THE SYNTHESIS OF VINYLPHOSPHONATE-LINKED RNA

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Abstract

An introductory chapter discusses the steric block, RNase H and RNA interference antisense mechanisms and the application of antisense nucleic acids as therapeutic agents. Examples of existing chemical modifications of the sugar and backbone regions of nucleic acids are given, followed by the introduction of the vinylphosphonate modification. The vinylphosphonate has previously been examined in DNA and has been synthesised by either Pd(0) catalysed cross-coupling of an *H*-phosphonate with a vinyl bromide, or by the cross-metathesis of a vinylphosphonate with a terminal olefin.

This thesis details the first examples of the vinylphosphonate modification in RNA. The initial aim of this project was the synthesis of a range of nucleosides where the 5'-C-O was replaced by a vinyl bromide carbon-carbon double bond.

Starting from α-D-glucose, acid catalysed formation of the 1,2:5,6-diisopropylidene α-D-glucofuranose was carried out followed by protection of the 3'-OH as an acetate. The 5,6-isopropylidene was then subjected to H_5IO_6 mediated one-pot hydrolysis-oxidative cleavage to obtain the 5-aldehyde. Wittig olefination using CBr_4 and Ph_3P led to the dibromo olefin which was then stereoselectively reduced using dimethyl phosphite and diisopropylamine to obtain the pure *trans*-vinyl bromide. Following hydrolysis of the acetate, the stereochemistry of the 3-OH was then inverted by sequential oxidation and reduction. With the correct stereochemistry, the 3-OH

was protected as the 2-methylnaphthyl ether. The 1,2-isopropylidene moiety was then hydrolysed and acetylated to the *bis*-acetate which was subjected to Vorbrüggen conditions obtaining the uridine (93%), adenosine (77%), cytidine (30) and guanosine (63%) vinyl bromide nucleosides. The 2'-OAc of the nucleosides were hydrolysed to the 2'-OH in yields of 74-92%. The uridine 2'-OH was protected as the 2'-OTBS ether (98%), analogous to the commercially available phosphoramidites used in automated oligonucleotide synthesis. Similarly, the adenosine and uridine nucleosides could also be blocked as the 2'-OMe (59% and 73% respectively). In the case of the uridine vinyl bromide, the 3'-*O*-(2-methylnaphthyl) protecting group was cleaved using DDQ, this then enabled the vinylphosphonate-linked uridine dinucleotides to be functionalised at the 3'-OH as the cyanoethyl phosphoramidite using *N,N*-diisopropyl-2-cyanoethyl-chlorophosphoramidite, DIPEA and DMAP in dichloromethane (2'-OTBS 74%, 2'-OMe 41%). These could then be used in automated solid phase oligonucleotide synthesis.

The *H*-phosphonates were prepared in a single step form the commercially available phosphoramidites using a tetrazole. These were then coupled to the vinyl bromide nucleosides using standard conditions of $Pd(OAc)_2$ (0.2 eq.), dppf (0.4 eq.) and propylene oxide (20 eq.) in THF at 70 ^{o}C in a sealed vial for 6 hours. A range of vinylphosphonate-linked dinucleotides were accessed in yields of 61-99%.

A detailed experimental section at the end of this thesis describes the procedures used in the synthesis and the analysis of the structures obtained.

Acknowledgements

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Abbreviations

A	Adenine
AIBN	2,2'-Azoisobutyronitrile
AON	Antisense oligonucleotide
app	Apparent
aq	Aqueous
Bhoc	Benzyhydroxycarbonyl
borsm	Based on recovered starting material
BSA	*N*,*O*-*bis*(trimethylsilyl)acetamide
BSTFA	*N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide
BTEAC	Benzyltriethylammonium chloride
Bz	Benzoyl
C	Cytosine
CAN	Cerium ammonium nitrate
CE	Cyanoethyl
CMV	Cytomegalovirus
DAST	Diethylamino sulphurtrifluoride
DBU	1,8-Diazobicyclo[5.4.0]undec-7-ene
DCA	Dichloroacetic acid
DCM	Dichloromethane
dd	Double doublet
ddd	Double double doublet
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DIPEA	Diisopropylethyl amine
DMP	Dess Martin periodinane
DMT	Dimethoxytrityl

dppf	1,1'-*bis*(diphenylphosphino)-ferrocene
dsRNA	Double stranded RNA
ENA	Ethylene Nucleic Acid
Fmoc	Fluorenylmethoxycarbonyl
G	Guanine
HPLC	High Performance Liquid Chromatography
imid.	Imidazole
KHMDS	Potassium hexamethydisilazide
LNA	Locked Nucleic Acid
MBHA	4-Methyl benzhydrylamine
mRNA	Messenger RNA
Nap	Naphthyl
nt	Nucleotide
PDC	Pyridinium Dichromate
PMB	*para*-methoxy benzyl
PNA	Peptide nucleic acid
pyr	Pyridine
RISC	RNA Induced Silencing Complex
RNAi	RNA Interference
rsm	Recovered starting material
siRNA	Short interfering RNA
ssRNA	Single stranded RNA
T	Thymine
TBAI	Tetra *n*-butylammonium iodide
TBHP	*tert*-Butyl hydroperoxide
TIPDS	tetraisopropyldisiloxane

T_m	Melting temperature
TMS	Trimethylsilyl
TOCSY	Total correlation spectroscopy
TPAP	Tetra *n*-propylammonium perruthenate
U	Uracil

- INTRODUCTION -

1. Introduction

1.1 Therapeutic Agents

Starting in 1990, the Human Genome Project saw one of the greatest landmarks in scientific history realised in 2003 when the sequence of the 3.7 billion base pairs that make up our human DNA was reported.[1] Previous to this the two groups striving to unravel the genetic code; the International Human Genome Project (HGP) and the biotechnology firm Celera both reported outline sequences of the human genome in 2001.[2,3] Identifying the 30,000 to 40,000 genes within human DNA, separated by non-coding regions also embraced new technologies.[2] Building on this knowledge, it is anticipated that understanding of the proteome and other molecular machinery will increase, enabling scientists to better identify, diagnose and treat disease.[4]

Traditionally, disease is treated by directing small molecule drugs to target proteins such as enzymes and receptors. Drug discovery is a lengthy, expensive process; in 1997 it was estimated that 100,000 compounds were screened every day and that several million compounds screened, identified a few thousand with the desired characteristic.[5] It is estimated that for every 5000-7000 compounds screened, five may progress to clinical trials and only one of may get approval and be marketed.[5,6] The average cost of developing and taking a drug to market is generally considered to exceed $800 million dollars.[7] Drug molecules may be natural

products, their analogues or molecules designed and synthesised using medicinal chemistry.

Protein structure must be carefully considered when designing molecules which can bind to it. The structure depends on the unique amino acid sequence and this information is carried in DNA (deoxyribonucleic acid); the genetic code of an organism. The process of protein synthesis involves the transcription of DNA to messenger RNA (mRNA); the information contained in mRNA is then read during translation and the polypeptide chain is synthesised to form the protein (Figure 1).

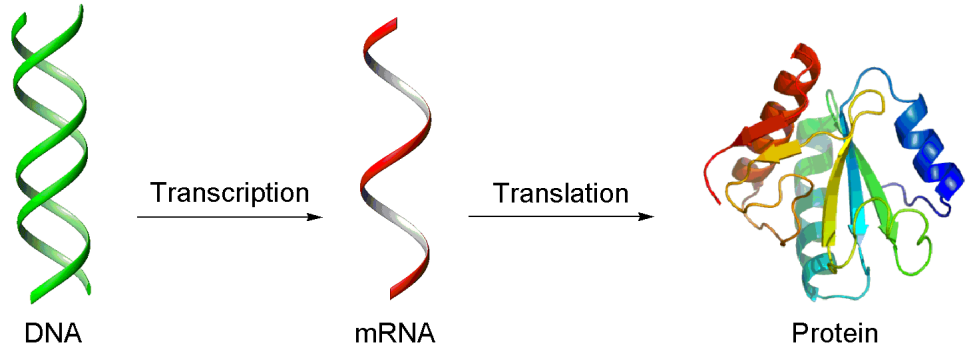


Figure 1.

Knowledge of the human genome however, does not automatically translate into a more successful programme of drug development. A gene sequence alone can only be used to predict the primary structure of a protein (*i.e.* the amino acid sequence). Predicting the secondary and tertiary structure, how it folds, is difficult.

1.2 Targeting the Nucleic Acids

An alternative to targeting a protein is to target the nucleic acids which contain the information to make the proteins. One of the advantages of this strategy is the structure of RNA is more highly conserved and is potentially more accessible than the active sites of enzymes.[8] Many small molecules have been developed to bind to nucleic acids and the area has been extensively reviewed.[9,10,11,12,13,14,15] One common drug target is the Type 1 HIV (HIV-1) where the virus has rapidly developed resistance to small molecules designed to inhibit enzyme function. The RNA responsible for gene regulation in HIV-1, the *trans*-activating region (TAR) has been targeted using small molecules (Figure 2).[8]

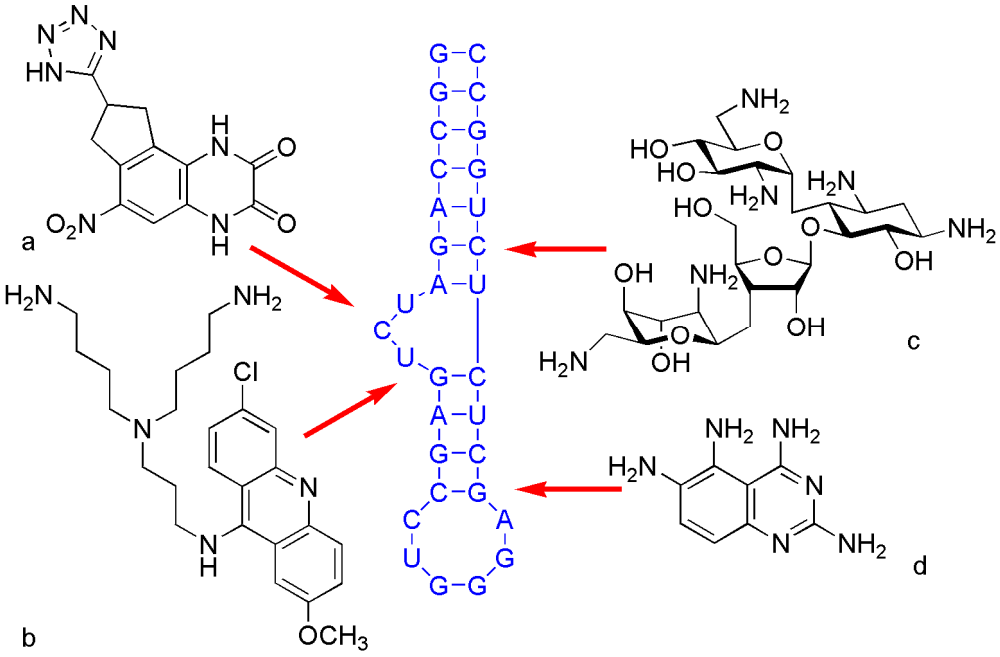


Figure 2. Examples of small molecules which target the HIV-1 TAR RNA: a. Quinoxaline-2,3-diones; b. CPG40336A (aminoalkyl linked acridines).; c. Neomycin B (aminoglycoside); d. 2,4-diaminoquinozalines.

Similar to the targeting proteins, the targeting of DNA or RNA using small molecules has the downside that a vast number of molecules must be screened to find a suitable candidate and the therapeutic agents are often not selective. Direct interaction with the genetic material can provide an alternative to the random, expensive and lengthy process of developing small molecule drugs.

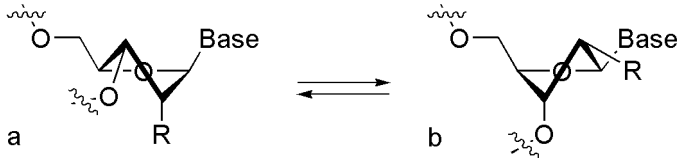
1.3 The Antisense Strategy

To target genetic material as a therapeutic strategy requires direct interaction with the genetic material. This can be achieved using the antisense principle, where the interaction of complementary base pairs results in specific binding between the therapeutic agent and the target genetic material.

DNA is a double helix of two complementary strands; the strand containing the genetic information is called the sense strand, and the strand which binds to it is the antisense strand. During transcription, the antisense strand of DNA is transcribed to a molecule of messenger RNA (mRNA) in the cell nucleus. Binding of a complementary antisense oligonucleotide (AON) to the mRNA is an alternative to using small molecules. A single strand of nucleic acid can bind to mRNA in a highly specific manner due to the hydrogen bonding between base pairs; the resulting duplex can interfere with gene expression. The strength of the interaction between oligonucleotide strands is characterised by the melting temperature, T_m of an oligonucleotide; a measure of the

temperature at which 50% of the duplex structure is dissociated into its constituent strands.

Nucleic acids can exist in different conformations depending on the degree of puckering of the sugar moiety. Different puckering is caused by the presence of the 2'-OH in the RNA compared with DNA results in differences in the helix conformations; RNA exists in the A-form whereas DNA exists in the B-form (Scheme 1).



Scheme 1. a. A-form (C-2'-*exo*) and b. B form (C-3'-*exo*).

1.3.1 Mechanisms of Antisense Action

There are three main mechanisms through which antisense mechanisms can be harnessed; (i) steric block, (ii) RNase H and (iii) RNA interference.

1.3.1.1 Steric Block

Translation involves the reading of the mRNA code and the subsequent protein synthesis; this process is facilitated by the movement of a ribosome along the mRNA strand in an ATP dependent process. Steric block is a none destructive mechanism of inhibiting protein synthesis; simply involving the binding of an antisense oligonucleotide fragment to the mRNA to block progression of the ribosome along the mRNA strand, thus halting

translation and protein synthesis (Figure 3). Translation in eukaryotic organisms commences with the recognition and binding on the initiation complex to the 5'-cap of the mRNA.[16] Translation then proceeds by the scanning down the mRNA sequence to the start codon (AUG) where ribosome assembly completes and translation of the amino acid coding section occurs. Blocking the complete ribosome assembly is vital to translational arrest.

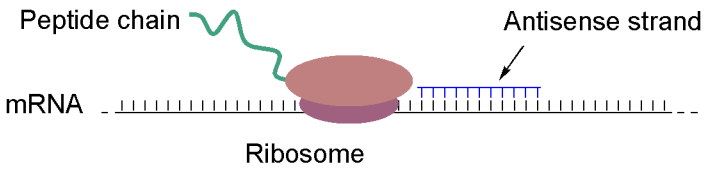


Figure 3. Mechanism of steric block.

The antisense strand is in equilibrium between the bound and dissociated state and the ATP driven progression of the ribosome along the mRNA can be sufficient to dislodge the weakly bound antisense strand. Targeting the 5'-end of the target mRNA provides more successful steric block.[17] Steric block is stoichiometric; gene knock down is only achieved while the antisense oligonucleotide is bound to the target mRNA. Therefore successful steric block requires oligonucleotides with a high T_m.

1.3.1.2 RNase H

An alternative antisense mechanism of involves the RNase H pathway involved in translation. RNase H is an endogenous nuclease which recognises DNA-mRNA duplexes and specifically degrades the mRNA strand of the duplex in a catalytic mechanism

(Figure 4). RNases H are found in many diverse organisms *e.g.* viruses and humans. Oligonucleotides as short as tetramers can successfully inhibit RNase H[18] however; the oligonucleotides must possess the DNA-like B-form to achieve activation of the RNase H.[19]

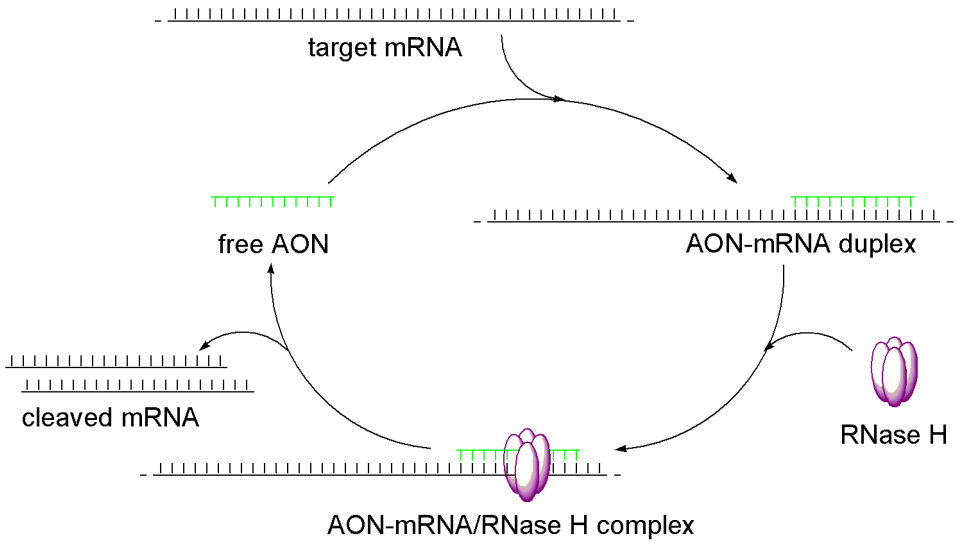


Figure 4. Mechanism of the RNase H.

Factors influencing whether a duplex will activate the RNase H have been extensively studied and reviewed.[20] The catalytic mechanism relies on the presence of Mg^{2+} and Mn^{2+} cations as cofactors.[21] The accessibility, size and shape of the minor groove within the helix are vital to the activation of the RNase H.[22,23] The RNase H mechanism of gene knockdown has been studied for forty years[21] and thus has many candidates being put forward in clinical trials.

1.3.1.3 RNA Interference (RNAi)

First observed in 1990 by Jorgensen *et al*. in the study of the petunia *Neurospora crassa*[24], RNAi is another method of post-transcriptional gene silencing. The implications were only fully realised in the seminal paper published by Fire and Mello in 1998, which led to a revolution in understanding and application of RNAi.[25] This was recognised by the award of the 2006 Nobel Prize in Medicine to these two scientists.

Double stranded RNA (dsRNA) is significantly more stable to nuclease degradation than the lone single strand (ssRNA) RNA, and this is advantageous in achieving successful gene knockdown.[26] In mammalian cells, short interfering RNA (siRNA) strands, typically 21-23 nucleotides in length has been shown to offer the optimal compromise between specificity and avoiding an interferon response.[27]

The RNA induced silencing complex (RISC) is formed by the duplex siRNA and a protein aggregate which then triggers an ATP-dependent process, unwinding the sense strand and leaving the antisense strand complexed with the protein. This active RISC binds to the complementary sequence of the target mRNA and the mRNA within the duplex is then cleaved by endonucleases contained within the RISC in an ATP independent hydrolysis.[28,29] It is thought that exonucleases then cleave the remaining target

mRNA. Cleavage of the mRNA releases the active RISC, enabling a catalytic process (Figure 5).

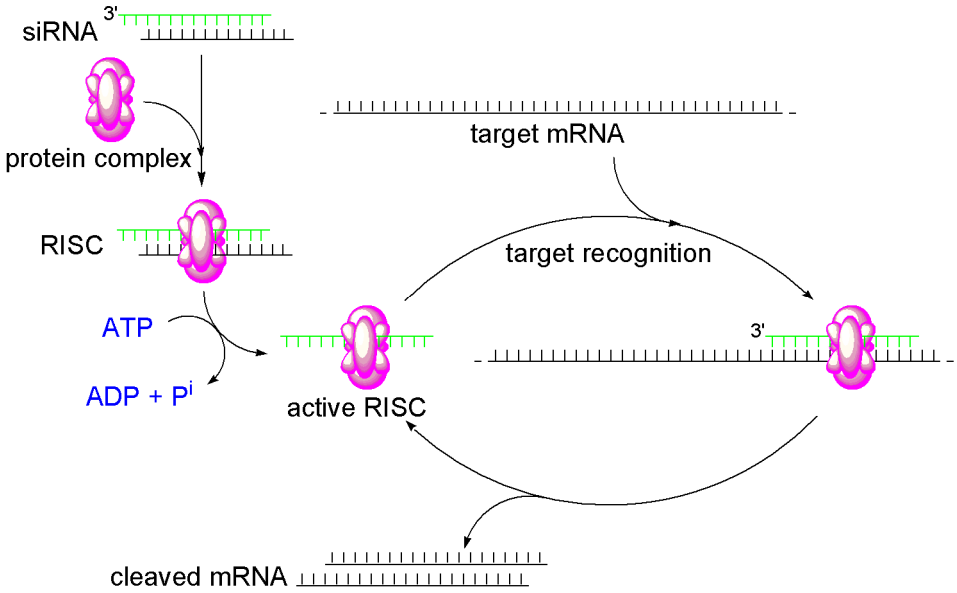


Figure 5. Mechanism of RNA interference.

The exact structure and make up of the RISC is unknown but some components and structural domains and their functions have been identified.[30,31,32,33,34] The presence of an protein from the Argonaute family is a key feature; this is the catalytic component of the RISC, responsible for binding the siRNA and cleavage of the mRNA by endonuclease activity.[35,36] The Argonaute-2 protein contains both PAZ and PIWI domains; the PAZ domain is essential for the nuclease activity of the RISC.[35,37] Recently, Hannon *et al.* obtained the crystal structure of the Argonaute protein from achaebacterium *Pyrococcus furiosis*, showing the PAZ and PIWI domains (Figure 6).[38]

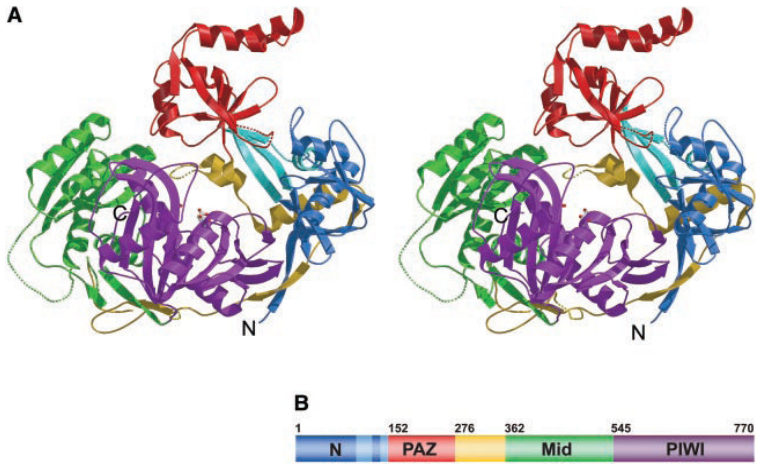


Figure 6. Crystal structure representation of *P. furiosus* Argonaute. (A) N-terminal domain (blue), "stalk" (light blue), PAZ domain (red), middle domain (green), PIWI domain (purple), and the interdomain connector (yellow). (B) Schematic diagram of the domain borders.

The 5'-end of the siRNA interacts with the PIWI domain of the Argonaute protein.[39,40] The 5'-end can exist as the 5'-monophosphate or the 5'-OH but does not tolerate chemical modification. However, capping of the siRNA 3'-OH is tolerated without loss of function.[41] RNAi requires an A-form helix, ruling out the use of antisense DNA.

Short interfering RNA's are already being investigated as therapeutic agents to target cancers, viral diseases, autoimmune and inflammatory disorders, neurological conditions, genetic diseases and other illnesses.[42,43] There has also been considerable investigation of drug delivery and the identification of the most effective sequences.[44,45,46]

1.3.2 Nucleic Acid Structure

Using the antisense strategy is not a problem-free approach; phosphodiester-linked oligonucleotides are impractical for three main reasons. The phosphodiester backbone is vulnerable to the rapid degradation by nucleases. Binding of the antisense strand is a reversible process; any desired effect of the antisense strategy is only observed when the duplex is formed. Therapeutic application requires good delivery and bioavailability of the antisense oligonucleotide. The anionic phosphodiester linkage has good aqueous solubility and therefore distribution, however, the hydrophilic nature results in poor transport across the hydrophobic cell membrane.

The most effective antisense sequences are those that are least self-complementary (minimising undesired tertiary structures). Specificity is important; mismatching reduces the effectiveness and can cause undesired, potentially toxic interactions.

Native RNA and DNA and modified oligonucleotides have been developed in antisense applications. There are three types of modification: backbone, sugar and nucleoside base modification (Figure 7). Modifications have been designed to increase nuclease stability and increase the binding affinity between strands.[47]

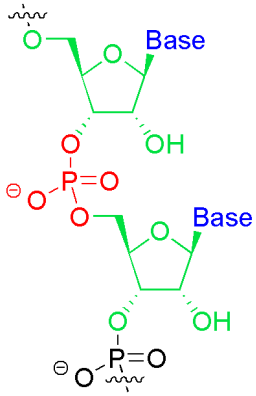


Figure 7. Possible modification of nucleic acids a. sugar (green), b. nucleoside base (blue) and c. backbone (red).

Modifications can lead to alternative conformations which can affect the three-dimensional geometry of the structure and lead to greater or lesser binding. The A-form, adopted by RNA is more stable than the 2'-*endo* B-form of DNA; hybrid RNA-DNA duplex stability depends on the extent of A-form characteristics. This depends on composition and proportion of ribonucleotides present in the chimera. Duplex stability is also affected by external factors *e.g.* base composition, mismatches in base pairs ionic strength, and the presence of denaturing agents.

Casey and Davidson reported that, depending on the sequence, RNA-DNA hybrids were typically 10-30 ^{o}C more stable than the analogous DNA-DNA hybrids in 80% formamide solution.[48,49] A comprehensive study by Kankia and Marky, also compared DNA, RNA and DNA-RNA hybrid duplexes and the effect of cation concentration and hydration on stability.[50] These results show that there is a wide variation in the relative stability of the hybrid duplexes.

The catalytic nature of the RNase H and RNAi mechanisms offer significant advantages over steric block; in particular lower concentrations are required to achieve a therapeutic effect. Caplan *et al.* reported that siRNA was more effective at gene knockdown than single stranded DNA.[51] However, a comparison of siRNA and RNaseH using phosphorothioate DNA showed that the potency, efficacy, specificity and duration of gene knockdown were equivalent.[52] In another comparative study the application of sequences of antisense DNA and siRNA were compared; 10-20% of the antisense DNA and 20-40% of the siRNA sequences exhibited greater than 70% gene knockdown, indicating that the siRNA's were more efficient.[53] Endonucleases such as RNases H also have limited sequence specificity for single strand DNA which activates the RNase H pathway and this can cause many off-target effects.

1.4 Nucleic Acid Modifications

1.4.1 Modified DNA

The many examples of modified nucleic acids have been extensively reviewed.[54,55,56,57] Chemical modification of nucleic acid structure can change the nature of the interactions in the antisense mechanisms, and this can be exploited to improve the binding affinity and nuclease resistance; these have been extensively explored in DNA.

The steric block mechanism, requires a strong binding affinity to achieve translational arrest. In the steric block mechanism,

phosphodiester-linked oligonucleotides only weakly inhibit protein synthesis. Since the mechanism does not involve the interaction of the antisense oligomer with proteins, highly modified oligonucleotide structures have been developed. The two most widely used modifications in the steric block mechanism are the morpholino oligonucleotides **1** which has the sugar replaced with a morpholine moiety and peptide nucleic acid (PNA) **2** oligonucleotides, where the sugar-phosphate backbone is completely replaced with an amide-linked backbone (Figure 8).[58]

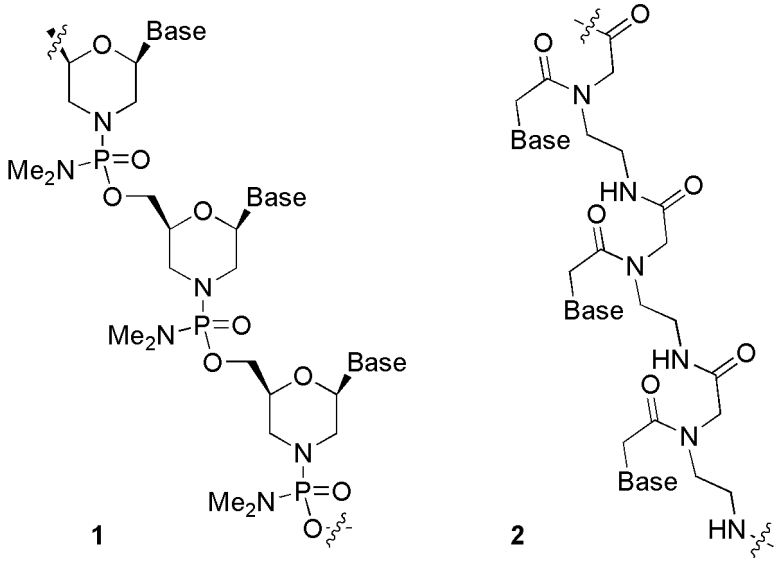
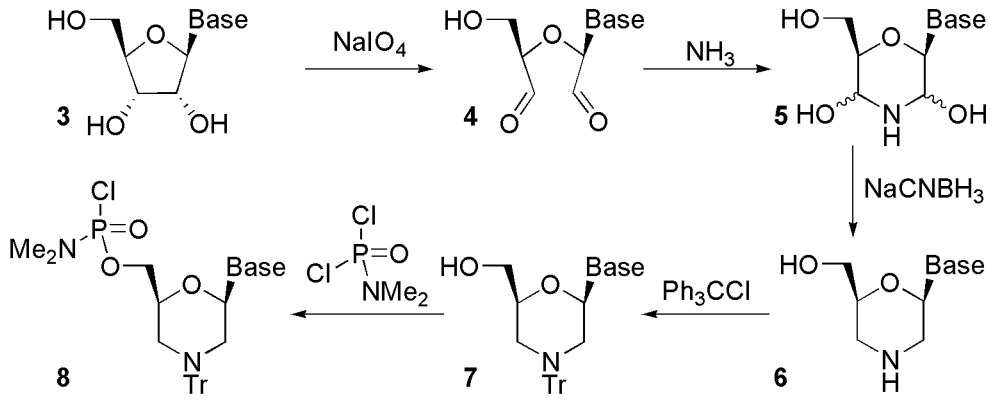


Figure 8. a. Morpholino and b. Peptide Nucleic Acid.

