vic} = 7.5 Hz, 1H, Ar-H), 7.50 – 7.40 (m, 4H, Ar-H), 7.33 – 7.27 (m, 1H, Ar-H), 6.05 (d, $J_{H1\text{-}F}$ = 15.6 Hz, 1H, H1), 5.48 (dd, $J_{H3\text{-}F}$ = 21.9 Hz, $J_{H3\text{-}H4}$ = 9.1 Hz, 1H, H3), 4.64 (dd, $J_{H5'a\text{-}H5'b}$ = 11.5 Hz, $J_{H5'a\text{-}H4'}$ = 5.8 Hz, 1H, H5′a), 4.45 (dd, $J_{H5'b\text{-}H5'a}$ = 11.5 Hz, $J_{H5'b\text{-}H4'}$ = 6.1 Hz, 1H, H5′b), 4.09 (app. dt, $J_{H4'\text{-}H3'}$ = 9.4 Hz, $J_{H4'\text{-}H5'a/b}$ = 6.0 Hz, 1H, H4′), 2.20 (s, 3H, Ac-CH_3), 1.52 (d, $J_{CH3\text{-}F}$ = 21.6 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.2 (C=O, Ac), 165.9 (C=O, Bz), 165.6 (C=O, Bz), 133.8 (C_q, Ar-C), 133.1 (C_q, Ar-C), 130.1 (CH, Ar-C), 130.0 (CH, Ar-C), 129.7 (CH, Ar-C), 129.6 (CH, Ar-C), 129.4 (CH, Ar-C), 128.8 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.2 (CH, Ar-C), 101.5 (d, $J_{C2\text{-}F}$ = 184.1 Hz, C_q, C2), 80.8 (d, $J_{C1\text{-}F}$ = 35.8 Hz, CH, C1), 77.0 (d, $J_{C3\text{-}F}$ = 17.1 Hz, CH, C3) 65.0 (CH_2, C5), 44.9 (d, $J_{C4\text{-}F}$ = 3.3 Hz, CH, C4), 21.0 (Ac-CH_3), 17.8 (d, $J_{CH3\text{-}F}$ = 23.9 Hz, 2-CH_3)**;** ^{19}F NMR (376 MHz, $CDCl_3$) δ -171.03 – -171.70 (m); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -171.37; ESI HRMS *m/z* found: $(M+H)^+$ 455.0932 $C_{19}H_{24}O_5S$, requires $(M+H)^+$ 455.0935.

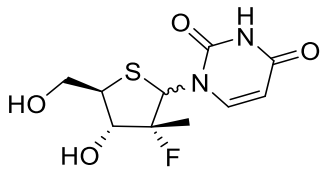
3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-α,β-(4′-thio-D-ribofuranosyl)-N^4-benzoylcytosine **265** †



Nucleoside **265** was prepared according to general procedure H using HMDS (6.2 mL, 29.8 mmol, 1.4 equiv.), N^4-benzoyl cytosine (0.906 g, 4.21 mmol, 1.4 equiv.), pyridine (2.0 mL), **263** (1.30 g, 3.01 mmol, 1.0 equiv.), TMSOTf (0.42 mL, 2.35 mmol, 0.78 equiv.) and MeCN (30 mL). Reaction time = 24 h. Purification: the crude product orange syrup (2.4/1 α/β anomer ratio) was purified on silica gel *via* automated flash chromatography (0 – 100% EtOAc/hexanes) to obtain **265** (1.6/1 α/β ratio, 0.750 g, 1.32 mmol, 41% crude product yield) and unreacted **263** (366 mg, 0.846 mmol, 36%). The anomeric mixture of **265** was further purified by fractional precipitation from a minimum of boiling EtOH or boiling MeOH to furnish **265β** (70.0 mg, 0.119 mmol, 4%), **265α** (56.0 mg, 95.0 μmol, 3%) and **265** (1.8/1 α/β ratio, 479 mg, 0.815 mmol, 27%). **α-anomer:** R_f 0.21 (1/1 EtOAc/hexanes); $[\alpha]_D^{25.2}$ +11.5 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H, NH), 8.14 – 8.01 (m, 2H, Ar-H), 8.01 – 7.81 (m, 2H, Ar-H), 7.77 – 7.29 (m, 10H, Ar-H), 7.24 – 7.06 (m, 2H, Ar-H), 6.83 (d, $J_{H1'\text{-}F}$ = 22.4 Hz, 1H, H1′), 5.65 (dd, $J_{H3'\text{-}F}$ = 24.6 Hz, $J_{H3'\text{-}H4'}$ = 9.8 Hz, 1H, H3′), 4.69 (dd, $J_{H5'a\text{-}H5'b}$ = 8.9 Hz, $J_{H5'a\text{-}H4'}$ = 5.1 Hz, 1H, H5′a), 4.55 – 4.42 (m, 2H, H4′ and H5′b), 1.49 (d, $J_{CH3\text{-}F}$ = 22.3 Hz, 2H, 2′-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -168.80 – -170.59 (m); ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$) δ -169.73; **β anomer:** R_f 0.30 (1/1 EtOAc/hexanes); $[\alpha]_D^{23.8}$ +138.0 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H, NH), 8.45 (d, $J_{H6\text{-}H5}$ = 7.1 Hz, 1H, H6), 8.21 – 7.99 (m, 4H, Ar-H), 7.99 – 7.81 (m, 2H, Ar-H), 7.78 – 7.34 (m, 10H, Ar-H and H5), 6.63 (d, $J_{H1'\text{-}F}$ = 13.8 Hz, 1H, H1′), 5.64 (dd, $J_{H3'\text{-}F}$ = 26.3 Hz, $J_{H3'\text{-}H4'}$ = 9.9 Hz, 1H, H3′), 4.90 (dd, $J_{H5'a\text{-}H5'b}$ = 12.1 Hz, $J_{H5'a\text{-}H4'}$ = 3.4 Hz, 1H, H5′a), 4.52 (dd, $J_{H5'b\text{-}H5'a}$ = 12.2 Hz, $J_{H5'b\text{-}H4'}$ = 4.0 Hz, 1H), 4.31 – 4.10 (m, 1H, H4′), 1.39 (d, $J_{CH3\text{-}F}$ = 22.1 Hz, 1H, 2′-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O Bz), 165.5 (C=O Bz), 162.2 (C=O Bz), 162.2 (C-NH, C4), 145.6 (C=O, C2), 145.4 (CH, C6), 134.1 (C_q Ar-C), 133.8 (C_q, Ar-C), 133.4 (C_q, Ar-C), 130.2 (CH, Ar-C), 129.8 (CH, Ar-C), 129.6 (CH, Ar-C), 129.4 (CH, Ar-C), 129.1 (CH, Ar-C), 128.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.3 (CH, Ar-C), 127.6 (CH, Ar-C), 102.6 (d, $J_{C2'\text{-}}$

$_F$ = 189.2 Hz, C_q, C2′), 97.7 (CH, C5), 75.0 (d, $J_{C3'-F}$ = 18.1 Hz, CH, C3′), 65.0 (d, $J_{C1'-F}$ = 37.0 Hz, CH, C1′), 61.9 (CH_2, C5′), 45.8 (d, $J_{C4'-F}$ = 3.2 Hz, CH, C4′), 17.4 (d, J_{CH3-F} = 24.2 Hz, 2′-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -150.78 – -160.97 (m); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$) δ -155.51; NSI HRMS *m/z* found: $(M+Na)^+$ 610.1416, $C_{31}H_{26}F_1N_3O_6S$, requires $(M+Na)^+$ 610.1419.

2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-α,β-(4′-thio-D-ribofuranosyl)uracil **240** †



A suspension of **264** (0.685 g, 1.41 mmol, 1.0 equiv.) in 7M NH_3/MeOH (2.7 mL, 18.9 mmol, 13 equiv.) and MeOH (1.8 mL) was stirred at rt for 2 days. The solvents were removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 50% acetone/toluene) to obtain **240** as an orange syrup (262 mg, 0.948 mmol, 67%, 1.9/1.0 α/β) alongside recovered **264** (109 mg, 0.226 mmol, 16%). The anomeric mixture of **240** (100 mg) was further purified on octadecyl modified silica gel *via* automated flash chromatography (0 – 100% MeCN/H_2O) and then *via* preparative HPLC (Table 11).

Time (minutes)	**%A (10 mM ammonium acetate)**	**%B (MeOH)**
0.0	96	4
17.0	96	4
23.0	75	25
29.0	75	25
33.0	0	100
36.0	0	100
36.1	96	4

Table 11. Preparative HPLC linear gradient system for purification of **240**. A 250 x 21.2 mm column packed 5 µ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 15 mL/minute. A solution of **240α/β** in H_2O (100 mg/mL) was prepared and 50 µL injected into the prep HPLC system.

Retention times α-anomer = 25.7 minutes (25.8 mg, 93.4 μmol, 26%, 95% pure), β-anomer = 26.7 minutes (17.1 mg, 61.9 μmol, 17%, 87% pure). The β-anomer was then further purified *via* preparative HPLC (Table 12).

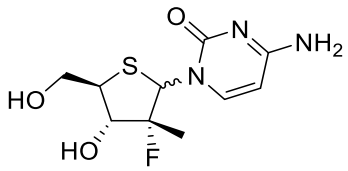
Time (minutes)	**%A (10 mM ammonium acetate)**	**%B (MeOH)**
0.0	96	4
3.0	75	25
8.0	75	25
12.0	0	100
15.0	0	100
15.1`	96	4

Table 12. Preparative HPLC linear gradient system for purification of **240β**. A 250 x 21.2 mm column packed 5 μ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 15 mL/minute. A solution of **240** in H_2O (100 mg/mL) was prepared and 50 μL injected into the prep HPLC system.

Retention time = 9.7 minutes (13.3 mg, 48.1 μmol, 15%). Purity was determined using general procedure L. R_f 0.27 (1/1, acetone/toluene); **α-anomer:** $[\alpha]_D^{22.6}$ **–**11.1 (*c* 0.9, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 8.07 (dd, $J_{H6\text{-}H5}$ = 8.2 Hz, $J_{H6\text{-}F}$ = 3.5 Hz, 1H, H6), 6.29 (d, $J_{H1'\text{-}F}$ = 23.2 Hz, 1H, H1′), 5.82 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 3.97 (dd, $J_{H5'a\text{-}H5'b}$ = 11.7 Hz, $J_{H5'a\text{-}H4'}$ = 2.9 Hz, 1H, H5′a), 3.91 (dd, $J_{H3'\text{-}F}$ = 25.7 Hz, $J_{H3'\text{-}H4'}$ = 10.2 Hz, 1H, H3′), 3.85 – 3.78 (m, 1H, H4′), 3.73 (dd, $J_{H5'b\text{-}H5'a}$ = 11.6 Hz, $J_{H5'b\text{-}H4'}$ = 6.2 Hz, 1H, H5′b), 1.37 (d, $J_{CH3\text{-}F}$ = 22.7 Hz, 3H, CH_3, 2′-CH_3); ^{13}C NMR (101 MHz, D_2O) δ 166.1 (C=O, C4), 152.4 (C=O, C2), 145.1 (d, $J_{C6\text{-}F}$ = 7.4 Hz, CH, C6), 101.9 (d, $J_{C2'\text{-}F}$ = 187.4 Hz, C_q, C2′), 101.7 (CH, C5), 76.0 (d, $J_{C3'\text{-}F}$ = 17.9 Hz, CH, C3′), 61.3 (CH_2, C5′), 60.6 (d, $J_{C1'\text{-}F}$ = 15.5 Hz, CH, C1′), 51.6 (d, $J_{C4'\text{-}F}$ = 2.4 Hz, CH, C4′), 18.13 (d, $J_{CH3\text{-}F}$ = 25.2 Hz, CH_3, 2′-CH_3); ^{19}F NMR (377 MHz, D_2O) δ -172.76 – -173.23 (m); **β-anomer:** $[\alpha]_D^{23.4}$ +18.1 (*c* 1.2, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 8.09 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 6.12 (d, $J_{H1'\text{-}F}$ = 15.2 Hz, 1H, H1′), 5.85 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H), 3.97 (dd, $J_{H5'a\text{-}H5'b}$ = 12.2, $J_{H5'a\text{-}H4'}$ = 2.9 Hz, 1H, H5′a), 3.88 (ov. dd, $J_{H5'b\text{-}H5'a}$ = 12.2 Hz, $J_{H5'b\text{-}H4'}$ = 5.1 Hz, 1H, H5′b), 3.87 (ov. dd, $J_{H3'\text{-}F}$ = 28.7 Hz, $J_{H3'\text{-}H4'}$ = 9.8 Hz, 1H, H3′), 3.50 (ddd, $J_{H4'\text{-}H3'}$ = 9.8 Hz, $J_{H4'\text{-}H5'b}$ = 5.1 Hz, $J_{H4'\text{-}H5'a}$ = 3.0 Hz, 1H, H4′), 1.31 (d, $J_{CH3\text{-}F}$ = 23.0 Hz, 3H, 2′-CH_3); ^{13}C NMR (101 MHz, D_2O) δ 165.8 (C=O, C4), 152.1 (C=O, C2) 142.9 (CH, C6), 104.1 (d, $J_{C2'\text{-}F}$ = 181.6 Hz, C_q,

C2′), 102.7 (CH, C5), 74.9 (d, $J_{C3'\text{-}F}$ = 19.8 Hz, CH, C3′), 64.1 (d, $J_{C1'\text{-}F}$ = 39.2 Hz, CH, C1′), 60.0 (CH_2, C5′), 50.4 (CH, C4′), 16.6 (d, $J_{CH3\text{-}F}$ = 23.8 Hz, CH_3, 2′-CH_3); ^{19}F NMR (376 MHz, D_2O) δ -166.91 – -167.23 (m); ^{19}F {^{1}H} NMR (376 MHz, D_2O) δ -167.05; ESI HRMS *m/z* found: $(M+H)^+$ 277.0654 $C_{10}H_{14}N_2O_4FS$, requires $(M+H)^+$ 277.0653.

2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-α,β-(4′-thio-D-ribofuranosyl)cytosine **242** †



A suspension of **265** (1/9 α/β ratio, 27.0 mg, 45.6 μmol, 1.0 equiv.) in neat 7M NH_3/MeOH (0.22 mL, 1.54 mmol, 34 equiv.) was stirred at rt for 72 h. The solvents were removed *in vacuo* and the crude product residue purified on octadecyl modified silica gel *via* automated flash chromatography (0 – 90% MeOH/H_2O) to obtain **242** as a colourless glass which was further purified to 99% *via* preparative HPLC (Table 13).

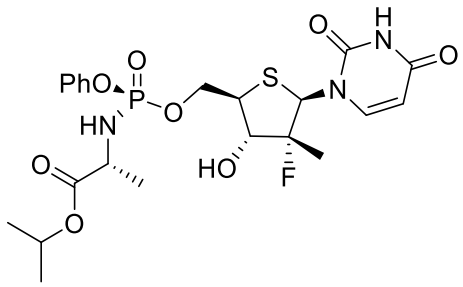
Time (minutes)	**%A (0.1% (*v*/*v*) HCOOH/H_2O)**	**%B (MeCN)**
0.0	96	4
12.0	5	95
13.0	5	95
13.1	96	96

Table 13. Preparative HPLC linear gradient system for purification of **242**. A 250 x 10.5 mm column packed 5 µ particle size with Zorbax C18-A was employed to load the sample The flow rate was 10 mL/minute. A solution of **242** in H_2O (100 mg/mL) was prepared and 50 μL injected into the prep HPLC system.

Retention times α-anomer = 2.95 minutes, white solid (1.3 mg, 4.7 μmol, 10%), β-anomer = 3.24 minutes, white solid (10.9 mg, 39.6 μmol, 87%). Purity was determined using general procedure L. R_f 0.51 (1/9, H_2O/MeCN); **α-anomer:** $[\alpha]_D^{22.6}$ **–**10.1 (*c* 0.9, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 8.02 (dd, $J_{H6\text{-}H5}$ = 7.8 Hz, $J_{H6\text{-}F}$ = 3.6 Hz, 1H, H6), 6.27 (d, $J_{H1'\text{-}F}$ = 23.3 Hz, 1H, H1′), 5.97 (d, $J_{H5\text{-}H6}$ = 7.4 Hz, 1H, H5), 3.89 (dd, $J_{H5'a\text{-}H5'b}$ = 11.2 Hz, $J_{H5'a\text{-}H4'}$ = 2.3 Hz, 1H, H5′a), 3.83 (dd, $J_{H3'\text{-}F}$ = 25.2 Hz, $J_{H3'\text{-}H4'}$ = 9.5 Hz, 1H, H3′), 3.74 (ddd, $J_{H4'\text{-}H3'}$ = 10.1 Hz, $J_{H4'\text{-}H5'b}$ = 6.3 Hz, $J_{H4'\text{-}H5'a}$ = 2.2 Hz, 1H, H4′), 3.64 (dd,

$J_{H5'b\text{-}H5'a}$ = 11.6 Hz, $J_{H5'b\text{-}H4'}$ = 6.8 Hz, 1H, H5′b), 1.26 (d, $J_{CH3\text{-}F}$ = 22.8 Hz, 3H, 2-CH_3); ^{13}C NMR (101 MHz, D_2O) δ 163.7 (C-NH_2, C4), 155.4 (C=O, C2), 145.9 (d, $J_{C6\text{-}F}$ = 7.4 Hz, CH, C6) 101.9 (d, $J_{C2'\text{-}F}$ = 187.6 Hz, C_q, C2′), 95.6 (d, $J_{C5\text{-}F}$ = 7.8 Hz, CH, C5), 76.0 (d, $J_{C3'\text{-}F}$ = 18.0 Hz, CH, C3′), 61.39 (CH_2, C5′), 61.02 (d, $J_{C1'\text{-}F}$ = 15.5 Hz, CH, C1′), 51.5 (d, $J_{C4'\text{-}F}$ = 2.5 Hz, CH, C4′), 18.2 (d, $J_{CH3\text{-}F}$ = 25.2 Hz, 2′-CH_3); ^{19}F NMR (376 MHz, D_2O) δ -172.83 – -173.26 (m); ^{19}F {^{1}H} NMR (376 MHz, D_2O) δ -173.06; **β-anomer:** ^{1}H NMR (400 MHz, D_2O) δ 8.08 (d, $J_{H6\text{-}H5}$ = 7.5 Hz, 1H, H6), 6.23 (d, $J_{H1'\text{-}F}$ = 15.7 Hz, 1H, H1′), 6.04 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 4.00 (dd, *J* = 12.0 Hz, 2.3 Hz, 1H, H5′a), 3.90 (dd, *J* = 11.2 Hz, 5.6 Hz, 1H, H5′b), 3.87 (dd, $J_{H3'\text{-}F}$ = 10.9 Hz, $J_{H3'\text{-}H4'}$ = 6.4 Hz, 1H, H3′), 3.56 – 3.48 (m, 1H, H4′), 1.26 (d, $J_{CH3\text{-}F}$ = 23.0 Hz, 1H, 2′-CH_3); ^{13}C NMR (101 MHz, D_2O) δ 165.6 (C-NH_2, C4), 157.8 (C=O, C2) 143.0 (CH, C6), 104.3 (d, $J_{C2'\text{-}F}$ = 181.6 Hz, C2′), 96.8 (CH, C5), 75.0 (d, $J_{C3'\text{-}F}$ = 19.6 Hz, CH, C3′), 64.6 (d, $J_{C1'\text{-}F}$ = 39.1 Hz, CH, C1′), 60.2 (CH_2, C5′), 50.4 (CH, C4′), 16.6 (d, $J_{CH3\text{-}F}$ = 23.9 Hz, 2′-CH_3); ^{19}F NMR (376 MHz, D_2O) δ -157.53 – -158.07 (m); ^{19}F {^{1}H} NMR (376 MHz, D_2O) δ -157.76; NSI HRMS *m/z* found: $(M\text{-}H)^-$ 274.0667 $C_{10}H_{14}FN_3O_3S$, requires $(M\text{-}H)^-$ 274.0667.