The morpholino modification **1** provides good metabolic stability and a higher binding affinity due to the absence of electrostatic interactions in the neutral phosphorodiamidite backbone. This also increases the lipophilic character however the poor aqueous solubility leads to the formation of aggregates. Morpholino oligomers have similar binding affinities to those of DNA-DNA duplexes and they do not activate the RNase H or siRNA pathways,

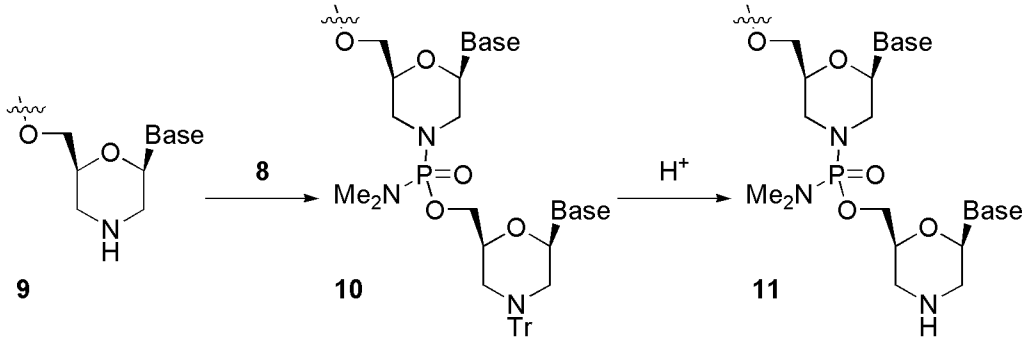
reducing off-target effects.[16] They have been extensively studied and have even progressed to clinical trials.[58]

Morpholinos are synthesised from the corresponding nucleoside building blocks in a simple, efficient synthesis from the nucleoside **3**, as described by Summerton and Wheeler (Scheme 2).[59]



Scheme 2.

The monomer morpholino units are coupled together, this is carried out in the absence of catalysts with high yields and deprotection of the trityl group is carried out with ease (Scheme 3).

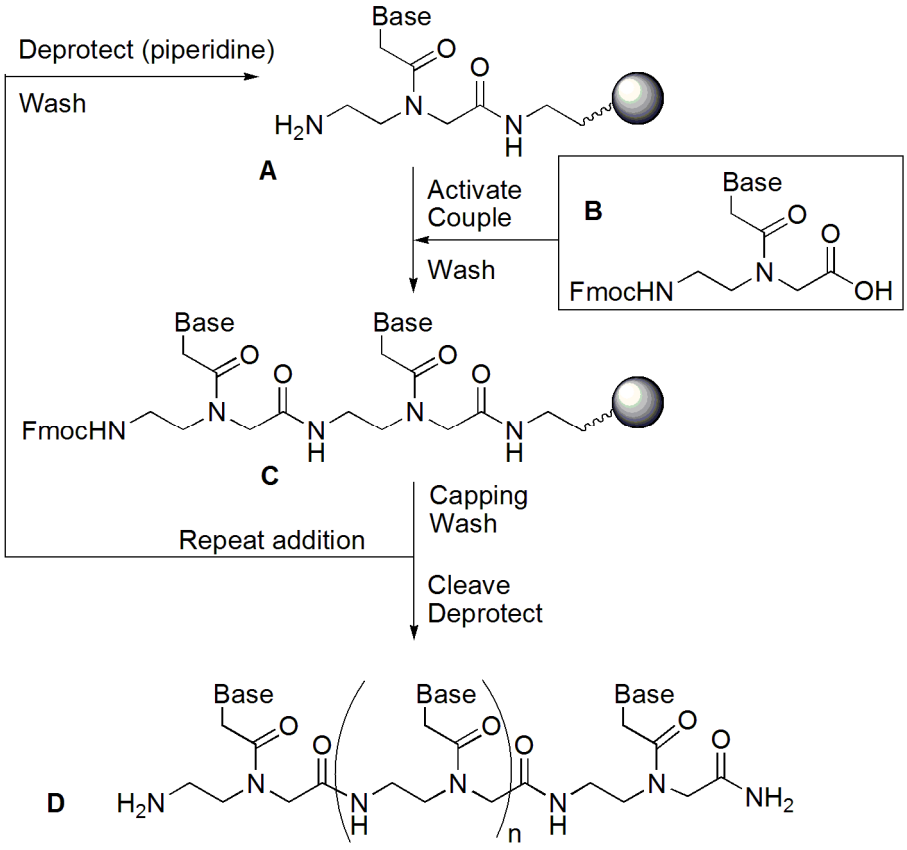


Scheme 3. Synthesis of morpholino oligonucleotides.

Peptide nucleic acid (PNA) **2**, is another example of a highly modified structure that still utilises the Watson-Crick base pairing of nucleoside bases to create complementary strands.[60,61,62] First

synthesised by Nielsen *et al.*,[63] PNA is resistant to nuclease and protease degradation and form very stable heteroduplexes with DNA or RNA.[64] As with the morpholinos, PNA does not activate the RNase H or siRNA pathways. Although the PNA is neutral, transport across cellular membranes does not readily occur; they have low solubility and do not readily bind to proteins. Biological studies have shown that PNA not only inhibits translation,[65,66] but can also activate[67] or inhibit[68] transcription.

Early synthesis of PNA monomers utilised the standard Boc protecting group chemistry.[69] Oligomer synthesis uses protocols similar to the Merrifield solid-phase peptide synthesis with solid support polystyrene beads with 4-methyl benzhydrylamine functional groups (MBHA). Monomer assembly uses automated synthesis protocols to obtain the desired oligomer.[70] Recently PNA has utilised milder reagents due to using alternative protecting groups; fluorenylmethoxycarbonyl (Fmoc) for the nucleoside primary amines and benzyhydroxycarbonyl (Bhoc) for the peptidic amines of the monomer units (Scheme 4).[71,72]



Scheme 4. Solid-phase synthesis of PNA.

In the RNase H and RNAi pathways, the duplex structure requires the antisense oligonucleotide to retain characteristics similar to the natural nucleic acid; thus requiring a more subtle modification than PNA. For effective response, within the RNA-DNA duplex, the antisense strand should retain a DNA-like B-form helix (2'-*endo* sugar) while the target mRNA retains the RNA A-form helix geometry (3'-*endo* sugar).

In the RNase H pathway, there are many examples of modified bases and modified backbone antisense oligonucleotides. Two of the most prevalent backbone modifications are the phosphorothioate **12** and boranophosphate **13** (Figure 9).[73]

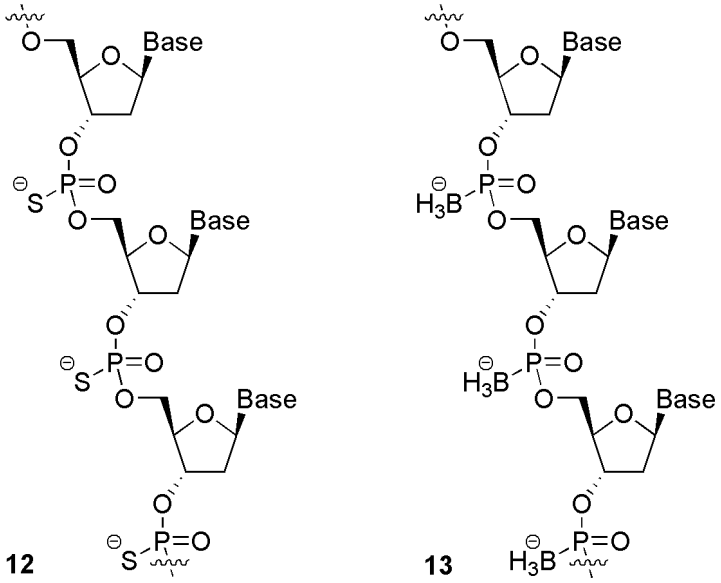
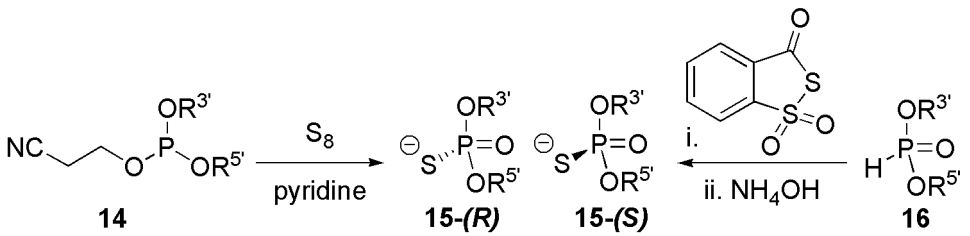


Figure 9. RNase H active modifications: a. Phosphorothioate, b. Boranophosphate.

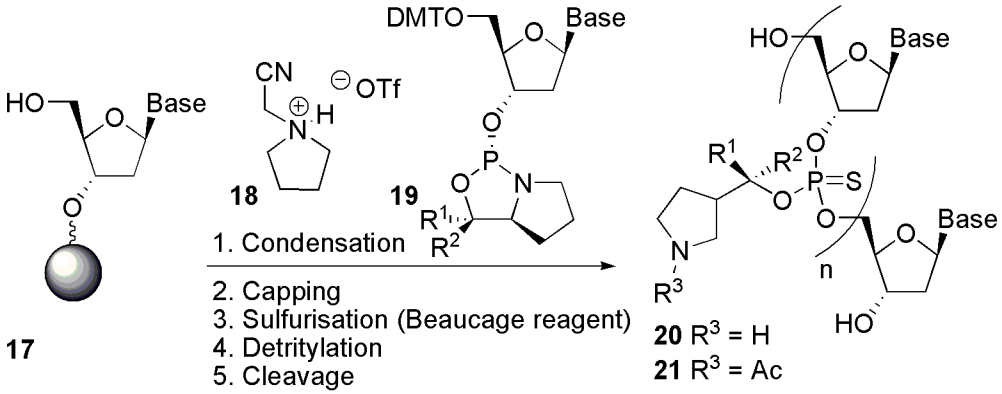
First investigated by Eckstein, the phosphorothioate **12** is the most common modification in the RNase H.[74] The modification involves the substitution of one of the phosphate oxygen atoms with a sulphur atom. The phosphorus is a chiral centre and early syntheses produced mixtures of diastereoisomers;[75,76] early work used elemental sulphur (S_8) to carry out sulphurisation. More recently the Beaucage reagent (3*H*-1,2-benzodithole-3-one-1,1-dioxide)[77] has been used (Scheme 5).



Scheme 5.

Stereoselective syntheses of phosphorothioates have recently been developed by Stec *et al.*[75] and Lesnikowski.[76] Oka *et al.* recently

reported a stereoselective synthesis of phosphorothioates on a solid support (Scheme 6).[78]

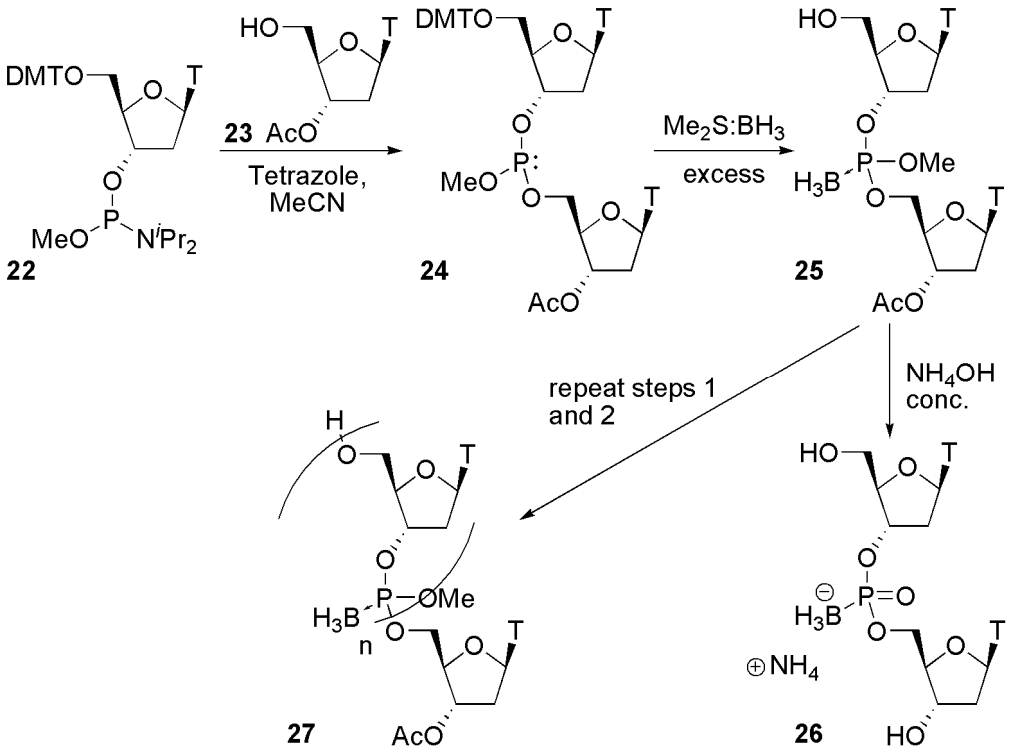


Scheme 6. Stereocontrolled synthesis of phosphorothioate DNA.

Phosphorothioates are more resistant to nucleases than the natural phosphodiester-linked nucleic acids. However, phosphorothioate oligonucleotides with a mixture of P(*R*)- and P(*S*) centres bind more weakly to the target RNA. Stec *et al.* showed that *R*-phosphorothioates oligonucleotides have stronger, tighter binding to the target RNA.[79]

Phosphorothioates have led to the development of many antisense oligomers. Evidence that antisense therapeutics are a viable strategy for targeting disease is possible and is highlighted by the successful marketing and FDA approval of Vitravene®, the first commercially available antisense drug. Developed by Isis Pharmaceuticals, Vitravene® is used to treat cytomegalovirus (CMV) retinitis in AIDS patients *via* the RNase H mechanism. The 21mer phosphorothioate, 5'-GCGTTTGCTCTTCTTCTTGTG-3' is a mixture of diastereoisomers.

Synthesis of the boranophosphate as a diastereoisomeric mixture was first described by Shaw (Scheme 7).[80] A non-bridging oxygen atom is replaced by a borano moiety, the structure is isoelectronic and isosteric with the natural phosphodiester. The boranophosphate modification has increased lipophilic character is resistant to nucleases.

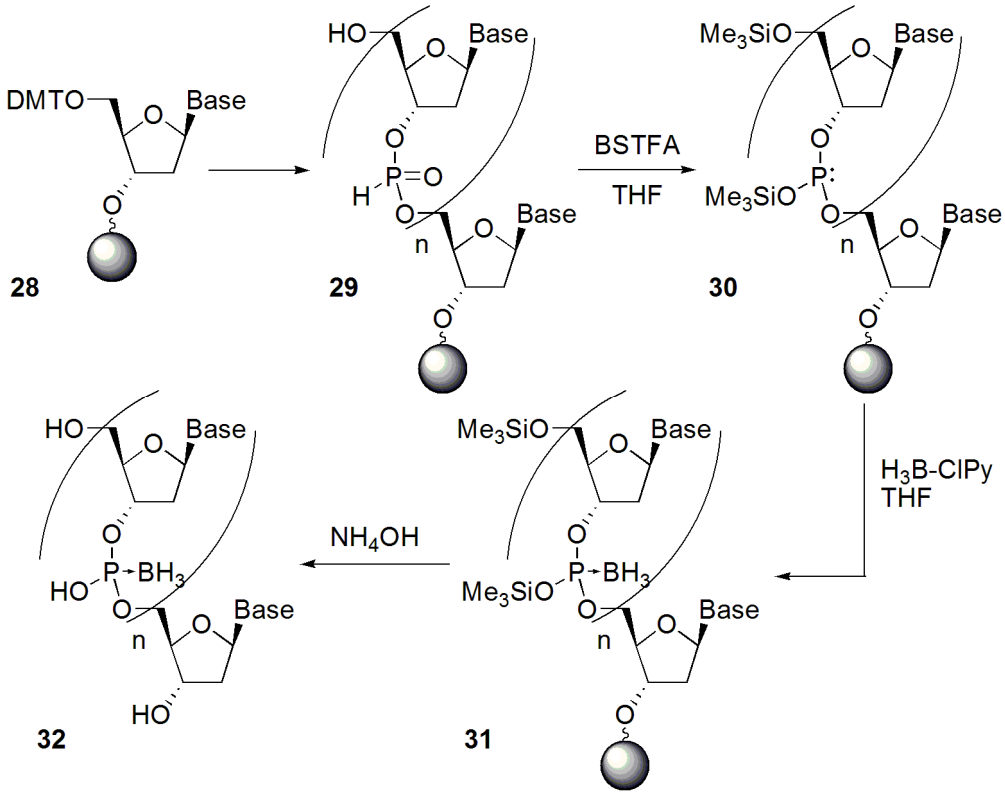


Scheme 7. Synthesis of boranophosphate oligonucleotides.

The boranophosphate, like the phosphorothioate is able to activate the RNase H mediated cleavage of the complementary RNA. Analysis of the individual diastereoisomers of the T*T boranophosphate dimer indicates that both structures have the DNA-like B-form.[81] Studies by Shaw *et al.*[82] using a single boranophosphate modification in a 14mer and by Matteuci *et al.*[83] of a 15mer containing all boranophosphate linkages showed that

boranophosphate binding to natural DNA and RNA was weaker than those of unmodified controls.

The solid-phase synthesis of boranophosphates using *H*-phosphonates was recently reported as a more facile method of oligomer synthesis.[84] Using standard protocols, the *H*-phosphonate oligomer was synthesised on the solid support then silylated. The borano moiety introduced using borane-2-chloropyridine. Deprotection and cleavage of the oligomer from the solid support used NH_4OH (Scheme 8.).

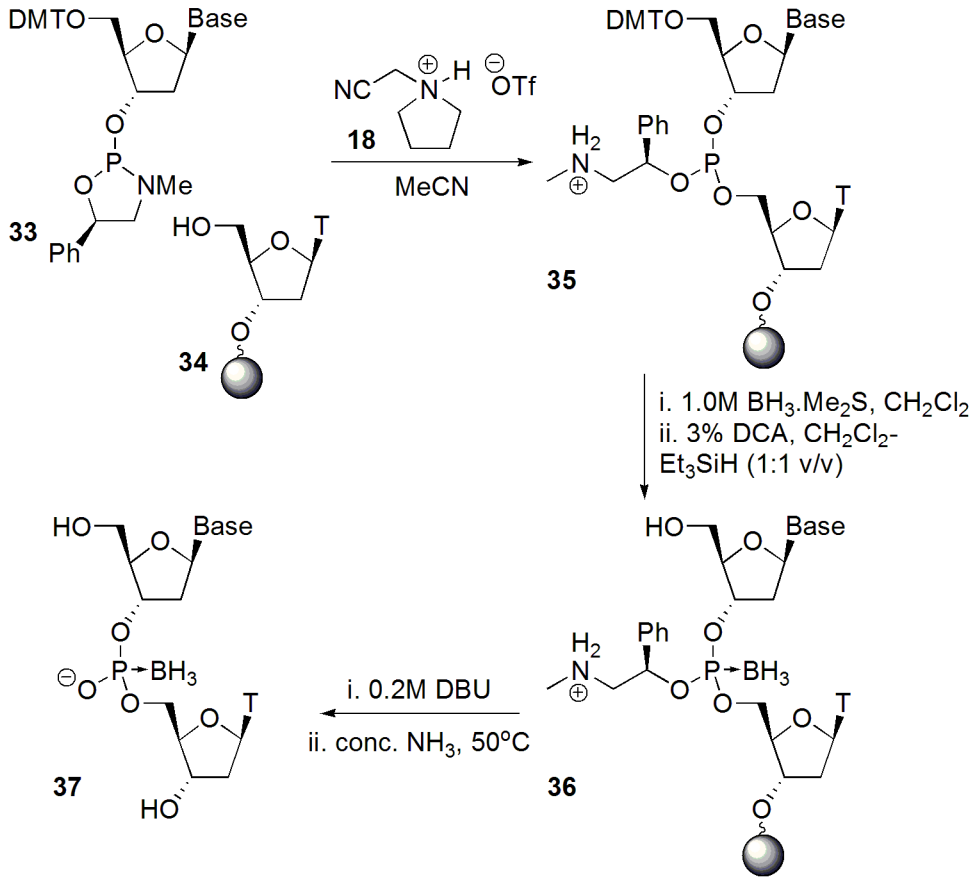


Scheme 8. Solid-phase synthesis of boranophosphates (BSTFA: *N,O*-bis(trimethylsilyl)trifluoroacetamide).

In 2000, Shaw *et al.* reported that the P*(S)* configuration was approximately 5 x 10^2 more stable to snake venom

phosphodiesterase, than the natural phosphodiester linkage and the P*(R)* was completely resistant to enzyme hydrolysis, even after prolonged incubation.[85] Shaw also reported the stereospecific enzymatic synthesis of the P*(S)* boranophosphate; synthesis of the analogous P*(R)* boranophosphate was not possible due to substrate specificity of the enzyme.[82,86,87]

Wada *et al.* recently reported the synthesis of the P*(R)* and P*(S)* boranophosphates with excellent diastereoselectivity using a chiral oxazaphospholidine and solid phase synthesis (Scheme 9). This synthesis has been carried out in solution and on solid support.[88]



Scheme 9. Stereoselective synthesis of boranophosphates.

1.4.2 Modified RNA

There are many examples of DNA modifications reported in the literature. Comparatively fewer have been reported for RNA although these are well reviewed.[26,89,90,91,92]

1.4.2.1 Backbone Modifications

Various modified backbone structures and their properties in siRNA have been investigated (Figure 10.). In comparison to the natural phosphodiester (**A**), in general modifications have increased nuclease resistance.[92] The phosphorothioate (**B**) and the boranophosphate (**C**) are two examples of phosphorus containing backbones. Positional changes of the phosphodiester linkage connecting to the sugar; the 2',5'-phosphodiester linkages (**D** and **E**) are tolerated in the sense strand on siRNAs but not the antisense strand.[93]

More diverse structures include capping the 5'- and 3'-termini with inverted deoxy sugars without a nucleoside base (**F**, **G**); increasing exonuclease resistance.[94] The 2-hydroxyethyl-phosphate (**H**) is also a successful end-cap when located at the 3'-end of the sense strand[95] and hydrophobic 5'-3' and 3'-5' amide backbones (**I**, **J**) are being investigated in RNAi.[96] Generally, chemical modification is better tolerated in the sense strand than the antisense strand of siRNA.[97]

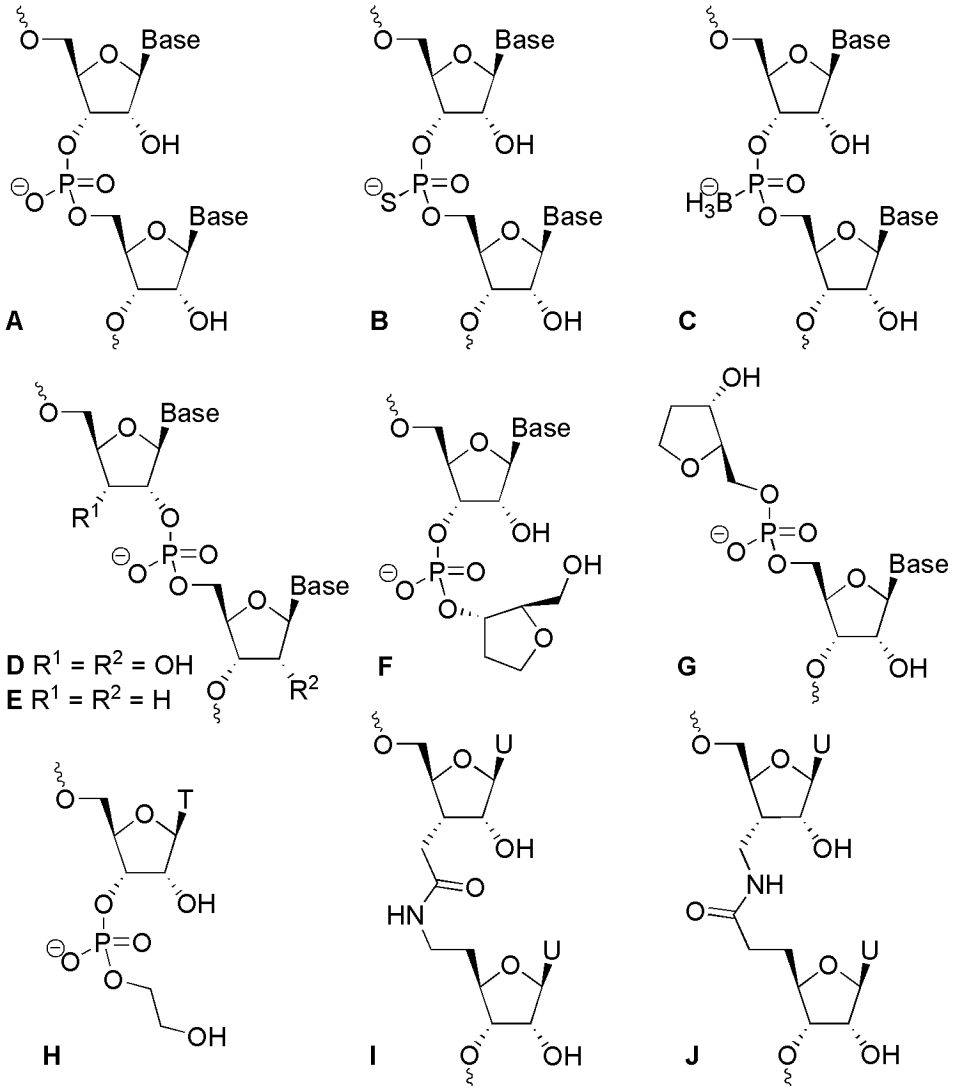
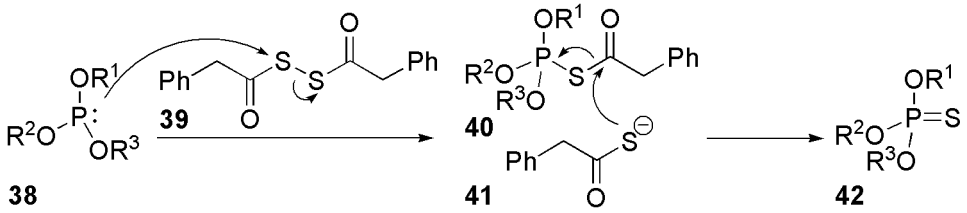


Figure 10. Backbone modifications of RNA.

Independent results published by Corey *et al.*[26] and Choi *et al.*[98] show that partial or complete incorporation of the phosphorothioate modification (**B**) to RNAi does not increase in nuclease resistance in human serum. Phosphorothioate oligonucleotides show slightly reduced binding affinities (*i.e.* the duplex is thermodynamically less stable); typically the T_m decreases by 0.5-0.8 °C per phosphorothioate modification within an oligomer.[99] This reduced binding affinity does not seem to have a detrimental effect on the potency of the siRNA.

Phosphorothioates have a tendency to exhibit non-specific binding to nucleic acids and binding to certain proteins which leads to cytotoxicity.[26] Different syntheses of phosphorothioate RNA have been reported in the literature; oligomers synthesised by Corey *et al.* were made using traditional solid-phase synthesis protocols on an automated ABI synthesiser, the phosphorothioate linkages were introduced by using 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (the Beaucage reagent) as the oxidiser rather than iodine.[26] Similarly, Ravikumar *et al.* have used a similar strategy and report a successful sulfurisation step using phenylacetyl disulfide in automated synthesis (Scheme 10.).[100]



Scheme 10.

Studies of boranophosphate siRNA analogues by Shaw *et al*[101,102] have used the same method of synthesis as they reported for the DNA series, and the P*(R)* diastereoisomers was isolated by chromatographic separation.[103] Although still negatively charged, these oligonucleotides have increased lipophilic properties and are more resistant to nuclease degradation than both the native phosphodiester and the phosphorothioate RNA.[102,104] Boranophosphate analogues show increased siRNA activity compared to both native siRNA and phosphorothioate siRNA and increased nuclease resistance unless the modification was present

in the middles of the antisense strand.[102] In addition the boranophosphate modification is non-toxic to cells.[102]

1.4.2.2 2'-Modifications

Modification at the 2'-position provides a solution to the non-specificity of natural phosphodiester and phosphorothioate oligonucleotides. The 2'-modifications are not tolerated in the RNase H mechanism because the steric bulk can alter the conformation of the antisense oligonucleotide, away from the DNA-like B-form conformation required to activate the RNase H mechanism. Steric bulk also affects whether an oligonucleotide is active in the RNAi mechanism if it distorts the structure away from the A-form conformation.

Research into RNA modifications has found that the 2'-OH is not necessary for RNAi however, the A-form helix is essential.[105] Electronegative elements at the 2'-position strongly stabilise the C3'-*endo* conformation of the ring. Complete modification of the oligonucleotide often leads to complete loss of siRNA activity due to reduced A-form character caused by steric bulk. Partial substitution however, can produce siRNA activity and increase nuclease resistance. Modifications also tend to exhibit positional effects relating to the siRNA activity of the oligomer.[106]

Deoxy residues, (**K**) are commonly used at the 3'-termini of siRNA strands; it is known that they are more resistant to nucleases, and

this contributes to increased stability of the siRNA (Figure 11).[106] As with most modifications however, the deoxy residues also result in decreased binding affinity.[106] The 2'-methoxy (**L**) and the 2'-deoxy-2'-fluoro (**M**) have been partially or wholly incorporated into siRNA oligomers and show increased stability in the plasma and increased binding due to stronger base pair interactions.[107] They are most effective when the gapmer strategy is used and modifications are located at the 3' and 5'-ends of the siRNA's and not in the middle. Fully modified strands of the 2'-methoxy (**L**) exhibit increased stability but complete loss of siRNA function due to deviation from the A-form. Partial substitution leads to comparable increases in stability as fully modified siRNAs but still active in RNAi pathway.[106] The modification is tolerated in both the sense and the antisense strand but in general the 3'-substitutions result in greater siRNA activity than 5'-substitutions.[106]

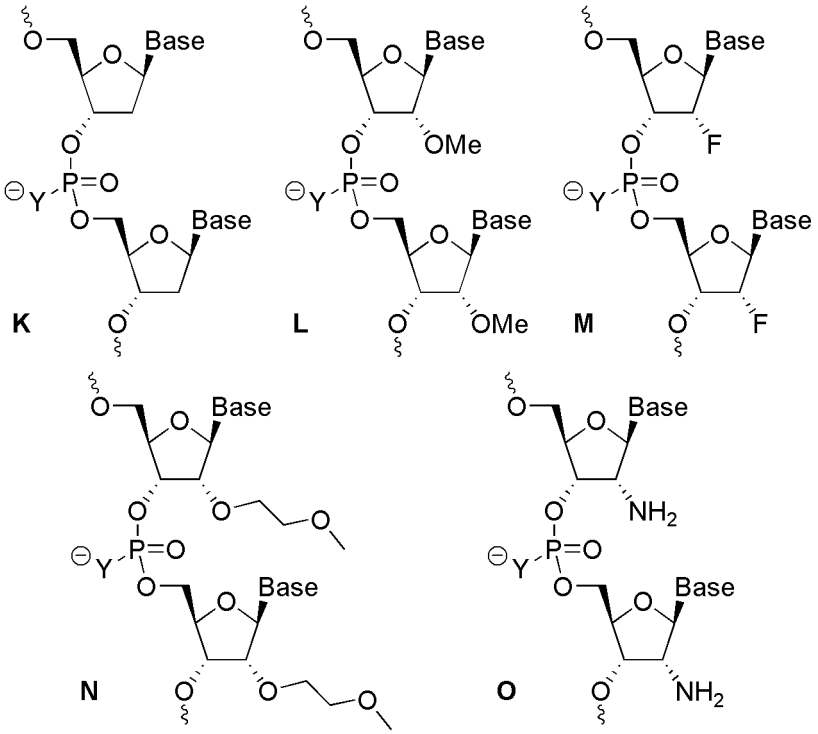


Figure 11. 2'-modifications used in RNAi. K. deoxyribose; L. methoxy; M. fluoro; N. methoxy ethyl (MOE); amino. Y = O or S.

The 2'-deoxy-2'-fluoro (**M**) has good thermodynamic stability, nuclease resistance and gene silencing comparable to non-modified sequences (both sense and antisense).[106] The bulkier the modification at the 2'-position, the less well tolerated it is in terms of RNAi activity. The 2'-methoxyethyl (**N**) is only tolerated in the sense strand; when located at 3'-end of the antisense strand significant loss of siRNA activity is observed.[106] The 2'-deoxy-2'-amino (**O**) is well tolerated in the sense and the antisense strand but has reduced siRNA activity.

1.4.2.3 Sugar Modifications and Locked Structures

Three types of sugar modification that have been applied to RNAi; the 2'-deoxy-2'-fluoro-arabinonucleotide (2'-araF, **P**) and the 4'-

thioribose (**Q**) are two examples (Figure 11). In 2'-araF (**P**), the stereochemistry of the 2'-F changes the sugar from being a ribose to an arabinose. In 4'-thioribose (**Q**) the ring oxygen is replaced by a sulphur atom. This modification has been incorporated into phosphodiester-linked RNA oligonucleotides.

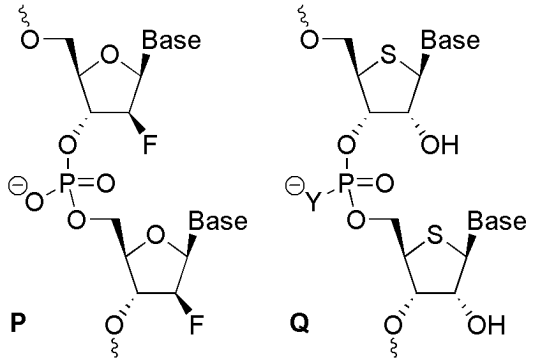
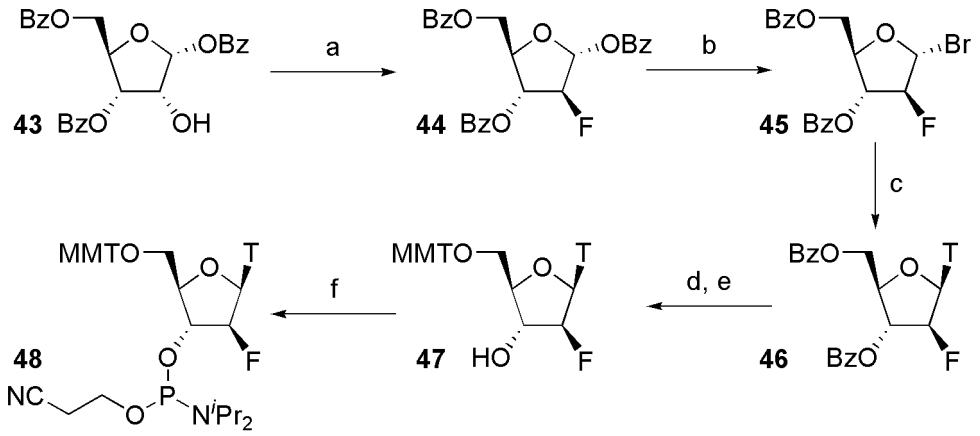


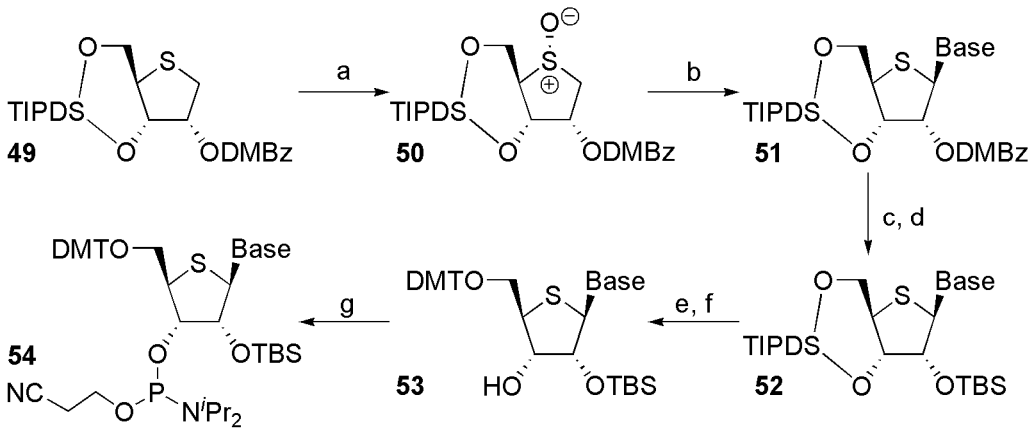
Figure 11. Modified sugar structures.

The 2'-araF thymidine phosphoramidite **48**, was synthesised in six steps from 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **43** (Scheme 11).[108] This was then used to make oligomers using the automated ABI synthesiser protocols.[108] The 2'-araF, **P**, has shown increased RNAi activity and stability in comparison to the unmodified siRNA. Fully modified 2'-araF sense strands and 2'-araF modifications at the 3'-end of the antisense strand are tolerated in the siRNA.[109] The 2-fluoro-D-arabinose raises the T_m of duplexes by ca. +0.5-1.0 °C/nt.[108,110]



Scheme 11. Reagents & conditions: a. DAST, CH_2Cl_2, 40 °C (71%); b. HBr, AcOH (97%); c. *bis*-silylated thymidine, CCl_4, reflux (48%); d. concentrated NH_4OH, MeOH (75%); e. MMTCl, pyridine, DMAP (94%); f. Cl-$P(OCH_2CH_2CN)(N^iPr_2)$, THF (93%).

The 4'-thio nucleosides were first reported in 1966 by Whistler *et al.*[111] Later, Matsuda *et al.* reported that 4'-thioribose RNA was approximately 600 times more stable than unmodified RNA.[112] Egli *et al.* obtained X-ray crystallographic data showing that the 4'-thio modification adopted a conformation very similar to the C3'-*endo* form of natural RNA; thus enabling good interaction with mRNA.[113] Mixed oligonucleotides containing combinations of 4'-thio, 2'-OMe and the 2'-methoxyethyl nucleosides been shown to be effective in siRNA.[114,115] Recently, Dande *et al.* have improved the synthesis of the 4'-thioribonucleosides and their phosphoramidites (Scheme 12.).[114] These oligomers are then assembled using automated oligonucleotide synthesis protocols.



Scheme 12. Reagents & conditions: a. diethyl L-tartrate, Ti(O^{i}Pr)$_4$, TBHP, CH_2Cl_2, -25 °C; b. TMSOTf, Et_3N, uracil, toluene/CH_2Cl_2 (1:1 v/v); c. $MeNH_2$, MeOH; d. TBSOTf, 2,6-lutidine, CH_2Cl_2; e. TBAF, AcOH, THF; f. DMT-Cl, pyridine; g. *N,N*-diisopropylmethyl-phosphonamidic chloride, DIPEA, CH_2Cl_2. TIPDS: tetraisopropyldisiloxane.

The third significant modification is the conformationally locked oligonucleotides of the one-carbon bridged locked nucleic acid (LNA), **R** and the two-carbon bridged ethylene nucleic acid (ENA), **S**, which significantly increase the thermodynamic stability of the duplex (Figure 12.).

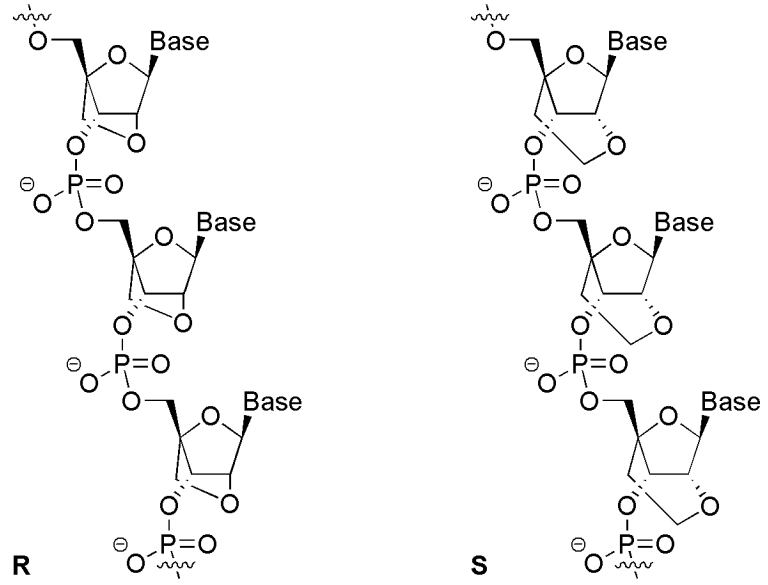
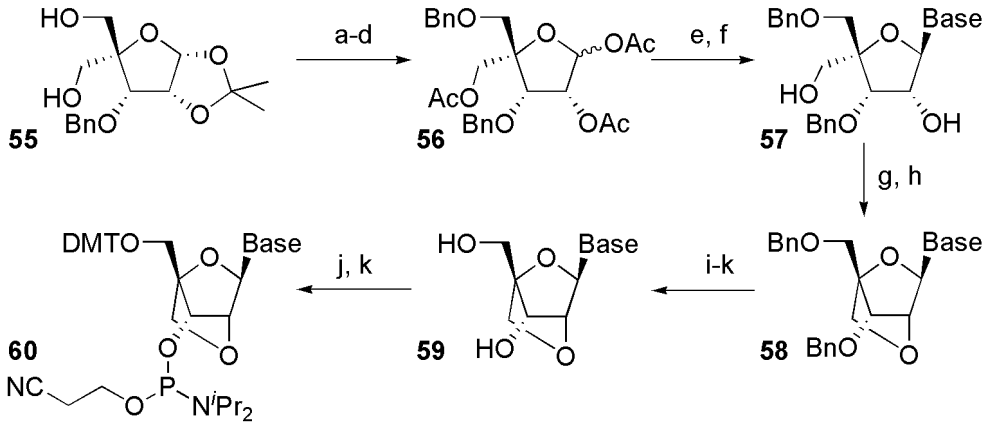


Figure 12. Conformationally locked structures: R. LNA; S. ENA.

Complete modification of the oligonucleotide results in total loss of siRNA activity but partial substitution results in significant increases in siRNA activity.[116] The LNA, **R** developed by Wengel is accepted at both ends of the siRNA, maintaining activity.[26] However when ENA residues are located at both ends of the siRNA, activity in the RNAi pathway is completely lost.[116] The application of modified LNA in the RNase H pathway is comparably efficient at gene knockdown as siRNA.[117]

Wengel *et al.* have achieved the synthesis of the five different nucleoside base monomers of LNA, **60** (Scheme 13)[118] and oligomers are constructed from these units using automated synthesis.[119]



Scheme 13. Reagents & conditions: a. BnBr, NaH, DMF; b. Ac_2O, pyridine; c. 80% AcOH; d. Ac_2O, pyridine (55%, steps a-d); e. nucleobase, BSA, TMSOTf, CH_3CN or CH_2ClCH_2Cl; f. $NaOCH_3$, MeOH (38-74% over steps e-f); g. TsCl, pyridine; h. NaH, DMF (30-51%, steps g-h); i. $Pd(OH)_2/C$, EtOH, H_2; or 1,4-cyclohexadiene, 10% $Pd(OH)_2/C$, MeOH; or BCl_3, CH_2Cl_2, hexane (36-98%); j. DMTCl, pyridine; k. $NCCH_2CH_2OP(Cl)N(^iPr)_2$, DIPEA, CH_2Cl_2, (37-65%, steps j-k).

Oligonucleotides containing LNA have increased T_m values of between 3 °C and 8 °C per LNA unit when binding to both RNA and DNA.[120] LNA adopts the A-type conformation, mimicking RNA in terms of its helical structure. Further modification of the LNA oligonucleotides have been made using the phosphorothioate backbone, the 2'-thio, replacing the 2'-oxygen with a sulphur[121] and the methylphosphonate backbone.[122] Other bridged nucleic acid structures have also been reported.[118]

1.5 Vinylphosphonate-Linked Nucleic Acids

With the knowledge that modified nucleic acids often have increased nuclease resistance, one backbone modification of interest is the vinylphosphonate linkage. This modification replaces the C5'-O5' with a carbon-carbon double bond which has a similar geometry to that of the native internucleotide phosphodiester linkage (Figure 13). The torsion angle, β is restricted to 180° but there is no additional steric bulk which may affect the conformation.

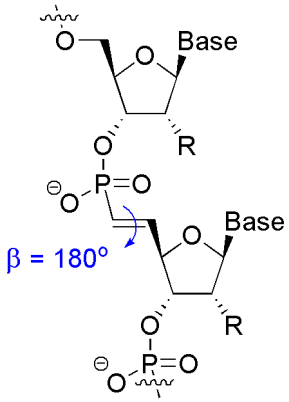
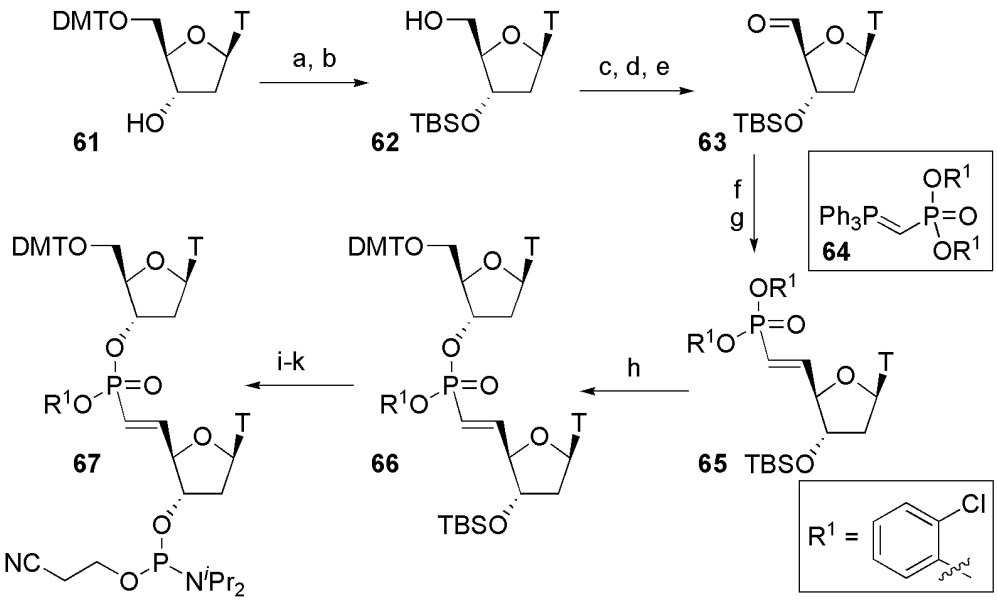


Figure 13. Vinylphosphonate linkage

The first vinylphosphonate-linked dinucleotide was reported by Zhao and Caruthers in 1996; synthesis of a vinylphosphonate-linked T*T dimer which was incorporated into oligonucleotides using solid phase synthesis.[123] The application of triphenylphosphoranylidene methylphosphonate **64**, in the synthesis of vinylphosphonates was first reported by Moffatt *et al.* in 1968.[123,124] The vinylphosphonate was installed using a Wittig reaction and the dinucleotide was then formed by the condensation of vinylphosphonate **65** with the 3'-OH of thymidine **61** (1.0 eq.) using triisopropylbenzenesulfonyl chloride (5.0 eq.) and 1-methyl imidazole (11 eq.). Successful coupling required manipulation of the protecting groups on the phosphorus to obtain thymidine dinucleotide **67** as a mixture of diastereoisomers at the phosphorus centre (Scheme 14.).



Scheme 14. Reagents & conditions: a. TBSOTf, pyridine (95%); b. 80% AcOH (82%); c. Pfitzner Moffatt conditions; d. imidazoline protection (93%); e. *p*TsOH; f. **64** (64%); g. 2-nitrobenzaldoxime, $HN=C(NMe_2)_2$, dioxane aq. (75%); h. **61**, 2,4,6-triisopropylbenzenesulfonyl chloride, 1-methylimidazole (61%, 1:1 mixture); i. 0.5 M HCl, MeOH (50%); j. DMTCl, $Bu_4N^+ClO_4^-$, pyridine (66%); k. 2-cyanoethylchlorophosphoramidite, DIPEA, THF (56%, 1:1:1:1 mixture).

The T*T dimer, **67** was incorporated into an oligomer, however, to be compatible with the solid-phase protocols, extensive protecting group manipulation was once again required. Studies showed that oligomers containing the vinylphosphonate linkage were readily soluble, and were sufficiently stable to tolerate storage over several months in aqueous media at pH 7.0.[123] Preliminary studies of the biological activity showed that the vinylphosphonate-linkage was considerably more resistant degradation by snake venom phosphodiesterase, even when only one vinylphosphonate modification was contained in a 14mer.[123] The initial studies also showed that the presence of the vinylphosphonate modification

reduced the T_m. An unmodified 14mer of all thymidine residues had a T_m of 36 °C however, a single modification in the centre of the oligomer had a T_m of 33 °C and the 14mer containing six modifications had a T_m of less than 20 °C.

More recently, the vinylphosphonate modification has since received detailed investigation through the work of Hayes *et al*.[125] It was first found that the presence of a single modification in a T*T dimer, incorporated into a DNA oligonucleotide was sufficient to inhibit the activity of PcrA helicase. This enzyme is non-specific DNA-binding and mostly targets single strand DNA.[126] It can also target double stranded DNA but with a significantly weaker interaction.[127] Studies indicated that rotational flexibility of the DNA was essential to allow the helicase to interact and process the single strand DNA. Due to the carbon-carbon double bond, the vinylphosphonate, while having similar geometry to the natural phosphodiester, has more limited rotational flexibility. In the process of translocation, ATP driven helicases unwind double stranded DNA and move along the single-stranded DNA. A vinylphosphonate modification on the backbone of the translocating strand inhibits the translocation. This suggests that the backbone must have a degree of rotational flexibility of the backbone to enable efficient unwinding. This finding has helped confirm that the helicase enzyme operates using an "inch-worm" translocation mechanism.

Hayes *et al.* also investigated other helicases; the Bloom (BLM) and Werner (WRN) syndrome proteins.[128] Positioning the vinylphosphonate on the translocating strand, just outside the duplex region, resulted in partial inhibition of the BLM-mediated unwinding of the 25 base pair duplex was observed. Positioning the vinylphosphonate modifications in the duplex region of the translocating strand the unwinding was more strongly inhibited. However, location of the vinylphosphonate modifications on the displaced strand had no inhibitory effect on the helicase. The human replication protein A (RPA) is known to specifically promote unwinding of long DNA duplexes by BLM and WRN. Using a 39 base pair forked duplex as the substrate, the presence of four consecutive modifications located inside the duplex on the translocating strand were compared to an unmodified version.[128]

In the absence of the RPA, the BLM helicase could only partially unwind the duplex in the modified version, however in the presence of the RPA, both the modified and the unmodified duplexes were completely unwound, indicating that the RPA reduced the inhibitory effect of the vinylphosphonate modification (Figure 14).[128]

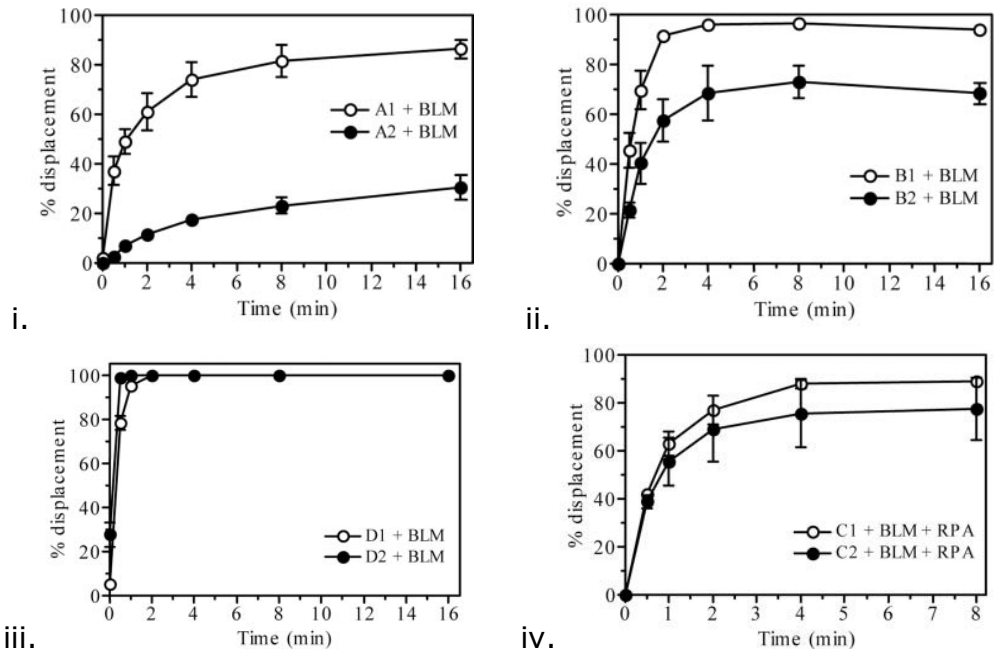


Figure 14. Kinetics of unwinding of i. A1 (unmodified), A2 (modified); ii. B1 (unmodified), B2 (modified); iii. D1 (unmodified) and D2 (modified) strands (1.0 nM concentration) by BLM (20 nM) and iv. C1 (unmodified), C2 (modified) by 20 nM BLM in the presence of 24 nM RPA.

The vinylphosphonate had a similar effect to the BLM on the WRN helicase. Location of vinylphosphonate modifications in the duplex region of the oligonucleotide, resulted in translocation inhibition, however, outside the duplex region, unwinding was observed as being more facile in the presence of the vinylphosphonate than in the non-modified version (Figure 15). Once again, the inhibition of the helicase unwinding activity was removed upon addition of the RPA. In comparison to the effect of RPA, when the BLM and WRN were inhibited by the presence of the vinylphosphonate, addition of the single-stranded DNA binding protein from *E. coli* (SSB) only partially restored helicase action.

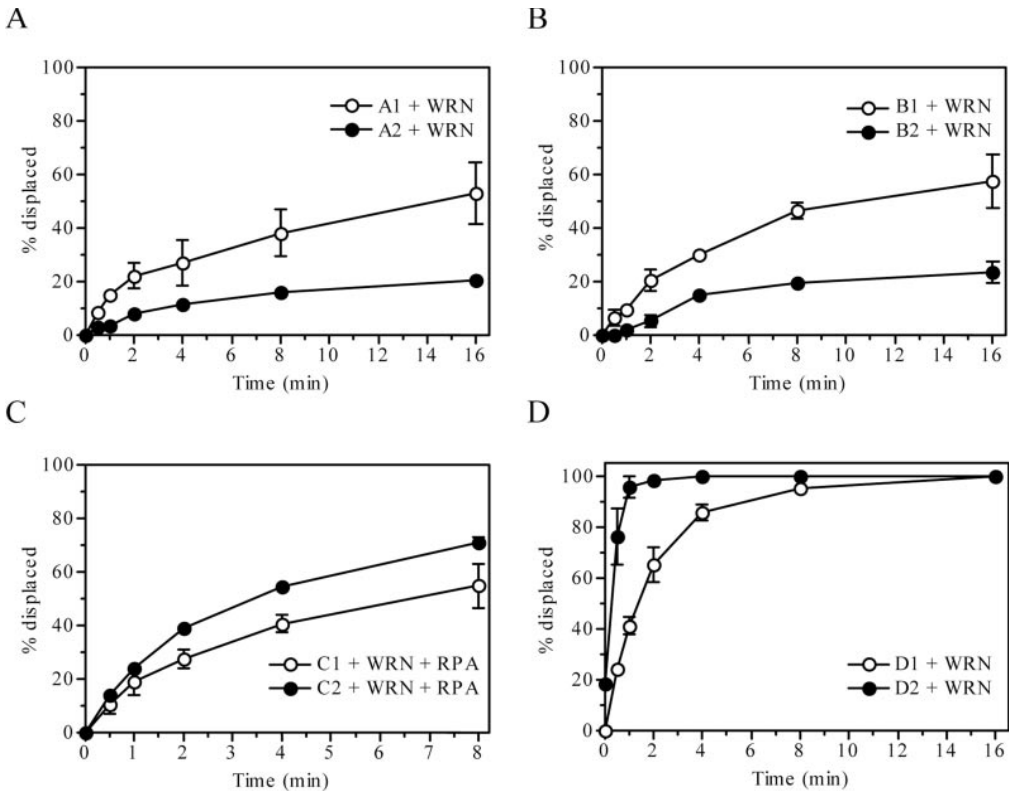


Figure 15. Effect of vinylphosphonate internucleotide linkages on WRN helicase activity in the absence and the presence of RPA. (A) Kinetics of unwinding of 1 nM A1 and 1 nM A2 by 5 nM WRN. (B) Kinetics of unwinding of 1 nM B1 and 1 nM B2 by 5 nM WRN. (C) Kinetics of unwinding of 1nM C1 and 1nM C2 by 5 nM WRN in the presence of 24nM RPA. (D) Kinetics of unwinding of 1 nM D1 and 1 nM D2 by 5 nM WRN. (Relative concentration of products expressed as % of total DNA).