P(*S*)-*N*-(2′-Deoxy-2′-fluoro-2′-methyl-1′-(4′-thio)-β-uridin-5′-O-yl)(phenoxy)phosphoryl-L-alanine-1-methylethyl ester **241** †



A suspension of **240β** (63.0 mg, 0.228 mmol, 1.0 equiv.) in THF (2.3 mL) was cooled to –20 °C. ^{t}BuMgCl (0.25 mL, 0.251 mmol, 1.1 equiv.) was added and the suspension stirred for 30 minutes at –20 °C. *N*-[(*R*)-(2,-3,-4,-5,-6-pentafluorophenoxy)-phenoxyphosphinyl]-1-methylethyl ester-D-alanine (124 mg, 0.274 mmol, 1.2 equiv.) was added and the solution stirred at rt for 24 h. MeOH (2 mL) was added and the solution stirred for 10 minutes, the solvents removed *in vacuo* and the crude product residue passed through a silica plug (EtOAc) and the solvents removed *in vacuo*. The crude product was partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL), the organic phase separated, and

the aqueous phase extracted with CH_2Cl_2 (2 × 10 mL). The organic phases were combined, washed with H_2O (10 mL), dried over $MgSO_4$ and the solvent removed *in vacuo* to obtain crude product **241** as a white solid (36.0 mg, 66.0 μmol, 29% crude product yield). The solvents were removed from the aqueous phase to recover **240β** as a colourless glass (40.1 mg, 0.144 mmol, 63%). Nucleoside **241** was further purified to 99% *via* preparative HPLC (Table 14).

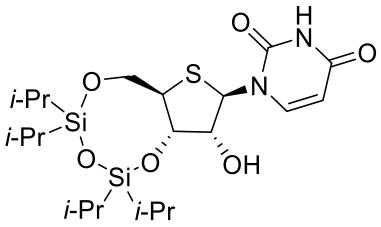
Time (minutes)	**%A (10 mM ammonium acetate)**	**%B (MeOH)**
0.0	96	4
10.0	96	4
15.0	75	25
21.0	75	25
30.0	0	100
35.0	0	100
35.1	96	4

Table 14. Preparative HPLC linear gradient system for purification of **241**. A 250 x 21.2 mm column packed 5 µ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 20 mL/minute. A solution of **241** in MeOH (100 mg/mL) was prepared and 150 μL injected into the prep HPLC system.

Retention time = 32.3 minutes (21.9 mg, 40.1 μmol, 18% overall yield). Purity was determined using general procedure L. R_f 0.48 (1/1, acetone/toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 7.37 – 7.29 (m, 2H, Ar-H), 7.24 – 7.15 (m, 3H, Ar-H), 6.22 (d, $J_{H1'\text{-}F}$ = 15.2 Hz, 1H, H1′), 5.79 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.99 (sep, $J_{CH\text{-}CH3}$ = 6.2 Hz, 1H, ^{i}Pr CH), 4.58 (ddd, $J_{H5'a\text{-}H5'b}$ = 11.8 Hz, $J_{H5'a\text{-}P}$ = 8.5 Hz, $J_{H5'a\text{-}H4'}$ = 3.5 Hz, 1H, H5′a), 4.47 – 4.34 (m, 2H, H5′b and NH alanine), 3.99 – 3.86 (m, 1H, H2″), 3.84 (dd, $J_{H3'\text{-}F}$ = 27.4 Hz, $J_{H3'\text{-}H4'}$ = 9.8 Hz, 1H, H3′), 3.80 (d, $J_{OH\text{-}H3'}$ = 9.7 Hz, 1H, 3′-OH), 3.69 – 3.59 (br m, 1H, H4′), 1.37 (d, $J_{CH3\text{-}F}$ = 17.3 Hz, 3H, 2′-CH_3), 1.34 (d, $J_{CH3\text{-}H2''}$ = 1.5 Hz, 3H, alanine 2″-CH_3), 1.23 (s, 3H, ^{i}Pr CH_3), 1.22 (s, 3H, ^{i}Pr CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3 (d, $J_{C3''\text{-}P}$ = 5.4 Hz, alanine C=O, C3″), 163.1 (C=O, C4), 151.0 (C=O, C2), 150.4 (d, $J_{C\text{-}P}$ = 6.6 Hz, C_q, Ar-C), 141.1 (CH, C6), 130.0 (CH, Ar-C), 125.3 (CH, Ar-C), 119.8 (CH, Ar-C), 119.8 (CH, Ar-C), 103.2 (CH, C5), 102.8 (d $J_{C2'\text{-}F}$ = 185.1 Hz, C_q, C2′) 75.1 (d, $J_{C3'\text{-}F}$ = 19.6 Hz, CH, C3′), 69.7 (CH, ^{i}Pr), 64.1 (d, $J_{C5'\text{-}P}$ = 5.3 Hz, CH_2, C5′), 63.9 (d, $J_{C1'\text{-}F}$ = 41.9 Hz, CH, C1′), 50.3 (alanine CH, C2″), 48.8 (d, $J_{C4'\text{-}P}$ = 3.6 Hz, CH, C4′), 21.8 (CH_3, ^{i}Pr), 21.6 (CH_3, ^{i}Pr), 20.7 (d, $J_{CH3\text{-}P}$ = 6.5 H, 2″-

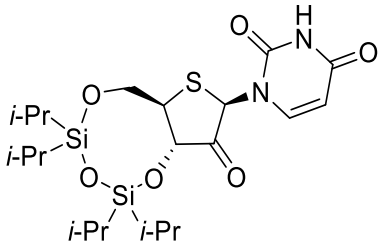
CH_3), 17.4 (d, $J_{CH3\text{-}F}$ = 24.4 Hz, 2′-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -158.6 – -160.0 (m); ^{19}F {^1H} NMR (376 MHz, $CDCl_3$) δ -159.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.81 (app. s); NSI HRMS *m/z* found: $(M+Na)^+$ 568.1280, $C_{22}H_{29}FN_3O_8PS$, requires $(M+Na)^+$ 568.1289.

3′,5′-*O*-(1,1,3,3-Tetraisopropyl-disiloxane)-1′-β-(4′-thio-D-ribofuranosyl)uracil **245** †



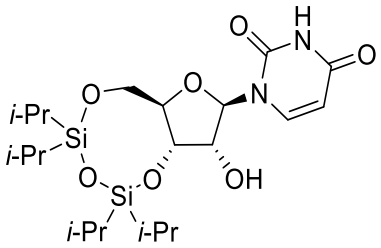
To a solution of **196** (1.00 g, 3.84 mmol, 1.0 equiv.) in pyridine (25.0 mL) was added $TIPDSCl_2$ (1.35 mL, 4.23 mmol, 1.1 equiv.). The solution was heated to 40 °C for 18 h, quenched with H_2O (10 mL) and the solvent removed *in vacuo* to obtain a brown oil which was partitioned between EtOAc (100 mL) and H_2O (80 mL). The organic layer was washed with saturated $NaHCO_3$ solution (2 × 80 mL) and brine (80 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a beige foam which was purified on silica gel *via* automated flash chromatography (0 – 20% EtOAc/petroleum ether) to afford **245** as a white foam (1.08 g, 2.21 mmol, 58%). R_f 0.58 (1/1, EtOAc/pet ether); $[\alpha]_D^{24.3}$ +33.0 (*c* 1.9, CH_2Cl_2); ^1H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H, N-H), 8.12 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 5.91 (d, $J_{H1'\text{-}H2'}$ = 4.4 Hz, 1H, H1′), 5.59 – 5.48 (m, 2H, H5 and H2′), 4.07 – 3.99 (m, 4H, H3′, H4′, H5′a, H′5b), 3.54 (d, $J_{OH\text{-}H2'}$ = 8.7 Hz, 1H, 2′-OH), 1.20 – 0.85 (m, 28H, ^{i}Pr, 4 × CH and 8 x CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.5 (C=O, C4), 151.1 (C=O uracil), C2), 141.4 (CH, C6), 101.2 (CH, C5), 77.5 (CH, C2′) 72.0 (CH_2, C5′), 65.7 (CH, C1′), 58.81 (CH, C4′), 49.3 (CH, C3′), 17.9 (CH_3, ^{i}Pr), 17.7 (CH_3, ^{i}Pr), 17.7 (CH_3, ^{i}Pr), 17.7 (CH_3, ^{i}Pr), 17.6 (CH_3, *i*Pr), 17.3 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 13.2 (CH, ^{i}Pr), 13.1 (CH, ^{i}Pr), 13.0 (CH, ^{i}Pr), 12.3 (CH, ^{i}Pr); ESI HRMS *m/z* found: $(M+H)^+$ 503.2073 $C_{21}H_{38}N_2O_6SSi_2$, requires $(M+H)^+$ 503.2062.

2′-Keto-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxane)-1′-β-(4′-thio-D-ribofuranosyl)uracil **246** †



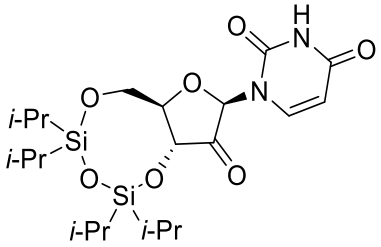
To a solution of **245** (1.67 g, 3.32 mmol, 1.0 equiv.) in DMSO (4.0 mL) was added Ac_2O (3.1 mL, 33.2 mmol, 10 equiv.) and the solution stirred vigorously at rt for 48 h. The solution was poured onto H_2O (200 mL), extracted with EtOAc (2 × 200 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (200 mL), H_2O (200 mL) and brine (200 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a colourless oil which was purified on silica gel *via* automated flash chromatography (0 – 30% EtOAc/CH_2Cl_2) to obtain **246** as a pale yellow foam (1.25 g, 2.48 mmol, 75%). R_f 0.86 (1/1, CH_2Cl_2/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H, NH), 7.09 (d, $J_{H6\text{-}H5}$ = 8.0 Hz, 1H, H6), 5.84 (br s, 1H, H1′), 5.77 (dd, $J_{H5\text{-}H6}$ = 8.0, $J_{H5\text{-}NH}$ = 1.8 Hz, 1H, H5), 4.71 (d, $J_{H3'\text{-}H4'}$ = 10.7 Hz, 1H, H3′), 4.19 (dd, $J_{H5'a\text{-}H5'b}$ = 13.0, $J_{H5'a\text{-}H4'}$ = 3.0 Hz, 1H, H5a′), 3.98 – 3.79 (m, 2H, H5′b and H4′), 1.19 – 0.96 (m, 28H, ^{i}Pr, 4 × CH and 8 x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.1 (C=O, C2′), 163.0 (C=O, C4), 150.1 (C=O, C2), 142.6 (CH, C6), 103.6 (CH, C5), 74.7 (CH, C3′), 61.2 (CH, C1′), 58.6 (CH_2, C5′), 48.0 (CH, C4′), 17.3 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 16.9 (CH_3, ^{i}Pr), 16.9 (CH_3, ^{i}Pr), 16.8 (CH_3, ^{i}Pr), 13.6 (CH, ^{i}Pr), 13.2 (CH, ^{i}Pr), 12.7 (CH, ^{i}Pr), 12.4 (CH, ^{i}Pr); ESI HRMS found: $(M+H)^+$ 501.1901 $C_{21}H_{36}N_2O_6SSi_2$, requires $(M+H)^+$ 501.1911.

3′,5′-*O*-(1,1,3,3-Tetraisopropyl-disiloxane)-1-β-(D-ribofuranosyl)uracil **248**



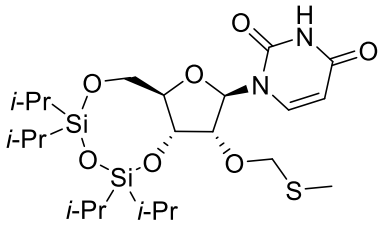
Uridine (2.00 g, 8.19 mmol, 1.0 equiv.) was dissolved in pyridine (25 mL) and $TIPDSCl_2$ (2.6 mL, 9.01 mmol, 1.1 equiv.) was added dropwise. The solution was heated to 40 °C for 18 h, quenched with ice-cold water (50.0 mL) and evaporated to dryness and the crude product residue partitioned between EtOAc (200 mL) and H_2O (100 mL). The layers were separated and the organic layer washed with saturated $NaHCO_3$ solution (2 × 200 mL) and brine (200 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a white foam which was purified on silica gel *via* automated flash chromatography (5 – 40% EtOAc/petroleum ether) to afford **248** as a white foam (3.11 g, 6.39 mmol, 78 %). R_f 0.83 (1/1, EtOAc/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H, NH), 7.73 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 5.73 (app. s, 1H, H1′), 5.69 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.35 – 4.09 (br m, 4H, H2′, H3′, H4′, H5′a), 4.00 (dd, $J_{H5'b\text{-}H5'a}$ = 13.2 Hz, $J_{H5'b\text{-}H4'}$ = 2.7 Hz, 1H, H5′b), 3.43 (br s, 1H, 2′-OH), 1.09 – 1.01 (m, 28H, ^{i}Pr, 4 × CH and 8 x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7 (C=O, C4), 150.4 (C=O, C2), 139.9 (CH, C6), 102.0 (CH, C5), 91.0 (CH, C1), 81.9 (CH, C2′ or C3′), 75.2 (CH, C2′ or C3′), 68.7 (CH, C4′), 60.1 (CH_2, C5′), 17.5 (CH_3, ^{i}Pr), 17.4 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 16.9 (CH_3, ^{i}Pr), 16.8 (CH_3, ^{i}Pr), 13.4 (CH, ^{i}Pr), 13.0 (CH, ^{i}Pr), 12.9 (CH, ^{i}Pr), 12.5 (CH, ^{i}Pr); NSI HRMS *m/z* found: $(M+Na)^+$ 509.2103 $C_{21}H_{38}N_2O_7Si_2$, requires $(M+Na)^+$ 509.2110. NMR data is consistent with literature values.[173]

2′-keto-3′,5′-*O*-(1,1,3,3-Tetraisopropyl-disiloxane)-1′-β-(D-ribofuranosyl)uracil **250**



A solution of **248** (0.780 g, 1.61 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (11 mL) and cooled to 0 °C. Dess-Martin periodinane (1.09 g, 2.57 mmol, 1.6 equiv.) was added and the solution stirred at 0 °C for 10 minutes before removing the ice bath and stirring at rt for 3 days. The reaction was quenched *via* addition of saturated aqueous $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ solution (3 × 100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered through Celite and the solvent removed *in vacuo* to obtain **250** as a white foam (0.680 g, 1.40 mmol, 87%) which was used without further purification. R_f 0.48 (3/7, acetone/toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 5.72 (d, $J_{H5\text{-}H6}$ = 8.0 Hz, 1H, H5), 5.03 (d, $J_{H3'\text{-}H4'}$ = 9.0 Hz, 1H, H3′), 4.95 (s, 1H, H1′), 4.12 – 4.05 (m, 2H, H5′a and H5′b), 3.95 – 3.80 (m, 1H, H4′), 1.21 – 0.94 (m, 28H, ^{i}Pr, 4 × CH and 8 x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.7 (C=O, C2′), 162.4 (C=O, C4), 149.2 (C=O, C2), 143.6 (CH, C6), 103.3 (CH, C5), 85.6 (CH, C1′), 79.6, (CH, C4′), 71.8 (CH, C3′), 62.3 (CH_2, C5′), 17.4 (CH_3, ^{i}Pr), 17.28 (CH_3, ^{i}Pr), 17.25 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 16.9 (CH_3, ^{i}Pr), 16.84 (CH_3, ^{i}Pr), 16.81 (CH_3, ^{i}Pr), 16.77 (CH_3, ^{i}Pr), 13.4 (CH, ^{i}Pr), 13.1 (CH, ^{i}Pr), 12.5 (CH, ^{i}Pr), 12.4 (CH, ^{i}Pr). NSI HRMS *m/z* found: $(M+H)^+$ 485.2120 $C_{21}H_{36}N_2O_7Si_2$, requires $(M+H)^+$ 485.2134. NMR data is consistent with literature values.[174]

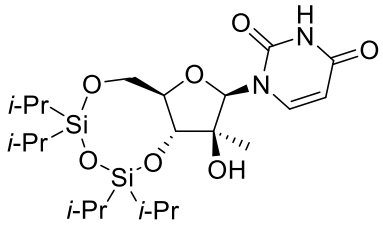
2′-*O*-(Methylsulfanyl)methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxane)-1′-β-(4′-D-ribofuranosyl)uracil **249** †



To a solution of **248** (0.500 g, 1.03 mmol, 1.0 equiv.) in DMSO (3.0 mL) was added acetic anhydride (3.3 mL, 10.3 mmol, 10 equiv.) and the solution stirred vigorously at rt for 24 h. The solution was diluted with EtOH (2.7 mL) and stirred at rt for 1 h. The solution was poured onto H_2O (100mL) and extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (5 × 80 mL) and brine (80 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product **249** as a colourless syrup (0.500 g, 1.03 mmol, *quant.*).

R_f 0.57 (3/7, acetone/toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (br s, 1H, N-H), 7.90 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 5.73 (s, 1H, H1′), 5.67 (dd, $J_{H5\text{-}H6}$ = 8.1 Hz, $J_{H5\text{-}NH}$ = 1.9 Hz, 1H, H5), 4.98 (s, 2H, OCH_2-S), 4.36 (d, $J_{H2'\text{-}H3'}$ = 4.5 Hz, 1H, H2′), 4.30 – 4.20 (m, 2H, H5′a, H4′), 4.14 – 4.10 (m, 1H, H3′), 3.98 (dd, $J_{H5'b\text{-}H5'a}$ = 13.6 Hz, $J_{H5'b\text{-}H4'}$ = 2.3 Hz, 1H, H5′b), 2.19 (s, 3H, S-CH_3), 1.14 – 0.99 (m, 28H, 4x CH and 8x CH_3 ^{i}Pr); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (C=O, C4), 149.7 (C=O, C2), 139.3 (CH, C6), 101.6 (CH, C5), 88.9 (CH, C1′), 77.3 (CH, C3′), 74.2 (OCH_2-S), 67.9 (CH, C4′), 59.3 (CH, C5′), 17.5 (CH_3, *i*Pr), 17.4 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 16.8 (CH_3, ^{i}Pr), 13.5 (CH, ^{i}Pr), 13.2 (CH, ^{i}Pr), 13.1 (CH, ^{i}Pr), 12.9 (S-CH_3), 12.2 (CH, ^{i}Pr); HRMS *m/z* found: $(M\text{-}H)^-$ 545.2180 $C_{23}H_{42}N_2O_7SSi_2$, requires $(M\text{-}H)^-$ 545.2178.