A nuclease resistance study was used to establish whether the vinylphosphonate modification could be used in antisense applications.[129,130] The nucleases studied were the commercially availably exonuclease III, a dsDNA specific nuclease and mung bean nuclease, which acts on ssDNA and as an RNA endonuclease. The exonuclease cleaves stepwise from the 3'-end of dsDNA, cleaving at the P-$O^{3'}$ position however, the results showed that when the exonuclease III encountered the vinylphosphonate

modification, the progress was slowed and cleavage also occurred at the P-O$^{5'}$ position, altering the pattern of usual cleavage. The vinylphosphonate-linkage also exhibited partial resistance to the mung bean nuclease, however only cleavage at the usual P-O$^{3'}$ position was observed.

A single vinylphosphonate modification in an oligonucleotide was not sufficient to inhibit the action of DNA polymerase I but the incorporation of four modifications did successfully inhibit the polymerase.[130] DNA polymerase plays an important role in DNA replication, building new DNA strands alongside the existing complementary strand. Hence it is possible that DNA polymerase could be used to introduce vinylphosphonate linkages into DNA.

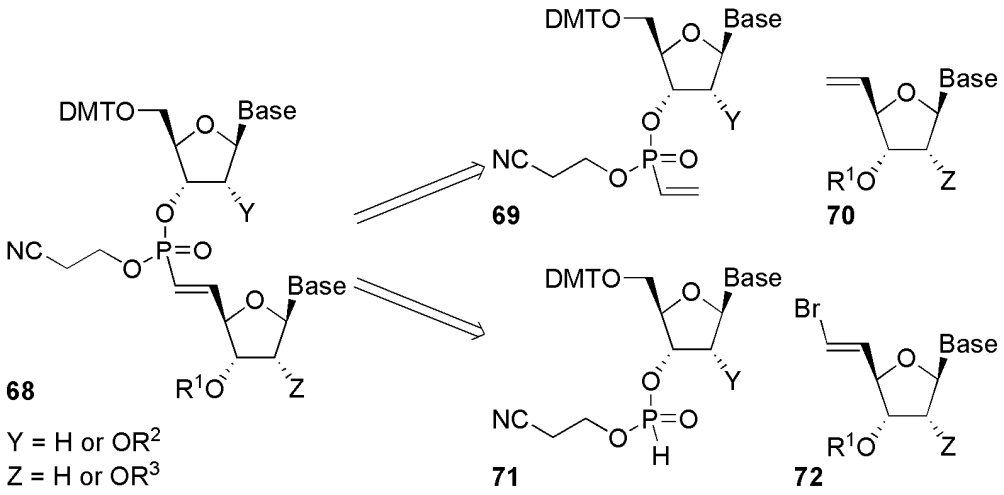
The above mentioned biological results indicate that the vinylphosphonate modification can inhibit a range of enzymes involved in the processing of nucleic acids. There is evidence of nuclease resistant although, like many modifications discussed, the binding affinity is slightly weaker than the non-modified version. In order to investigate this further it is first necessary to investigate the synthesis of the vinylphosphonates and their assembly into oligonucleotides.

1.6 The Synthesis of Vinylphosphonates

Within the literature there are many examples of different synthetic strategies used to make vinylphosphonates. There are

both catalytic and non-catalytic examples using different synthetic strategies and these have been extensively reviewed.[131,132,133]

While exploring the synthesis of vinylphosphonate-linked nucleic acids **68**, the Hayes group has investigated two different catalytic strategies. Firstly, formation of the carbon-carbon double bond by the cross metathesis of a vinylphosphonate **69** and a terminal olefin **70**. Secondly, palladium-catalysed carbon-phosphorus bond formation between an *H*-phosphonate **71** and a vinyl halide **72**. Both these strategies require the synthesis of the two building blocks that are then assembled using the appropriate method (Scheme 15).

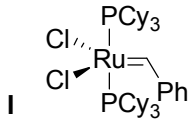


Scheme 15. Retrosynthetic analysis of the vinylphosphonate modification.

1.6.1 Carbon-Carbon Bond formation – Metathesis

Recently, cross metathesis has provided a method for the synthesis of functionalised vinylphosphonates.[134] Grubbs *et al.* reported that olefin cross-metathesis proceeded with excellent *E/Z* selectivity and in high yields.[135] Hanson reported the use of the

first generation Grubbs catalyst **I** in the synthesis of cyclic vinylphosphonates (Table 1.).[136]



I

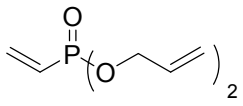
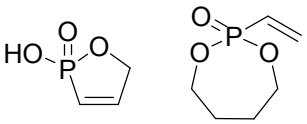
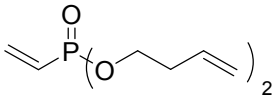
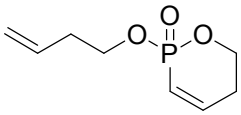
Substrate	Product(s)	Conditions	Yield/%
		6 mol%, 6 hours	44, 31
		3 mol%, 2 hours	79

Table 1.

The second generation Grubbs catalyst **II**, has been reported to give superior results compared to the first generation catalyst. A comparative study reported by van Boom *et al.* showing that the second generation catalyst gave significantly higher yields in a shorter time and at a lower catalyst loading (Table 2).[137]

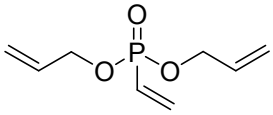
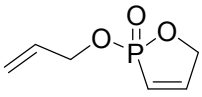
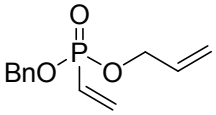
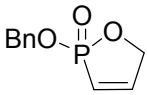
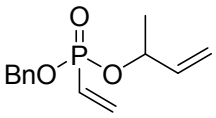
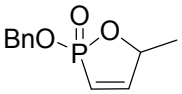
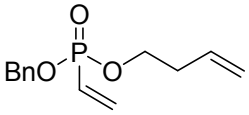
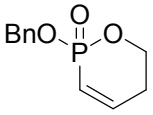
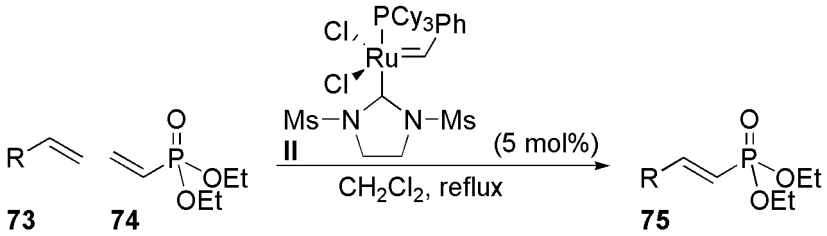
Substrate	Product	Grubbs I Yield/%	Grubbs II Yield/%
		44 (6 hours)	100 (30 min)
		25 (4 days)	100 (15 min)
		65 (2 days)	92 (30 min)
		85 (4 days)	100 (20 min)

Table 2. Comparison of the different Grubbs catalysts. Conditions: 0.01 mol substrate, CH_2Cl_2, reflux; catalyst: Grubbs I (2 mol%) or Grubbs II (1 mol%).

Grubbs *et al.* reported the synthesis of alkyl and aryl vinylphosphonates **75** using the second generation catalyst **II** (Scheme 16, Table 3.).[138]



Scheme 16.

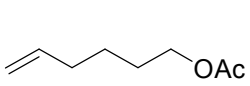
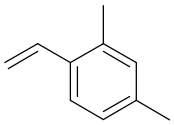
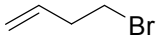
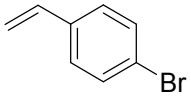
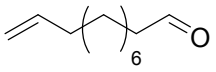
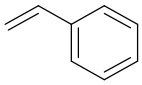
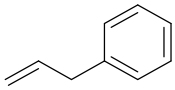
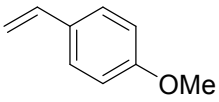
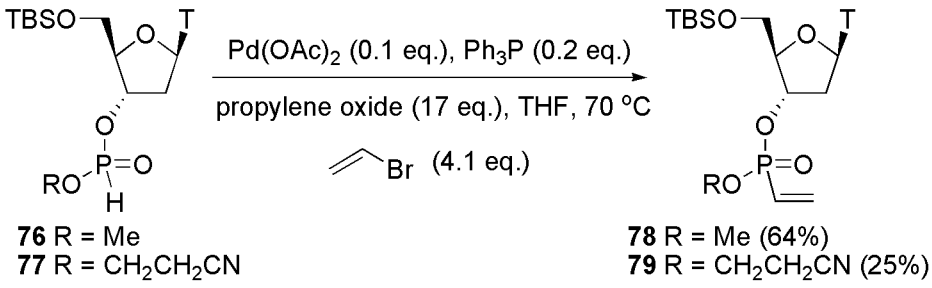
Alkene 73	Yield/%	Alkene	Yield/%
	95		93
	82		77
	88		97
	90		97

Table 3.

1.6.1.1 Previous work – Synthesis of the cross-metathesis building blocks

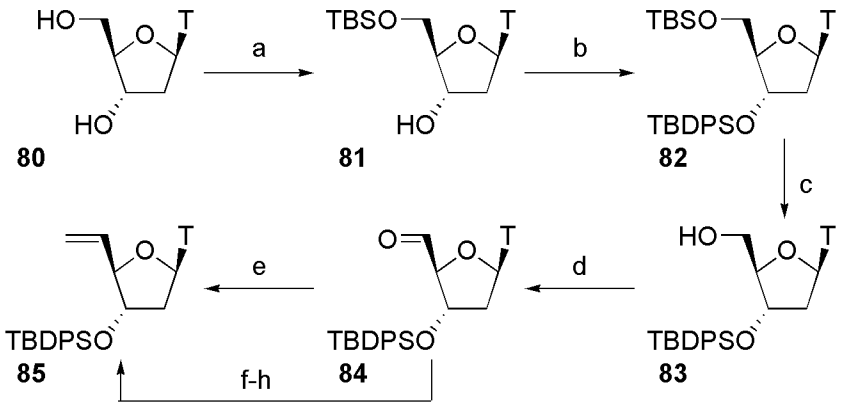
Using metathesis the synthesis of vinylphosphonate-linked nucleic acids, vinylphosphonate building blocks, **73** and **74** were synthesised from the *H*-phosphonates **71** and **72** respectively as a mixture of diastereoisomers using a palladium cross-coupling (Scheme 17).



Scheme 17.

The thymidine terminal olefin nucleoside **85** was accessed from the commercially available thymidine **80** using a Wittig reaction

(Scheme 18).[139] The disappointing yield of the direct Wittig reaction (step e) led Solesbury to investigate the synthesis *via* the vinyl bromide (f-h).[140]

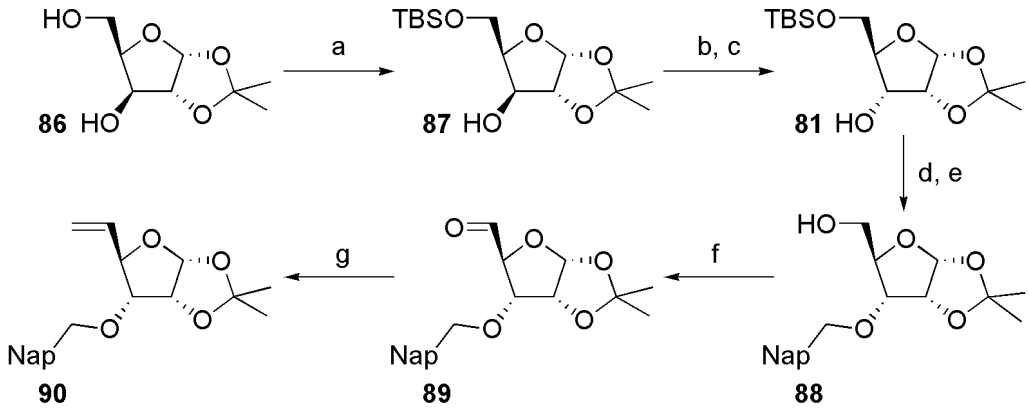


Scheme 18. Reagents & conditions: a. TBSCl (1.3 eq.), imidazole (1.5 eq.), DMF, 0 °C to r.t. (98%); b. TBDPSCl (1.3 eq.), imidazole (1.5 eq.), DMF, 0 °C to r.t. (98%); c. HF aq. (12 eq.), MeCN, r.t. (90%); d. Dess Martin periodinane, (1.2 eq.), CH_2Cl_2, r.t. (83%); e. $Ph_3PCH_3^+Br^-$ (4.0 eq.), *n*BuLi (4.0 eq.), THF, 0 °C to r.t. (24%); f. CBr_4 (2.0 eq.), Ph_3P (4.0 eq.), CH_2Cl_2, 0 °C to r.t. (51%); g. $(MeO)_2P(O)H$ (4.0 eq.), Et_3N (4.5 eq.), DMF, r.t. (83%, *E/Z* 2.4:1); nBu_3SnH (1.6 eq.), $Pd(PPh_3)_4$ (0.04 eq.), C_6H_6, 75 °C (22%).

The disappointing low yield of the Wittig reaction was attributed to the acidic protons of the thymidine and the 4'-H adjacent to the aldehyde; which can be abstracted in basic conditions. Investigation of KHMDS and *t*BuOK bases failed to improve this yield any further. Lera[139] and Solesbury[140] both investigated the methylenation reaction using the Tebbe ($Cp_2TiCH_2ClAl(CH_3)_2$)[141] and the Nysted (cyclo-dibromodi-μ-methylene[μ-(tetrahydro-furan)]trizinc) reagents. The Tebbe reagent gave a slightly higher yield of olefin **85** (37%) whereas using the Nysted, the reaction failed to go to completion. Unfortunately, the purification of these

reaction mixtures proved problematic so the Wittig strategy was kept.

Due to the low yield of the terminal olefin obtained, Solesbury investigated accessing the terminal olefin from a generic sugar. It was anticipated the starting from 1,2-isopropylidene-α-D-xylofuranose **86**, olefination could be performed prior to addition of the nucleoside base, of which the acidic N*H* proton is thought to interfere with the Wittig reaction. Olefin **90** was obtained in seven steps, requiring inversion of stereochemistry at the 3-position (Scheme 19). Wittig olefination of aldehyde **89** using methyltriphenylphosphonium bromide and butyl lithium produced olefin **90** in a disappointing yield (27% over 2 steps).

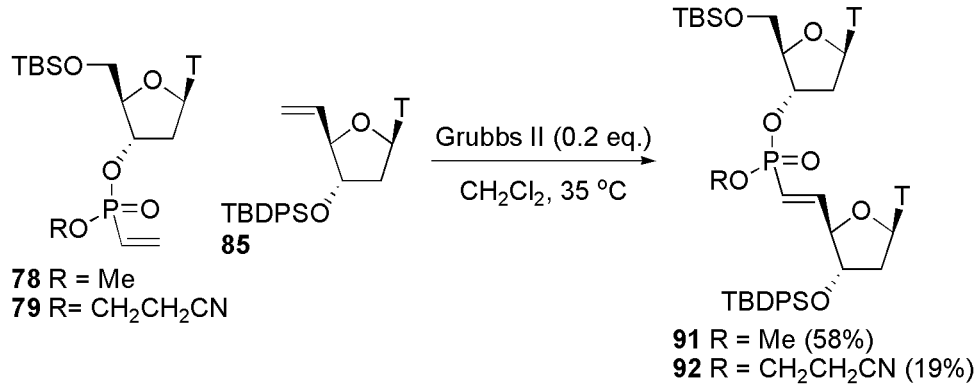


Scheme 19. Reagents & conditions: a. TBSCl (1.4 eq.), imidazole (2.5 eq.), DMF, 0 °C to r.t. (92%); b. $(ClCO)_2$ (1.1 eq.), DMSO (2.2 eq.), Et_3N (3.3 eq.), -65 °C to r.t. (85%); c. $NaBH_4$ (1.0 eq.), MeOH, 0 °C, (99%); d. $NapCH_2Br$ (1.2 eq.), KOH (1.8 eq.), 18-crown-6 (0.04 eq.), THF, r.t. (82%); e. TBAF, THF, r.t. (86%); f. Dess Martin periodinane (1.4 eq.), CH_2Cl_2, r.t.; g. CH_3PPh_3Br (4.0 eq.), *n*BuLi (4.0 eq.), THF, 0 °C to r.t. (27%, 2 steps).

Due to the low yield observed in the Wittig reaction, the Peterson reaction and application of the Tebbe, Nysted and Petasis (Cp_2TiMe_2)[142] reagents were once again explored as alternative methods of forming the terminal olefin however there was no improvement in the yield of the olefin.

1.6.1.2 Previous work – Synthesis of vinylphosphonate-linked nucleic acids by cross-metathesis

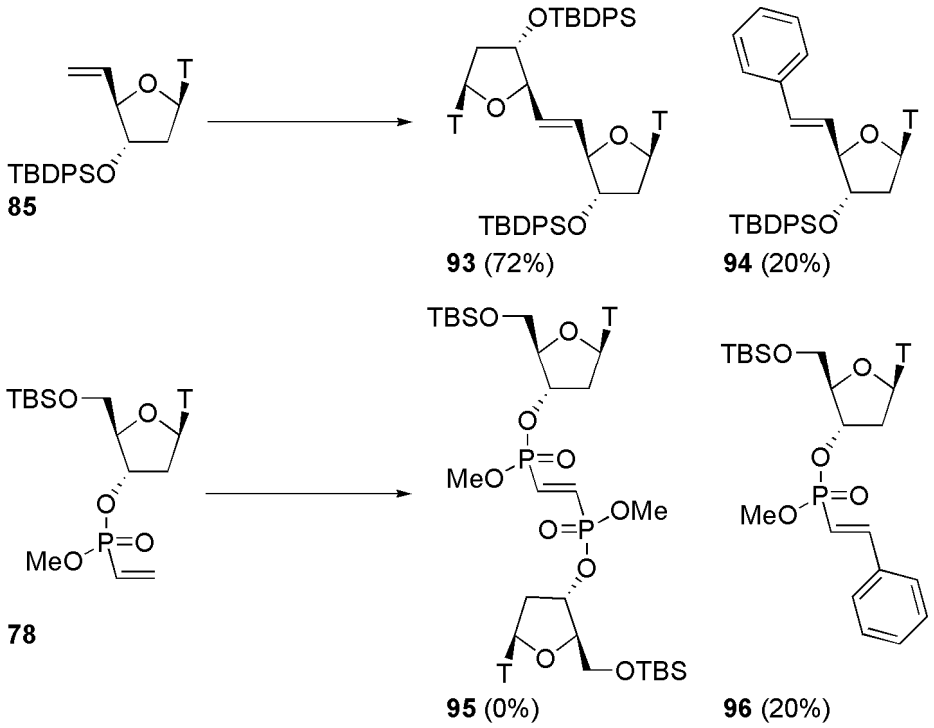
Synthesis of the dinucleotide using cross-metathesis reaction was first tried using the first generation Grubbs catalyst **I**; however, only unreacted starting material was recovered. The more electron rich second generation Grubbs catalyst **II** led to the formation of the desired *trans*-vinylphosphonate dinucleotides **91** and **92** in 58% and 19% yields respectively with no trace of the *cis*-vinylphosphonate (Scheme 20).



Scheme 20.

In the metathesis reaction, it is possible for both olefin components to undergo self-metathesis to form homo-dimerised products. A

study by Lera found that terminal olefin **85** underwent self metathesis, forming the homo-dimer **93** (72%) and the byproduct of benzylidene transfer from the catalyst **94** (20%); present in a quantitative amount in relation to the amount of catalyst used (Scheme 21). Vinylphosphonate **78** did not undergo homo-dimerisation to form **95** under the same conditions; however the quantitative byproduct of benzylidine transfer **96** was observed (20%).

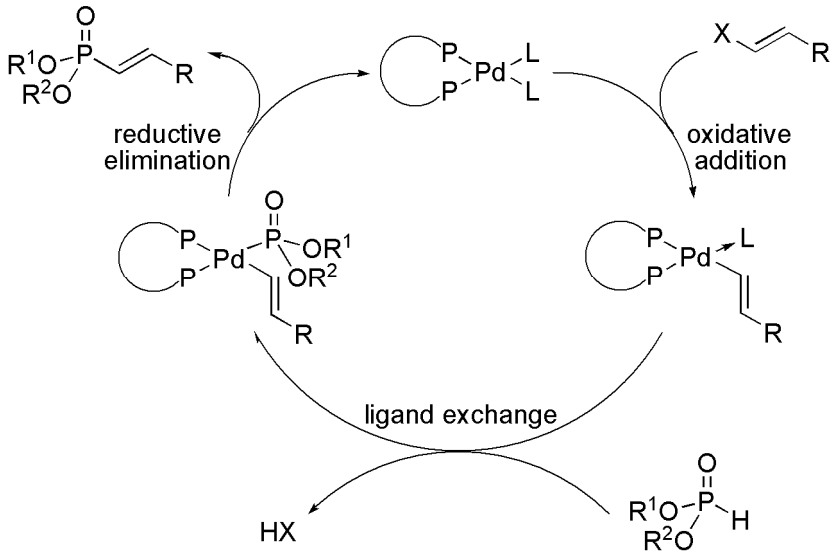


Scheme 21. Reagents & conditions: Grubbs II (0.2 eq.), CH_2Cl_2, 35 °C.

1.6.2 Carbon-Phosphorus Bond Formation – Pd(0)

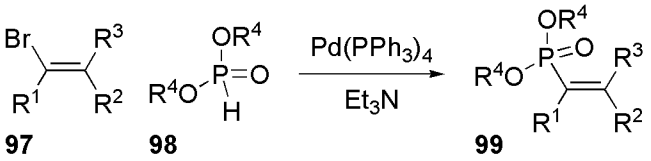
The seminal work in carbon-phosphorus bond formation by palladium catalysed cross-coupling reactions was carried out by Hirao *et al*[143] and the use of palladium chemistry to form carbon-phosphorus bonds is well reviewed.[144]

The catalytic cycle involved in the carbon-phosphorus bond-forming reaction involves the oxidative addition of the Pd(0) with the halide, the nucleophilic phosphorus species then attacks the organopalladium complex, eliminating the HX which is removed from the reaction by a scavenger (typically an amine base). Finally, the cross-coupled product is eliminated by the reductive elimination which regenerates the Pd(0) species (Scheme 22). The C-P bond formation occurs with complete retention of stereochemistry of the vinyl moiety.



Scheme 22. Catalytic cycle for the Pd(0) mediated cross-coupling reaction.

Hirao reported the coupling of arylbromides[145] and vinylbromides[146] with dialkyl phosphites leading to the dialkyl aryl phosphonates and dialkyl vinylphosphonates. Reaction of vinyl bromide **97** (1.1 eq.) with dialkylphosphite **98** (1.0 eq.) using catalytic $Pd(PPh_3)_4$ (5 mol%) and Et_3N (1.1 eq.) at 90 °C, obtained the vinylphosphonate **99** in good yield (Scheme 23, Table 4).[146]



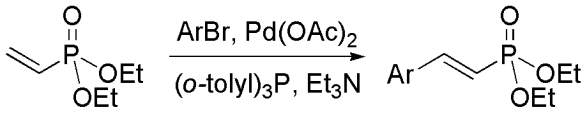
97 **98** **99**

Scheme 23.

R^1	R^2	R^3	R^4	Yield/%
Ph	H	H	Et	66
H	Ph	H	Et	93
H	H	Ph	Et	91
H	H	Ph	*i*Pr	81
H	H	Ph	*n*Bu	92
H	H	Me	Et	98
H	H	Me	*i*Pr	92

Table 4.

Xu *et al.* prepared (2-arylvinyl)phosphonate **101** from diethyl vinylphosphonate **100** and aryl bromides using $Pd(OAc)_2$ (1 mol%) and a phosphine ligand (2 mol%) to generate the Pd(0) *in situ* (Scheme 24, Table 5).[147] The products obtained were exclusively (*E*)-diethyl 2 arylvinylphosphonates in yields of 60-78%. This work also shows that simple vinylphosphonates can be further elaborated to more complex vinylphosphonates using the Heck reaction.



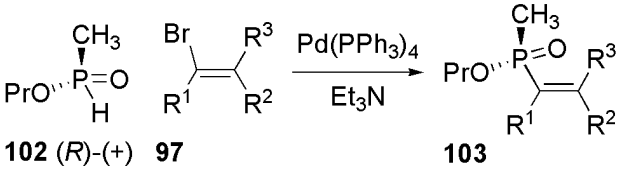
100 **101**

Scheme 24.

Ar	Yield/%	Ar	Yield/%
Ph	78	2-Nap	61
p-Me-Ph	72	*p*-CHO-Ph	60
o-Me-Ph	67	*p*-NO_2-Ph	63
p-MeO-Ph	76	*p*-CH_3CONH-Ph	62
p-Me_2N-Ph	74	Ph-Ph	74

Table 5.

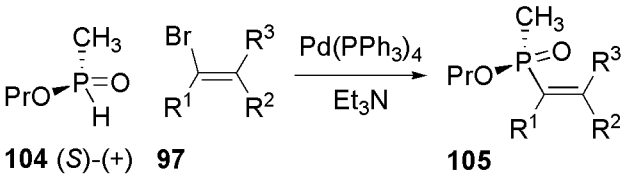
Xu *et al.* also showed that not only is the vinyl moiety stereochemistry retained, but there is also retention of stereochemistry (>97% e.e.) at the phosphorus when enantiomerically pure phosphinates **102** and **104** (>97% e.e.) was coupled with a vinyl bromide (Scheme 25, Scheme 26).[148]



102 (*R*)-(+) **97** **103**

R^1	R^2	R^3	Yield/%
H	H	H	64
Me	H	H	71
H	Ph	H	98

Scheme 25.



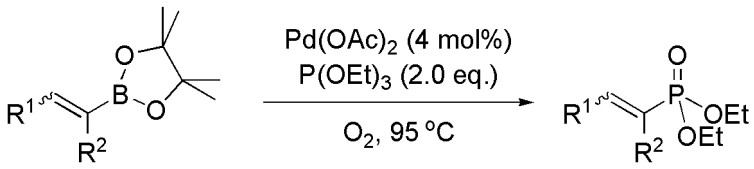
104 (*S*)-(+) **97** **105**

R^1	R^2	R^3	Yield/%
H	H	H	71
H	Ph	H	98
H	H	Ph	98

Scheme 26.

The stereoselective reaction of trialkylphosphites and vinylboronates in an oxygen atmosphere can be catalysed to obtain

vinylphosphonates in high yield and excellent selectivity (Scheme 27, Table 6).[149]



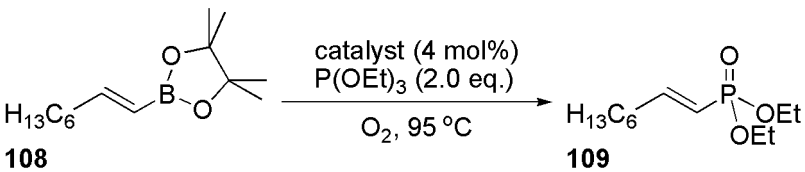
106 **107**

Scheme 27.

R^1	R^2	Time	Yield/%
Ph	H	9	78
Ph	H	7	75
p-Cl-Ph	H	9	79
p-Cl-Ph	H	6	60
n-C_6H_{13}	H	8	84
n-C_6H_{13}	H	6	63
p-F-Ph	H	9	81
Ph	Me	120	55

Table 6.

Other catalysts were also investigated in this reaction including $PdCl_2$, $NiCl_2$, $Pd(OAc)_2$-PPh_3 and $Pd(dppf)Cl_2$ but the $Pd(OAc)_2$ was shown to give the highest yields (Scheme 28, Table 7).



108 **109**

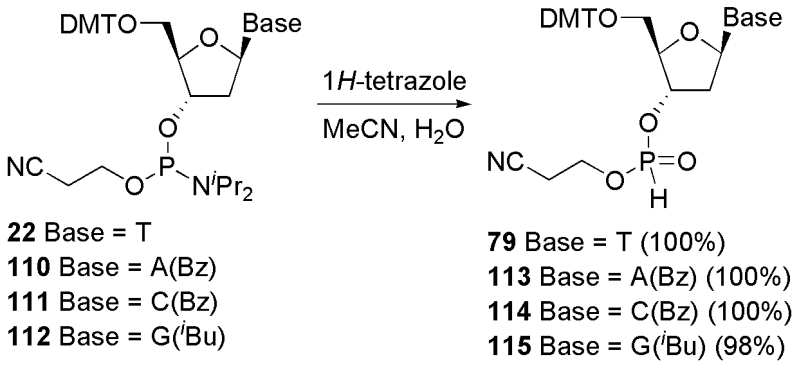
Scheme 28.

Catalyst	Time/hours	Yield/%
$Pd(OAc)_2$	8	85
$PdCl_2$	8	75
$NiCl_2$ (anhydrous) 6 mol%	25	43
$Pd(OAc)_2$, PPh_3 (4.0 eq.)	12	55
$Pd(dppf)Cl_2$	40	incomplete

Table 7.

1.6.2.1 Previous work – Synthesis of the Pd(0) coupling building blocks

The *H*-phosphonate building blocks **79**, **113**, **114** and **115**, used in the palladium catalysed cross-coupling reaction were obtained in a single step as mixtures of diastereoisomer from the commercially available phosphoramidites (**22**, **110**, **111** and **112** respectively) used in solid phase oligonucleotide synthesis (Scheme 29). The phosphoramidites were also synthesised from the commercially available 5'-ODMT-3'-OH nucleosides and the phosphoramidite using literature procedures.[129,150,151,152]

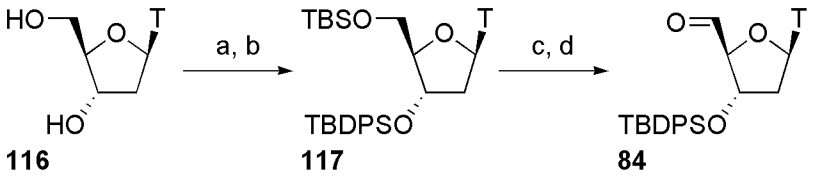


22 Base = T
110 Base = A(Bz)
111 Base = C(Bz)
112 Base = G(^{i}Bu)

79 Base = T (100%)
113 Base = A(Bz) (100%)
114 Base = C(Bz) (100%)
115 Base = G(^{i}Bu) (98%)

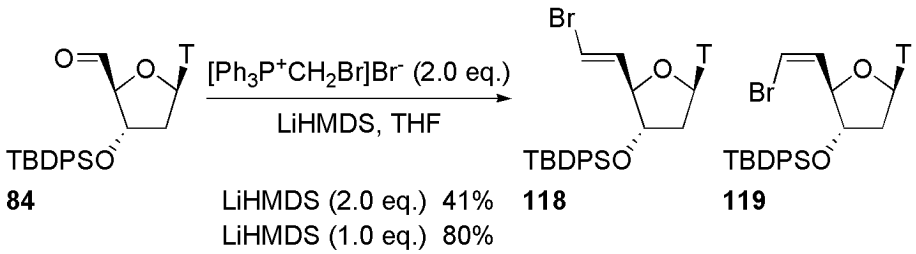
Scheme 29.

Initial studies involved the synthesis of the vinyl bromide nucleoside from the commercially available nucleoside **116**. Initially focussing on thymidine, aldehyde **118**, was obtained in four steps (Scheme 30).[154]



Scheme 30. Reagents & conditions: a. TBSCl, imidazole, DMF (86%); b. TBDPSCl, imidazole, DMF (90%); c. HF, MeCN (88%); d. Dess Martin periodinane, CH_2Cl_2 (80%).

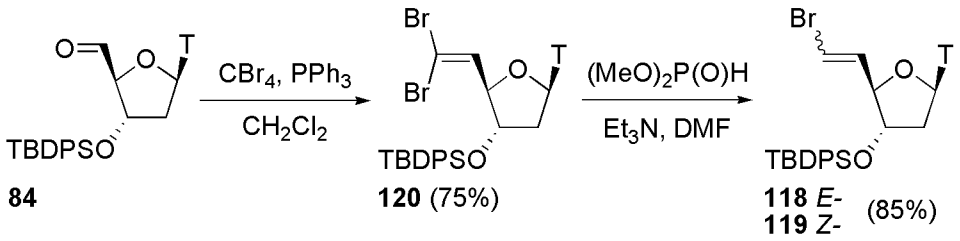
The Wittig reaction obtained a mixture of the *E*- and *Z*-vinyl bromides **118** and **119**. Bromomethyl(triphenylphosphonium)-bromide and LiHMDS generates a non-stabilised ylide; leading to the desired *E*-isomer forming as the minor product (Scheme 31).[154,125]



Scheme 31.

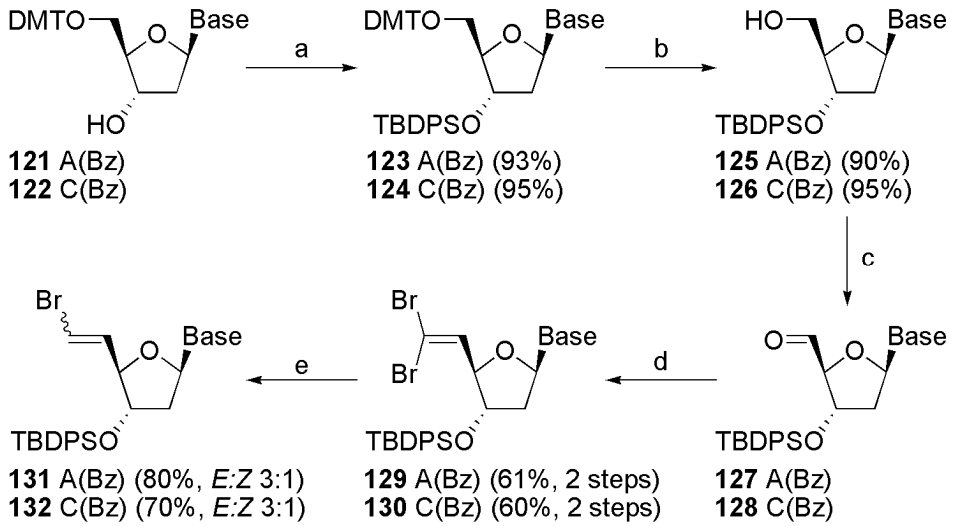
Since *E*-vinyl bromide **118** is the desired isomer, the two-step synthesis of the vinyl bromide from the aldehyde was investigated by Abbas.[156,154] Dibromo olefin **120** was synthesised using the procedure of Ramirez *et al.*[153] and was then reduced stereoselectively using the method of Hirao *et al.*[143] (Scheme 32).

This two step process obtained the vinyl bromide as a 3:1 mixture of *E*- and *Z*-isomers **118** and **119** respectively in a combined 84% yield. The *E*- and *Z*- isomers were readily separated by chromatography.



Scheme 32.

Later, Bertram investigated the synthesis of the other nucleoside vinyl bromides. Adenosine and cytosine dibromo olefins **129** and **130** were synthesised in four steps from the commercially available nucleosides **121** and **122**, using the same methodology. However, unlike the thymidine, the *E*- and *Z*- isomers of the adenosine and cytosine vinyl bromides **131** and **132** could not be separated (Scheme 33).

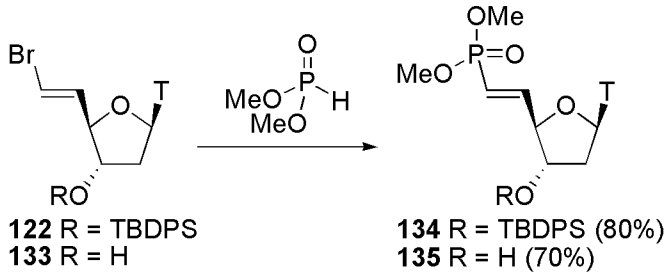


Scheme 33. Reagents & conditions: a. TBDPSCl, imidazole, DMF; b. 70% AcOH, THF; c. Dess Martin periodinane, CH_2Cl_2, r.t.; d. $[Ph_3P^+CHBr_2]Br^-$, KHMDS, CH_2Cl_2, r.t.; e. $(MeO)_2P(O)H$, Et_3N, DMF.

Encouragingly, this work shows that the vinyl bromide nucleosides can be accessed in five steps from the commercially available nucleosides.

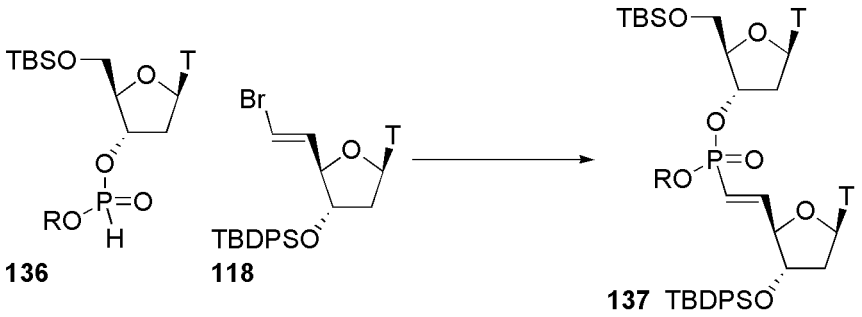
1.6.2.2 Previous work – Synthesis of vinylphosphonate-linked nucleic acids by Pd(0) cross-coupling

Bertram and Abbas have successfully demonstrated the application of palladium catalysis in the synthesis of the vinylphosphonate-linked DNA.[125,129,139,154,155,157] The synthesis of the vinylphosphonate using the methodology of Hirao obtained the 5'-vinylphosphonate (Scheme 34).[156,139]



Scheme 34. Reagents & conditions: R=TBDPS: $Pd(PPh_3)_4$, Et_3N, DMF, 70 °C;[154,156] R=H: $Pd(OAc)_2$, PPh_3, propylene oxide, THF, 70 °C.[139]

Using the palladium catalysed cross-coupling procedure, the thymidine vinylphosphonate dinucleotide was synthesised. It was found that the choice of catalyst, HBr scavenger and solvent had a strong effect on the reaction but it is seen that the reaction tolerates different functional groups (Scheme 35, Table 8).[157] The use of the sterically crowded *bis*(diphenylphosphino)ferrocene ligand greatly accelerated the reaction and the use of THF as the solvent removed the requirement of an aqueous work up procedure.[154]



Scheme 35.

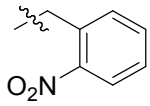
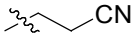
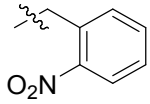
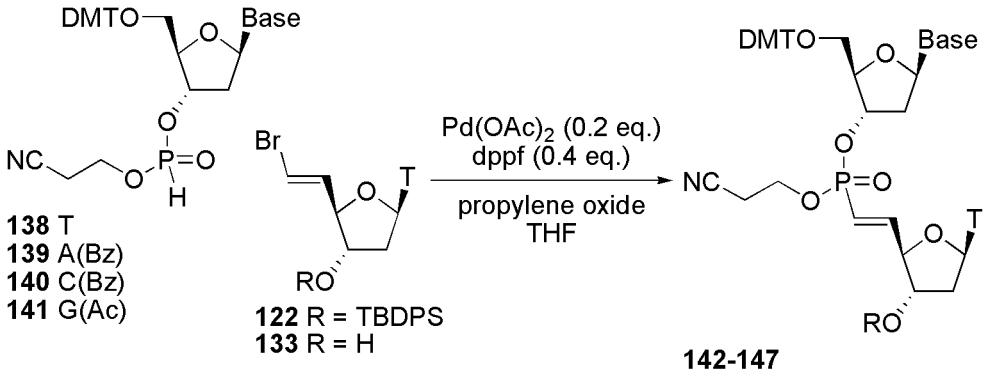
R	Catalyst	Ligand	Solvent	Scavenger	Yield/%
Me	$Pd(PPh_3)_4$	-	DMF	Et_3N	42
Me	$Pd(OAc)_2$	dppf	THF	Et_3N	78
Ph	$Pd(OAc)_2$	dppf	THF	Et_3N	21
	$Pd(OAc)_2$	dppf	THF	Et_3N	24
	$Pd(OAc)_2$	dppf	THF		72
Me	$Pd(OAc)_2$	dppf	THF		60
Ph	$Pd(OAc)_2$	dppf	THF		53
	$Pd(OAc)_2$	dppf	THF		65

Table 8. Conditions: Catalyst (0.02 eq.), ligand (0.04 eq.), Et_3N (4.5 eq.) or propylene oxide (1.2 eq.).

The palladium cross-coupling reaction was also applied to a system containing a thymidine vinyl bromide and the 5'-TBS *N*-6-benzoyl adenosine *H*-phosphonate using the same strategy.[157] The formation of the A*T dinucleotide was successfully carried out in a 92% yield indicating that the palladium chemistry is applicable to purines and pyrimidines. Later, Bertram utilised the dimethoxytrityl protecting group at the 5'-position of the *H*-phosphonate, thus being compatible with the protecting groups used in the automated solid phase synthesis protocols for oligonucleotides. The four different DNA *H*-phosphonate nucleosides were coupled to a thymidine vinyl bromide in good

yield (Scheme 36, Table 9). Pleasingly the coupling reaction was also compatible with a free hydroxyl group on the vinyl bromide and no loss of yield was observed.

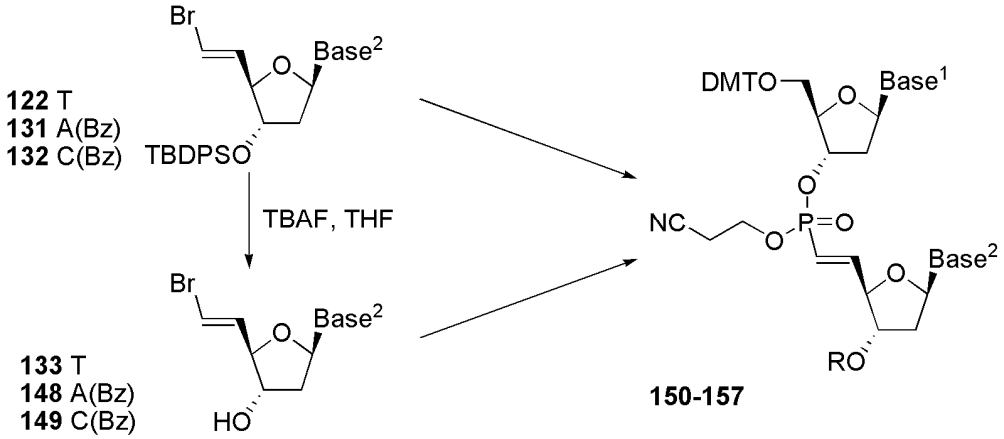


Scheme 36.

	142	**143**	**144**	**145**	**146**	**147**
Base	T	A(Bz)	C(Bz)	G(Ac)	T	A(Bz)
R	TBDPS	TBDPS	TBDPS	TBDPS	OH	OH
Yield/%	73	85	61	67	59	57

Table 9.

Although the adenosine and cytosine vinyl bromides were obtained as inseparable *E/Z* mixtures, they successfully underwent cross-coupling with *H*-phosphonates although this led to complex mixtures of isomers, which were also inseparable. Removing the TBDPS protecting group to obtain the 3'-OH did not aid separation, either at the vinyl bromide stage or the vinylphosphonate dinucleotide (Scheme 37, Table 10).



Scheme 37. Reagents & conditions: *H*-phosphonate **79** or **119** (1.2 eq.), $Pd(OAc)_2$ (0.2 eq.), dppf (0.4 eq.), propylene oxide (20 eq.), THF.

	Base[1]	**Base[2]**	**R**	**Yield/%**
150	T	C(Bz)	TBDPS	0
151	A(Bz)	C(Bz)	TBDPS	35
152	T	A(Bz)	TBDPS	45
153	A(Bz)	A(Bz)	TBDPS	53
154	T	T	H	0
155	A(Bz)	A(Bz)	H	0
156	T	T	H	51
157	A(Bz)	A(Bz)	H	47

Table 10.

These results gave further examples of the successful cross-coupling of *H*-phosphonates with vinyl bromides, indicating that it is tolerant of different protecting groups. Alongside this work, Lera also found that using the same coupling protocols on a dibromo olefin gave rise to the alkynylphosphonate compound.[139,158]

1.6.3 Comparison of cross-metathesis and Pd(0) coupling

Throughout most of the previous work on the DNA series, the building blocks for cross-metathesis or Pd(0) cross-coupling were accessed from the commercially available nucleosides. Although the cross-metathesis strategy avoids the problem of obtaining pure pure *E*-vinyl bromides, at this time, the palladium-catalysed route has proven to be the most successful. Unfortunately, the cross metathesis approach has only given success in the synthesis of the thymidine and uridine nucleosides. The olefin cross metathesis route results in not only the desired vinylphosphonate-linked dimer but also the homo-dimer generated by the competing self-metathesis of the terminal olefin.[140]

Vinylphosphonate-linked dinucleotides can be synthesised then incorporated into oligonucleotides using standard automated solid-phase synthesis protocols. To date, all biological investigation of the vinylphosphonate-linked nucleic acids has focussed on the T*T vinylphosphonate modification.

1.7 Aims of Research

Previous investigation of vinylphosphonate-linked nucleic acids has focussed on the thymidine DNA nucleotides with the synthesis from commercially available DNA nucleosides and the application to RNase H mediated antisense strategies. Further exploration of the biology of vinylphosphonate-linked oligonucleotides requires that

methodology is developed that enables all the different nucleoside building blocks to be synthesised as single stereoisomers. Synthesis of the vinylphosphonate-linked dinucleotides and then oligomeric structures can then be carried out.

Using nucleosides in, the Wittig olefination leads to mixtures of *E*- and *Z*-vinyl bromides which cannot be separated and obtained in stereochemically pure form with the exception of thymidine. It is the intention of this research to investigate the synthesis of a generic vinyl bromide building block as a single diastereoisomer from a simple sugar starting material.

A generic sugar building block can then be elaborated to access the different diastereomerically pure vinyl bromide nucleosides. Since the commercially available sugars posses the 2-OH moiety, the nucleosides produced will be the in the RNA series. This convergent strategy will then enable access to the 16 different combinations of 3'-5' vinylphosphonate-linked dinucleotides. This will provide the first examples of vinylphosphonate-linked RNA which can then be incorporated into RNA oligonucleotides using solid phase synthesis protocols.

Starting from a generic vinyl bromide gives direct access to the RNA series of nucleosides. Previous work has shown that the 2'-position can be deoxygenated to allow access to the DNA series.[140] If successful, it is hoped that in the future this can be used to

synthesise both DNA and RNA vinylphosphonate-linked dinucleotides which can be incorporated into oligonucleotides using solid phase synthesis.

- RESULTS & DISCUSSION -

2. Results and Discussion

2.1 Synthesis of the Vinyl Bromide Precursor

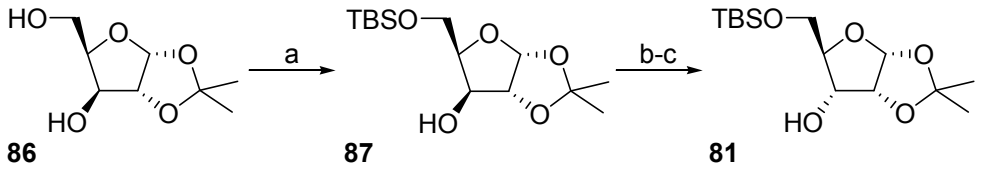
2.1.1 First Generation Synthesis

In the design and synthesis of the vinyl bromide component, the initial aim was investigate the installation of the vinyl bromide moiety prior to addition of the nucleoside base. It was hoped that this strategy would overcome the problems experienced in the DNA series where the vinyl bromide could not be isolated as a single isomer when olefination was performed in the presence of the nucleoside base (see section 1.6.2.1).[129,154]

At the start of the project, the commercially available 1,2-isopropylidene xylofuranose **86** was identified as a good starting point. Unfortunately, the analogous ribofuranose is not commercially available so the first steps involved inversion of the stereochemistry of the 3-OH.

The initial steps of this route followed work developed by R. Bertram.[129] The selective protection of the primary alcohol, 5-OH as the TBS ether using standard conditions (imidazole, TBS-Cl, DMF), gave xylofuranose **87** with a pleasing 72% yield (Scheme 38).[159] The oxidation with Dess Martin periodinane (DMP) in dichloromethane[160,161] gave the ketone which was then reduced with $NaBH_4$ (1.2 eq.) in the presence of methanol (20 eq.) in dry ether. The reduction was entirely selective as the hydride addition occurs

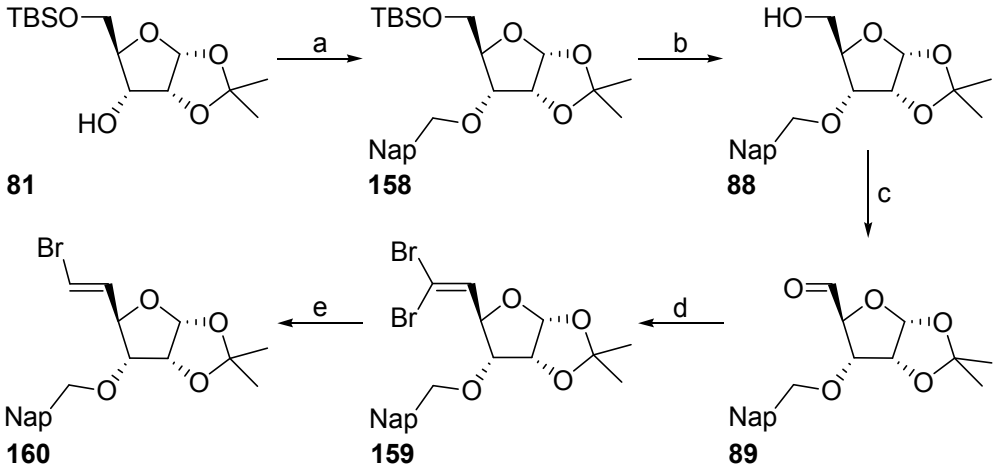
in an *exo* fashion, at the more accessible top face, to access ribofuranose **81** in an excellent 89% yield.



86 **87** **81**

Scheme 38. Reagents & conditions: a. TBSCl, imidazole, DMF, 0 ^{o}C (72%); b. DMP, CH_2Cl_2, 0 ^{o}C (91%); c. $NaBH_4$, MeOH, Et_2O, 0 ^{o}C (89%).

Previously, Bertram had used the PMB protecting group at the 3-position,[129] however, this proved to be too acid-labile at a later stage when hydrolysis of the 1,2-isopropylidene was carried out using acidic conditions. Therefore, at this stage the decision was made to use the 2-methylnaphthyl protecting group at the 3-OH position. A protecting group that is both acid and base stable is necessary for later steps in the synthesis. As first reported by Spencer *et al*.,[162] Bertram has shown that the 2-methylnaphthyl could be utilised as an acid stable protecting group in the synthesis of the vinyl bromide nucleosides. This protecting group can be cleaved reductively using hydrogenation or oxidatively using DDQ or CAN.[163] Alcohol **81** was protected using the method of Bessodes *et al.*:[164] thus 2-bromomethyl naphthalene (1.2 eq.) in the presence of potassium hydroxide (1.8 eq.) and 18-crown-6 (0.04 eq.) in dry THF afforded **158** in a yield of 70% (Scheme 39). The TBS ether of **158** was then cleaved using TBAF (86%) and the resulting alcohol **88** was cleanly oxidised to aldehyde **89** using hypervalent iodine oxidising agent DMP in CH_2Cl_2 (79%).[129]



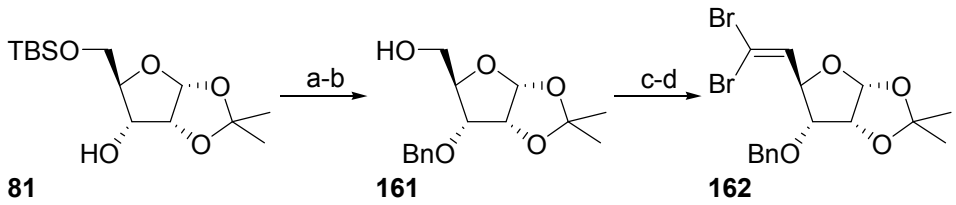
Scheme 39. Reagents & conditions: a. 2-NapCH_2Br, KOH, 18-c-6, THF, r.t. (70%); b. TBAF, THF, r.t. (86%); c. DMP, CH_2Cl_2, r.t. (79%); d. Ph_3P, CBr_4, CH_2Cl_2, 0 °C (22%); e. $(MeO)_2P(O)H$, Et_3N, DMF, 110 °C (84%).

Our next task was to form the *E*-vinyl bromide, **160**; this was achieved using our previously developed two-step procedure. Work in the Hayes group has developed a modified Hirao reduction[165] of a dibromo olefin to form the bromo olefin, favouring the *trans* geometry.[156] Dibromo olefin **159** was generated using the method of Ramirez *et al.*; by reaction of the aldehyde **89** with the Ph_3P-CBr_4 ylid, generated *in situ* by the reaction of Ph_3P (4.0 eq.) and CBr_4 (2.0 eq.) in CH_2Cl_2 at r.t. in a disappointing 22% yield (Scheme 39).[153,166,167] Further attempts to optimise this reaction were unsuccessful.[129] The dibromo olefin **159** was then reduced using dimethyl phosphite and Et_3N in DMF at 110 °C to obtain a 3:1 mixture of *E*- and *Z*-vinyl bromides isomers in 84% combined yield.

Due to the disappointing yields in the Wittig reaction to form the aldehyde **89**, the decision was made to revert to the benzyl protecting group previously used by Solesbury in the development

of a similar route from the 1,2-isopropylidene xylofuranose **86** to an analogous terminal olefin (Scheme 56).[140] Similar to the 2-methylnaphthyl, the benzyl protecting group is stable in acid and base environments and can be removed under neutral conditions.

Benzylation of the 3-OH of ribofuranose **81** was achieved using BnBr (1.3 eq.), TBAI (0.05 eq.) and NaH (1.6 eq.) (Scheme 40).[168] This was followed by deprotection of the TBS ether using TBAF as a fluoride source to give alcohol **161** in yield of 83% over the two steps. The intermediate aldehyde was generated by oxidation of the alcohol **161** using Dess Martin periodinane and this was isolated in an excellent 96% yield. The dibromo olefin **162** was obtained from this aldehyde in 69% yield using the protocol of Ramirez *et al.*[153] This shows a marked improvement in yield compared to the 22% yield when the 2-methylnaphthy protecting group was used presumably due to reduced steric bulk.

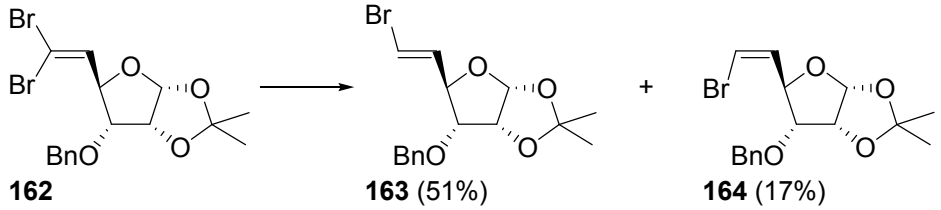


81 **161** **162**

Scheme 40. Reagents & conditions: a. BnBr, NaH, TBAI, THF, 0 °C; b. TBAF, THF (83%, 2 steps); c. DMP, CH_2Cl_2, 0 °C (96%); d. Ph_3P, CBr_4, CH_2Cl_2, 0 °C (69%).

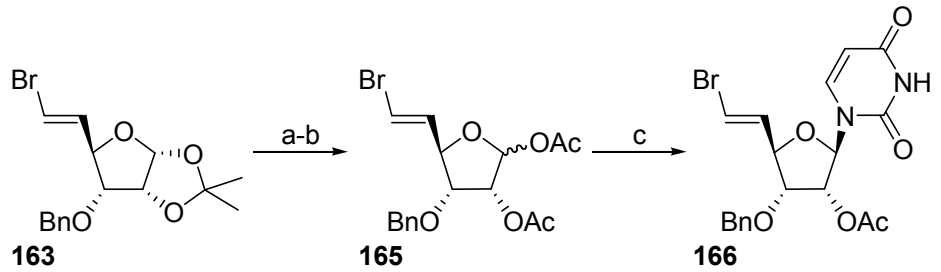
The dibromo olefin **162** was reduced using dimethyl phosphite (4.0 eq.) and Et_3N (2.0 eq.) in dry DMF at 110 °C to produce *E*- and *Z*-vinyl bromides **163** and **164** in a 3:1 ratio respectively (Scheme

41).[165,156] The two isomers were separated by column chromatography and the desired *E*-vinyl bromide **163** was isolated in 51% yield. This is an improvement on the previous work in the DNA series of installing the vinyl bromide with the nucleotide base already present.



Scheme 41. Reagents & conditions: $(MeO)_2P(O)H$, Et_3N, DMF, 110 °C.

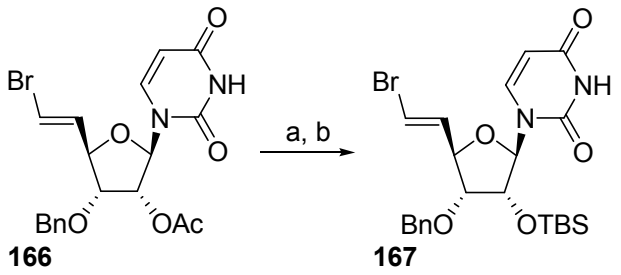
Following the generation of *E*-vinyl bromide **163**, the 1,2-acetonide was hydrolysed in refluxing 60% v/v aqueous acetic acid (Scheme 42). The diol was acetylated to form the *bis*-acetate **165** using Ac_2O and pyridine as a 10:1 mixture of anomers with a yield of 95% over the two steps. Using the modified Vorbrüggen glycosylation conditions of Yang *et al.*; uracil was added with complete facial selectivity to form the uridine vinyl bromide **166** in 85% yield.[169]



Scheme 42. Reagents & conditions: a. 60% AcOH, reflux; b. Ac_2O, pyridine, r.t. (95%, 2 steps); c. Uracil, BSA, MeCN, 65 °C *then* TMSOTf (85%).