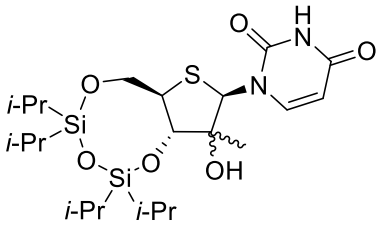
2′-*C*-Methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxane)-1′-β-(4′-D-ribofuranosyl)uracil **251**



Crude product **250** (0.735 g, 1.51 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (20 mL) and the solution cooled to 0 °C. $AlMe_3$ (2.9 mL, 5.76 mmol, 5.0 equiv.) was added dropwise and the solution stirred for a further 10 minutes at 0 °C before warming to rt and stirring vigorously. After 5 h, the solution was cooled to <5 °C and quenched *via* addition of saturated aqueous $NaHCO_3$ (350 mL) until pH = 7, and extracted with CH_2Cl_2 (5 × 75 mL), the combined organic layers washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as yellow syrup which was purified on silica gel *via* automated flash chromatography (0 – 12% acetone/toluene) to obtain **251** as a yellow syrup (312 mg, 0.815 mmol, 54%). R_f 0.55 (3/7, acetone/toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 5.74 – 5.72 (m, 2H, H1′ and H5), 4.15 – 4.11 (m, 2H, H5′a and H3′), 3.97 (dd, $J_{H5'b\text{-}H5'a}$ = 13.4 Hz, $J_{H5'b\text{-}H4'}$ = 2.7 Hz, 1H, H5′b), 3.85 – 3.59 (m, 1H, H4′), 1.53 (s, 3H, 2′-CH_3), 1.14 – 0.95 (m, 28H, ^{i}Pr, 4x CH and 8x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1 (C=O, C4), 152.3 (C=O, C2), 137.8 (CH, C6), 100.9 (CH, C5), 90.5 (CH, C1′), 81.5 (CH, C4′), 78.5 (CH, C2′) 72.7 (CH, C3′), 60.2 (CH_2, C5′), 20.8 (2′-CH_3), 17.5 (CH_3, ^{i}Pr), 17.4 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 17.1 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 16.8 (CH_3,

^{i}Pr), 16.8 (CH_3, ^{i}Pr), 13.5 (CH, ^{i}Pr), 13.0 (CH, ^{i}Pr), 13.0 (CH, ^{i}Pr), 12.4 (CH, ^{i}Pr); ESI HRMS *m/z* found: $(M+H)^+$ 501.2463 $C_{22}H_{40}N_2O_7Si_2$, requires $(M+H)^+$ 501.2447. ^{1}H NMR data is consistent with literature values.[145]

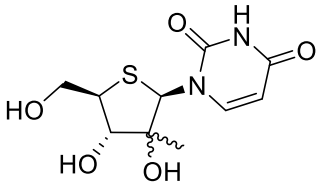
(2′*R*,*S*)-2′-*C*-Methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxane)-1′-β-(4′-thio-D-ribo/arabinofuranosyl)uracil **247** †



A solution of **246** (388 mg, 0.775 mmol, 1.0 equiv.) in CH_2Cl_2 (13 mL) was cooled to 0 °C. $AlMe_3$ (2.7 mL, 5.42 mmol, 7.0 equiv.) was added dropwise and the solution stirred for a further 10 minutes at 0 °C before warming to rt and stirring vigorously. After 18 h, the solution was cooled to 0 °C and quenched *via* addition of saturated aqueous $NaHCO_3$ (350 mL), and extracted with CH_2Cl_2 (5 × 75 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow oil which was purified on silica gel *via* automated flash chromatography (0 – 12% acetone/toluene) to obtain **247** as a colourless glass (196 mg, 0.380 mmol, 49%), and recovered **246** (0.188 g, 0.388 mmol, 26%). R_f 0.34 (3/7, EtOAc/$CHCl_3$); 1.5/1.0 ratio diastereoisomers; **major diastereoisomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H, N-H), 7.94 (d, $J_{H6\text{-}H5}$ = 8.3 Hz, 1H, H6), 6.19 (d, $J_{H1'\text{-}OH}$ = 1.1 Hz, 1H, H1′), 5.72 (dd, $J_{H5\text{-}H6}$ = 8.3 Hz, $J_{H5\text{-}NH}$ = 2.3 Hz, 1H, H5), 4.16 – 4.01 (m, 2H, H3′ and H5′a) 3.93 (dd, $J_{H5'b\text{-}H5'a}$ = 12.9 Hz, $J_{H5'b\text{-}H4'}$ = 2.7 Hz, 1H, H5′b), 3.73 (dt, $J_{H4\text{-}H3'}$ = 9.4, $J_{H4'\text{-}H5'a/b}$ = 2.8 Hz, 1H, H4′), 2.39 (d, $J_{OH\text{-}H1'}$ = 1.2 Hz, 1H, 2′-OH), 1.35 (s, 3H, 2′-CH_3), 1.14 – 1.04 (ov. m, 28H, ^{i}Pr, 4 × CH and 8 x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2 (C=O, C4), 151.6 (C=O, C2), 144.4 (CH, C6), 101.1 (CH, C5), 83.6 (C_q, C2′), 78.8 (CH, C3′), 61.4 (CH, C1′), 58.8 (CH_2, C5′), 51.9 (CH, C4′), 24.6 (C2′-CH_3), 17.4 (CH_3, ^{i}Pr), 17.4 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.3 (CH_3, ^{i}Pr), 17.2 (CH_3, ^{i}Pr), 17.1 (CH_3, ^{i}Pr), 13.8 (CH_3, ^{i}Pr), 13.6 (CH_3, ^{i}Pr), 13.5 (CH_3, ^{i}Pr), 13.2 (CH_3, ^{i}Pr), 12.9 (CH, ^{i}Pr), 12.9 (CH, ^{i}Pr), 12.8 (CH, ^{i}Pr), 12.6 (CH, ^{i}Pr); **minor diastereoisomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, NH), 8.06 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 6.02 (s, 1H, H1′), 5.78 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.31 (d, $J_{H3'\text{-}H4'}$ = 8.8 Hz, 1H, H3′), 4.15 – 4.04 (ov. m, 1H, H5′a), 3.88 (dd, $J_{H5'b\text{-}H5'a}$ =

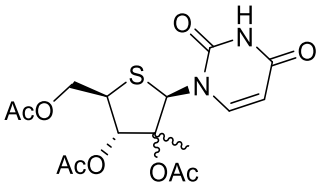
12.6 Hz, $J_{H5'b\text{-}H4'}$ = 3.7 Hz, 1H, H5′b), 3.31 (app. dt, $J_{H4'\text{-}H3'}$ = 8.8 Hz, $J_{H4'\text{-}H5'a/b}$ = 3.3 Hz, 1H), 2.05 (s, 1H, 2′-OH), 1.60 (s, 3H, 2′-CH_3), 1.18 – 0.91 (ov. m, 28H, ^{i}Pr, 4x CH and 8x CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6 (C=O, C4), 151.6 (C=O, C2), 141.9 (CH, C6), 102.0 (CH, C5), 79.9 (C_q, C2′), 78.8 (CH, C3′), 67.9 (CH, C1′), 60.6 (CH_2, C1′), 52.3 (CH, C4′), 29.3 (C2′-CH_3) 17.6 (CH_3, ^{i}Pr), 17.5 (CH_3, ^{i}Pr), 17.1 (CH_3, ^{i}Pr), 17.1 (CH_3, ^{i}Pr), 17.0 (CH_3, ^{i}Pr), 13.6 (CH, ^{i}Pr), 13.5 (CH, ^{i}Pr), 13.3 (CH, ^{i}Pr), 13.1 (CH, ^{i}Pr), 12.9 (CH, ^{i}Pr), 12.9 (CH, ^{i}Pr), 12.6 (CH, ^{i}Pr), 12.5 (CH, ^{i}Pr); ESI HRMS *m/z* found: $(M+H)^+$ 517.2242 $C_{22}H_{40}N_2O_6SSi_2$, requires $(M+H)^+$ 517.2218.

(2*R*,*S*)-2′-*C*-Methyl-1′-β-(4′-thio-D-ribo/arabinofuranosyl)uracil **252** †



To a solution of **247** (270 mg, 0.522 mmol, 1.0 equiv.) in THF (8.0 mL) was added glacial AcOH (0.06 mL, 1.04 mmol, 1.0 equiv.) and TBAF (1.0 mL, 1.04 mmol, 2.0 equiv.) and the solution stirred at rt for 4 h. The solvent was removed *in vacuo* and the crude product residue purified on silica gel *via* automated flash chromatography (0 – 6% MeOH/CH_2Cl_2) to obtain **252** as a colourless oil (86.0 mg, 0.314 mmol, 60%). R_f 0.49 (15/85, MeOH/CH_2Cl_2); 1.5/1 ratio diastereomers; **major diastereoisomer:** ^{1}H NMR (400 MHz, MeOD) δ 8.16 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 6.15 (s, 1H, H1′), 5.69 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.04 (dd, $J_{H5'a\text{-}H5'b}$ = 11.1 Hz, $J_{H5'a\text{-}H4'}$ = 3.1 Hz, 1H, H5′a), 3.70 – 3.65 (m, 1H, H5′b), 3.61 (d, $J_{H3'\text{-}H4'}$ = 9.8 Hz, 1H, H3′), 3.28 – 3.20 (m, 1H, H4′), 1.27 (s, 3H, 2′-CH_3); ^{13}C NMR (101 MHz, MeOD) δ 164.9 (C=O, C4), 151.9 (C=O, C2), 145.1 (CH, C6), 99.7 (CH, C5), 79.9 (C_q, C2′), 78.3 (CH, C3′), 62.9 (CH_2, C5′), 62.2 (CH, C1′), 53.0 (CH, C4′), 21.9 (2′-CH_3); NSI HRMS *m/z* found: $(M+H)^+$ 275.0697 $C_{10}H_{14}N_2O_5S$, requires $(M+H)^+$ 275.0696.

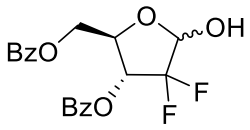
(2*R*,*S*)-2,3,5-Tri-*O*-acetyl-2′-*C*-methyl-1′-β-(4′-thio-D-ribo/arabinofuranosyl)uracil **253** †



Ac_2O (0.60 mL, 6.21 mmol, 4.5 equiv.) was added to a solution of **252** (375 mg, 1.38 mmol, 1.0 equiv.) in pyridine (9.2 mL) and the solution stirred at rt. After 1.5 h, the solution was poured onto 1M aqueous HCl solution (80 mL) and extracted with EtOAc (2 × 40 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (3 × 80 mL) and brine (80 mL), dried over anhydrous Na_2SO_4, filtered and dried *in vacuo* to obtain the crude product as a yellow residue which was purified on silica gel *via* automated flash chromatography (0 – 12% Acetone/toluene then 0 – 100% EtOAc/CH_2Cl_2) to obtain **253** as a colourless glass (521 mg, mmol, 1.30 mmol, 94%). R_f 0.44 (EtOAc); 1.2/1 ratio diastereoisomers; **major diastereoisomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H, N-H), 7.99 (d, $J_{H6\text{-}H5}$ = 8.3 Hz, 1H, H6), 6.30 (s, 1H, H1′), 5.72 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 5.14 (d, $J_{H3'\text{-}H4'}$ = 9.6 Hz, 1H, H3′), 4.36 (dd, $J_{H5'a\text{-}H5'b}$ = 11.5 Hz, $J_{H5'a\text{-}H4'}$ = 4.3 Hz, 1H, H5′a), 4.24 – 4.08 (m, 1H, H5′b), 4.09 – 3.98 (m, 1H, H4′), 2.17 (s, 3H, CH_3, 2′-CH_3), 2.09 (s, 3H, Ac-CH_3), 1.31 (s, 3H, Ac-CH_3), 1.26 (s, 3H, Ac-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5 (C=O, Ac), 169.4 (C=O, Ac), 162.8 (C=O, Ac), 144.0 (uracil C=O, C4), 101.3 (uracil C=O, C2), 79.8 (CH, C3′), 77.9 (C_q, C2′), 63.8 (CH_2, C5′), 61.5 (CH, C1′), 46.9 (CH, C4′), 29.3 (2′-CH_3) 23.4 (Ac-CH_3), 20.7 (Ac-CH_3), 20.7 (Ac-CH_3); ESI HRMS *m*/*z* found: $(M+Na)^+$ 423.0846 $C_{16}H_{20}N_2O_8S$, requires $(M+Na)^+$ 423.0833.

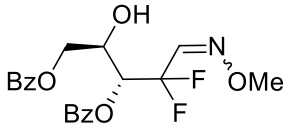
10.9. 2′-Deoxy-2′-*gem*-difluoro-1′-(4′-thio-D-*ribo*) nucleoside analogues

3,5-Di-*O*-benzoyl-2-deoxy-2-*gem*-difluoro-1-α,β-D-ribofuranose **318**



Hemiacetal **318** was prepared according to general procedure B using 2-deoxy-2-*gem*-difluoro-3,5-di-*O*-benzoyl-D-ribono-1,4-lactone (20.0 g, 58.1 mmol, 1.0 equiv.), Li(O^{t}Bu)$_3$AlH (16.2 g, 63.8 mmol, 1.2 equiv.) and THF (110 mL). Reaction time = 15 minutes. Obtained the crude product as a yellow syrup which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **318** as a yellow syrup (19.1 g, 50.5 mmol, 95%). R_f 0.18 (1/4, Et_2O/petroleum ether); 1.7/1 ratio α/β; **α-anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.01 (m, 4H, Ar-H), 7.66 – 7.36 (m, 6H, Ar-H), 5.54 – 5.44 (m, 2H, H1 and H3), 4.80 – 4.73 (m, 1H, H4), 4.69 – 4.65 (m, 1H, H5a), 4.60 (dd, $J_{H5b-H5a}$ = 12.0 Hz, J_{H5b-H4} = 4.4 Hz, 1H, H5b), 3.33 (d, J_{OH-F} = 4.3 Hz, 1H, 1-OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2 (C=O, Bz), 165.2 (C=O, Bz), 133.9 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 121.5 (dd, J_{C2-F} = 271.9 Hz, J_{C2-F} = 249.3 Hz, C_q, C2′), 96.1 (dd, J_{C1-F} = 42.0 Hz, J_{C1-F} = 23.5 Hz, CH, C1), 79.6 (t, J_{C4-F} = 3.3 Hz, CH C4), 71.9 (dd, J_{C3-F} = 36.0 Hz, J_{C3-F} = 18.0 Hz, CH, C3), 63.2 (CH_2, C5); ^{19}F NMR (376 MHz, $CDCl_3$) δ -109.33 (ddd, J_{F-F} = 252.0 Hz, J = 16.3 Hz, J = 6.7 Hz), -125.24 (app. d, J_{F-F} = 251.8 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 379.0970 $C_{19}H_{16}F_2O_4$, requires $(M+H)^+$ 379.0988. NMR data is consistent with literature values.[175,176]

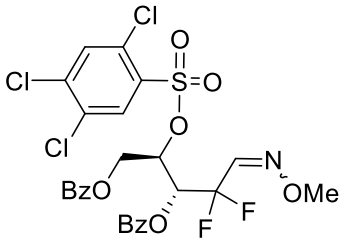
(2*R*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-2-*gem*-difluoro-4-hydroxy-1-(methoxyimino)pentane (*E*/*Z*) **319**



A solution of **318** (19.1 g, 50.5 mmol, 1.0 equiv.) and H_2NOMe·HCl (6.65 g, 79.7 mmol, 1.5 equiv.) in 3/1 (*v*/*v*) solution of MeCN/H_2O (410 mL) was cooled to 0 °C. Et_3N (11 mL, 79.7 mmol, 1.5 equiv.) and pyridinium *p*-toluenesulfonate (8.67 g, 34.5 mmol, 0.65 equiv.) was added and the solution stirred for a further 5 minutes at 0 °C and allowed to warm to rt, stirring vigorously. After 4 days, the solvent

was removed *in vacuo* and the residue partitioned between EtOAc (1 L) and H_2O (900 mL). The organic layer was separated and washed with H_2O (3 × 900 mL) and brine (900 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product **319** as a white foamy syrup (19.4 g, 47.6 mmol, 90%) which was used without further purification. R_f 0.57 (1/1, Et_2O/petroleum ether); 10/1 isomer ratio; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 7.96 (m, 4H, Ar-H), 7.66 – 7.49 (m, 3H, Ar-H and H1), 7.48 – 7.38 (m, 4H, Ar-H), 5.86 (ddd, J_{H3-F} = 13.1 Hz, J_{H3-F} = 10.5 Hz, J_{H3-H4} = 5.9 Hz, 1H, H3), 4.68 (dd, $J_{H5a-H5b}$ = 11.9 Hz, J_{H5a-H4} = 2.8 Hz, 1H, H5a), 4.60 – 4.52 (m, 1H, H4), 4.46 (dd, $J_{H5b-H5a}$ = 11.9 Hz, J_{H5b-H4} = 5.9 Hz, 1H, H5b), 3.90 (s, 3H, OCH_3), 2.98 (s, 1H, 4-OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9 (C=O, Bz), 165.1 (C=O, Bz), 142.5 (dd, J_{C1-F} = 32.4 Hz, J_{C1-F} = 28.9 Hz, C=N, C1), 133.9 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.1 (CH, Ar-C), 129.8 (CH, Ar-C), 129.5 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.4 (CH, Ar-C), 116.3 (dd, J_{C2-F} = 246.5 Hz, J_{C2-F} = 244.2 Hz, C_q, C2), 73.0 (dd, J_{C3-F}= 27.1 Hz, J_{C3-F} = 24.6 Hz, CH, C3), 68.3 (CH, C4), 65.6 (CH_2, C5), 63.0 (OCH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -104.57 (ddd, J_{F-F} = 277.2 Hz, J_{F-H3} 10.4 Hz, J_{F-H1} = 6.8 Hz), -106.98 (ddd, J_{F-F} = 277.2 Hz, J_{F-H3} = 13.1 Hz, J_{F-H1} = 6.2 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 408.1267 $C_{20}H_{19}F_2NO_6$, requires $(M+H)^+$ 408.1253. This compound was reported in the literature but not characterised.[109]

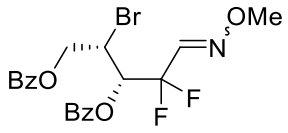
(2*R*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-4-*O*-(2′,4′,5′-trichlorophenylsulfonyl)-2-*gem*-difluoro-4-hydroxy-1-(methoxyimino)pentane (*E*/*Z*) **315** †



Compound **315** was prepared according to general procedure E using **319** (19.4 g, 47.6 mmol, 1.0 equiv.), 2,4,5-trichlorobenzenesulfonyl chloride (14.7 g, 52.4 mmol, 1.1 equiv.), *N*-methylimidazole (4.2 mL, 52.4 mmol, 1.1 equiv.) and MeCN (125 mL). Reaction time = 3 h. Purification: the crude product beige solid was triturated from rt Et_2O (500 mL) and the white precipitate collected by suction filtration and the filtrate washed with ice-cold Et_2O (100 mL). The

mother liquor was dried *in vacuo* and triturated from boiling Et_2O (60 mL) and the white solid collected by suction filtration, the filtrate washed with ice-cold Et_2O (40 mL) and the solids combined, obtaining **315** as a white solid (19.3 g, 29.6 mmol, 62%). R_f 0.51 (1/4, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ –33.9 (*c* 0.3, CH_2Cl_2) for isomeric mixture; 11/1 isomer ratio; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.01 (m, 3H, Ar-H), 7.91 – 7.85 (m, 2H), Ar-H, 7.67 – 7.54 (m, 2H, Ar-H), 7.52 – 7.38 (m, 4H, Ar-H and H1), 6.14 (ddd, $J_{H3\text{-}F}$ = 13.2 Hz, $J_{H3\text{-}F}$ = 10.3 Hz, $J_{H3\text{-}H4}$ = 2.8 Hz, 1H, H3), 5.63 (app. dt, $J_{H4\text{-}H5b}$ = 8.5 Hz, $J_{H4\text{-}H3/H5a}$ = 2.6 Hz, 1H, H4), 4.81 (dd, $J_{H5a\text{-}H5b}$ = 12.8 Hz, $J_{H5a\text{-}H4}$ = 2.5 Hz, 1H, H5a), 4.68 (dd, $J_{H5b\text{-}H5a}$ = 12.8 Hz, $J_{H5b\text{-}H4}$ = 8.5 Hz, 1H, H5b), 3.90 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8 (C=O, Bz), 164.1 (C=O, Bz), 140.9 (app. t, $J_{H1\text{-}F}$ = 31.8 Hz, C1), 139.1 (C_q Ar-C) 134.3 (C_q, Ar-C), 134.1 (C_q, Ar-C), 133.6 (C_q, Ar-C), 133.3 (C_q, Ar-C), 132.0 (C_q, Ar-C), 131.8 (CH, Ar-C), 131.7 (CH, Ar-C), 130.1 (CH, Ar-C), 129.6 (CH, Ar-C), 128.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.2 (CH, Ar-C), 115.6 (dd, $J_{C2\text{-}F}$ = 247.3 Hz, $J_{C2\text{-}F}$ = 243.1 Hz, C_q, C2), 78.5 (CH, C4), 71.3 (dd, $J_{C3\text{-}F}$ = 29.8 Hz, $J_{C3\text{-}F}$ = 25.3 Hz, C3), 63.3 (OCH_3), 62.2 (CH_2, C5); ^{19}F NMR (376 MHz, $CDCl_3$) δ -102.28 (ddd, $J_{F\text{-}F}$ = 283.7 Hz, $J_{F\text{-}H3}$ = 10.1 Hz, $J_{F\text{-}H1}$ = 5.4 Hz), -105.24 (ddd, $J_{F\text{-}F}$ = 283.7 Hz, $J_{F\text{-}H3}$ = 13.3 Hz, $J_{F\text{-}H1}$ = 6.2 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 649.9996 $C_{26}H_{20}{}^{35}Cl_3F_2NO_8S$, requires $(M+H)^+$ 650.0016.