Although the 2'-OAc serves as an adequate protecting group, it was decided to manipulate this to have the 2'-OTBS group as this would

be required later on, for the solid-phase synthesis of oligonucleotides. The 2'-OAc species **166** was deprotected using K_2CO_3 in MeOH to form the alcohol which was then protected to form TBS ether **167** in 66% yield using TBSCl, and imidazole in dry DMF (Scheme 43).[159]



166 **167**

Scheme 43. Reagents & conditions: a. K_2CO_3, MeOH, r.t. (95%); b. TBSCl, imid., NaH, DMF, 0 ^{o}C (66%).

2.1.1.1 Troubleshooting the First Generation Synthesis

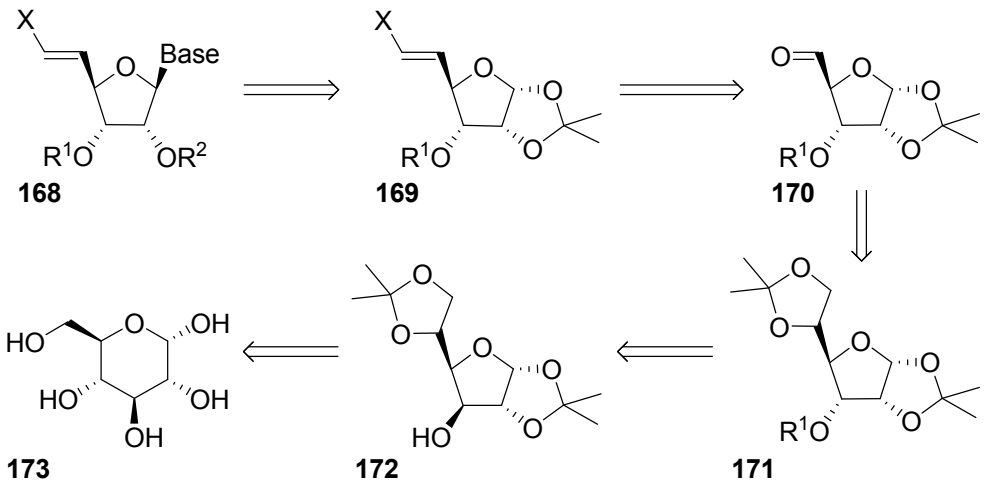
The initial synthesis of the vinyl bromide nucleoside from the isopropylidene starting material contains problems and needs improvement. In the ten steps it takes to get from the starting xylofuranose **86** to the 2'-OTBS uridine vinyl bromide **167**, two oxidations are performed that require DMP. On its own, Dess Martin periodinane is a good, clean oxidising agent. However, it is required in a stoichiometric amount and use of the reagent at such early stages in the synthesis is not economical. The protecting group strategy must also be revised to use a protecting group at the 3'-position that can be removed at the desired stage. Parallel work in the Hayes' group showed that the benzyl protecting group could not be removed without loss of the carbon-carbon double bond moiety of the vinyl bromide, so a second generation approach was required.

2.2 Second Generation Synthesis

Taking into consideration the weaker points of the first generation synthesis, a second generation synthesis was designed. The aim is to achieve the efficient synthesis of the generic vinyl bromide as a single stereoisomer. From this key building block, it is the aim of this project to be able to add the different nucleoside bases so that all the different vinylphosphonate-linked dinucleotides combinations can be synthesised by the Pd(0) cross-coupling reaction.

2.2.1 Retrosynthetic Analysis

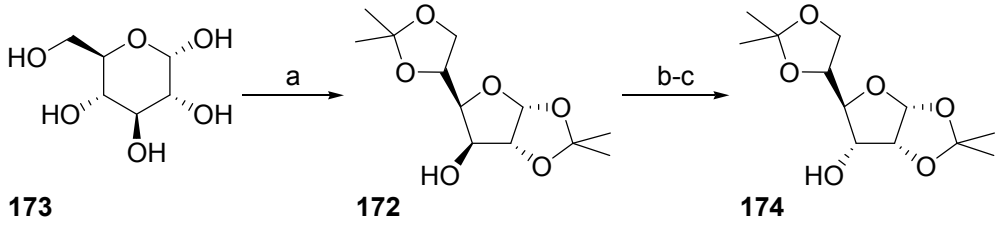
It was decided to use α-D-glucose **173** as a starting material for the synthesis of the vinyl halides. Retrosynthetic analysis showed that although the 3-OH must still be inverted; the 5-aldehyde **170** can be accessed without the use of Dess Martin periodinane but by the oxidative cleavage of a 1,2-diol (Scheme 44).



Scheme 44. Retrosynthetic analysis.

2.2.2 Synthesis

Using the procedure of Kartha,[170] α-D-glucose **173** was stirred vigorously as a suspension in refluxing acetone in the presence of iodine (0.2 eq.) for 4.5 hours (Scheme 45). The 1,2:5,6-diisopropylidene-α-D-glucofuranose **172** was obtained in 66% yield based on recovered starting material (borsm).

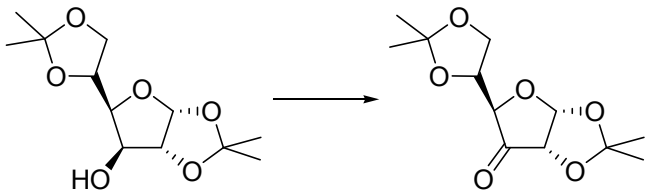


173 **172** **174**

Scheme 45. Reagents & conditions: a. acetone, I_2, 80 °C (66%); b. PDC, Ac_2O, CH_2Cl_2, 45 °C; c. $NaBH_4$, MeOH, Et_2O, 0 °C (84%, 2 steps).

Once again the inversion of the 3'-OH must be carried out in this approach and based on a survey of the literature, several different methods for the oxidation of the 3-OH of **172** to ketone **175** were investigated (Scheme 46, Table 11). There are several examples in the literature of the use of catalytic ruthenium tetroxide used to oxidise the alcohol.[171] This is generated *in situ* from $RuCl_3$ or RuO_2 and uses biphasic solvent system of chloroform and water. Sharpless reported that the three-solvent system of CCl_4-MeCN-H_2O gave improved yields in ruthenium tetroxide catalysed oxidations.[172] Morris and Kiely also reported the use of the phase transfer catalyst benzyltriethylammonium chloride (BTEAC) to improve yields and rates of reaction times alcohols with low water solubility.[173] Despite literature examples of this, we failed to get the reaction to perform effectively. Similarly, the mild reaction conditions of Ley *et al.* using

TPAP were unsuccessful.[174] The use of the Swern reaction was not investigated since previous work in the Hayes group had found this to give unacceptably low yields.



172 **175**

Scheme 46. Oxidation of the 3'-OH of the glucofuranose.

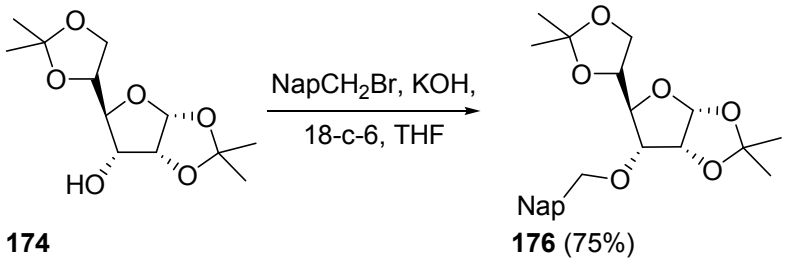
Method	Yield / %		Ref.
	172	**175**	
$RuCl_3$ (2 mol%.), K_2CO_3 (0.27 eq.), $NaIO_4$ (1.5 eq.), 1:1 v/v $CHCl_3$:H_2O, 20 h	19	55	171
RuO_2 (2 mol%), K_2CO_3 (0.27 eq.), $NaIO_4$ (1.5 eq.), 1:1 v/v $CHCl_3$:H_2O, 19½ h	71	0	171
$RuCl_3$ (2.2 mol%), $NaIO_4$ (4.1 eq.), 2:2:3 v/v CCl_4:MeCN:H_2O, 72 h	60	0	172
$RuCl_3$ (15 mol%), BTEAC (0.01 eq.), $NaIO_4$ (1.2 eq.), 1:1 v/v $CHCl_3$:H_2O, 19½ hours	64	0	173
TPAP (5 mol%), NMO (1.5 eq.), 4Å m.s., CH_2Cl_2, 24 h	91	0	174
PDC (0.9 eq.), AcOH (0.45 eq.), 3Å m.s., CH_2Cl_2, 3½ h	2	12	175
PDC (0.6 eq.), Ac_2O (3.3 eq.), CH_2Cl_2, 3½ h	0	84	176

Table 11. Summary of methods investigated in the oxidation of **26**.

The best method uncovered for the oxidation of glucofuranose **175** was the use of pyridinium dichromate (0.6 eq.) with acetic anhydride (3.0 eq.) and Celite® in refluxing CH_2Cl_2 for 3½ hours.

The Celite® prevented the formation of a tar-like substance in the reaction mixture, enabling a cleaner isolation of the ketone **175**.[175] Following filtration of the reaction slurry through a plug of silica, and eluting with petrol-EtOAc (2:1 ratio v/v), the ketone was isolated as the hydrate. In our hands, the oxidation is a somewhat capricious reaction resulting in incomplete oxidation. However, longer reaction times lead to a rapid decrease in yield. Unfortunately, the glucofuranose **172** and allofuranose **174** are difficult to purify by chromatography due to poor separation. Following the oxidation, the reduction was carried out in a stereoselective manner using $NaBH_4$ and MeOH to allofuranose **174** in a yield of 84% over the two steps.

Persevering with the allofuranose, the 3-OH of **174** was protected as the 2-methylnaphthyl ether using the previously used method.[164] The 2-methylnaphthyl ether **176** was obtained as a white solid in 75% yield (Scheme 47). Despite the poor yields previously obtained in the key Wittig reaction, this was the obvious choice while investigating the feasibility of this route due to the fact that it can be removed under neutral conditions and it is not vulnerable to acidic conditions.

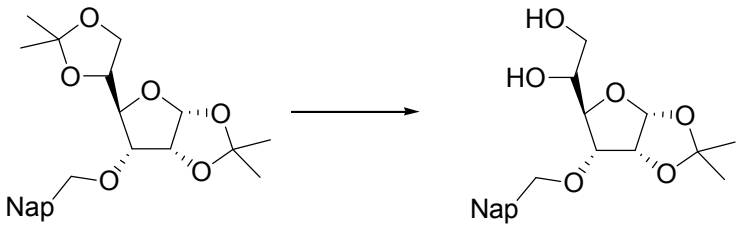


174 **176** (75%)

Scheme 47. Addition of 2-methylnaphthyl protecting group.

To obtain the aldehyde, hydrolysis of the 5,6-acetonide followed by oxidative cleavage of the diol was required. The oxidative cleavage is advantageous over the first generation synthesis where the stoichiometric use of Dess Martin periodinane was necessary due to the fact that it uses a cheaper reagent, $NaIO_4$.

It is known that acid hydrolysis of the 5,6-acetonide of the hexofuranose sugar **176** occurs preferentially over the 1,2-acetonide.[177,178,179,180,] Initially, hydrolysis using 80% v/v aqueous acetic acid at room temperature was attempted (Scheme 48). This reaction was very slow but unfortunately promotion by gentle heating resulted in several different products (presumably from hydrolysis of the 1,2-acetonide). Stirring the mixture at r.t. for up to 3 days was necessary to obtain reasonable yields without formation of the tetraol. Purification of diol **177** was problematic due to its viscous, syrupy nature and difficulties in removing acetic acid. Following column chromatography, diol **177** was obtained in a poor 33% yield. Traces of the acetic acid remained despite co-evaporation with copious quantities of toluene. The reaction was difficult to follow by TLC, since the acetonide **176** and diol **177** were co-polar.



176 **177**

Scheme 48. Reagents & conditions: a. 80% AcOH, r.t. (33%).

Several other methods of hydrolysis were also attempted but they also led to very slow reactions or hydrolysis of 1,2-acetonide **176** (Table 11). Once again the purification and handling of the reaction mixtures was challenging.

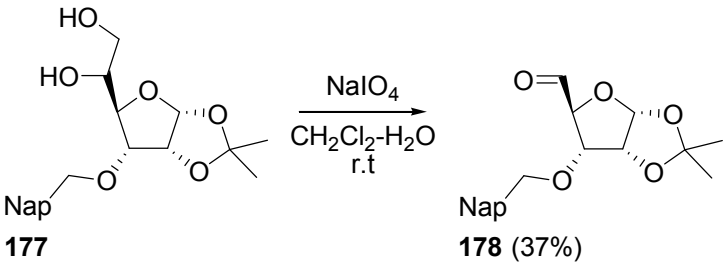
Conditions	Observations*
80% v/v AcOH aq., r.t., 3 d.[181,182,183]	Recovered **176** (33%)
H_2SO_4 (0.08 M)/MeOH 1:1 v/v, r.t., 4 d.[184]	Recovered **176** (98%)
$NaHSO_4.SiO_2$, 8:3 v/v CH_2Cl_2-^{i}PrOH, r.t., 6 d.[185,186]	Recovered **176** (97%)
80% v/v HCO_2H aq., r.t., 90 mins	Complex mixture of products, hydrolysis of both acetonides
80% v/v $ClCH_2CO_2H$ aq., r.t., 90 mins	Complex mixture of products, hydrolysis of both acetonides
0.1 eq. $ClCH_2CO_2H$, 9:1 v/v MeCN-H_2O, r.t., 19 h.	Recovered **176** (81%)
0.1 eq. HCO_2H, 9:1 v/v MeCN-H_2O, r.t., 19 h.	Recovered **176** (83%)

Table 12. Summary of attempted hydrolysis conditions.

*All reactions were difficult to purify and obtain dry due to the viscous and syrup-like nature of the diol.

Unfortunately, synthesis of aldehyde **178** was also problematic; the reactions were incomplete or yielded a mixture of products. Despite these challenges, the aldehyde was generated using sodium periodate (1.05 eq.) in 1:1 v/v CH_2Cl_2:H_2O to achieve the oxidative

cleavage of diol **177** to attain aldehyde **178** in a 37% yield (Scheme 49).

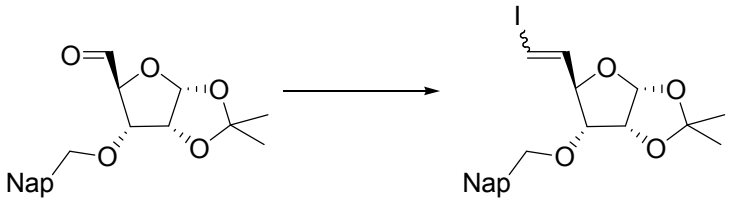


Scheme 49. Oxidative cleavage of the diol.

Although we had reverted to the 2-methylnaphthyl protecting group, we were aware that the yield from the Wittig reaction of aldehyde **178** was low (22%).[129] Since the desired target vinylphosphonate is formed by the palladium-catalysed cross-coupling of the *H*-phosphonate with a vinyl halide there are alternatives to the vinyl bromide and hence alternative strategies for accessing this coupling partner were considered. One commonly procedure for preparing *E*-vinyl iodides is the Takai reaction.[187] An additional advantage of using the vinyl iodide is that it should undergo the coupling reaction more efficiently than the vinyl bromide.

2.2.2.1 The Takai Reaction

Chromium(II) chloride (6.0 eq.) and iodoform (2.0 eq.) in dry THF were used to generate vinyl iodide **179** as a 2:1 mixture of *E*- and *Z*-isomers from aldehyde **178** (Scheme 50).[187] Attempted purification by column chromatography obtained the *E*-vinyl iodide **179** in 32% yield but impurities from the $CrCl_2$ were still present and could not be removed.

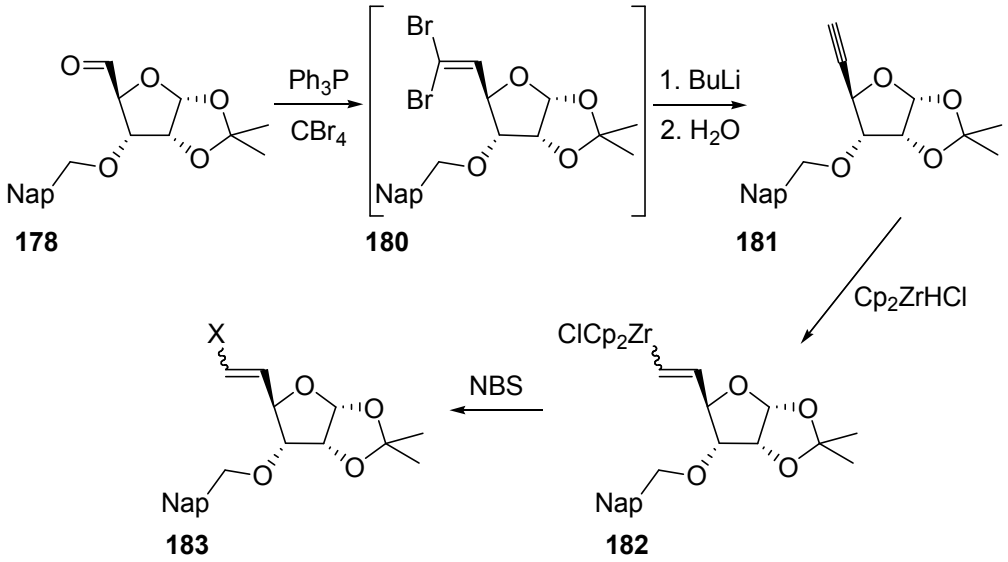


Scheme 50. Reagents & conditions: $CrCl_2$, CHI_3, THF, 0 °C (*E*:*Z* 2:1, combined 32%).

The 2:1 *E:Z* ratio, the low yields and purification difficulties of the vinyl bromide **179** were disappointing and further work using the Takai reaction was abandoned. Another deleterious factor is that $CrCl_2$ is used in significant excess. This is an expensive and highly moisture sensitive reagent. This project would require multi-gram quantities and large scale handling of such material is not feasible and the economics of the process is not viable. Although the Wittig pathway requires an additional step, the chemistry is more robust, cheaper and offers better yields than Takai chemistry.

Another widely used method of obtaining vinyl halides is through the use of hydrozirconation.[188] However, for the following reasons this approach was not investigated in the progress of this project. Currently, the challenging part of this synthesis was the formation of the aldehyde. Using the hydrozirconation chemistry still requires formation of aldehyde **178** from which alkyne **181** is synthesised *via* the Corey-Fuchs reaction and the intermediate dibromo olefin **180** (Scheme 51). Furthermore, the hydrozirconation requires stoichiometric use of a zirconocene species *e.g.* Schwartz reagent, which is then displaced by the addition of an electrophilic source of

bromine or iodine (*e.g.* I_2, Br_2 or NBS) to obtain the *E*-vinyl halide **183** as the major product.



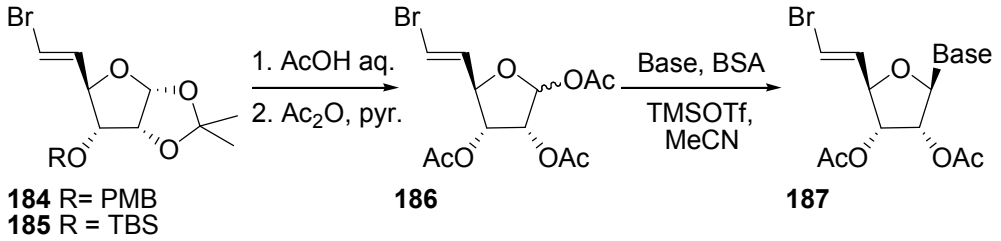
Scheme 51. Synthetic steps required to obtain the vinyl halide using hydrozirconation methodology.

2.2.2.2 Protecting Group Strategy

The 2-methylnaphthyl group produces significantly lower yields in the Wittig reaction; however, this route from the α-D-glucose is still the most viable path for generation of the vinyl bromide. At this stage in the project it became necessary to investigate a different protecting group for the 3-OH moiety.

Previous work has shown that the 3-OPMB and the 3-OTBS protected alcohols **184** and **185** are not appropriate for use at this stage since they would not survive the acidic conditions required to hydrolyse the 1,2-isopropylidene moiety. If they were used then the 1,2,3-triacetate **186** would be generated prior to addition of the nucleoside base thus making it necessary to be able to discriminate

between the 2'- and the 3'-position of the *bis*-acetate **187** at a later stage (Scheme 52).



184 R= PMB
185 R = TBS

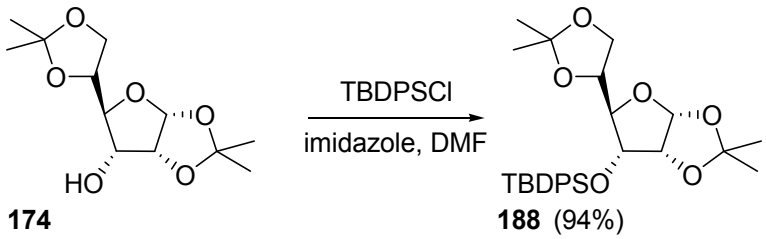
186

187

Scheme 52. Use of the acid sensitive PMB or TBS protecting group leads to the *bis*-acetate nucleoside.

The *tert*-butyldiphenylsilane (TBDPS) group was the first option to be considered as it is an acid stable protecting group which can be removed by treatment with TBAF. Previous work by N. Solesbury has shown that the TBDPS group cannot be removed from the vinylphosphonate dimers[140] therefore it would be necessary to deprotect and change the protecting group prior to the formation of the vinylphosphonate bond. Although this adds two extra steps to the synthesis, the TBDPS could be used, then cleaved and reprotected as the 2-methylnaphthyl ether after the vinyl halide has been installed but prior to the cross-coupling reaction.

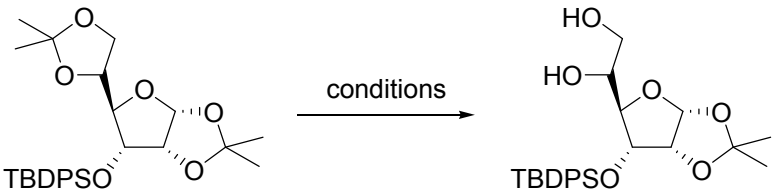
Allofuranose **174** was protected using TBDPSCl and imidazole in dry DMF to form the TBDPS ether **188** in an excellent 94% yield (Scheme 53).[189,190,191] The TBDPS ether is approximately 100 times more stable to acid hydrolysis than the TBS ether so should remain intact in the acid hydrolysis at a later stage.[192]



174 **188** (94%)

Scheme 53. Addition of the TBDPS protecting group.

Unfortunately, as previously observed with the 2-methylnaphthyl compound **176** (Scheme 48), acid hydrolysis of the 5,6-isopropylidene of **188** using the standard aqueous acetic acid (80% v/v) conditions[181,182,183] was very slow so different methods were investigated. Previous work in the group has shown that if the reaction mixture was heated or left for too long (four days) then hydrolysis of the 1,2-isopropylidene would occur.[140] Investigating different methods of hydrolysis led to the use of a catalytic amount (0.1 eq.) of dichloro-dicyanobenzoquinone (DDQ) in 9:1 v/v MeCN:H_2O at r.t.[184] In aqueous media, this is reduced to the *bis*-phenol (pK_a of 3.42) by the acetone byproduct of the acetal hydrolysis.[193] Hydrolysis did occur however, the reaction was slow and low yielding. Hydrolysis of the TBDPS acetonide **188** using acids with a similar pK_a to the reduced DDQ was also investigated but once again the diol, **189** was a viscous syrup and was difficult to handle and purify by column chromatography or repeated evaporation with toluene.



188 **189**

Scheme 54. Acetal hydrolysis.

The hydrolysis was attempted using of 0.1 eq. of chloroacetic acid at r.t. in 9:1 v/v MeCN:H_2O, however, after 19 hours only the unreacted acetonide **188** was observed. Warming the reaction to 40 ^{o}C or increasing the amount of acid (0.2 eq.) led to a complex mixture of products. Higher levels of the chloroacetic acid (0.5 eq.) had the undesired effect of cleavage of the TBDPS protecting group.

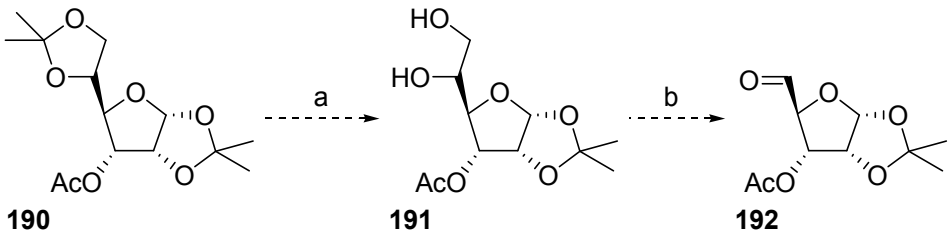
It is inferred from these results that the large steric bulk of the 2-methylnaphthyl and the TBDPS protecting groups is responsible for the very slow rates of hydrolysis of the diols. A solution to this problem is the use of a smaller protecting group.

Fortunately, a solution to the 3-protection was found with the acetate group. The 3-OH of the allofuranose **174** was protected using acetic anhydride (1.56 eq.) in the presence of triethylamine (3 eq.) and a catalytic quantity of DMAP (0.1 eq.), stirring at room temperature in dry CH_2Cl_2 to obtain **190** in a 86% yield as a crystalline solid (Scheme 55).[194]

174 **190** (86%)

Scheme 55. Acetate protection of the 3-OH.

The next challenge was the hydrolysis of the 5,6-acetonide **190** to form diol **191** and oxidative cleavage to access aldehyde **192** (Scheme 56); hydrolysis using a 70% aqueous solution v/v of acetic acid is a reliable method of cleaving the 5,6-acetonide.[182] However, in the subsequent oxidative cleavage process, it was not desirable to use sodium periodate which requires an aqueous reaction mixture (*e.g.* EtOH:H_2O).[195] Due to the high polarity of the diol **38**, and aldehyde **39**, an aqueous reaction mixture resulted in the significant loss of product due to its solubility in the aqueous phase and formation of the aldehyde hydrate.

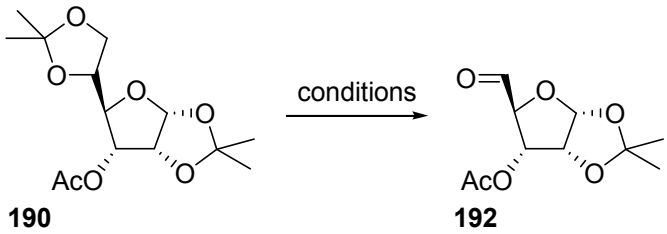


190 **191** **192**

Scheme 56. Reagents & conditions: a. 70% AcOH, r.t.; b. $NaIO_4$, 4:1 v/v EtOH:H_2O, r.t.

To reduce the problem of hydrate formation, a single organic phase could be used instead of the aqueous system. However, this approach would impede the progress of the $NaIO_4$ mediated oxidative cleavage of diol **191** due to the poor solubility. The

presence of silica is often reported to aid such reactions but in this case, no benefit was seen.[196] One-pot procedures for the hydrolysis and subsequent oxidative cleavage of the diol to the aldehyde **192** have also been published in the literature (Scheme 57).[169,196] Different methods were attempted with varying degrees of conversion (Table 13).



190 **192**

Scheme 57. One-pot hydrolysis-oxidative cleavage of the 5,6-isopropylidene.

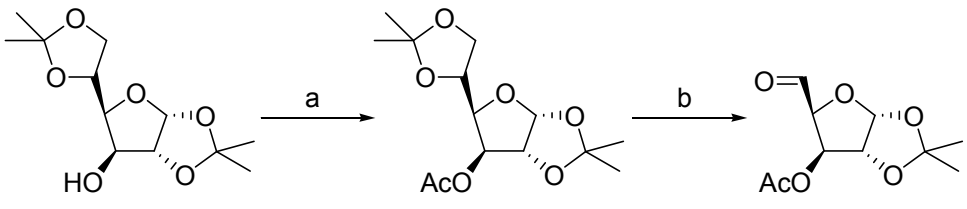
Conditions	**Observations**	**Ref.**
$NaIO_4.SiO_2$, CH_2Cl_2, 4 ½ d.	Recovered **190** (50%)	196
$NaIO_4$ (1.0 eq.), H_5IO_6 (0.5 eq.), EtOAc, 5 d.	Aldehyde **192** and hydrate (92%)	169
H_5IO_6 (1.1 eq.), EtOAc, 4 h.	Aldehyde **192** and hydrate (98%)	176 197
H_5IO_6 (1.25 eq.), THF, 4 h.	Aldehyde **192** and hydrate (71%)	

Table 13. Conditions used in the one pot hydrolysis-oxidative cleavage of the 5,6-acetonide of **190**.

Using the method of periodic acid in THF, spectroscopic analysis ^1H NMR of the crude product showed a mixture of the aldehyde **192** and the corresponding hydrate. The method of Yang *et al.* uses a combination of periodic acid and sodium periodate in dry ethyl acetate (dried over $CaCl_2$) to obtain the aldehyde **192**.[169] However, the most successful results were obtained using a variation of the

method of Agrofoglio *et al.* using solely periodic acid (1.2 eq.) in dry EtOAc.[197] Once the aldehyde **192** was obtained, it was used without further purification; it was found that purification did not increase the yield of the Wittig reaction and column chromatography often led to loss of material.

While investigating the formation of aldehyde **192** and the subsequent olefination and regioselective reduction steps, the unreliable nature of the earlier PDC oxidation prompted the exploration of a model system. Using the glucofuranose **172**, this approach would involve the inversion of the 3-OH stereochemistry at a later stage in the synthesis; removing the capricious PDC oxidation of the 1,2:5,6-diisopropylidene-α-D-glucofuranose (Scheme 10). Glucofuranose **172** was protected as the acetate **193** using the same conditions in an excellent 93% yield (Scheme 58).[194] Aldehyde **194** was then produced using the one-pot hydrolysis-oxidative cleavage procedure using 1:1 v/v EtOAc-THF as the solvent.



172 **193** **194**

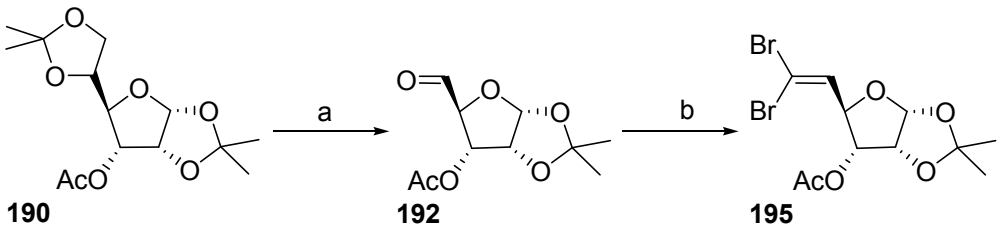
Scheme 58. Reagents & conditions: a. Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t. (93%); b. H_5IO_6, EtOAc-THF, r.t. (83%).

Conditions	Observations
$NaIO_4.SiO_2$, CH_2Cl_2, 7 d.[196]	Recovered **193** (34%)
$NaIO_4$ (1.0 eq.), H_5IO_6 (0.5 eq.), EtOAc, 3 d.[169]	Aldehyde **194** (73%)
H_5IO_6 (1.5 eq.), EtOAc, 3 h.	Aldehyde **194** (75%)
H_5IO_6 (1.25 eq.), 1:1 v/v EtOAc-THF, 2½ h.	Aldehyde **194** (83%)

Table 14. Conditions used in the one pot hydrolysis-oxidative cleavage of the 5,6-acetonide of **193**.

2.2.2.3 Wittig Olefination

After identifying the acetate as a good protecting group at the 3-OH the olefination reaction was then explored. Previous synthesis of the *gem*-dibromo olefin found using the Wittig reaction and generating the ylid *in situ* was the most successful method. Triphenylphosphine (4.0 eq.) and carbon tetrabromide (2.0 eq.) were combined and stirred at 0 °C for 1 hour 15 minutes. The ylid was then added to a solution of aldehyde **192** in dry CH_2Cl_2 (Scheme 59). It was essential that the hygroscopic CBr_4 was dried prior to use in order to obtain respectable yields in the olefination.



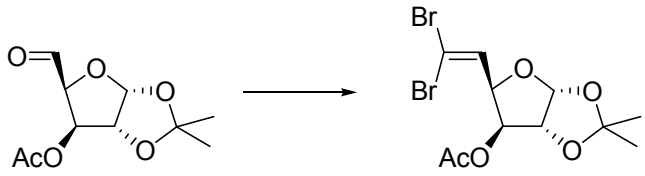
190 **192** **195**

Scheme 59. Reagents & conditions: a. H_5IO_6, EtOAc, r.t.; b. Ph_3P, CBr_4, CH_2Cl_2, 0 °C (36%, 2 steps).

Following purification, dibromo olefin **195** was obtained as a white solid in 36% yield (over 2 steps from **190**). A potential solution to

the low yields of this reaction was the synthesis and isolation of the ylid phosphonium salt $Ph_3P^+CHBr_2Br^-$ according to the procedure of Dolhem *et al*.[198] However, using the isolated phosphonium salt did not increase the yield of the Wittig reaction and so was not pursued further.

Investigating the model system, the aldehyde **194** obtained directly from the glucofuranose acetate **193** was also found to readily form the hydrate. In order to minimise formation of the undesired *gem*-diol it was desirable to use aldehyde **194** immediately after the hydrolysis-oxidative cleavage. It was hoped that further drying the aldehyde using freshly activated molecular sieves would push the aldehyde-hydrate equilibrium towards a higher percentage of the aldehyde **194**, thus increasing the yield of the Wittig reaction (Scheme 60). Although this failed to increase the yield of the Wittig reaction, a yield of 45% over the two steps from glucofuranose **193** to dibromo olefin **196** was considered acceptable and enabled us to pursue the synthetic path further.



194 **196**

Scheme 60. Reagents & conditions: Ph_3P, CBr_4, CH_2Cl_2, 0 °C (45%, 2 steps from glucofuranose acetate **190**).

The low yields of the Wittig reaction are partially due to the tendency of the aldehyde to form the hydrate. Although the use of

4Å molecular sieves was unsuccessful at dehydrating the hydrate of aldehyde **194**, it was found that improved yields in the Wittig reaction were observed when the aldehyde hydrate was dehydrated using calcium hydride.[199] The aldehyde was stirred as a solution in dichloromethane with CaH_2 until evolution of H_2 had ceased. Due to its insoluble nature $Ca(OH)_2$ could be removed by filtration while the acetate protecting group was left intact. The difference in quality of the aldehyde **194** was obvious from observing the ^1H NMR spectra before and after the addition of the CaH_2 (Figure 16).

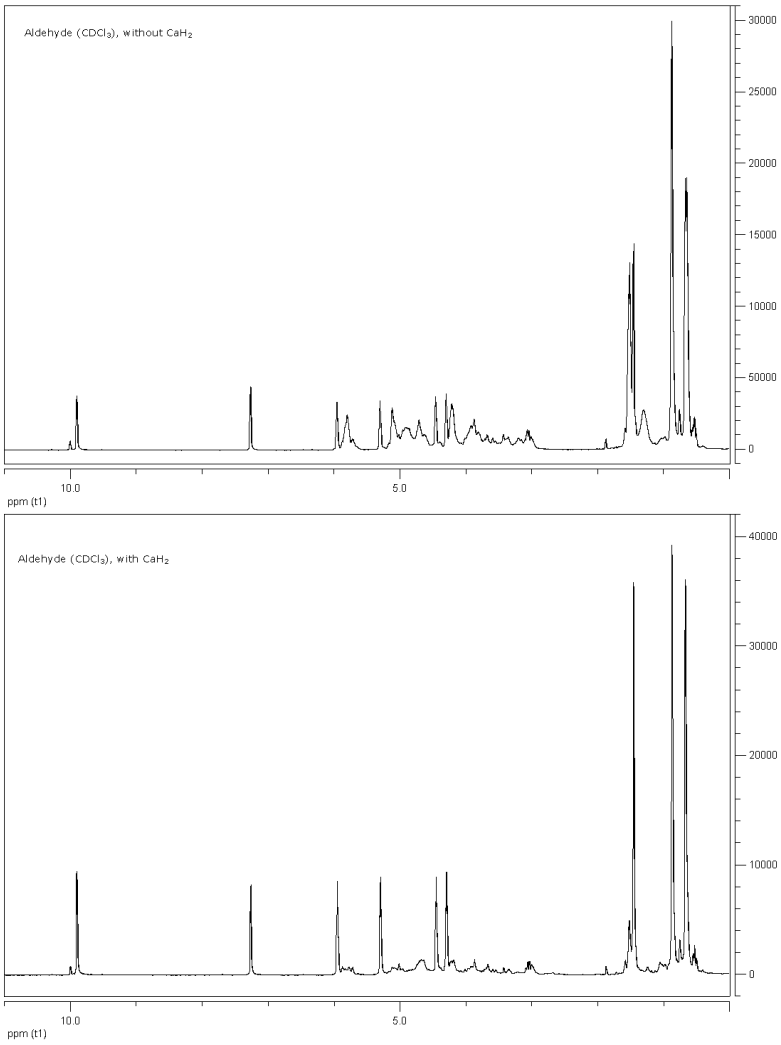
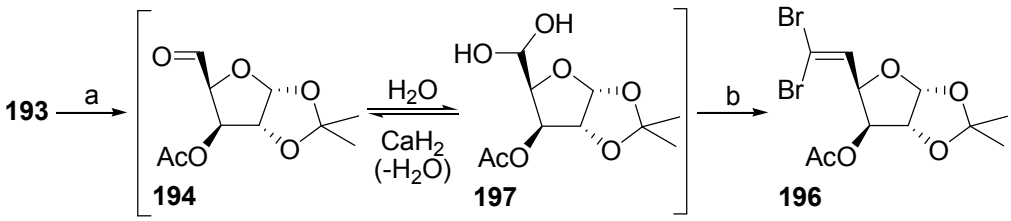


Figure 16. Comparison of aldehyde **194** ^1H NMR before and after the addition of CaH_2.

A solution of the aldehyde was stirred over CaH_2 then transferred *via* canula into the Ph_3P-CBr_4 solution leaving the solid behind. Alternatively the Ph_3P-CBr_4 solution could be transferred *via* canula to the flask containing the aldehyde and the calcium hydride. Both strategies were investigated but the yield did not seem to be adversely affected by the presence of the CaH_2 in the reaction mixture. Using the CaH_2 to dry aldehyde **194** and dehydrate the *gem*-diol **197** led to a very pleasing increase in the yield of the Wittig reaction, obtaining the dibromo olefin **196** with yields consistently over 60% for the two step process of forming the aldehyde and the olefination.



Scheme 61. Reagents & conditions: a. H_5IO_6, EtOAc-THF, r.t., *then* CaH_2, CH_2Cl_2, r.t.,; b. Ph_3P, CBr_4, CH_2Cl_2, 0 °C (45%, over the 2 steps).

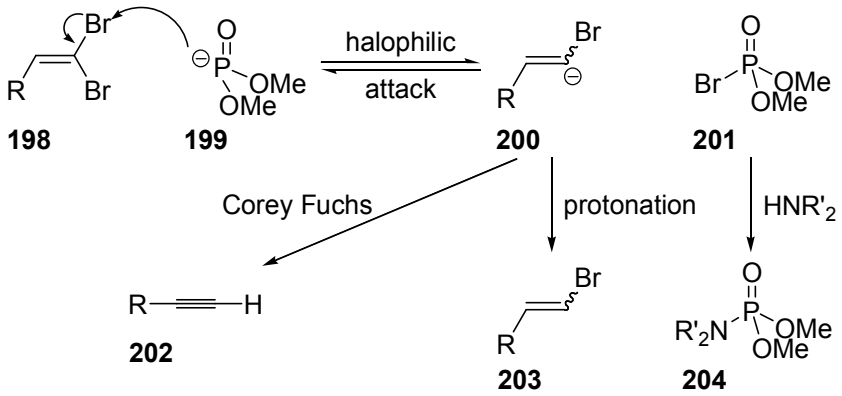
It has also been reported by Kerr *et al.* that the prolonged exposure of a ketal functionality to the Ph_3P-CBr_4 reaction media can result in poor yields due to the loss of the 1,2-isopropylidene protecting group.[200,201] The standard Ramirez procedure to generate the dibromo olefin uses four equivalents of the Ph_3P and two equivalents of CBr_4. Kerr slightly reduced the quantity of reagents, using Ph_3P (3.6 eq.) and CBr_4 (1.7 eq.). In addition, the aldehyde and phosphine were combined in solution and the carbon tetrabromide was added slowly while constantly monitoring the reaction.

Similarly we were able to reduce the relative quantities of phosphine and carbon tetrabromide used, however, no improvement in yield was observed using the strategy of the slow addition of carbon tetrabromide.

2.2.2.4 Investigating the Hirao Reduction

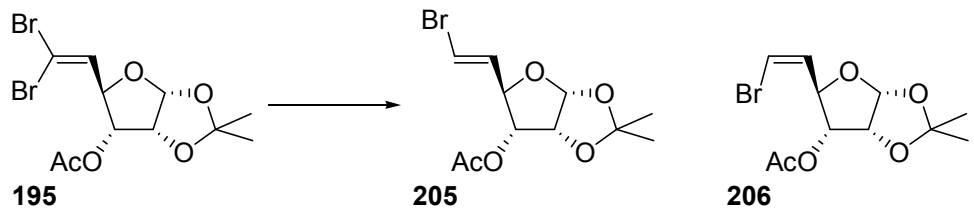
The conditions used for the Hirao reduction used in the first generation synthesis (Scheme 41) were based on the reduction of the dibromo olefin of the DNA nucleotides used by Abbas in previous work and were never fully optimised.[156,158] At this stage it was opportune to investigate and optimise this reaction. Both the ribofuranose and the xylofuranose vinyl bromides were also investigated to determine if the stereochemistry of the 3-OH had any effect on the selectivity of the reaction.

Although the mechanism of the Hirao reduction is not fully known, studies widely support the theory is that it proceeds *via* a vinyl anion intermediate **200** (Scheme 62).[156,202] The phosphoryl bromide species **201** that forms as a product of the halophilic attack is very reactive. To push the equilibrium to the right hand side, it is necessary to remove this from the reaction media; this can be achieved by the reaction with a secondary amine, forming the stable phosphoramide **204**.



Scheme 62. Proposed mechanism of the Hirao reduction and possible side products.

Initial studies showed that unlike the first generation synthesis, the reduction could be carried out at r.t. over a shorter reaction time. The reduction reactions were carried out at three different temperatures using the reaction conditions previously optimised within the group; dimethyl phosphite (4.0 eq.) and an amine (2.0 eq.) in dry DMF for 16 hours (Scheme 63, Scheme 64).[156] Following on from some work in the Hayes group by C. Gonzalez-Rodriguez a different amine was also tried.[203] In agreement with the suggested mechanism (Scheme 62), it was found that secondary amines led to faster rates of reaction than tertiary amines, diisopropylamine was found to be particularly effective. In the case of both dibromo olefin isomers **195** and **196**, the rate of reaction was increased but there was no increase in the *E:Z* ratio (Table 15, Table 16).



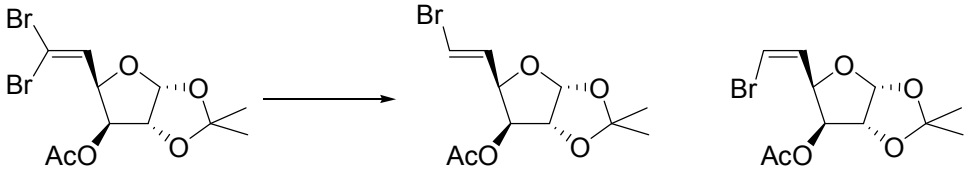
Scheme 63. Reagents & conditions: $(MeO)_2P(O)H$, Et_3N OR $^{i}Pr_2NH$, DMF.

Base	Temp / °C	Ratio* 195:205:206	Combined yield / % **
Et_3N	-60	70:24:6	95
Et_3N	r.t.	0:81:19	84
Et_3N	70	0:82:18	68
HN^iPr_2	-60	30:40:14	72
HN^iPr_2	r.t.	0:76:24	85
HN^iPr_2	70	0:84:16	76

Table 15. Hirao reduction conditions for the reduction of ribofuranose **195**.

* Ratios based on ^{1}H NMR spectra of crude reaction mixture.

** Combined yield after purification by column chromatography.



Scheme 64. Reagents & conditions: $(MeO)_2P(O)H$, Et_3N OR $^iPr_2NH$, DMF.

Base	Temp / °C	Ratio* 196:207:208	Combined yield / %
Et_3N	-60	70:24:6	77
Et_3N	r.t.	2:71:27	60
Et_3N	70	5:63:32	75
HN^iPr_2	-60	12:70:18	43
HN^iPr_2	r.t.	0:72:28	72
HN^iPr_2	70	5:66:29	81

Table 16. Hirao reduction conditions for the reduction of xylofuranose **196**.

* Ratios based on ^{1}H NMR spectra of crude reaction mixture

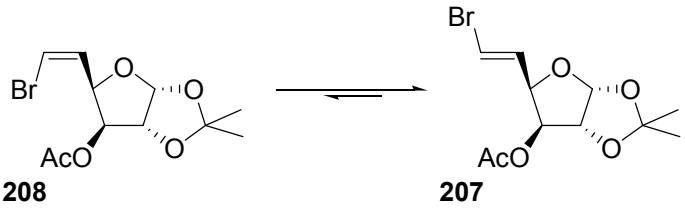
** Combined yield after purification by column chromatography.

These results show that the temperature and the nature of the amine base had no effect on the selectivity of the reaction. However, the shorter reaction time required when using diisopropylamine was advantageous so this was used in place of the triethylamine in all subsequent reactions.

Further optimisation of the reaction involved altering the quantities of the dimethyl phosphite and the amine used. It was found that dimethyl phosphite (2.0 eq.) and diisopropylamine (2.0 eq.) were sufficient to allow the reaction to proceed to completion.

Although this reaction provided us with a mixture of *E*- and *Z*-isomers, the efficiency of the synthetic route could be further increased by isomerism of the *Z*-vinyl bromide **208** to the desired *E*-vinyl bromide **207** under radical conditions. Initially thiophenol and 2,2'-azobisisobutyronitrile (AIBN) in refluxing carbon tetrachloride was used, however the reaction was slow and after 48 hours the *E*:*Z* ratio was still 1:4. Similarly, benzoyl peroxide (0.1 eq.) and *N*-bromosuccinimde (NBS, 1.1 eq.) in refluxing carbon tetrachloride for 22 hours led to a 2:1 ratio of *E*- and *Z*-isomers **207** and **208** respectively.[204] The most successful conditions were NBS (1.1 eq.), AIBN (0.1 eq.), in gently refluxing CCl_4 for 2 hours, longer reaction times led to lower yields (Scheme 65). Subjecting the pure *Z*-vinyl bromide **207**, to the optimised conditions of NBS (1.0 eq.), AIBN (0.1 eq.), in CCl_4 at 80 ^{o}C for two hours led to a 2:1 ratio of *E*- and

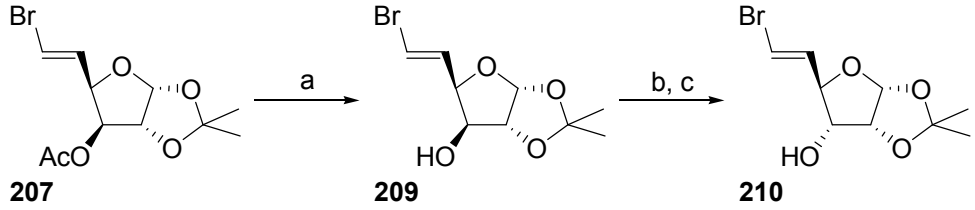
Z-isomers **207** (62%) and **208** (31%) respectively in a combined 93% yield.



Scheme 65. Reagents & conditions: NBS (1.1 eq.), AIBN (0.1 eq.), CCl_4, 78 °C (combined: 93%; **207** 62%, **208** 31%)

2.2.2.5 Inversion of the 3-OH

Following optimisation of the Hirao reduction, it was then necessary to investigate whether the 3-OH could be inverted. The acetate protecting group on **207** was removed by base hydrolysis using K_2CO_3 (0.5 eq.) in MeOH to produce alcohol **209** in an excellent 93% yield (Scheme 66). Dess Martin periodinane oxidation of alcohol **209** provided the corresponding C3 ketone. The ketone was then reduced with complete selectivity using $NaBH_4$ (1.2 eq.) and MeOH in Et_2O at 0 °C to afford the desired ribofuranose **210** in a yield of 73% over the two steps.



Scheme 66. Reagents & conditions: a. K_2CO_3 (0.5 eq.), MeOH, r.t. (93%); b. DMP (1.2 eq.), CH_2Cl_2, r.t.; $NaBH_4$ (1.2 eq.), MeOH, Et_2O, 0 °C, (73%, 2 steps).

The relative and absolute stereochemistry of **210** was confirmed by X-ray crystallography (Figure 17 and Appendix 1).

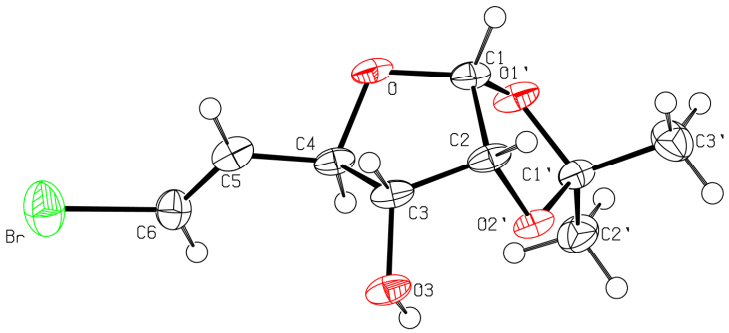
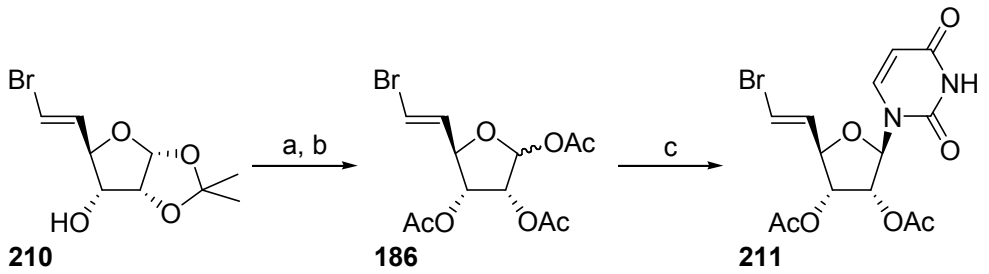


Figure 17. Structure of **59** obtained by X-ray crystallography.

2.2.2.6 Addition of Nucleoside Base

With the successful inversion of the 3-OH, the 1,2-isopropylidene of furanose **210** was hydrolysed and acetylated to form triacetate **186** in a 3:1 mixture of anomers (Scheme 67). Using the same glycosylation conditions as used in the first generation synthesis (Scheme 42), uracil was then added with complete facial selectivity to form uridine **211** in 99% yield.[169]



210 **186** **211**

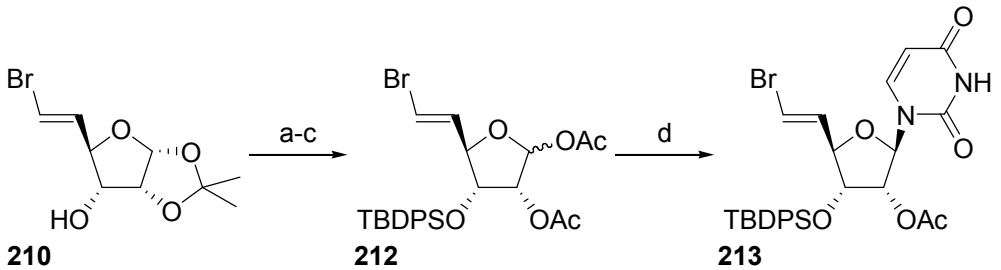
Scheme 67. Reagents & conditions: a. 70% AcOH reflux; b. Ac_2O (30 eq.), pyridine (33 eq.), r.t. (82%, 2 steps); c. Uracil (3.0 eq.), BSA (3.0 eq.), MeCN, *then* TMSOTf (1.48 eq.), 65 °C (99%).

Although the formation of the uridine vinyl bromide **211** was a pleasing result, it is, however, not the ideal building block; it is desirable to be able to discriminate between the 2'-OH and the 3'-OH at a later stage. In order to achieve this from uridine **211** the

bis-acetate would need to be deprotected and then selectively protected as the TBS ether at the 2'-OH. There is literature precedent for this to be possible; the 2'-OH is more acidic than the 3'-OH.[205,206] However, these literature examples are of nucleotides containing a bulky 5'-ODMT group which may exert a steric influence.[207,208,209] For the purposes of the palladium-catalysed cross-coupling reaction, it is thought that the free 3'-OH will not affect the coupling reaction.

2.2.2.7 Accessing the desired building blocks

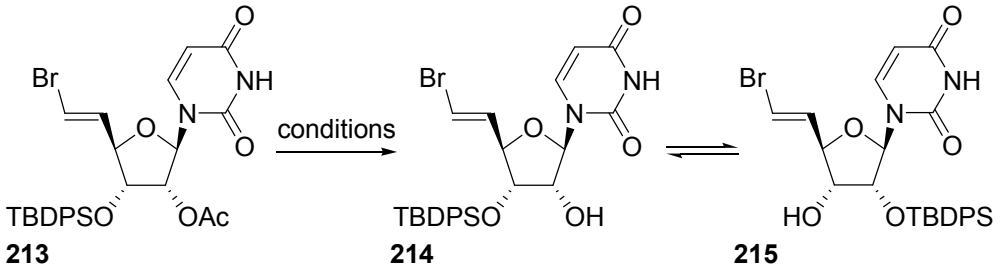
To enable the discrimination between the 2'-OH and 3'-OH, we resorted to using a protecting group following the inversion of the 3'-OH. Thus alcohol **210** was protected using TBDPSCl, imidazole in DMF at r.t. to form the TBDPS ether in 99% yield (Scheme 68).[189,190] Once again the 1,2-isopropylidene was hydrolysed and then acetylated to obtain the *bis*-acetate **212** in an 86% yield over the two steps. The TBDPS uridine **213** was then formed using standard conditions of uracil, BSA and TMSOTf in MeCN in 84% yield.



210 **212** **213**

Scheme 68. Reagents & conditions: a. TBDPSCl, imidazole, DMF, r.t. (99%); b. 70% AcOH, reflux; c. Ac_2O, pyr., r.t., (86%, 2 steps); d. uracil, BSA, MeCN *then* TMSOTf, 65 ^{o}C (84%).

Following successful addition of the nucleoside base, it was desirable to manipulate the functionality at the 2'-position. The base hydrolysis of the uridine acetate **213** using K_2CO_3 in MeOH and an acid work up (1N HCl) resulted in a 1:1 mixture of products. Chromatography was used to separate the products, however during this process the two isomers re-equilibrated to a 7:3 ratio. The products were identified by ^{1}H COSY NMR spectrocopy as the 2'-OH **214** and 3'-OH **215** isomers respectively in a combined yield of 21% (Scheme 69). This mixture of products was caused by the base mediated migration of the TBDPS group following the hydrolysis of the acetate.[192] The procedure was repeated using K_2CO_3 and MeOH hydrolysis but the reaction mixture was dry loaded onto silica and purified without an aqueous work up. This also led to a 1:2 ratio of the 2'-OH **214** and 3'-OH **215** isomers respectively in a combined yield of 76%, indicating a base-promoted process.



Scheme 69. Deprotection of the 2'-OAc.

An alternative method of hydrolysis using NaOMe in CH_2Cl_2 at r.t. also led to the same problems. After 6 hours a 1:1:1 ratio of starting material acetate **213** and the two different TBDPS isomers **214** and **215** were observed. Further attempts to remove the

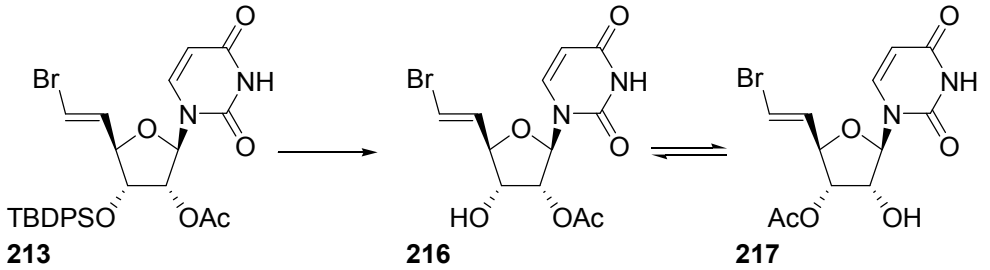
acetate selectively using DIBAL-*H* and methanolic ammonia also resulted in the migration of the silyl group (Table 17).

Conditions	**Ratio 213:214:215 (combined yield)**
K_2CO_3 (0.5 eq.), MeOH, r.t., SiO_2 dry loaded	0:1:2 (21%)
K_2CO_3 (0.5 eq.), MeOH, r.t., 21 h., aq. work up	0:7:3 (76%)
K_2CO_3 (0.5 eq.), MeOH, r.t., 3 h., aq. work up	4:1:1 (96%)
NaOMe, CH_2Cl_2, r.t., 6 h.	1:1:1 (54%)
DIBAL-*H* (2.5 eq.), CH_2Cl_2, -78 ^oC, 2 ¼ h.	0:1:1 (83%)
2M NH_3-MeOH, r.t., 23 h.	5:1:1 (54%)

Table 17. Summary of reagents and conditions used to deprotect 2'-position.