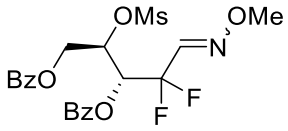
(2*S*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-4-bromo-4-*O*-(2′,4′,5′-trichlorophenylsulfonyl)-2-*gem*-difluoro-4-hydroxy-1-(methoxyimino)pentane (*E*/*Z*) **316**



To a solution of **315** (19.3 g, 29.6 mmol, 1.0 equiv.) in 2-butanone (100 mL) was added LiBr (12.9 g, 148 mmol, 5.0 equiv.). The solution was heated to 80 °C for 18 h, poured onto ice-water (1 L) and extracted with CH_2Cl_2 (5 × 200 mL). The combined organic layers were washed with H_2O (2 × 700 mL) and brine (700 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product which was purified on silica gel *via* automated flash chromatography (0 – 30% Et_2O/petroleum ether) to obtain **316** as a yellow oil (13.5 g, 28.7 mmol, 97%). R_f 0.77 (1/1, Et_2O/petroleum ether); 6.3/1.0 ratio isomers; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 –

8.11 (m, 2H, Ar-H), 8.10 – 8.04 (m, 2H, Ar-H), 7.68 – 7.54 (m, 2H, Ar-H), 7.54 – 7.40 (m, 5H, Ar-H and H1), 6.10 (ddd, $J_{H3\text{-}F}$ = 11.2 Hz, $J_{H3\text{-}F}$ = 9.8 Hz, $J_{H3\text{-}H4}$ = 2.8 Hz, 1H, H3), 4.83 – 4.68 (m, 2H, H5a and H4), 4.48 (dd, $J_{H5b\text{-}H5a}$ = 13.5 Hz, $J_{H5b\text{-}H4}$ = 9.4 Hz, 1H, H5b), 3.83 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6 (C=O, Bz), 164.6 (C=O, Bz), 141.4 (dd, $J_{C1\text{-}F}$ = 32.0 Hz, $J_{C1\text{-}F}$ = 30.3 Hz, C=N, C1), 134.0 (C_q, Ar-C), 133.4 (C_q, Ar-C), 130.3 (CH, Ar-C), 130.1 (CH, Ar-C), 130.0 (CH, Ar-C), 129.9 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 115.8 (dd, $J_{C2\text{-}F}$ = 246.7 Hz, $J_{C2\text{-}F}$ = 245.7 Hz, C_q, C2), 69.8 (dd, $J_{C3\text{-}F}$ = 28.8 Hz, $J_{C3\text{-}F}$ = 28.1 Hz, CH, C3), 64.7 (CH_2, C5), 63.0 (OCH_3), 44.3 (t, $J_{C4\text{-}F}$ = 2.0 Hz, C-Br, C4); ^{19}F NMR (376 MHz, $CDCl_3$) major isomer δ -102.7 (ddd, $J_{F\text{-}F}$ = 279.74 Hz, $J_{F\text{-}H3}$ = 11.1 Hz, $J_{F\text{-}H1}$ = 5.7 Hz), -105.10 (ddd, $J_{F\text{-}F}$ = 279.7 Hz, $J_{F\text{-}H3}$ = 9.4 Hz, $J_{F\text{-}H1}$ = 7.1 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 470.0414 $C_{20}H_{18}{}^{79}BrF_2NO_5$, requires $(M+H)^+$ 470.0409. This compound was reported in the literature but not characterised.[109]

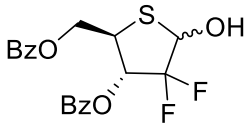
2*R*,3*R*,4*S*)-3,5-Di-*O*-benzoyl-4-*O*-(2′,4′,5′-trichlorophenylsulfonyl)-2-*gem*-difluoro-4-hydroxy-4-*O*-mesyl-1-(methoxyimino)pentane (*E*/*Z*) **320**



A solution of **319** (256 mg, 0.527 mmol, 1.0 equiv.) in THF (3.7 mL) was cooled to 0 °C. MsCl (53 μL, 0.691 mmol, 1.1 equiv.) and Et_3N (0.10 mL, 0.691 mmol, 1.1 equiv.) were added over 5 minutes. The solution was stirred at rt for 24 h, diluted with EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (50 mL), H_2O (50 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product **320** as a colourless syrup (328 mg, 0.527 mmol, *quant.*) which was used without further purification. R_f 0.48 (1/1, Et_2O/petroleum ether); 13/1 isomer ratio; **major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (m, 4H, Ar-H), 7.69 – 7.54 (m, 2H, Ar-H), 7.53 – 7.40 (m, 5H, Ar-H and H1), 6.18 (ddd, $J_{H3\text{-}F}$ = 13.0 Hz, $J_{H3\text{-}F}$ = 10.9 Hz, $J_{H3\text{-}H4}$ = 3.4 Hz, 1H, H3), 5.65 – 5.59 (m, 1H, H4), 4.89 (dd, $J_{H5a\text{-}H5b}$ = 12.6 Hz, $J_{H5a\text{-}H4}$ = 2.6 Hz, 1H, H5a), 4.60 (dd, $J_{H5b\text{-}H5a}$ = 12.6 Hz, $J_{H5b\text{-}H4}$ = 8.3 Hz, 1H, H5b), 3.90 (s, 3H, OCH_3), 3.11 (s, 3H, Ms-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 164.4 (C=O, Bz), 140.1 (app. t, $J_{C1\text{-}F}$ = 32.5 Hz, C1), 134.1 (C_q, Ar-C), 133.4 (C_q, Ar-C), 130.2 (CH, Ar-C), 130.0 (CH, Ar-C), 129.9 (CH, Ar-C), 129.8 (CH, Ar-

C), 129.2 (CH, Ar-C), 128.8 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 115.6 (dd, $J_{C2\text{-}F}$ = 246.6 Hz, $J_{C2\text{-}F}$ = 243.0 Hz, C_q, C2), 75.8 (CH, C4), 71.0 (dd, $J_{C3\text{-}F}$ = 28.9 Hz, $J_{C3\text{-}F}$ = 24.7 Hz, CH, C3), 63.3 (OCH_3), 62.4 (app. t, $J_{C5\text{-}F}$ = 3.5 Hz, C5), 39.2 (Ms-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -103.03 (ddd, $J_{F\text{-}F}$ = 284.8 Hz, $J_{F\text{-}H3}$ = 10.8 Hz, $J_{F\text{-}H1}$ = 5.2 Hz), -104.78 (ddd, $J_{F\text{-}F}$ = 284.8 Hz, $J_{F\text{-}H3}$ = 13.0 Hz, $J_{F\text{-}H1}$ = 5.8 Hz); ESI HRMS *m/z* found: (M+H)+ 486.1028 $C_{21}H_{21}F_2NO_8S$, requires $(M+H)^+$ 486.1029. ^{1}H NMR data is consistent with literature values.[109]

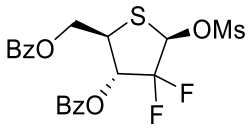
3,5-Di-*O*-benzoyl-2-deoxy-2-*gem*-difluoro-1-α,β-(4-thio-D-ribofuranose) **317** †



Aldehyde **322** was prepared according to general procedure D using **316** (13.5 g, 28.7 mmol, 1.0 equiv.), 50% (*w*/*v*) glyoxylic acid solution (112 mL, 201 mmol, 7.0 equiv.) and MeCN (59 mL). reaction time = 18 h. Furnished crude product **322** as a brown syrup (13.2 g, ~28.7 mmol *quant.*) which was used immediately in the next step without further purification. R_f 0.11 (1/1, Et_2O/petroleum ether); ESI HRMS *m/z* found: $(M+H)^+$ 441.0415 $C_{19}H_{15}{}^{79}BrF_2O_5$, requires $(M+H)^+$ 441.0415. Thiohemiacetal **317** was prepared according to general procedure G using crude product **322** (8.43 g, 19.1 mmol, 1.0 equiv.), $NaSH{\cdot}H_2O$ (1.84 g, 24.8 mmol, 1.3 equiv.), DMF (24 mL) and H_2O (2.5 mL). Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 100% Et_2O/petroleum ether) to obtain **317** as a yellow oil (7.32 g, 18.6 mmol, 97%). R_f 0.48 (1/1, Et_2O/petroleum ether); 1/3 anomer ratio; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.01 (m, 2H, Ar-H), 7.97 – 7.90 (m, 2H, Ar-H), 7.65 – 7.27 (m, 6H, Ar-H), 6.04 (ddd, $J_{H3\text{-}F}$ = 17.7 Hz, $J_{H3\text{-}F}$ = 7.6 Hz, $J_{H3\text{-}H4}$ = 4.6 Hz, 1H, H3), 5.34 (dd, $J_{H1\text{-}F}$ = 7.0 Hz, $J_{H1\text{-}F}$ 2.4 Hz, 1H, H1), 5.30 (s, 1H, 1-OH), 4.64 (dd, $J_{H5a\text{-}H5b}$ = 11.6 Hz, $J_{H5a\text{-}H4}$ = 6.6 Hz, 1H, H5a), 4.60 – 4.53 (m, 1H, H5b), 3.89 – 3.83 (m, 1H, H4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 165.0 (C=O, Bz), 133.9 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.1 (CH, Ar-C), 130.1 (CH, Ar-C), 129.8 (CH, Ar-C), 129.7 (CH, Ar-C), 129.2 (CH, Ar-C), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 123.5 (dd, $J_{C2\text{-}F}$ = 211.4 Hz, $J_{C2\text{-}F}$ = 125.9 Hz, C_q, C2), 76.2 (dd, $J_{C1\text{-}F}$ = 34.5 Hz, $J_{C1\text{-}F}$ = 22.1 Hz, CH, C1), 72.2 (dd, $J_{C3\text{-}F}$ = 27.7 Hz, $J_{C3\text{-}F}$ = 19.0 Hz, CH, C3), 65.0 (CH_2, C5), 41.8 (d, $J_{C4\text{-}F}$ = 5.8

Hz, CH, C4); ^{19}F NMR (376 MHz, $CDCl_3$) δ -119.43 (app. d, $J_{F\text{-}F}$ = 234.1 Hz), -123.72 (ddd, $J_{F\text{-}F}$ = 233.9 Hz, $J_{F\text{-}H3}$ = 17.7 Hz, $J_{F\text{-}H1}$ = 7.0 Hz); ESI HRMS *m/z* found: $(M+H)^+$ 395.0764, $C_{19}H_{15}F_2O_5S$, requires $(M+H)^+$ 395.0765. ^{1}H NMR data is consistent with literature values.[109]

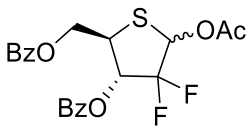
3,5-Di-*O*-benzoyl-2-deoxy-2-*gem*-difluoro-1-*O*-mesyl-1-β-(4-thio-D-ribofuranose) **323** †



To a solution of **317** (0.695 g, 1.76 mmol, 1.0 equiv.) in CH_2Cl_2 (8.8 mL) was added MsCl (0.20 mL, 2.64 mmol, 1.5 equiv.) and Et_3N (0.40 mL, 2.64 mmol, 1.5 equiv.). The solution was stirred at rt for 3.5 h, diluted with CH_2Cl_2 (100 mL) and washed with H_2O (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow syrup which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/pet/ether) to obtain **323** as a yellow foam (0.744 g, 1.57 mmol, 89%), a mixture of anomers (1/4 ratio α/β). The β-anomer was separated by crystallisation from hot Et_2O (15 mL) to obtain **323β** as colourless needles. The solvent from the mother liquor was removed *in vacuo* and the residue crystallised by vapour diffusion from CH_2Cl_2/hexane (5 mL each) and the two sets of crystalline solids combined to obtain **323β** (0.603 g total, 1.28 mmol, 72% total). R_f 0.28 (1/1 Et_2O/petroleum ether); $[\alpha]_D^{25.8}$ –52.6 (*c* 1.4, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.01 (m, 2H, Ar-H), 8.01 – 7.93 (m, 2H, Ar-H), 7.61 (t, J_{vic} = 7.5 Hz, 1H, Ar-H), 7.57 – 7.40 (m, 3H, Ar-H), 7.41 – 7.29 (m, 2H, Ar-H), 6.04 (br d, $J_{H1\text{-}F}$ = 6.4 Hz, 1H, H1), 6.00 (ddd, $J_{H3\text{-}F}$ = 20.6 Hz, $J_{H3\text{-}H4}$ = 8.5 Hz, $J_{H3\text{-}F}$ = 3.9 Hz, 1H), 4.69 (dd, $J_{H5a\text{-}H5b}$ = 11.8 Hz, $J_{H5a\text{-}H4}$ = 5.5 Hz, 1H, H5a), 4.53 (dd, $J_{H5b\text{-}H5a}$ = 11.8 Hz, $J_{H5b\text{-}H4}$ 5.5 Hz, 1H, H5b), 3.92 (dt, $J_{H4\text{-}H3}$ = 8.5 Hz, $J_{H4\text{-}H5a/h5b}$ = 5.5 Hz, 1H, H4), 3.07 (s, 3H, Ms-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8 (C=O, Bz), 164.8 (C=O, Bz), 134.1 (C_q, Ar-C), 133.5 (C_q, Ar-C), 130.2 (CH, Ar-C), 129.7 (CH, Ar-C), 129.1 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.1 (CH, Ar-C), 121.7 (dd, $J_{C2\text{-}F}$ = 269.9 Hz, $J_{C2\text{-}F}$ = 253.3 Hz, C2), 81.6 (dd, $J_{C1\text{-}F}$ = 38.0 Hz, $J_{C1\text{-}F}$ = 21.9 Hz, CH, C1), 71.4 (dd, $J_{C3\text{-}F}$ = 25.8 Hz, $J_{C3\text{-}F}$ = 18.2 Hz, CH, C3), 64.0 (CH_2, C5), 42.3 (d, $J_{C4\text{-}F}$ = 6.2 Hz, CH, C4), 40.1 (Ms-CH_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ -116.33 (dd, $J_{F\text{-}F}$ = 233.9 Hz, $J_{F\text{-}H3}$ = 3.3 Hz), -123.91 (ddd, $J_{F\text{-}F}$ = 233.9 Hz, $J_{F\text{-}H3}$ =

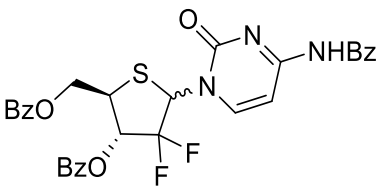
20.6 Hz, $J_{F\text{-}H1}$ = 6.4 Hz); ESI HRMS *m/z* found: $(M+Na)^+$ 495.0372 $C_{20}H_{18}F_2O_7S_2$, requires $(M+Na)^+$ 495.0354.

1-*O*-Acetyl-3,5-di-*O*-benzoyl-2-deoxy-2-difluoro-1-α,β-(4-thio-D-ribofuranose) **325** †



Acetyl glycoside **325** was prepared according to general procedure F using **317** (0.990 g, 2.51 mmol, 1.0 equiv.), Ac_2O (0.28 mL, 3.01 mmol, 1.2 equiv.) Et_3N (0.42 mL, 3.01 mmol, 1.2 equiv.) and CH_2Cl_2 (13 mL). Reaction time = 5h. Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 30% Et_2O/petroleum ether) to obtain **325** as a colourless syrup (0.963 g, 2.21 mmol, 88%). R_f 0.50 (1/1, Et_2O/pet ether); 3/2 ratio anomers; **major anomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.00 (m, 2H, Ar-H), 7.96 – 7.90 (m, 2H, Ar-H), 7.65 – 7.52 (m, 1H, Ar-H), 7.54 – 7.39 (m, 4H, Ar-H), 7.34 – 7.27 (m, 1H, Ar-H), 6.04 (d, $J_{H1\text{-}F}$ = 7.7 Hz, 1H, H1), 6.05 – 5.95 (ov. m, 1H, H3), 4.66 (dd, $J_{H5a\text{-}H5b}$ = 11.6 Hz, $J_{H5a\text{-}H4}$ = 6.0 Hz, 1H, H5a), 4.48 (dd, $J_{H5b\text{-}H5a}$ = 11.6 Hz, $J_{H5b\text{-}H4}$ = 5.7 Hz, 1H, H5b), 3.89 (app. dt, $J_{H4\text{-}H3}$ = 8.7, $J_{H4\text{-}H5a/H5b}$ = 5.9 Hz, 1H, H4), 2.13 (s, 3H, Ac-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9 (C=O, Ac), 165.8 (C=O, Bz), 165.0 (C=O, Bz), 134.0 (C_q, Ar-C), 133.3 (C_q, Ar-C), 130.2 (CH, Ar-C), 129.7 (CH, Ar-C), 129.2 (CH, Ar-C), 128.6 (CH, Ar-C), 128.3 (CH, Ar-C), 123.5 (dd, $J_{C2'\text{-}F}$ = 370.5 Hz, $J_{C2'\text{-}F}$ = 119.4 Hz, C_q, C2′), 75.0 (dd, $J_{C1\text{-}F}$ = 38.2 Hz, $J_{C1\text{-}F}$ 20.6 Hz, CH, C1), 72.0 (dd, $J_{C3\text{-}F}$ = 25.8 Hz, $J_{C3\text{-}F}$ = 18.6 Hz, CH, C3), 64.5 (CH_2, C5), 41.5 (d, $J_{C4\text{-}F}$ = 6.2 Hz, CH, C4), 20.8 (Ac-CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ -117.94 (dd, $J_{F\text{-}F}$ = 236.0 Hz, $J_{F\text{-}H3}$ = 3.6 Hz), -123.92 (ddd, $J_{F\text{-}F}$ = 235.8 Hz, $J_{F\text{-}H3}$ = 20.5 Hz, $J_{F\text{-}H1}$ = 7.9 Hz); ESI HRMS *m/z* found: $(M+Na)^+$ 459.0697 $C_{21}H_{18}F_2O_6SNa^+$, requires $(M+Na)^+$ 459.0684.

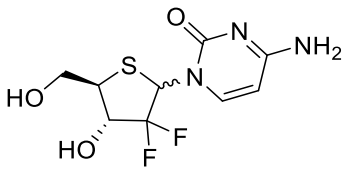
3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-*gem*-difluoro-1′-α,β-(4′-thio-D-ribofuranosyl)-*N*^{4}-benzoyl-cytosine **324** †



Nucleoside **324** was prepared according to general procedure I using N^4-benzoylcytosine (0.711 g, 3.30 mmol, 1.4 equiv.), HMDS (4.9 mL, 22.7 mmol, 9.9 equiv.), pyridine (1.8 mL), **325** (1.03 g, 2.36 mmol, 1.0 equiv.), $SnCl_4$ (0.83 mL, 7.08 mmol, 3.0 equiv.) and DCE (24 mL). Reaction time = 3 h. Purification: the crude product was purified on silica gel *via* automated flash chromatography (0 – 50% then 50 – 100% EtOAc/hexanes) to obtain **324** as a yellow syrup (1/1 α/β ratio, 376 mg, 0.636 mmol, 28%), and recovered **325** (262 mg, 0.600 mmol, 25%). The anomeric mixture of **324** was then further purified *via* fractional precipitation from boiling EtOH or boiling EtOAc to furnish **324α** as a white solid (130 mg, 0.220 mmol, 10%), **324β** as a white solid (88.0 mg, 149 mmol, 6%) and **324** as a yellow syrup (1.2/1 α/β ratio, 128 mg, 0.216 mmol, 10%). R_f 0.18 (1/9, acetone/toluene); **β-anomer:** $[\alpha]_D^{24.7}$ **–**38.9 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H, NH), 8.31 (d, $J_{H6\text{-}H5}$ = 7.6 Hz, 1H, H6), 8.12 – 8.05 (m, 4H, Ar-H), 7.96 – 7.87 (m, 2H, Ar-H), 7.67 – 7.59 (m, 3H, Ar-H), 7.56 – 7.44 (m, 7H, Ar-H and H5), 6.99 (app. t, $J_{H1'\text{-}Fa/Fb}$ = 9.7 Hz, 1H, H1′), 5.93 – 5.81 (br m, 1H, H3′), 4.76 (dd, $J_{H5'a\text{-}H5'b}$ = 11.8 Hz, $J_{H5'a\text{-}H4'}$ = 6.6 Hz, 1H), 4.66 (dd, $J_{H5'b\text{-}H5'a}$ = 11.8 Hz, $J_{H5'b\text{-}H4'}$ = 6.1 Hz, 1H, H5′b), 3.97 (br. dt, $J_{H4'\text{-}H3'}$ = 11.6 Hz, $J_{H4'\text{-}H5'a/H5'b}$ = 5.8 Hz, 1H, H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 165.9 (C=O, Bz), 164.6 (C=O, Bz), 162.5 (C-NH, C4), 146.1 (CH, C6), 146.0 (C=O, C2), 134.2 (C_q, Ar-C), 133.7 (C_q, Ar-C), 133.4 (C_q, Ar-C), 132.9 (CH, C5), 130.2 (CH, Ar-C), 129.8 (CH, Ar-C), 129.1 (CH, Ar-C), 128.7 (CH, Ar-C), 128.7 (CH, Ar-C), 128.0 (CH, Ar-C), 127.7 (CH, Ar-C), 123.0 (dd, $J_{C2'\text{-}F}$ = 264.6 Hz, $J_{C2'\text{-}F}$ = 260.0 Hz, C_q, C2′), 72.3 (dd, $J_{C3'\text{-}F}$ = 29.7 Hz, $J_{C3'\text{-}F}$ = 23.5 Hz, CH, C3′), 63.1 (d, $J_{C5'\text{-}F}$ = 1.2 Hz, CH_2, C5′), 59.9 (dd, $J_{C1'\text{-}F}$ = 29.7 Hz, $J_{C1'\text{-}F}$ = 22.9 Hz, CH, C1′), 44.5 (CH, C4′); ^{19}F NMR (376 MHz, $CDCl_3$) δ -114.01 – -114.64 (ov. m); **α-anomer:** $[\alpha]_D^{23.9}$ +15.9 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H, NH), 8.32 (dd, $J_{H6\text{-}H5}$ = 7.7 Hz, $J_{H6\text{-}F}$ = 1.6 Hz, 1H, Ar-H), 8.04 – 7.95 (m, 4H, Ar-H), 7.94 – 7.82 (m, 2H, Ar-H), 7.71 – 7.31 (m, 10H, Ar-H and H5), 7.02 (app. t, $J_{H1'\text{-}Fa/Fb}$ = 9.6 Hz, 1H, H1′), 5.94 (app. dt, $J_{H3'\text{-}Fa/Fb}$ = 12.8 Hz, $J_{H3'\text{-}H4'}$ = 6.5 Hz, 1H, H3′), 4.67 (dd, $J_{H5'a\text{-}H5'b}$ = 11.7 Hz, $J_{H5'a\text{-}H4'}$ = 6.6 Hz, 1H), 4.55 (dd, $J_{H5;b\text{-}H5'a}$ = 11.7 Hz, $J_{H5'b\text{-}H4'}$ = 6.4 Hz, 1H), 4.25 (app. q, $J_{H4'\text{-}H5'a/H5'b/H3'}$ = 6.4 Hz, 1H, H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 165.9 (cytosine C-NH, C4), 164.5 (C=O, Bz), 162.6 (C=O, Bz), 146.6 (C=O cytosine, C2), 146.6 (CH, C6), 134.2 (C_q, Ar-C), 133.5 (C_q, Ar-C), 133.4 (C_q, Ar-C), 132.9 (CH, C5), 130.1 (CH, Ar-C), 129.8 (CH, Ar-C), 129.1 (CH, Ar-C), 129.0 (CH, Ar-C), 128.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.0

(CH, Ar-C), 127.7 (CH, Ar-C), 122.7 (app. t, J = 262.8 Hz, C_q, C2′), 73.5 (dd, $J_{C3'\text{-}F}$ = 30.9 Hz, $J_{C3'\text{-}F}$ = 19.4 Hz, CH, C3′), 63.9 (CH_2, C5′), 59.5 (dd, $J_{C1'\text{-}F}$ = 32.4 Hz, $J_{C1'\text{-}F}$ = 19.2 Hz, CH, C1′), 45.1 (d, $J_{C4'\text{-}F}$ = 1.7 Hz, CH, C4′); ^{19}F NMR (376 MHz, $CDCl_3$) δ -106.35 (app d, $J_{F\text{-}F}$ = 239.4 Hz), -120.10 (app. dt, $J_{F\text{-}F}$ = 239.5 Hz, $J_{F\text{-}H1/H3}$ = 10.6 Hz); NSI HRMS *m/z* found: $(M+H)^+$ 592.1344 $C_{30}H_{24}N_3O_6F_2S$, requires $(M+H)^+$ 592.1348.

2′-Deoxy-2′-*gem*-difluoro-1′-α,β-(4′-thio-D-ribofuranosyl)cytosine **156**



MeOH (0.50 mL) was added to a suspension of **324** (87.0 mg, 0.148 mmol, 1.0 equiv.) in neat 7M NH_3/MeOH (0.50 mL, 3.47 mmol, 23 equiv.) until a homogenous solution was obtained. The solution was stirred at rt for 18 h, the solvents removed *in vacuo* and the crude product residue purified on octadecyl modified silica gel *via* flash chromatography (0/100, 10/90, 100/0 H_2O/MeOH) to obtain crude product **156** as a yellow glass, a mixture of anomers (39.8 mg, 0.142 mmol, 96%) and the anomers separated and purified *via* preparative HPLC (Table 15).

Time (minutes)	**%A (10 mM ammonium acetate)**	**%B (MeOH)**
0.0	96	4
17.0	96	4
22.0	60	40
28.0	60	40
30.0	0	100
33.0	0	100
33.1	96	4

Table 15. Preparative HPLC linear gradient system for purification of **156**. A 250 x 21.2 mm column packed 5 µ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 15 mL/minute. A solution of **156** in H_2O (100 mg/mL) was prepared and 150 μL injected into the prep HPLC system.

Retention times α-anomer = 25.3 minutes (18.1 mg, 64.8 μmol, 98% purity, 44% yield), β-anomer = 26.3 minutes (14.1 mg, 50.5 μmol, 87% purity, 34% yield). The β-anomer fraction was then further purified to 99% *via* preparative HPLC (Table 16).

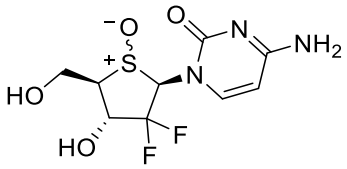
Time (minutes)	**%A (10 mM ammonium acetate)**	**%B (MeOH)**
0.0	96	4
1.0	96	4
7.0	60	40
8.0	60	40
12.0	0	100
12.1	96	4

Table 16. Preparative HPLC linear gradient system for purification of **156β**. A 250 x 21.2 mm column packed 5 µ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 15 mL/minute. A solution of **156β** in H_2O (100 mg/mL) was prepared and 100 μL injected into the prep HPLC system.

Retention time = 24.7 minutes (8.1 mg, 29.0 μmol, 20% overall yield). Purity was determined using general procedure L. R_f 0.57 (1/9 H_2O/MeCN); **α-anomer:** $[\alpha]_D^{22.8}$ +5.1 (*c* 1.3, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 8.00 (dd, $J_{H6\text{-}H5}$ = 7.6 Hz, $J_{H6\text{-}F}$ = 2.6 Hz, 1H, H6), 6.50 (dd, $J_{H1'\text{-}F}$ = 12.6 Hz, $J_{H1'\text{-}F}$ = 8.9 Hz, 1H, H1′), 5.99 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 4.31 (ddd, $J_{H3'\text{-}F}$ = 16.6 Hz, $J_{H3'\text{-}H4'}$ = 8.5 Hz, $J_{H3'\text{-}F}$ = 5.7 Hz, 1H, H3′), 3.87 (dd, $J_{H5'a\text{-}H5'b}$ = 11.7 Hz, $J_{H5'a\text{-}H4'}$ = 3.7 Hz, 1H, H5′a), 3.75 – 3.67 (ov. m, 1H, H5′b), 3.68 – 3.61 (m, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 180.4 (C-NH_2, C4), 157.5 (C=O, C2), 144.1 (d, $J_{C6\text{-}F}$ = 3.6 Hz, CH, C6), 123.5 (dd, $J_{C2'\text{-}F}$ = 261.1 Hz, $J_{C2'\text{-}F}$ = 256.6 Hz, C_q, C2′), 96.2 (CH, C5), 72.2 (dd, $J_{H3'\text{-}F}$ = 27.0 Hz, $J_{H3'\text{-}F}$ = 20.5 Hz, CH, C3′), 60.9 (CH_2, C5′), 58.4 (dd, $J_{H1'\text{-}F}$ = 30.1 Hz, $J_{H1'\text{-}F}$ = 19.2 Hz, CH, H1′), 48.5 (d, $J_{C4'\text{-}F}$ = 5.1 Hz, CH, C4′); ^{19}F NMR (377 MHz, D_2O) δ -110.10 (app. br d, $J_{F\text{-}F}$ = 231.3 Hz), -123.73 – -124.70 (app dt, $J_{F\text{-}F}$ = 230.5 Hz, $J_{F\text{-}H3'/H1'}$ = 14.5 Hz); ^{19}F NMR {^{1}H} (377 MHz, D_2O) δ -110.10 (app. br dd, $J_{F\text{-}F}$ = 232.1 Hz, $J_{F\text{-}H1'\text{-}H3'}$ = 5.4 Hz), -124.21 (d, $J_{F\text{-}F}$ = 231.9 Hz, $J_{F\text{-}H1'/H3'}$ = 14.6 Hz); **β-anomer:** ^{1}H NMR (400 MHz, D_2O) δ 8.14 (d, $J_{H6\text{-}H5}$ = 7.6 Hz, 1H, H6), 6.39 (dd, $J_{H1'\text{-}F}$ = 11.9 Hz, $J_{H1'\text{-}F}$ = 2.3 Hz, 1H, H1′), 6.00 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 4.23 (ddd, $J_{H3'\text{-}F}$ = 18.4 Hz, $J_{H3'\text{-}H4'}$ = 8.6 Hz, $J_{H3'\text{-}F}$ = 6.1 Hz, 1H, H3′), 3.89 (dd, $J_{H5'a\text{-}H5'b}$ = 12.3 Hz, $J_{H5'a\text{-}H4'}$ = 3.8 Hz, 1H, H5′a), 3.84 (dd, $J_{H5'b\text{-}H5'a}$ = 12.3 Hz, $J_{H5'b\text{-}H5'a}$ = 5.1 Hz, 1H, H5′b), 3.40 (app dt, $J_{H4'\text{-}H3'}$ = 8.7 Hz, $J_{H4'\text{-}H5}$ = 4.4 Hz, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 181.0 (C-NH_2, C4), 157.9 (C=O, C2),

142.6 (CH, C6), 123.8 (dd, $J_{C2'\text{-}F}$ = 262.3 Hz, $J_{C2'\text{-}F}$ = 255.6 Hz, C_q, C2′), 96.6 (CH, C5), 70.5 (dd, $J_{C3'\text{-}F}$ = 26.9 Hz, $J_{C3'\text{-}F}$ = 21.4 Hz, CH, C3′), 59.8 (CH_2, C5′), 59.7 – 59.1 (m, CH, C1′), 46.3 (d, $J_{C4'\text{-}F}$ = 6.1 Hz, CH, C4′); ^{19}F NMR (376 MHz, D_2O) δ -115.37 – -116.20 (m), -117.05 – -119.14 (m); NSI HRMS *m/z* found: $(M+H)^+$ 280.0561 $C_9H_{11}F_2N_3O_3S$, requires $(M+H)^+$ 280.0562.

2′-Deoxy-2′-*gem*-difluoro-1′-β-(4′-sulfinyl-D-ribofuranosyl)cytosine **313** †



Nucleoside **313** was prepared according to general procedure K using **156** (20.0 mg, 71.6 μmol, 1.0 equiv.), *m*-CPBA (14.0 mg, 78.8 μmol) and 1/1 (*v*/*v*) H_2O/MeCN solution (0.72 mL). Reaction time = 18 h. Purification to 99% *via* preparative HPLC (Table 17).

Time (minutes)	**%A (H_2O)**	**%B (MeOH)**
0.0	96	4
5.0	96	4
15.0	0	100
18.0	0	100
18.1	96	4

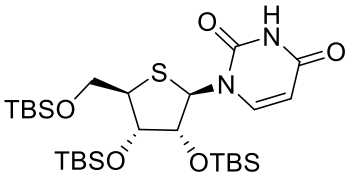
Table 17. Preparative HPLC linear gradient system for purification of **313**. A 250 x 21.2 mm column packed 5 μ particle size with Polaris 5 C18-A was employed to load the sample The flow rate was 20 mL/minute. A solution of **313** in H_2O (100 mg/mL) was prepared and 50 μL injected into the prep HPLC system.

Retention time = 7.7 minutes (3.2 mg, 10.8 μmol, 15%). Purity was determined using general procedure M. R_f 0.41 (1/9, H_2O/MeCN); 4/1 ratio diastereoisomers; **major diastereoisomer:** ^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, $J_{H6\text{-}H5}$ = 7.5 Hz, 1H, H6), 6.05 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 5.11 (app. d, $J_{H1'\text{-}F}$ = 18.7 Hz, 1H, H1′), 4.61 (ddd, $J_{H3'\text{-}F}$ = 18.5 Hz, $J_{H3'\text{-}F}$ = 12.6 Hz, $J_{H3'\text{-}H4'}$ = 7.2 Hz, 1H, H3′), 4.25 (dd, $J_{H5'a\text{-}H5'b}$ = 12.3 Hz, $J_{H5'a\text{-}H5'b}$ = 4.3 Hz, 1H, H5′a), 4.10 (dd, $J_{H5'b\text{-}H5'a}$ = 12.3 Hz, $J_{H5'b\text{-}H4'}$ = 8.8 Hz,

1H, H5′b), 3.22 – 3.02 (m, 1H, H4′); ^{13}C NMR (101 MHz, D_2O) δ 167.0 (C-NH_2, C4), 156.9 (C=O, C2), 146.5 (CH, C6), 129.3 (dd, $J_{C2'\text{-}F}$ = 220.9 Hz, $J_{C2'\text{-}F}$ = 127.9 Hz, C_q, C2′), 97.2 (CH, C5), 84.6 (dd, $J_{C1'\text{-}F}$ = 61.9 Hz, $J_{C1'\text{-}F}$ = 25.0 Hz, CH, C1′), 72.9 (dd, $J_{C3'\text{-}F}$ = 12.5 Hz, $J_{C3'\text{-}F}$ = 8.4 Hz, CH, C3′), 72.8 (CH, C4′), 58.3 (CH_2, C5′); ^{19}F NMR (377 MHz, D_2O) δ -103.0 (app. dt, $J_{F\text{-}F}$ = 241.3 Hz, $J_{F\text{-}H1'/H3'}$ = 18.7 Hz), -110.3 (app. dd, $J_{F\text{-}F}$ = 241.3 Hz, $J_{F\text{-}H3'}$ = 7.4 Hz); NSI HRMS *m/z* found: $(M+H)^+$ 296.0516 $C_9H_{11}F_2N_3O_4S$, requires $(M+H)^+$ 296.0517.

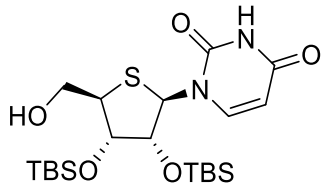
10.10. Towards 4′-substituted 1′-(4′-thio-D-*ribo*) nucleoside analogues

2′,3′,5′-Tri-*O*-*tert*-butyldimethylsilyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **223** †



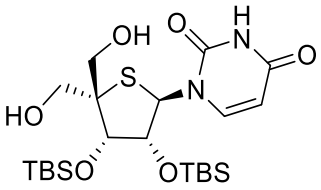
To a solution of **196** (3.05 g, 11.7 mmol, 1.0 equiv.) in DMF (80 mL) was added DMAP (2.14 g, 17.5 mmol, 1.5 equiv.) and 2,6-lutidine (8.1 mL, 70.2 mmol, 6.0 equiv.) and the solution cooled to 0 °C. TBSOTf (16 mL, 70.2 mmol, 6.0 equiv.) was added to the solution dropwise over 10 minutes, and the solution warmed to 40 °C, stirring for 18 h. The reaction was quenched with H_2O (20 mL), and the solvent removed *in vacuo* to obtain an orange oil which was dissolved in EtOAc (300 mL), washed with saturated aqueous $NaHCO_3$ (3 × 200 mL) and brine (200 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 20% EtOAc/hexane) to afford **223** as a white foam (5.15 g, 8.54 mmol, 73%). R_f 0.86 (1/1, EtOAc/hexane); $[\alpha]_D^{23.0}$ +41.9 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (br s, 1H, NH), 8.20 (d, $J_{H6\text{-}H5}$ = 7.9 Hz, 1H, H6), 5.64 (d, $J_{H1'\text{-}H2'}$ = 2.9 Hz, 1H, H1′), 5.60 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 3.99 – 3.88 (m, 2H, H2′ and H3′), 3.80 (dd, $J_{H5'a\text{-}H5'b}$ = 11.2 Hz, $J_{H5'a\text{-}H4'}$ = 2.7 Hz, 1H, H5′a), 3.68 (dd, $J_{H5'b\text{-}H5'a}$ = 11.2 Hz, $J_{H5'b\text{-}H4'}$ = 3.9 Hz, 1H H5′b), 3.39 – 3.23 (m, 1H, H4′), 0.83 (s, 9H, Si-^{t}Bu), 0.79 – 0.77 (m, 18H, 2 × Si-^{t}Bu), 0.09 – -0.15 (m, 18H, 6 × Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8 (C=O, C4), 150.5 (C=O, C2), 142.0 (CH, C6), 101.8 (CH, C5), 76.7 (CH, C2′ or C3′), 73.5 (CH, C2′ or C3′), 65.2 (CH,C1′), 61.9 (CH_2 ,C5′), 52.0 (CH, C4′), 26.0 (Si-^{t}Bu), 26.0 (Si-^{t}Bu), 25.8 (Si-^{t}Bu), 25.7 (Si-^{t}Bu), 25.7 (Si-^{t}Bu), 18.6 (C_q, Si-^{t}Bu), 18.0 (C_q, Si-^{t}Bu), 17.9 (C_q, Si-^{t}Bu), -4.2 (Si-CH_3), -4.6 (Si-CH_3), -4.7 (Si-CH_3), -4.8 (Si-CH_3), -5.3 (Si-CH_3), -5.5 (Si-CH_3); ESI HRMS *m*/*z* found: $(M+H)^+$ 603.3161 $C_{27}H_{54}N_2O_5SSi_3$, requires $(M+H)^+$ 603.3134.

2′,3′-Di-*O*-*tert*-butyldimethylsilyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **224** †



To a suspension of **223** (4.52 g, 7.50 mmol, 1.0 equiv.) in 80% (*v*/*v*) aqueous AcOH solution (36 mL) and THF (50 mL) was added TFA (1.2 mL, 15.0 mmol, 2.0 equiv.) and the solution stirred at rt for 18 h. Anhydrous $NaHCO_3$ was added until pH = 7, and the solvents removed *in vacuo*. The resultant solid was dissolved in EtOAc (400 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 150 mL), brine (150 mL) and H_2O (100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to furnish the crude product as an orange foam which was purified by crystallisation from a minimum of boiling EtOAc (~10 mL) and ice-cold petroleum ether (~60 mL) to afford **224** as white crystalline solid (2.66 g, 5.44 mmol, 73%). R_f 0.50 (1/1, EtOAc/hexane); $[\alpha]_D^{24.2}$ +9.4 (*c* 1.7, CH_2Cl_2); mp 187 – 189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H, N-H), 8.08 (d, $J_{H6\text{-}H5}$ = 7.7 Hz, 1H, H6), 5.76 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 5.70 (d, $J_{H1'\text{-}H2'}$ = 4.6 Hz, 1H, H1′), 4.33 (app. br s, 1H, H2′), 4.06 (dd, $J_{H3'\text{-}H2'}$ = 5.4, $J_{H3'\text{-}H4'}$ = 3.1 Hz, 1H, H3′), 3.96 – 3.89 (m, 1H, H5′a) 3.81 – 3.75 (m, 1H, H5′b), 3.46 (app. dt, $J_{H4'\text{-}H5a/b}$ = 11.6 Hz, $J_{H4'\text{-}H3'}$ = 3.1 Hz, 1H, H4′), 1.01 – 0.85 (m, 18H, 3 × Si-^{t}Bu), 0.13 – 0.14 (m, 12H, 4 × Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7 (C=O, C4), 150.4 (C=O, C2), 142.5 (CH, C6), 102.1 (CH, C5), 78.5 (CH, C2′), 74.6 (CH, C3′), 66.8 (CH, C1′), 61.7 (CH_2, C5′), 52.6 (CH, C4′), 25.8, (Si-^{t}Bu) 25.7 (Si-^{t}Bu), 18.0 (C_q, Si-^{t}Bu), 17.9 (C_q, Si-^{t}Bu), -4.3 (Si-CH_3), -4.6 (Si-CH_3), -4.7 (Si-CH_3), -4.9 (Si-CH_3); ESI HRMS *m*/*z* found: $(M+H)^+$ 489.2285 $C_{21}H_{40}N_2O_5SSi_2$, requires $(M+H)^+$ 489.2269.

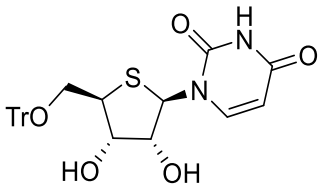
2′,3′-Di-*O*-tert-butyldimethylsilyl-4′-*bis*-hydroxymethyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **228** †



A solution of **224** (1.90 g, 3.89 mmol, 1.0 equiv.) in MeCN (26 mL) was cooled to 0 °C. Dess-Martin periodinane (2.47 g, 5.84 mmol, 1.5 equiv.) was added and the solution stirred at 0 °C for 5 minutes, then heated to 80 °C. After 15 minutes, the solution was cooled to 0 °C, diluted with EtOAc (150 mL) and saturated $Na_2S_2O_3$ solution (30 mL) and the mixture stirred vigorously for 10 minutes. The organic phase was separated and washed with H_2O (3 × 60 mL) and brine (60 mL), dried over anhydrous Na_2SO_4, cooled to 0 °C and filtered through Celite. The solvent was removed *in vacuo* to give the crude product aldehyde as a white foam which was dissolved in 1,4-dioxane (25 mL). 2M NaOH (1.95 mL, 3.89 mmol, 1.00 equiv.) and aqueous HCHO (37% *w/v*) (32.0 mL, 3.89 mmol, 10.0 equiv.) were added and the solution stirred at rt for 3 h. The reaction was quenched *via* addition of glacial AcOH (4 mL), the solvents removed *in vacuo* and the resultant residue suspended in H_2O (200 mL) and successively extracted with 1/9 (*v*/*v*) ^{i}PrOH/$CHCl_3$ solution (3 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a white solid which was suspended in MeOH (22.0 mL), cooled to 0 °C and $NaBH_4$ (0.790 g, 23.3 mmol, 6.00 equiv.) added in portions over 10 minutes. The mixture was warmed to rt and stirred for 2 h. The solvent was removed *in vacuo* to obtain a white solid which was suspended in 1/9 (*v*/*v*) ^{i}PrOH/$CHCl_3$ solution (200 mL) and washed with H_2O (2 × 80 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a white foam, which was purified on silica gel *via* automated flash chromatography (30 – 60% EtOAc/$CHCl_3$) to afford **228** as a white foam (0.515 g, 0.992 mmol, 26% over 3 steps). R_f 0.38 (5/95, MeOH/CH_2Cl_2); $[\alpha]_D^{24.3}$ **–**7.3 (*c* 1.4, MeOH); ^{1}H NMR (400 MHz, MeOD) δ 8.51 (d, $J_{H6\text{-}H5}$ = 8.0 Hz, 1H, H6), 5.99 (d, $J_{H1'\text{-}H2'}$ = 5.4 Hz, 1H, H1′), 5.71 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.48 (dd, $J_{H2'\text{-}H1'}$ = 5.3, $J_{H2'\text{-}H3'}$ 2.9 Hz, 1H, H2′), 4.27 (d, $J_{H3'\text{-}H2'}$ = 3.0 Hz, 1H, H3′), 4.02 (d, J_{gem} = 11.0 Hz, 1H, H5′ or H6′), 3.85 – 3.75 (m, 3H, H5′ or H6′), 0.94 (s, 9H, Si-^{t}Bu), 0.89 (s, 9H, Si-^{t}Bu),

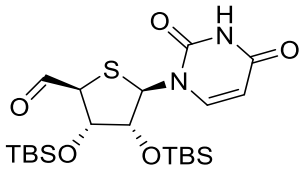
0.17 – 0.06 (m, 12H, 4 × Si-CH_3); ^{13}C NMR (101 MHz, MeOD) δ 164.6 (C=O, C4), 151.4 (C=O, C2), 143.0 (CH, C6), 101.2 (CH, C5), 79.9 (CH, C2′), 75.1 (CH, C3′), 64.5 (CH_2, C5′ or C6′), 63.6 (CH, C1′), 63.2 (CH_2, C5′ or C6′), 25.3 (Si-^{t}Bu), 17.9 (C_q, Si-^{t}Bu), 17.6 (C_q, Si-^{t}Bu), -4.8 (Si-CH_3), -5.4 (Si-CH_3), -5.4 (Si-CH_3), -5.8 (Si-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 519.2372, $C_{22}H_{42}N_2O_6SSi_2$, requires $(M+H)^+$ 519.2375.

5′-*O*-Trityl-1′-β-(4′-thio-D-ribofuranosyl)uracil **321**



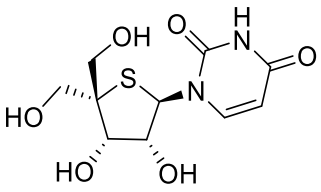
To a solution of **196** (480 mg, 1.84 mmol, 1.0 equiv.) in pyridine (12 mL) was added trityl chloride (1.13 g, 4.06 mmol, 2.2 equiv.) and DMAP (0.630 g, 5.16 mmol, 2.8 equiv.) and the solution warmed to 50 °C and stirred for 18 h. The reaction was cooled to rt, quenched with MeOH (7 mL) and stirred at rt for 30 minutes. The solvent was removed *in vacuo*, and the crude product diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 80 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (50 mL) and the combined organic phases washed with brine (60 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as yellow foam which was purified on silica gel *via* automated flash chromatography (0 – 15% MeOH/ CH_2Cl_2) to afford **321** as a white foam (0.850 g, 1.69 mmol, 92%). R_f 0.75 (15/85, MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 7.48 – 7.42 (m, 6H, Ar-H), 7.39 – 7.22 (m, 9H, Ar-H), 5.97 (d, $J_{H1'\text{-}H2'}$ = 3.7 Hz, 1H, H1′), 5.47 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.29 – 4.13 (ov. m, 2H, H2′ and H3′), 3.61 – 3.51 (m, 2H, H5′a and H4′), 3.51 – 3.40 (m, 1H, H5′b); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5 (C=O, C4), 151.7 (C=O, C2), 143.4 (C_q, Ar-C), 143.4 (C_q, Ar-C),141.7 (C_q, Ar-C), 128.8 (CH, Ar-C), 128.0 (CH, Ar-C), 127.4 (CH, Ar-C), 102.7 (CH, C5), 87.6 (C_q, trityl), 78.9 (CH, C2′ or C3′), 74.0 (CH, C2′ or C3′), 65.3 (CH, C1′), 63.4 (CH_2, C5′), 50.7 (CH, C4′); ESI HRMS *m/z* found: $(M+Na)^+$ 525.1474 $C_{28}H_{26}N_2O_5S$, requires $(M+Na)^+$ 525.1455. NMR data is consistent with literature values.[132]

2′,3′-Di-*O*-*tert*-butyldimethylsilyl-4′-formyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **225** †



A solution of **224** (220 mg, 0.454 mmol, 1.0 equiv.) in MeCN (3.0 mL) was cooled to 0 °C. Dess-Martin periodinane (289 mg, 0.681 mmol, 1.5 equiv.) was added and the solution stirred at 0 °C for 5 minutes then warmed to 80 °C. After 30 minutes, the solution was cooled to 0 °C, a 1/1 (*v*/*v*) EtOAc/saturated aqueous $Na_2S_2O_3$ solution (20 mL) was added, and the mixture stirred vigorously for 10 minutes. The organic phase was separated and washed with saturated aqueous $NaHCO_3$ solution (3 × 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain crude product **225** as a white foam (177 mg, 0.370 mmol, 81%) which was used immediately in the next step without further purification. R_f 0.48 (3/7, EtOAc/$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (d, $J_{H5'\text{-}H4'}$ = 1.7 Hz, 1H, H5′), 9.51 (s, 1H, NH), 7.53 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 5.96 (d, $J_{H1'\text{-}H2'}$ = 6.3 Hz, 1H, H1′), 5.75 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.30 (app. t, $J_{H3'\text{-}H2',\ H3\text{-}H4'}$ = 3.3 Hz, 1H, H3′), 4.12 (dd, $J_{H2'\text{-}H1'}$ = 6.3 Hz, $J_{H2'\text{-}H3'}$ = 3.0 Hz, 1H, H2′), 3.80 (dd, $J_{H4'\text{-}H3'}$ = 3.6 Hz, $J_{H4'\text{-}H5'}$ = 1.6 Hz, 1H, H4′), 0.82, (s, 9H, Si-^{t}Bu), 0.78 (s, 9H, Si-^{t}Bu), 0.02 – -0.03 (m, 12H, 4 × Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.9 (CHO, C5′), 163.1 (C=O, C4), 150.6 (C=O, C2), 141.1 (CH, C6), 103.2 (CH, C5), 78.9 (CH, C2′), 73.9 (CH, C3′), 65.7 (CH, C1′), 57.5 (CH, C4′), 25.7 (Si-^{t}Bu), 25.6 (Si-^{t}Bu), 18.0 (C_q, Si-^{t}Bu), 17.9 (C_q, Si-^{t}Bu), -4.4 (Si-CH_3), -4.6 (Si-CH_3), -4.9 (Si-CH_3), -4.9 (Si-CH_3); ESI HRMS *m*/*z* found: $(M+H)^+$ 487.2135 $C_{21}H_{39}N_2O_5SSi_2$, requires $(M+H)^+$ 487.2118.

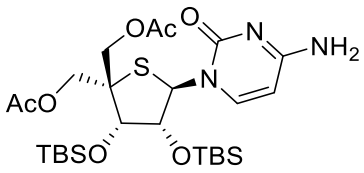
4′-*Bis*-hydroxymethyl-1′-β-(4′-thio-D-ribofuranosyl)uracil **229** †



A solution of **228** (200 mg, 0.385 mmol, 1.0 equiv.) in THF (2.6 mL) was added to a flask wrapped in aluminium foil, and TBAF (92 μL, 0.924 mmol, 2.4 equiv.) added. The solution was stirred at rt for 18 h, the solvent removed *in vacuo* and the crude product diluted in H_2O (30 mL) and washed with EtOAc (30 mL). The aqueous layer was separated and treated with Amberlyst 15(H^+) ion exchange

resin and $CaCO_3$, filtered and freeze-dried to afford **229** as a white foam (60.0 mg, 0.207 mmol, 54%). R_f 0.44 (1/4, MeOH/CH_2Cl_2); $[\alpha]_D^{26}$ +21.1 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 8.05 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 6.06 (d, $J_{H1'\text{-}H2'}$ = 8.2 Hz, 1H, H1′), 5.90 (d, $J_{H5\text{-}H6}$ = 8.1 Hz, 1H, H5), 4.51 (app. dd, $J_{H2'\text{-}H1'}$ = 8.2, $J_{H2'\text{-}H3'}$ = 3.9 Hz, 1H, H2′), 4.18 (d, $J_{H3'\text{-}H2'}$ = 3.9 Hz, 1H, H3′), 3.90 – 3.75 (m, 4H, H5′a, H5′b and H6′a, H6′b); ^{13}C NMR (101 MHz, D_2O) δ 165.9 (C=O, C4), 152.3 (C=O, C2), 142.6 (CH, C6), 102.8 (CH, C5), 77.1 (CH, C2′), 73.6 (CH, C3′), 64.0 (CH, C4′), 62.8 (CH_2, C5′ or C6′), 62.6 (CH_2, C5′ or C6′), 62.1 (CH, C1′); ESI HRMS *m/z* found: $(M+H)^+$ 291.0652 $C_{10}H_{14}N_2O_6S$, requires $(M+H)^+$ 291.0645.

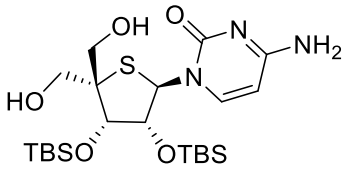
4′-*Bis*-acetoxymethyl-2′,3′-di-*O*-tert-butyldimethylsilyl-1′-β-(4′-thio-D-ribofuranosyl)cytosine **232** †



To a solution of **228** (330 mg, 0.636 mmol, 1.0 equiv.) in pyridine (3.2 mL) was added Ac_2O (0.18 mL, 1.91 mmol, 3.0 equiv.). The solution was stirred at rt for 1.5 h, poured onto 1M aqueous HCl solution (50 mL) and extracted with EtOAc (2 × 40 mL). The organic phase was washed with saturated $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and dried *in vacuo* to obtain the crude product as a colourless syrup which was purified on silica gel *via* automated flash chromatography (0 – 30% EtOAc/$CHCl_3$) to obtain **230** as a colourless glass (338 mg, 0.560 mmol, 88%). Nucleoside **232** was prepared according to general procedure J using **230** (338 mg, 0.560 mmol, 1.0 equiv.), 1,2,4-triazole (387 mg, 5.60 mmol, 10 equiv.), MeCN (5.6 mL) and $POCl_3$ (90 μL, 1.34 mmol, 2.4 equiv.), reaction time = 1.5 h; ii) 1,4-dioxane (5.00 mL) and 25% (*w/v*) aqueous NH_4OH solution (3.00 mL, 33.9 mmol, 60.0 equiv.), reaction time = 18 h; Crude product **232** was purified on silica gel *via* automated flash chromatography (0 – 100% EtOAc/$CHCl_3$) to obtain **232** as a colourless syrup (180 mg, 0.299 mmol, 53%). R_f 0.77 (1/4, MeOH/CH_2Cl_2); $[\alpha]_D^{23.1}$ +40.1 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (app. br s, 1H, H6), 5.94 – 5.70 (m, 2H, H1′ and H5), 4.91 (d, J_{gem}= 11.4 Hz, 1H, H5′a/b or H6′a/b), 4.48 (d, J_{gem} = 12.0 Hz, 1H, H5′a/b or H6′a/b), 4.38 – 4.24 (m, 2H, H6′b and H2′), 4.21 (d, J_{gem} = 12.0 Hz, 1H, H5′a/b or H6′a/b), 4.06 (d, $J_{H3'\text{-}H2'}$ = 3.2 Hz, 1H, H3′), 2.15 (s, 3H, Ac), 2.07 (s, 3H, Ac), 0.92 (s, 9H, Si-^{t}Bu), 0.90 (s, 9H, Si-^{t}Bu) 0.24 (s, 3H, Si-

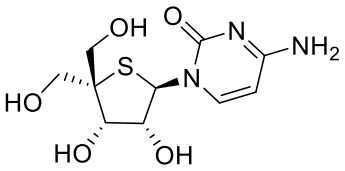
CH_3), 0.12 (s, 3H, Si-CH_3), 0.05 (s, 3H, Si-CH_3), 0.00 (s, 3H, Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4 (C=O, Ac), 170.0 (C=O, Ac), 165.6 (C=O, C4), 156.1 (C=O, C2), 142.9 (CH, C6), 94.2 (CH, C5), 80.5 (CH, C2′), 74.5 (CH, C3′), 67.4 (CH_2, C5′ or C6′), 66.7 (CH, C1′), 64.9 (CH_2, C5′ or C6′), 58.7 (C_q, C4′), 31.9 (Si-^{t}Bu), 29.7 (Si-^{t}Bu), 29.7 (Si-^{t}Bu), 29.4 (Si-^{t}Bu), 26.0 (Si-^{t}Bu), 25.9 (Si-^{t}Bu), 22.7 (Si-^{t}Bu), 20.9 (Ac-CH_3), 20.9 (Ac-CH_3), 18.1 (C_q, Si-^{t}Bu), 18.1 (C_q, Si-^{t}Bu), 14.1 (Si-^{t}Bu), -3.8 (Si-CH_3), -4.2 (Si-CH_3), -5.0 (Si-CH_3), -5.4 (Si-CH_3); ESI HRMS *m/z* found: $(M+Na)^+$ 624.2588 $C_{26}H_{47}N_3O_7SSi_2$, requires $(M+Na)^+$ 602.2565.

2′,3′-Di-*O*-tert-butyldimethylsilyl-4′-*bis*-hydroxymethyl-1′-β-(4′-thio-D-ribofuranosyl)cytosine **234** †



To a solution of **232** (180 mg, 0.299 mmol, 1.0 equiv.) in MeOH (2.0 mL) was added 7M NH_3/MeOH solution (0.21 mL, 1.50 mmol, 5.0 equiv.) and the solution stirred at rt. After 20 h, the solvent was removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 10% MeOH/CH_2Cl_2) to obtain **234** as a beige foam (139 mg, 0.268 mmol, 90%). R_f 0.17 (5/95, MeOH/CH_2Cl_2); $[\alpha]_D^{22.8}$ +16.4 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD) δ 8.65 (d, $J_{H6\text{-}H5}$ = 7.2 Hz, 1H, H6), 5.99 (d, $J_{H1'\text{-}H2'}$ = 3.6 Hz, 1H, H1′), 5.91 (d, $J_{H5\text{-}H6}$ = 7.5 Hz, 1H, H5), 4.59 (br s, 1H, OH), 4.40 – 4.39 (app. br t, $J_{H2'\text{-}H1'/H3'}$ = 3.5 Hz, 1H, H2′), 4.29 (d, $J_{H3'\text{-}H2'}$ = 3.3 Hz, 1H, H3′), 4.14 (d, J_{gem} = 11.1 Hz, 1H, H5′a/b or H6′a/b), 3.92 (d, J_{gem} = 11.6 Hz, 1H, H5′a/b or H6′a/b), 3.91 – 3.72 (m, 1H, H6′b and H5′b), 0.95 (s, 9H, Si-^{t}Bu), 0.93 (s, 9H, Si-^{t}Bu) 0.19 (s, 3H, Si-CH_3), 0.13 (s, 3H, Si-CH_3), 0.12 (s, 3H, Si-CH_3), 0.10 (s, 3H, Si-CH_3); ^{13}C NMR (101 MHz, MeOD) δ 166.0 (C=O, C4), 157.4 (C=O, C2), 143.6 (CH, C6), 94.3 (CH, C5), 80.8 (CH, C2′), 74.5 (CH, C3′), 64.8 (CH_2, C5′ or C6′), 63.7 (CH, C1′), 62.8 (CH_2, C5′ or C6′), 54.7 (C_q, C4′), 30.7 (Si-^{t}Bu), 29.3 (Si-^{t}Bu), 28.1 (Si-^{t}Bu), 25.3 (Si-^{t}Bu), 25.2 (Si-^{t}Bu), 17.8 (C_q, Si-^{t}Bu), 17.7 (C_q, Si-^{t}Bu), -4.9 (Si-CH_3), -5.3 (Si-CH_3), -5.8 (Si-CH_3), -6.1 (Si-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 518.2562 $C_{22}H_{43}N_3O_5SSi_2$, requires $(M+H)^+$ 518.2540.

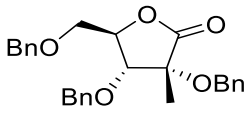
4′-*Bis*-hydroxymethyl-1′-β-(4′-thio-D-ribofuranosyl)cytosine **219** †



To a solution of **234** (139 mg, 0.268 mmol, 1.0 equiv.) in THF (1.8 mL) was added TBAF (0.80 mL, 0.804 mmol, 3.0 equiv.) and the solution was stirred at rt for 22 h. The solvent was removed *in vacuo*, the crude product dissolved in H_2O and amberlyst 15(H^+) ion exchange resin and $CaCO_3$ were added and the mixture stirred for 3 h. The mixture was filtered and the mother liquor freeze dried to obtain **219** as an unknown TBAF salt (177 mg crude) which was dissolved in pyridine (0.30 mL), Ac_2O (0.14 mL, 1.51 mmol, 10.0 equiv.) added and the solution stirred at rt for 14 h, diluted in EtOAc (10 mL) and washed with saturated aqueous $NaHCO_3$ solution (10 mL). The organic layer was separated and the aqueous extracted with EtOAc (5 mL). The combined organic phases were washed with brine (10 mL), dried over $MgSO_4$, filtered and the solvents removed *in vacuo* to obtain the crude product **235** as a yellow syrup (85 mg, ~0.151 mmol, 56%) which was dissolved in MeOH (0.3 mL) and 7M NH_3/MeOH (0.32 mL, 2.27 mmol, 15.0 equiv.) and the solution stirred at rt for 24 h. The solvents were removed *in vacuo* and the crude product purified on octadecyl modified silica gel *via* automated flash chromatography (0/100, 10/100, 100/0 MeCN/H_2O) to obtain **219** as a white solid (32.3 mg, 0.112 mmol, 42% over 3 steps). R_f 0.04 (1/4, MeOH/$CHCl_3$); $[\alpha]_D^{22.7}$ +7.9 (*c* 0.32, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 8.06 (d, $J_{H6\text{-}H5}$ = 7.6 Hz, 1H, H6), 6.14 (ov. d, $J_{H1'\text{-}H2'}$ = 8.7 Hz, 1H, H1′), 6.12 (ov. d, $J_{H5\text{-}H6}$ = 8.0 Hz, 1H, H5), 4.57 (dd, $J_{H2'\text{-}H1'}$ = 8.0 Hz, $J_{H2'\text{-}H3'}$ = 4.0 Hz, 1H, H2′), 4.25 (d, $J_{H3'\text{-}H2'}$ = 4.0 Hz, 1H, H3′), 3.96 (d, J_{gem} = 11.6 Hz, 1H, H5′a or H6′a), 3.89 (d, J_{gem} = 12.0 Hz, 1H, H5′a or H6′a), 3.84 (d, J_{gem} = 12.0 Hz, 1H, H5′b or H6′b), 3.82 (d, J_{gem} = 11.6 Hz, 1H, H5′b or H6′b); ^{13}C NMR (101 MHz, D_2O) δ 165.9 (C-NH_2, C4), 142.6 (CH, C6), 97.0 (CH, C5), 77.4 (CH, C2′), 73.9 (CH, C3′), 64.2 (CH_2, C5′ or C6′), 63.7 (CH, C1′), 62.4 (C_q, C4′), 62.2 (CH_2, C5′ or C6′); NSI HRMS *m*/*z* found: $(M\text{-}H)^-$ 288.0660 $C_{10}H_{15}N_3O_5S$,requires $(M\text{-}H)^-$ 288.0660.

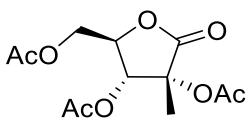
10.11. Towards 2′-*C*-Methyl-1′-(4′-thio-D-*ribo*) nucleoside analogues

2,3,5-Tri-*O*-benzyl-2-*C*-methyl-1,4-D-ribonolactone **184**



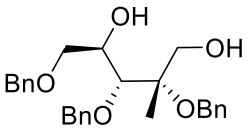
2-*C*-methyl-D-ribo-1,4-lactone (1.50 g, 9.25 mmol, 1.0 equiv.) was dissolved in DMF (37 mL) and the solution cooled to –10 °C. A 60% dispersion of NaH (370 mg, 9.25 mmol, 1.0 equiv.) was added and the solution stirred for 30 minutes. BnBr (1.7 mL, 1.39 mmol, 1.5 equiv.) was added and the solution stirred for 1 h. Repeat additions of NaH (370 mg, 1.0 equiv.) and BnBr (1.7 mL, 1.39 mmol, 1.5 equiv.) were made (3 times each) after a further 30 minutes and 1 h respectively) and the solution stirred vigorously at –10 °C for 4 days. The reaction was quenched with a few drops of MeOH and the solvent reduced *in vacuo* to <10 mL. The crude product was diluted with EtOAc (200 mL) and washed with H_2O (200 mL) and brine (200 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* and the crude product purified on silica gel *via* automated flash chromatography (0 – 100% Et_2O/petroleum ether) to obtain **284** as a colourless oil (1.52 g, 3.52 mmol, 38%). R_f 0.64 (1/1, Et_2O/petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.23 (m, 12H, Ar-H), 4.79 (d, J_{gem} = 11.7 Hz, 1H, Bn-CH_2), 4.63 – 4.49 (ov. m, 6H, Bn-CH_2, and H4), 4.04 (d, $J_{H3\text{-}H4}$ = 7.6 Hz, 1H, H3), 3.79 (dd, $J_{H5a\text{-}H5b}$ = 11.5 Hz, $J_{H5a\text{-}H4}$ = 2.4 Hz, 1H, H5a), 3.61 (dd, $J_{H5b\text{-}H5a}$ = 11.5 Hz, $J_{H5b\text{-}H4}$ = 3.5 Hz, 1H, H5b), 1.52 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4 (C=O lactone, C1), 137.9 (C_q, CH, Ar-C), 137.5 (C_q, CH, Ar-C), 137.3 (C_q, CH, Ar-C), 129.0 (CH, Ar-C), 128.8 (CH, Ar-C), 128.51 (CH, Ar-C), 128.48 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 128.20 (CH, Ar-C), 128.17 (CH, Ar-C), 127.9 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 80.4 (CH, C4), 80.1 (CH, C3), 73.6 (C_q, C2), 67.4 (CH_2, Bn), 67.4 (CH_2, Bn), 73.3 (CH_2, C5), 33.6 (CH_2, Bn), 19.9 (2-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 433.2015 $C_{27}H_{28}O_5$, requires $(M+H)^+$ 433.2010. NMR data is consistent with literature values.[177]

2,3,5-Tri-*O*-acetyl-2-*C*-methyl-1,4-D-ribonolactone **282** *



2-*C*-methyl-D-ribo-1,4-lactone (0.500 g, 3.08 mmol, 1.0 equiv.) and DMAP (38.0 mg, 0.308 mmol, 0.1 equiv.) was dissolved in acetone (15 mL) and Ac_2O (1.5 mL, 15.4 mmol, 5.0 equiv.) and the solution cooled to <5 °C. Et_3N (2.2 mL, 15.4 mmol, 5.0 equiv.) was added dropwise and the solution allowed to warm to rt and stirred vigorously for 18 h. The solution was reduced *in vacuo* the residue diluted with EtOAc (80 mL) and washed with saturated aqueous $NaHCO_3$ solution (3 × 80 mL) and brine (80 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product **282** as a colourless syrup (0.950 g, 2.83 mmol, 92%) which was used without further purification. R_f 0.73 (1/1, EtOAc/petroleum ether); $[\alpha]_D^{22.8}$ +162.1 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 (d, $J_{H3\text{-}H4}$ = 5.7 Hz, 1H, H3), 4.76 – 4.67 (m, 1H, H4), 4.48 (dd, $J_{H5a\text{-}H5b}$ = 12.5 Hz, $J_{H5a\text{-}H4}$ = 2.8 Hz, 1H, H5a), 4.24 (dd, $J_{H5b\text{-}H5a}$ = 12.5 Hz, $J_{H5b\text{-}H4}$ = 3.8 Hz, 1H, H5b), 2.13 (s, 3H, Ac-CH_3), 2.11 (s, 3H, Ac-CH_3), 2.10 (s, 3H Ac-CH_3), 1.69 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6 (C=O lactone, C1), 170.1 (C=O, Ac), 170.0 (C=O, Ac), 169.5 (C=O, Ac), 79.0 (CH, C4), 74.8 (C_q, C2), 71.8 (CH, C3), 62.6 (CH_2, C5), 23.2 (2-CH_3), 20.6 (Ac CH_3), 20.3 (Ac CH_3), 19.9 (Ac CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 247.0814 $C_{10}H_{13}O_7$, requires $(M+H)^+$ 247.0813. This compound was reported in the literature but not characterised.[178]

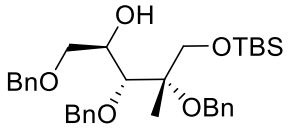
(2*S*,3*R*,4*R*)-2,3,5-Tri-*O*-benzyl-2-*C*-methyl-1,4-pentanediol **292** †



A solution of **284** (2.55 g, 5.84 mmol, 1.0 equiv.) in MeOH (40 mL) was cooled to 0 °C and $NaBH_4$ (490 mg, 12.9 mmol, 2.2 equiv.) added in small portions over a period of 30 minutes. The reaction was then stirred for 1 h at rt, and the solvent removed *in vacuo*. The crude product residue was dissolved in EtOAc (250mL), washed with water (2 × 200 mL) and brine (200 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to obtain the crude product as a colourless oil which was purified on silica gel *via* automated flash chromatography (0 – 100% Et_2O/petroleum ether) to obtain **292** as a colourless oil (2.48 g, 5.67 mmol, 96%). R_f 0.20 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.3}$ +19.3 (*c* 2.6, acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.19 (m, 15H, Ar-H), 4.70 (d, J_{gem} = 11.2 Hz, 1H, Bn-CH_2), 4.63 (ov. d, J_{gem} = 11.2 Hz, 1H, Bn-CH_2), 4.62 (ov. d, J_{gem} = 12.0 Hz, 1H, Bn-CH_2),

4.58 (d, J_{gem} = 10.8 Hz, 1H, Bn-CH_2), 4.54 (d, J_{gem} = 12.0 Hz, 1H, Bn-CH_2), 4.50 (d, J_{gem} = 10.8 Hz, 1H, Bn-CH_2), 4.09 (app. dt, $J_{H4\text{-}H3/H5a}$ = 8.0 Hz, $J_{H4\text{-}H5b}$ = 3.6 Hz, 1H, H4), 3.84 (d, $J_{H3\text{-}H4}$ = 8.2 Hz, 1H, H3), 3.79 – 3.62 (m, 4H, H1a/b and H5a/b), 1.34 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2 (C_q, Ar-C), 138.1 (C_q, Ar-C), 137.5 (C_q, Ar-C), 128.7 (CH, Ar-C), 128.4 (CH, Ar-C), 128.1 (CH, Ar-C), 128.0 (CH, Ar-C), 127.9 (CH, Ar-C), 127.7 (CH, Ar-C), 127.6 (CH, Ar-C), 82.2 (C_q, C2), 77.7 (CH, C3), 74.6 (CH_2, Bn), 73.7 (CH_2, Bn), 71.5 (CH_2, C1), 71.3 (CH, C4), 64.4 (CH_2, Bn), 63.5 (CH_2, C5), 15.3 (2-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 437.2341 $C_{27}H_{32}O_5$, requires $(M+H)^+$ 437.2323.

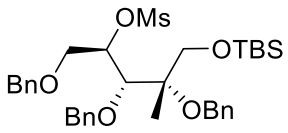
(2*S*,3*R*,4*R*)-2,3,5-Tri-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-*C*-methyl-pentane-4-ol **294** †



To a solution of **285** (0.870 g, 1.99 mmol, 1.0 equiv.) in MeCN (6.6 mL) was added 2,6-lutidine (0.23 mL, 1.99 mmol, 1.0 equiv.) and the solution cooled to 0 °C. TBSOTf (0.46 mL, 1.99 mmol, 1.0 equiv.) was added dropwise and the solution stirred vigorously at 0 °C for 1.5 h. The reaction was quenched *via* addition of saturated aqueous $NaHCO_3$ solution until pH = 7, diluted in EtOAc (100 mL) and washed with H_2O (100 mL). The aqueous layer was extracted with EtOAc and the combined organic layers washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a colourless oil which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **294** as a colourless oil (0.737 g, 1.34 mmol, 67%). R_f 0.77 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ +12.0 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.14 (m, 15H, Ar-H), 4.62 – 4.43 (ov. m, 6H, 3 × Bn-CH_2), 4.22 (s, 1H, 4-OH), 4.05 – 3.98 (m, 1H, H4), 3.86 (d, $J_{H3\text{-}H4}$ = 8.4 Hz, 1H, H3), 3.73 (d, $J_{H1a\text{-}H1b}$ = 11.2 Hz, 1H, H1a), 3.68 – 3.62 (m, 3H, H5a/b and H1b), 1.23 (s, 3H, 2-CH_3), 0.88 – 0.85 (m, 9H, Si-^{t}Bu), -0.00 (s, 3H, Si-CH_3), -0.02 (s, 3H, Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6 (CH, Ar-C), 138.4 (CH, Ar-C), 138.0 (CH, Ar-C), 128.5 (CH, Ar-C), 128.3 (CH, Ar-C), 128.3 (CH, Ar-C), 127.8 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 127.5 (CH, Ar-C), 127.4 (CH, Ar-C), 127.3 (CH, Ar-C), 82.2 (C_q, C2), 77.1 (CH, C3), 74.2 (CH_2, Bn), 73.5 (CH_2, Bn), 71.6 (CH_2, C1), 71.5 (CH, C4), 64.6 (CH_2 Bn), 63.9 (CH_2, C5), 26.0 (Si-^{t}Bu), 25.9 (Si-^{t}Bu), 25.6 (Si-^{t}Bu), 15.2 (2-CH_3), -3.6 (C_q, Si-^{t}Bu) -

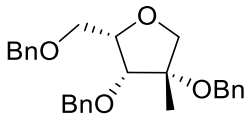
5.4 (Si-CH_3), -5.6 (Si-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 551.3203 $C_{33}H_{46}O_5Si$, requires $(M+H)^+$ 551.3188.

(2*S*,3*R*,4*R*)-2,3,5-Tri-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-4-*O*-mesyl-2-*C*-methyl-pentane **296** †



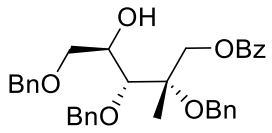
A solution of **294** (120 mg, 0.221 mmol, 1.0 equiv.) in pyridine (1.5 mL) was cooled to 0 °C. MsCl (30.0 μL, 0.332 mmol, 1.5 equiv.) was added dropwise and the solutions stirred for a further 5 minutes before removing the ice-bath and warming to rt, stirring vigorously. After 3 h, the solution was poured onto 1M aqueous HCl solution (20 mL) and extracted with EtOAc (25 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ solution (25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain **296** as a yellow oil (126 mg, 0.200 mmol, 91%) which was used in the next step without further purification. R_f 0.45 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.3}$ 15.7 (*c* 1.9, acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.26 (m, 12H, Ar-H), 5.34 (ddd, $J_{H4\text{-}H5a}$ = 8.7 Hz, $J_{H4\text{-}H5b}$ = 2.2 Hz, $J_{H4\text{-}H3}$ = 1.3 Hz, 1H, H4), 4.79 (d, J_{gem} = 11.2 Hz, 1H, Bn-CH_2), 4.63 – 4.48 (m, 2H, Bn-CH_2), 4.43 (d, J_{gem} = 11.8 Hz, 1H, Bn-CH_2), 4.37 (d, J_{gem} = 11.8 Hz, 1H, Bn-CH_2), 4.02 (d, $J_{H3\text{-}H4}$ = 1.3 Hz, 1H, H3), 3.89 (dd, $J_{H5b\text{-}H5a}$ = 11.8 Hz, $J_{H5b\text{-}H4}$ = 2.2 Hz, 1H, H5b), 3.77 (dd, $J_{H5a\text{-}H5b}$ = 11.8 Hz, $J_{H5a\text{-}H4}$ = 8.7 Hz, 1H, H5a), 3.66 (d, $J_{H1a\text{-}H1b}$ = 10.9 Hz, 1H, H1a), 3.59 (d, $J_{H1b\text{-}H1a}$ = 10.8 Hz, 1H, H1b), 2.92 (s, 3H, Ms-CH_3), 1.22 (s, 3H, 2-CH_3), 0.90 (s, 9H, Si-^{t}Bu), 0.01 (s, 3H, Si-CH_3), 0.00 (s, 3H, Si-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9 (C_q, Ar-C), 138.0 (C_q, Ar-C), 137.9 (C_q, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C) , 128.3 (CH, Ar-C), 128.3 (CH, Ar-C), 128.3 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 127.7 (CH, Ar-C), 127.5 (CH, Ar-C), 127.3 (CH, Ar-C), 127.3 (CH, Ar-C), 84.4 (CH, C4), 82.3 (CH, C3), 79.5 (C_q, C2), 74.7 (CH_2, Bn), 73.2 (CH_2, Bn), 70.1 (CH_2, C5), 64.8 (CH_2, Bn), 64.8 (CH_2, C1), 38.5 (Ms-CH_3), 25.9 (Si-^{t}Bu), 18.2 (C_q, Si-^{t}Bu) 16.6 (2-CH_3), -5.5 (Si-CH_3), -5.6 (Si-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 629.2949 $C_{34}H_{48}O_7SSi$, requires $(M+H)^+$ 629.2963.

(2*S*,3*R*,4*S*)- 2,3,5-Tri-*O*-benzyl-2-*C*-methyl-1-(4-oxo)cyclopentane **305** †



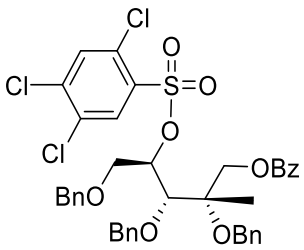
To a solution of **296** (120 mg, 0.191 mmol, 1.0 equiv.) in 2-butanone (0.80 mL) was added LiBr (83.0 mg, 0.954 mmol, 5.0 equiv.) and the solution stirred at 80 °C. After 2 h, the solution was cooled to rt and poured onto H_2O (20 mL) and extracted with EtOAc (25 mL). The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product which was purified on silica gel *via* automated flash chromatography (0 – 100% Et_2O/petroleum ether) to obtain **305** as a colourless syrup (20.0 mg, 40.0 μmol, 21%). R_f 0.72 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ +22.3 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.07 (m, 15H, Ar-H), 4.80 (d, J_{gem} = 11.7 Hz, 1H, Bn-CH_2), 4.63 (d, J_{gem} = 9.7 Hz, 1H, Bn-CH_2), 4.61 – 4.56 (m, 2H, Bn-CH_2), 4.53 (d, J_{gem} = 11.5 Hz, 1H, Bn-CH_2), 4.50 (d, J_{gem} = 11.9 Hz, 1H, Bn-CH_2), 4.27 (app dt, $J_{H4\text{-}H5a/b}$ = 6.8 Hz, $J_{H4\text{-}H3}$ = 5.1 Hz, 1H, H4), 4.12 (d, $J_{H1a\text{-}H1b}$ = 8.6 Hz, 1H, H1a), 3.81 (d, $J_{H3\text{-}H4}$ = 5.2 Hz, 1H, H3), 3.79 – 3.72 (m, 2H, H5a and H5b), 3.62 (d, $J_{H1b\text{-}H1a}$ = 8.6 Hz, 1H, H1b), 1.43 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1 (C_q, Ar-C), 138.4 (C_q, Ar-C), 138.3 (C_q, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 128.3 (CH, Ar-C), 128.1 (CH, Ar-C), 127.6 (CH, Ar-C), 127.9 (CH, Ar-C), 127.8 (CH, Ar-C), 127.3 (CH, Ar-C), 127.6 (CH, Ar-C), 127.3 (CH, Ar-C), 127.3 (CH, Ar-C), 84.0 (CH, C3), 82.8 (CH, C4), 79.9 (C_q, C2), 74.3 (CH_2, C1), 73.9 (CH_2, Bn), 73.5 (CH_2, Bn), 69.6 (CH_2, C5), 66.8 (CH_2, Bn), 21.6 (2-CH_3); ESI HRMS *m*/*z* found: $(M+H)^+$ 419.2206 $C_{27}H_{30}O_4$, requires $(M+H)^+$ 419.2217.

(2*S*,3*R*,4*R*)-1-*O*-Benzoyl-2,3,5-tri-*O*-benzyl-2-*C*-methyl-pentan-4-ol **297** †



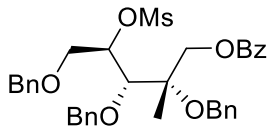
A solution of **285** (0.921 g, 2.11 mmol, 1.0 equiv.) in pyridine (11 mL) was cooled to <5 °C. BzCl (0.27 mL, 2.32 mmol, 1.1 equiv.) was added and the solution stirred at rt for 1 h. The reaction was quenched *via* addition of H_2O (10 mL), the solvent removed *in vacuo* and the residue partitioned between EtOAc (100 mL) and saturated aqueous $NaHCO_3$ solution (100 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (2 × 100 mL) and brine (100mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow oil which was purified on silica gel *via* automated flash chromatography (0 – 50% Et_2O/petroleum ether) to obtain **297** as a colourless oil (0.670 g, 1.24 mmol, 59%). R_f 0.51 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ **–**37.6 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.05 (m, 2H, Ar-H), 7.66 – 7.56 (m, 1H, Ar-H), 7.51 – 7.42 (m, 2H, Ar-H), 7.40 – 7.15 (m, 15H, Ar-H), 4.77 (d, J_{gem} = 12.4 Hz, 1H, Bn-CH_2), 4.72 – 4.53 (m, 6H, 2 × Bn-CH_2, H5a and H5b), 4.30 (d, J_{gem} = 12.4 Hz, 1H, Bn-CH_2), 4.17 – 4.12 (m, 1H, H4), 4.04 (s, 1H, 4-OH), 4.00 (d, $J_{H3\text{-}H4}$ = 8.0 Hz, 1H, H3), 3.74 (app. d, $J_{H1a\text{-}H1b}$ = 3.2 Hz, 1H, H1a and H1b), 1.45 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1 (C=O, Bz), 138.2 (C_q, Ar-C), 137.9 (C_q, Ar-C), 137.4 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.9 (CH, Ar-C), 129.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.4 (CH, Ar-C), 128.0 (CH, Ar-C), 127.9 (CH, Ar-C), 127.8 (CH, Ar-C), 127.8 (CH, Ar-C), 127.6 (CH, Ar-C), 80.8 (C_q, C2), 77.8 (CH, C3), 74.6 (CH_2, Bn), 73.7 (CH_2, Bn), 71.5 (CH, C4), 71.4 (CH_2, C1), 64.8 (CH_2, C5), 64.1 (CH_2, Bn), 15.4 (2-CH_3); ESI HRMS *m*/*z* found: $(M+H)^+$ 541.2606 $C_{34}H_{36}O_6$, requires $(M+H)^+$ 541.2585.

(2*S*,3*R*,4*R*)-1-*O*-Benzoyl-2,3,5-tri-*O*-benzyl-2-*C*-methyl-4-*O*-(2,4,5-trichlorobenzenesulfonyl)-pentane **298** †



To a solution of **297** (140 mg, 0.259 mmol, 1.0 equiv.) in MeCN (0.68 mL) was added 2,4,5-trichlorobenzenesulfonyl chloride (80.0 g, 0.285 mmol, 1.1 equiv.) and *N*-methyl imidazole (20.0 μL, 0.285 mmol, 1.1 equiv.). The resultant suspension was stirred at rt for 2 h, poured onto H_2O (30 mL) and extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with H_2O (15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product as a colourless oil which was purified on silica gel *via* automated flash chromatography (0 – 25% Et_2O/petroleum ether) to obtain **298** as a colourless oil (116 mg, 0.212 mmol, 82%). R_f 0.24 (1/4, Et_2O/petroleum ether); $[\alpha]_D^{22.8}$ +22.0 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.91 (m, 3H, Ar-H), 7.58 (t, J_{vic} = 7.4 Hz, 1H, Ar-H), 7.49 – 7.38 (m, 3H, Ar-H), 7.38 – 7.12 (m, 16H, Ar-H), 7.11 – 6.95 (m, 2H, Ar-C), 5.37 (d, $J_{H3\text{-}H4}$ = 7.7 Hz, 1H, H3), 4.91 (d, $J_{H1a\text{-}H1b}$ = 11.2 Hz, 1H, H1a), 4.63 (d, $J_{H1b\text{-}H1a}$ = 11.2 Hz, 1H, H1b), 4.60 – 4.49 (m, 3H, Bn-CH_2), 4.30 – 4.18 (m, 4H, Bn-CH_2 and H4), 3.89 (dd, $J_{H5a\text{-}H5b}$ = 11.9 Hz, $J_{H5a\text{-}H4}$ = 1.8 Hz, 1H, H5a), 3.79 (dd, $J_{H5b\text{-}H5a}$ = 11.9 Hz, $J_{H5b\text{-}H4}$ = 8.3 Hz, 1H, H5b), 1.30 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0 (C=O, Bz), 138.4 (C_q, Ar-C), 138.2 (C_q, Ar-C), 137.4 (C_q, Ar-C), 137.2 (C_q, Ar-C), 133.2 (C_q, Ar-C), 132.8 (CH, Ar-C), 132.4 (CH, Ar-C), 131.6 (CH, Ar-C), 131.4 (CH, Ar-C), 129.7 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.3 (CH, Ar-C), 128.3 (CH, Ar-C), 128.1 (CH, Ar-C), 127.8 (C-Cl, Ar-C), 127.5 (C-Cl, Ar-C), 127.2 (C-Cl, Ar-C), 86.1 (CH, C3), 81.4 (CH, C4), 78.1 (C_q, C2), 74.5 (CH_2, C1), 73.1 (CH_2, Bn), 70.0 (CH_2, C5), 64.7 (CH_2, Bn), 64.6 (CH_2, Bn), 16.9 (2-CH_3); ESI HRMS *m*/*z* found: $(M+Na)^+$ 805.1203 $C_{40}H_{37}{}^{35}Cl_3O_8S$, requires $(M+Na)^+$ 805.1172.

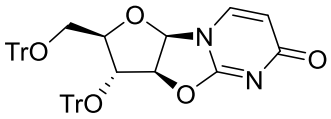
(2*S*,3*R*,4*R*)-1-*O*-Benzoyl-2,3,5-tri-*O*-benzyl-4-*O*-mesyl-2-*C*-methyl- -pentane **299** †



A solution of **297** (160 mg, 0.296 mmol, 1.0 equiv.) in THF (2.3 mL) was cooled to 0 °C. MsCl (90.0 μL, 1.18 mmol, 4.0 equiv.) and Et_3N (0.33 mL, 2.36 mmol, 8.0 equiv.) were added and the solution stirred at rt for 30 minutes. The reaction was quenched *via* addition of saturated aqueous $NaHCO_3$ until pH = 7, and the aqueous solution extracted with EtOAc (2 × 40 mL). The combined organic phases were washed with brine (80 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to obtain the crude product **299** as a yellow oil (201 mg, 0.325 mmol, 83%) which was used without further purification. R_f 0.60 (1/1, Et_2O/petroleum ether); $[\alpha]_D^{21.7}$ +6.0 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.91 (m, 2H, Ar-H), 7.62 – 7.52 (m, 1H, Ar-H), 7.49 – 7.37 (m, 2H, Ar-H), 7.37 – 7.10 (m, 15H, Ar-H), 5.41 (ddd, $J_{H4\text{-}H5a}$ = 8.3 Hz, $J_{H4\text{-}H5b}$ 2.5 Hz, $J_{H4\text{-}H3}$ 1.5 Hz, 1H, H4), 4.85 (d, J_{gem} = 11.1 Hz, 1H, Bn-CH_2), 4.62 – 4.54 (m, 4H, Bn-CH_2), 4.47 (s, 2H, H1a and H1b), 4.30 (d, J_{gem} = 12.2 Hz, 1H, Bn-CH_2), 4.11 (d, $J_{H3\text{-}H4}$ = 1.4 Hz, 1H, H3), 3.91 (dd, $J_{H5b\text{-}H4}$ = 11.8 Hz, $J_{H5b\text{-}H4}$ = 2.6 Hz, 1H, H5b), 3.85 (dd, $J_{H5a\text{-}H5b}$ = 11.8 Hz, $J_{H5a\text{-}H4}$ = 8.3 Hz, 1H, H5a), 2.90 (s, 3H, Ms-CH_3), 1.34 (s, 3H, 2-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9 (C=O, Bz), 138.2 (C_q, Ar-C), 137.7 (C_q, Ar-C), 137.1 (C_q, Ar-C), 133.2 (C_q, Ar-C), 129.8 (CH, Ar-C), 129.6 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.5 (CH, Ar-C), 128.4 (CH, Ar-C), 128.3 (CH, Ar-C), 128.1 (CH, Ar-C), 127.8 (CH, Ar-C), 127.8 (CH, Ar-C), 127.7 (CH, Ar-C), 127.5 (CH, Ar-C), 83.6 (CH, C4), 81.3 (CH, C3), 78.3 (C_q, C2), 74.4 (CH_2, Bn), 73.3 (CH_2, C1), 70.0 (CH_2, C5), 64.8 (CH_2, Bn), 64.7 (CH_2, Bn), 38.3 (Ms-CH_3), 16.7 (2-CH_3); ESI HRMS *m/z* found: $(M+H)^+$ 619.2412 $C_{35}H_{38}O_8S$, requires $(M+H)^+$ 619.2360.

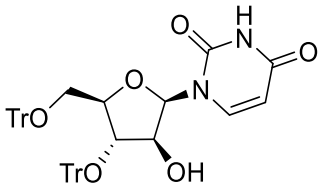
10.12. Towards 2-Deoxy-2(*R*)-fluoro-1′-(4′-thio-D-*ribo*) nucleoside analogues

3′,5′-*O*-Trityl-2′,2-anhydrouridine **215**



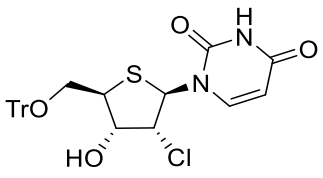
To a suspension of **206** (2.00 g, 8.26 mmol, 1.0 equiv.) in pyridine (55 mL) was added trityl chloride (6.90 g, 24.8 mmol, 3.0 equiv.) and DMAP (2.20 g, 18.0 mmol, 2.0 equiv.) and the mixture heated to 100 °C, stirring vigorously for 48 h. The reaction mixture was diluted in EtOAc (250 mL) and washed with saturated aqueous $NaHCO_3$ solution (3 × 200 mL) and brine (200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a brown foam which was purified on silica gel *via* automated flash chromatography (10 – 100% EtOAc/petroleum ether) to obtain **215** as a yellow foam (1.37 g, 1.93 mmol, 23%). R_f 0.46 (1/2, EtOAc/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 7.48 – 7.37 (m, 6H, Ar-H), 7.32 – 7.10 (m, 24H, Ar-H), 6.17 (d, $J_{H1'\text{-}H2'}$ = 4.3 Hz, 1H, H1′), 5.01 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.30 (app. t, $J_{H2'\text{-}H1'/H3'}$ = 5.1 Hz, 1H, H2′), 3.92 (dt, $J_{H3'\text{-}H2'}$ = 5.1 Hz, $J_{H3'\text{-}H4'}$= 2.4 Hz, 1H, H3′), 3.60 (dd, $J_{H5'a\text{-}H5'b}$ = 11.1 Hz, $J_{H5'a\text{-}H4'}$ = 2.2 Hz, 1H, H5′a), 3.41 – 3.18 (m, 2H, H5′b and H4′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5 (C=O, C4), 149.8 (C_q-trityl), 143.4 (C_q-trityl), 143.0 (C-O, C2), 139.3 (CH, C6), 129.0 (CH, Ar-C), 129.0 (CH, Ar-C), 128.0 (CH, Ar-C), 127.7 (CH, Ar-C), 127.6 (CH, Ar-C), 102.1 (CH, C5), 89.4 (CH, C1′), 82.0 (CH, C3′), 72.4 (CH, C2′), 62.4 (CH_2, C5′), 61.5 (CH, C4′); NSI HRMS *m*/*z* found: $(M+Na)^+$ 733.2673 $C_{47}H_{38}N_2O_5$, requires $(M+Na)^+$ 733.2673. ^{1}H NMR data is consistent with literature values.[179]

3′,5′-Di-*O*-trityl-1′-β-(D-arabinofuranosyl)uracil **216**



A 1M of aqueous NaOH solution was added (6.1 mL, 6.13 mmol, 3.6 equiv.) to a solution of **215** (1.00 g, 4.13 mmol, 1.0 equiv.) in MeOH (24 mL), and the solution heated to reflux. After 3 h the solution was cooled to rt and quenched with AcOH (2 mL), and the solvent removed *in vacuo*. The crude product residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (3 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain **216** as a beige foam which required no further purification (1.05 g, 1.44 mmol, 85%). R_f 0.10 (1/2, EtOAc/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (br s, 1H, NH), 7.60 (d, $J_{H6\text{-}H5}$ = 8.2 Hz, 1H, H6), 7.37 – 7.14 (m, 30H, Ar-H), 6.11 (d, $J_{H1'\text{-}H2'}$ = 2.9 Hz, 1H, H1′), 5.58 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.02 – 3.96 (m, 1H, H3′), 3.92 – 3.85 (m, 1H, H4′), 3.63 (app. br s, 1H, H2′), 3.44 (dd, $J_{H5'a\text{-}H5'b}$ = 10.8 Hz, $J_{H5'a\text{-}H4'}$ = 2.4 Hz, 1H, H5′a), 2.98 (dd, $J_{H5'a\text{-}H5'b}$ = 10.8 Hz, $J_{H5'b\text{-}H4'}$ = 3.5 Hz, 1H, H5′b); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0 (C=O, C4), 149.8 (C=O, C2), 143.6 (C_q, trityl), 142.7 (C_q, trityl), 141.8 (CH, C6), 128.7 (CH, Ar-C), 128.6 (CH, Ar-C), 128.2 (CH, Ar-C), 128.1 (CH, Ar-C), 127.9 (CH, Ar-C), 127.6 (CH, Ar-C), 127.6 (CH, Ar-C), 100.5 (CH, C5), 86.4 (CH, C1′), 83.6 (CH, C4′), 80.0 (CH, C3′), 74.8 (CH, C2′), 63.3 (CH_2, C5′); NSI HRMS *m*/*z* found: $(M+Na)^+$ 751.2779 $C_{47}H_{40}N_2O_6$, requires $(M+Na)^+$ 751.2779. NMR data is consistent with literature values.[179]

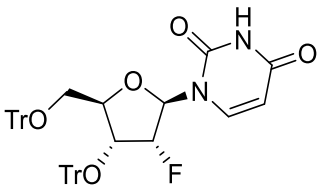
(2′*R*)-2′-Chloro-2′-deoxy-1′-*O*-trityl-1′-β-(4′-thio-D-ribofuranosyl)uracil **213** †



Trityl chloride (1.72 g, 6.18 mmol, 3.0 equiv.) was added to a suspension of **207** (0.500 g, 2.06 mmol, 1.0 equiv.) and DMAP (0.503 g, 4.12 mmol, 2.0 equiv.) in pyridine (14 mL) and the solution was heated to 100 °C. After 72 h, the solvent was removed *in vacuo* and the crude product residue dissolved

in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ solution (3 × 150 mL) and brine (150 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as an orange foam, which was purified on silica gel *via* automated flash chromatography (0 – 20% acetone/$CHCl_3$) to obtain **213** as a colourless oil (360 mg, 0.690 mmol, 34%). R_f 0.14 (1/9, acetone/$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H, N-H), 7.80 (d, $J_{H6\text{-}H5}$ = 8.1 Hz, 1H, H6), 7.55 – 7.41 (m, 6H, Ar-H), 7.39 – 7.27 (m, 9H, Ar-H), 6.22 (d, $J_{H1'\text{-}H2'}$ = 6.0 Hz, 1H, H2′), 5.48 (d, $J_{H5\text{-}H6}$ = 8.2 Hz, 1H, H5), 4.38 (dd, $J_{H2'\text{-}H1'}$ = 6.1 Hz, $J_{H2'\text{-}H3'}$ = 3.5 Hz, 1H, H2′), 4.28 (app. t, $J_{H3'\text{-}H2'/H4'}$ = 3.8 Hz, 1H), 3.68 – 3.57 (m, 2H, H5′a and H4′), 3.51 – 3.40 (m, 1H, H5′b); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0 (C=O, C4), 150.6 (C=O, C2), 143.6 (C_q, Ar-C), 143.1 (C_q, Ar-C), 143.0 (C_q, Ar-C), 140.5 (CH, C6), 129.0 (CH, Ar-C), 128.8 (CH, Ar-C), 128.8 (CH, Ar-C), 128.6 (CH, Ar-C), 128.2 (CH, Ar-C), 128.0 (CH, Ar-C), 127.7 (CH, Ar-C), 127.6 (CH, Ar-C), 127.5 (CH, Ar-C), 127.3 (CH, Ar-C), 103.2 (CH, C5), 88.1 (C_q, benzylic), 74.8 (CH, C3′), 66.2 (CH, C2′), 64.8 (CH, C1′), 63.3 (CH_2, C5′), 50.8 (CH, C4′); NSI HRMS *m/z* found: $(M+Na)^+$ 543.1127 $C_{28}H_{25}{}^{35}ClN_2O_4S$, requires $(M+Na)^+$ 543.116.

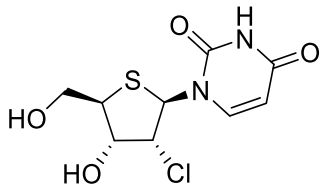
(2′*R*)-2′-Deoxy-2′-fluoro-3′,5′-*O*-trityl-1′-β-(D-ribofuranosyl)uracil **217**



A solution of **216** (0.500 g, 0.686 mmol, 1.0 equiv.) in CH_2Cl_2 (6.9 mL) and pyridine (0.11 mL, 1.37 mmol, 2.0 equiv.) was cooled to 0 °C. DAST (0.15 mL, 1.17 mmol, 1.7 equiv.) was added dropwise and the solution stirred at 0 °C for a further 10 minutes, then stirred at rt. After 24 h, the solution was cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$ until pH = 7, diluted with CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ solution (20 mL), the organic layer separated, and the aqueous layer extracted with CH_2Cl_2 (2 × 5 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to obtain the crude product as a yellow oil which was purified on silica gel *via* automated flash chromatography (0 – 10% acetone/$CHCl_3$) to obtain **217** as a yellow foam (376 mg, 0.514 mmol, 75%). R_f 0.86 (1/9, acetone/$CHCl_3$); ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.36 (s, 1H, NH), 7.58 (d, J_{H6-H5} = 8.2 Hz, 1H, H6), 7.41 – 7.16 (m, 30H, Ar-H), 6.06 (dd, $J_{H1'-F}$ = 14.5 Hz, $J_{H1'-H2'}$ = 2.8 Hz, 1H, H1′), 5.12 (d, J_{H5-H6} = 8.1 Hz, 1H, H5), 4.20 (ddd, $J_{H3'-F}$ = 15.3 Hz, $J_{H3'-H4'}$ = 6.4 Hz, $J_{H3'-H2'}$ = 4.2 Hz, 1H, H3′), 4.08 (ddd, $J_{H2'-HF}$ = 51.3 Hz, $J_{H2'-H3'}$ = 3.1 Hz, $J_{H2'-H1'}$ = 3.09 Hz, 1H, H2′), 4.01 – 3.96 (m, 1H, H4′), 3.54 (dd, $J_{H5'a-H5'b}$ = 11.1 Hz, $J_{H5'a-H4'}$ = 2.0 Hz, 1H, H5′a), 3.26 (dd, $J_{H5'b-H5'a}$ = 11.1 Hz, $J_{H5'b-H4'=}$ 3.0 Hz, 1H, H5′b); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.6 (C=O, C4), 149.6 (C=O, C2) , 143.4 (C_q, trityl), 143.1 (C_q, trityl), 139.9 (CH, C6), 128.9 (CH, Ar-C), 128.9 (CH, Ar-C), 128.0 (CH, Ar-C), 128.0 (CH, Ar-C), 127.6 (CH, Ar-C), 127.5 (CH, Ar-C), (CH, C5), 92.4 (CH, C4′), 91.5 (d, $J_{C2'-F}$ = 194.5 Hz, CH, C2′), 87.5 (d, $J_{C1'-F}$ = 33.9 Hz, CH, C1′), 71.3 (d, $J_{C3'-F}$ = 20.2 Hz, CH, C3′), 62.1 (CH_2, C5′); ^{19}F NMR (377 MHz, $CDCl_3$) δ -199.51 (app. dt, $J_{F-H2'}$ = 51.3 Hz, $J_{F-H1'/H3'}$ = 14.9 Hz); NSI HRMS *m/z* found: $(M+Na)^+$ 753.2734 $C_9H_9FN_2O_3$, 753.2735. requires $(M+Na)^+$ NMR data is consistent with literature values.[180]

(2′*R*)-2′-Deoxy-2′-chloro-1′-β-(4′-thio-D-ribofuransyl)uracil **218** *



To a solution of **213** (350 mg, 0.673 mmol, 1.0 equiv.) in 1/1 (*v*/*v*) solution of CH_2Cl_2/MeOH (4.0 mL), was added *p*-TsOH (26.0 mg, 0.135 mmol, 0.20 equiv.) and the solution stirred at rt. After 3 h, the reaction was quenched *via* addition of Et_3N (0.25 mL) and the solvents removed *in vacuo*. The residue was partitioned between EtOAc (10 mL) and H_2O (25 mL). The aqueous layer was separated, and the organic layer extracted with H_2O (2 × 10 mL). The aqueous layers were combined and the solvent removed *in vacuo* to obtain the crude product as a beige oil which was purified on silica gel *via* automated flash chromatography (0 – 10% MeOH/CH_2Cl_2) to obtain **218** as a colourless glass (68.0 mg, 0.246 mmol, 37%). R_f 0.70 (15/85, MeOH/CH_2Cl_2); $[\alpha]_D^{26}$ +20.3 (*c* 0.9, H_2O); ^{1}H NMR (400 MHz, MeOD) δ 8.17 (d, J_{H6-H5} = 8.2 Hz, 1H, H6), 6.26 (d, $J_{H1'-H2'}$ = 7.8 Hz, 1H, H1′), 5.78 (d, J_{H5-H6} = 8.1 Hz, 1H, H5), 4.66 (dd, $J_{H2'-H1'}$ = 7.8, $J_{H2'-H3'}$ = 3.5 Hz, 1H, H2′), 4.34 (app. t, $J_{H3'-H2'/H4'}$ = 3.3 Hz, 1H, H3′), 3.82 (dd, $J_{H5'a-H5'b}$ = 11.7 Hz, $J_{H5'a-H4'}$ = 4.5 Hz, 1H, H5′a), 3.76 (dd, $J_{H5'b-H5'a}$ = 11.7 Hz, $J_{H5'b-H4'}$ = 5.3 Hz, 1H, H5′b), 3.50 (app. td, $J_{H4'-H5'a/b}$ = 4.9 Hz, $J_{H4'-H3'}$ = 3.1 Hz, 1H, H4′); ^{13}C NMR

(101 MHz, MeOD) δ 164.3 (C=O, C4), 151.2 (C=O, C2), 141.4 (CH C6), 102.2 (CH, C5), 74.3 (CH, C3′), 64.7 (CH, C2′), 64.0 (CH, C1′), 62.4 (CH_2, C5′), 53.7 (CH, C4′); ESI HRMS *m/z* found: $(M+Na)^+$ 301.0025 $C_9H_{11}{}^{35}ClN_2O_4S$, requires $(M+Na)^+$ 302.0020. This compound was reported in the literature but not characterised.[181]

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