Due to the problems encountered, the acetate can be kept in place if necessary since it can be removed at a later stage in oligonucleotides synthesis by the global deprotection conditions at the end of oligonucleotide synthesis. With this in mind, the selective deprotection of the 3'-OTBDPS ether **213** was also attempted. Since the acetate is base sensitive, the standard conditions to remove a silyl group using TBAF were modified and the reaction mixture was buffered with 2.4 equivalents of glacial acetic acid (Scheme 70, Table 18). The reaction mixture was concentrated and directly loaded onto silica and purified by chromatography. A mixture of products, the 2'-OAc **216** and 3'-OAc products **217** were co-eluted in a 1:2 ratio respectively (94% combined yield). Repeating the reaction and using an aqueous work up procedure led

to the same 1:2 ratio of acetates **216** and **217** respectively in a combined 63% yield.



213 **216** **217**

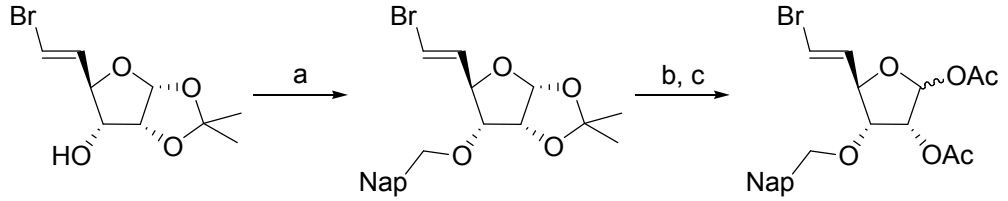
Scheme 70. Reagents & conditions: TBAF (1.2 eq.), AcOH (2.4 eq.), THF, r.t.

Conditions	Ratio 216:217 (Combined yield)
dry loaded onto SiO_2	1:2 (94%)
aqueous work up	1:2 (63%)

Table 18. Summary of methods used to deprotect 3'-TBDPS ether **213**.

This lack of success led to the route using the TBDPS ether **212** being abandoned. A suitable protecting group still had to withstand the acid hydrolysis however with the vinyl bromide installed, the previous problem of the 2-methylnaphthyl protecting group having very poor yields in the Wittig reaction were no longer relevant.

Vinyl bromide **210** was protected as the 2-methylnaphthyl ether using the previous conditions[164] to obtain vinyl bromide **160** in an excellent 98% yield (Scheme 71). Hydrolysis and peracetylation of the 1,2-isopropylidene **160** generated the *bis*-acetate **218** as a mixture of the α and β-anomers in an 85% yield over the two steps.



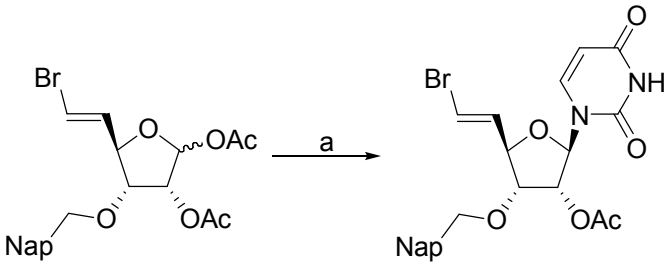
210 **160** **218**

Scheme 71. Reagents & conditions: a. $BrCH_2Nap$, KOH, 18-c-6, THF, r.t. (98%); b. 70% AcOH, reflux; c. Ac_2O, pyr., r.t. (85%, 2 steps).

2.3 Vinyl bromide nucleosides

2.3.1 Uridine vinyl bromides

With the *bis*-acetate **218** in hand, the task of generating the different vinyl bromide nucleosides was near to being achieved. Initially we investigated the uridine vinyl bromide since this was vital for our primary target of the uridine dinucleotide. Using the modified Vörbruggen conditions of Shi *et al.*,[169] the nucleoside base was added with excellent 93% yield to obtain the uridine vinyl bromide **219** (Scheme 72).



218 **219** (93%)

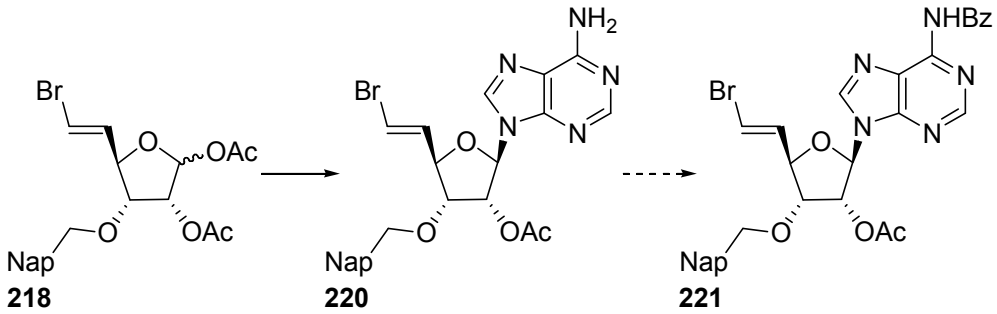
Scheme 72. Reagents & conditions: uracil, BSA, MeCN, 70 °C *then* TMSOTf.

2.3.2 Adenosine Vinyl Bromides

To date there has been no synthesis of the purine vinyl bromides in a stereochemically pure form. Synthesis of the adenosine vinyl

bromide also gives us the chance to explore the Pd(0) cross-coupling of the RNA purine nucleosides for the first time.

A review of the literature indicated that the different Lewis acids used in the Vörbruggen glycosylation can affect the ratio of *N*-7 and *N*-9 isomers of purine nucleosides.[210,211,212] The desired *N*-9 isomer is the kinetic product whereas the *N*-7 isomer is the thermodynamic product.[213,214] It is reported that the weaker Lewis acid $SnCl_4$ gives more favourable yields of the desired *N*-9 isomer. To participate in the Pd(0) coupling reaction it may be necessary to block the primary amine to avoid competing reactions. The most commonly used protecting group on the purine is the benzoyl moiety. There are two possible routes to the adenosine vinyl bromides; one is to synthesise the adenosine then protect the amine prior to the Pd(0) cross-coupling. Initially the adenosine vinyl bromide **220** was obtained in a 25% yield using adenine (1.5 eq.) and $SnCl_4$ (2.5 eq.) in MeCN at room temperature (Scheme 73).[215] Upon work up, the tin salts formed an emulsion and after chromatography the yield was disappointing even though only a single isomer was observed.

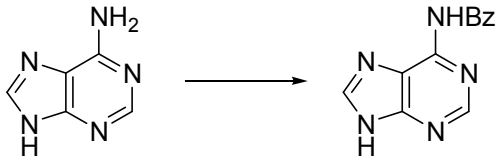


Scheme 73. Two step route to the adenosine vinyl bromide: a. $SnCl_4$, MeCN, r.t. (25%); b. benzoylation.

Although the literature has examples of the formation of the adenosine using Vörbruggen conditions used $SnCl_4$ it was decided to investigate the use of the TMSOTf to try and improve the yield.[211,212,213]

The alternative strategy, avoiding the second step necessary to protect the amine, utilised benzoyl adenine **223**. More recently, "ultra mild" protecting groups have been developed using the phenoxyacetyl group which can be removed under milder conditions.[216,217,218]

Taking this into consideration, *N*-6-benzoyl adenine was selected to be used instead of the unprotected adenine. The *N*-6-benzoyl adenine **223** was prepared by a modified procedure of Bullock *et al.*[219] using benzoyl chloride (2.5 eq.) and pyridine under reflux conditions for six hours. The product was cooled, triturated with $NaHCO_3$ solution then recrystallised from EtOH-H_2O (9:1 v/v) to obtain the desired product **223** in a 76% yield (Scheme 74).

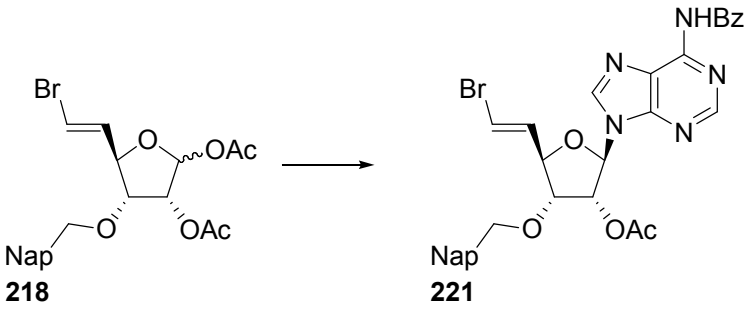


222 **223**

Scheme 74. Reagents & conditions: BzCl, pyridine, reflux (76%).

The desired *N*-9-benzoyl adenosine vinyl bromide **221** was obtained in an excellent 94% yield with no evidence of the *N*-7 isomer observed. Although this strategy involved heating the reaction to

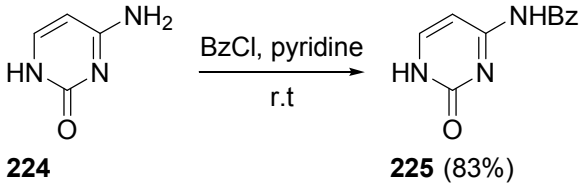
80 °C to solubilise the 6-benzoyl adenine allowing it to be silylated by the BSA. A cleaner, higher yielding reaction was also attained by using toluene as the solvent instead of MeCN (Scheme 75).[169]



Scheme 75. Reagents & conditions: *N*-6-benzoyl adenine, BSA, toluene, 80 °C then TMSOTf (94%).

2.3.3 Cytidine Vinyl Bromides

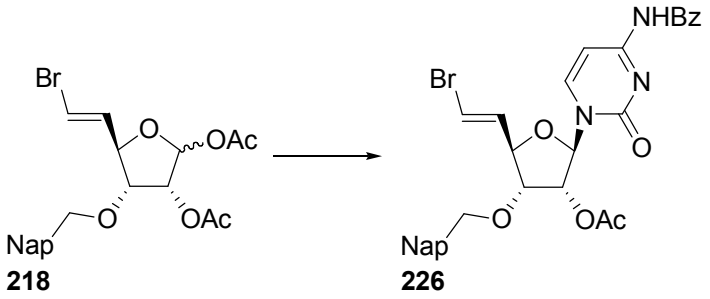
As with the adenosine, it was also necessary to protect the primary amine of the cytidine in order to carry out the Pd(0) coupling. The cytosine base **224** was protected using benzoyl chloride (3.75 eq.) in pyridine at r.t. to obtain benzoyl cytosine **225** in a 83% yield (Scheme 76).[220]



Scheme 76. Synthesis of benzoyl cytidine.

The Vörbruggen glycosylation was carried out using the *bis*-acetate **218**, *N*-4-benzoyl cytosine **225** and BSA in MeCN with TMSOTf as the Lewis acid (Scheme 77).[169] The desired cytidine vinyl bromide **226** was obtained in a 30% yield from the *bis*-acetate **218**.

Regrettably, due to time constraints within the project this was not further investigated or optimised.

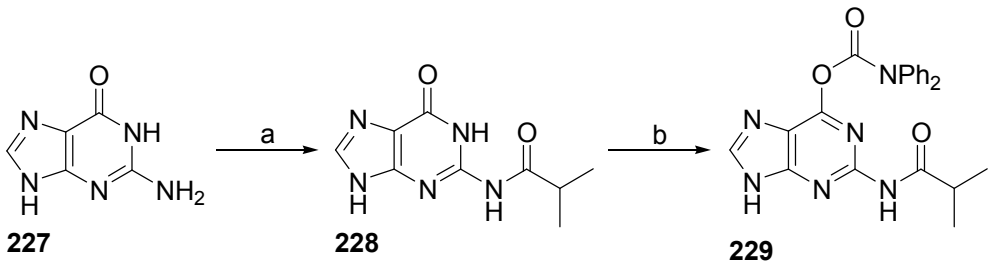


Scheme 77. Reagents & conditions: *N*-4-benzoyl cytosine, BSA, MeCN, 70 °C then TMSOTf (30%).

2.3.4 Guanosine Vinyl Bromides

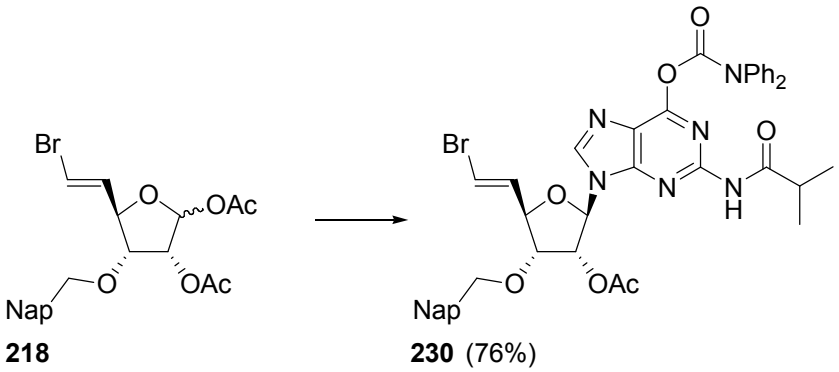
Synthesis of the vinyl bromide guanosine presents similar problems to that of the adenosine; there is the possibility of synthesising both the *N*-7 and the *N*-9 isomers.[210,221,222,223,224,225] A literature survey shows that the amine of guanine **227** is most often protected as the 2-*N*-acetyl[222] or the 2-*N*-isobutyryl[221] or the 2-*N*-dimethylformamidine (DMF) derivative. The isobutyryl amide **228** was formed using isobutyric anhydride (2.0 eq.) in DMF at 160 °C in a 88% yield (Scheme 78).[234] The carbonyl of the guanine was then also protected using the diphenylcarbamyl protecting group developed by Robins *et al*. (Scheme 78).[222,225] Following the procedure of Milecki *et* al., transient acetylation using Ac_2O (2.25 eq.) in DMF at 100 °C was followed by treatment with diphenycarbamoyl chloride (1.05 eq.) and DIPEA (1.75 eq.) in pyridine to obtain guanine **229** in 57% yield (43% over the two steps). The diphenylcarbamyl protecting group offers steric bulk to reduce the formation of the undesired *N*-7 isomer. Shaughnessy *et*

al. have also shown that it is possible for the oxygen of the guanosine to coordinate irreversibly with palladium species during Pd(0) coupling reactions.[226] The use of the diphenylcarbamyl group also prevents coordination of the purine oxygen with the palladium during the Pd(0) cross-coupling formation of the vinylphosphonate.



Scheme 78. Reagents & conditions: a. $(^{i}PrCO)_2O$, DMF, 160 °C (79%); b. Ac_2O, DMF, 100 °C then Ph_2NCOCl, DIPEA, pyridine, r.t. (54%).

The 2-*N*-isobutyryl-6-*O*-diphenylcarbamoyl guanine **229** was silylated *in situ* with BSA in toluene and reacted with the *bis* acetate **218** using TMSOTf to obtain the desired vinyl bromide guanosine **230** in a 76% yield (Scheme 79).



Scheme 79. Reagents & conditions: **229**, BSA, toluene, 80 °C then TMSOTf.

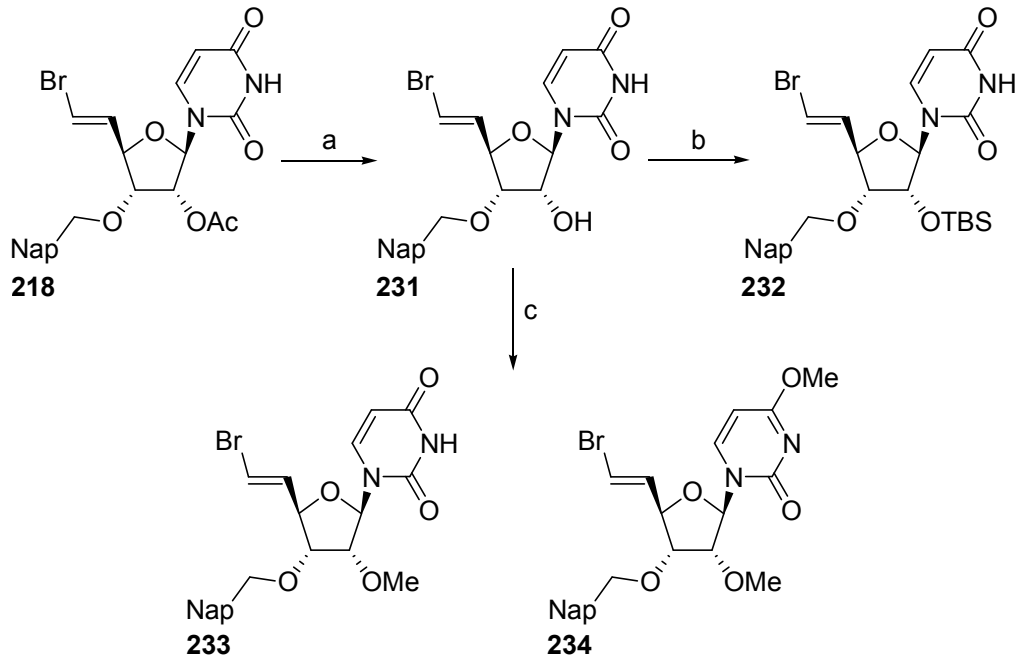
2.3.5 Manipulation of the 2'- and 3'-blocking groups

Following successful addition of the four different nucleoside bases, it was advantageous to manipulate the protecting group at the 2'-

position. Standard oligonucleotide synthesis protocols utilise the 2'-OTBS protecting group which can be cleaved in the global deprotection after oligomer synthesis. The alternative, widely used strategy is the use of 2'-OMe blocking group.

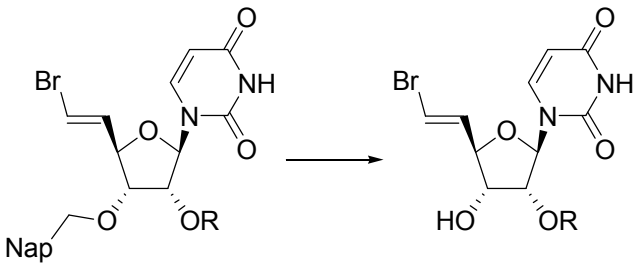
2.3.5.1 Uridine nucleosides

The acetate of uridine **219** was easily hydrolysed using K_2CO_3 and MeOH to obtain the alcohol **231** which could then be elaborated. At this stage we were interested in investigating the 2'-OTBS and the 2'-OMe nucleosides. Firstly the 2'-OTBS vinyl bromide uridine **232** could be obtained using the standard conditions of TBSCl and imidazole in DMF in a 98% yield (Scheme 80). The methylated uridine vinyl bromide **233** was achieved using the protocol of Wengel *et al.* of NaH (4 eq.), and MeI (2 eq.) in THF at 4 ^{o}C.[227] During the course of this reaction, a small amount (<5%) of the *bis*-methylated uridine **234** was obtained and this was readily separated from the desired compound by chromatography.



Scheme 80. Reagents & conditions: a. K_2CO_3, MeOH, r.t. (92%); b. TBSCl, imidazole, DMF, r.t. (98%); c. NaH, MeI, THF, 4 °C (59%).

Coupling of these vinyl bromide uridines showed that the Pd(0) cross-coupling worked well (see section 2.4). We were interested in investigating the Pd(0) cross-coupling with a free alcohol at the 3'-OH. This would enable further derivitisation at the 3'-OH of the vinylphosphonate dimer as the phosphoramidite, necessary for oligonucleotide synthesis. The 2-methylnaphthyl protecting group of uridines **232** and **234** could be removed using DDQ (3.0 eq.) in 4:1 v/v CH_2Cl_2-MeOH at a gentle reflux (Scheme 81). These conditions were suitably mild so that the TBS protecting group was left intact to obtain the alcohol **235** in an excellent 96% yield. The analogous 2'-OMe alcohol **236** was obtained using the same conditions in a similarly pleasing 81% yield.



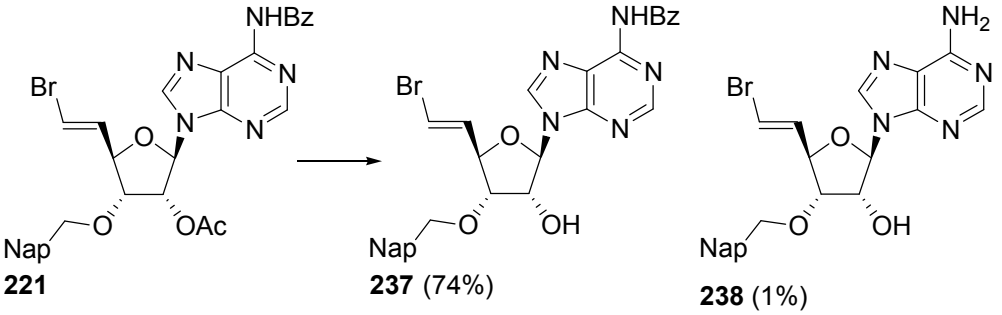
232 R = TBS
233 R = Me

235 R = TBS (96%)
236 R = Me (81%)

Scheme 81. Reagents & conditions: DDQ, 4:1 v/v CH_2Cl_2:MeOH, reflux.

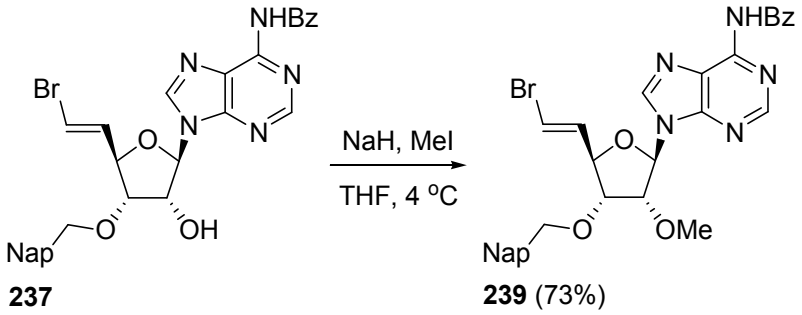
2.3.5.2 Adenosine nucleosides

As with the uridine vinyl bromide, the acetate on the 2'-position of the adenosine vinyl bromide **221** was exchanged for a methyl group. Alternative conditions were required to selectively hydrolyse the acetate of **221** leaving the benzoyl amide intact. Using potassium carbonate in methanol at 0 °C for two hours led to a mixture of products. However, sodium methoxide (0.2 eq.) in methanol at r.t. for 90 minutes led to recovered acetate starting material **221**;[228] longer reaction times (13 hours) led to mixtures of starting material acetate **221**, the desired 2'-OH **237** and the debenzoylated adenosine byproduct **238**. Methanolic ammonia (1.0 eq.) at 0 °C did not result in any deprotection, however, at r.t. for 2 hours led to a 1.7:1 mixture of acetate starting material **221** and alcohol **237** respectively.[229] The best conditions were found to be a short reaction time using ethanolic sodium hydroxide with which the desired *N*-6-benzoyl adenosine alcohol **237** was obtained in 74% yield (Scheme 82).[230,231] Minimising the reaction time (less than 15 minutes) reduced the amount of the debenzoylated product **238**.



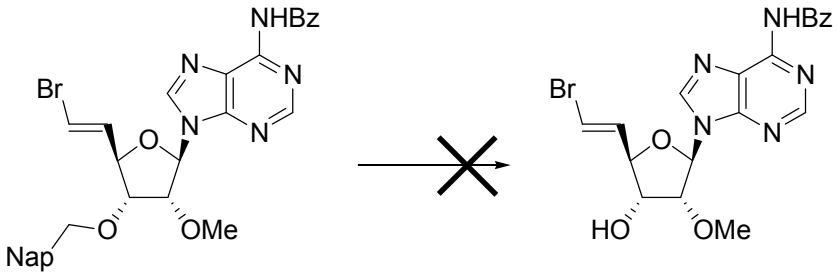
Scheme 82. Reagents & conditions: EtOH, 2M NaOH, r.t.

Using carefully anhydrous conditions, the 2'-OH of **237** could be methylated using the same conditions as for the uridine (NaH (5.0 eq.) followed by MeI (3.0 eq.) in THF at 4 °C) (Scheme 82).[227] The methylated adenosine **239** was obtained in 73% yield.



Scheme 83. Methylation of 2'-OH.

Unfortunately attempts to deprotect the 2-methynaphthyl of **237** to obtain the 3'-OH adenosine **240** were unsuccessful (Scheme 84). The use of DDQ resulted in a very messy reaction where unreacted starting material and a complex mixture of products were observed. Similarly using CAN as an alternative single electron oxidant, was also unsuccessful. One possible problem is the depurination caused by the acidic environment of the DDQ in the reaction mixture.[232]



239 **240**

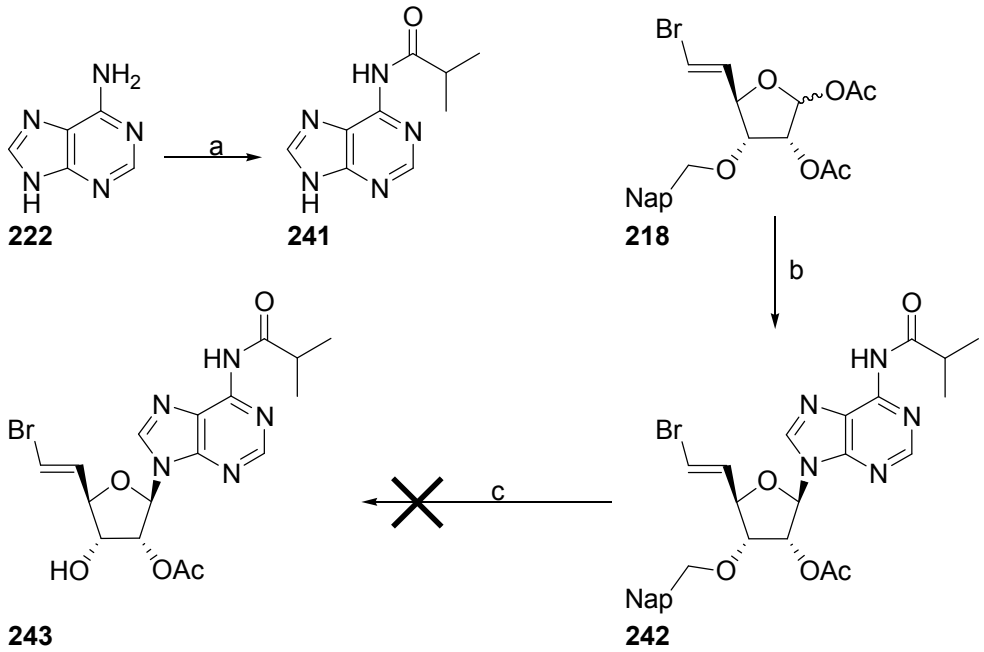
Scheme 84. Attempted deprotection of the 3'-OCH_2Nap protecting group.

It is known that benzyl protecting groups can be reductively cleaved using hydrogenation conditions and by dissolving metal reductions. Applying this knowledge to the 2-methylnapthyl group using lithium metal solely was unsuccessful. Using the conditions of Donohoe *et al.* of lithium di *tert*-butylbiphenyl (LiDBB) and *bis*(methoxyethyl)amine in THF at -78 ^{o}C resulted in a trace of the desired compound being observed but attempts to isolate it were unsuccessful.[233]

The reasons for the lack of success of the deprotection were unknown. It was considered that the aromatic nature of the purine may affect the system thus hindering the one electron oxidation. However, parallel work in the guanosine series (see section 2.2.5.4) showed that this was not necessarily the case. Another possibility was the aromatic benzoyl protecting group of the amine affecting the system. This was investigated using an alternative group to protect the amine of the purine.

The *N*-6-isobutyryl adenine **241** was investigated due to its steric bulk theoretically leading to a more favourable ratio of the *N*-9

isomer. Following the procedure of Shevlin *et al*,[234] the *N*-6-isobutyryl adenine **241** was prepared from adenine **222** using isobutyric anhydride (3.0 eq.) in DMF heating at 160 °C and was obtained in a pleasing 75% yield (Scheme 85).[235] Using the same conditions as previous, the nucleoside was prepared using BSA and TMSOTf in toluene obtained the isobutyryl adenosine **242** in an unoptimised 24% yield. Remaining with the acetate protecting group to minimise the number of steps, the DDQ mediated deprotection of the 2-methylnaphthyl of **242** to obtain the 3'-OH adenosine vinyl bromide **243** was investigated however this was unsuccessful.

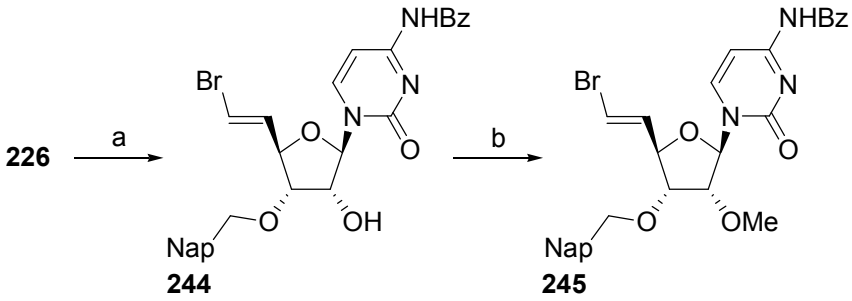


Scheme 85. Reagents & conditions: a. $^{i}Bu_2O$, DMF, 160 °C (75%); b. **241**, BSA, toluene, 80 °C then TMSOTf (24%); c. DDQ, 4:1 CH_2Cl_2:MeOH, reflux.

2.3.5.3 Cytidine nucleosides

A small scale test reaction also showed that the acetate **226** could be hydrolysed to alcohol **244** using sodium hydroxide in ethanol and

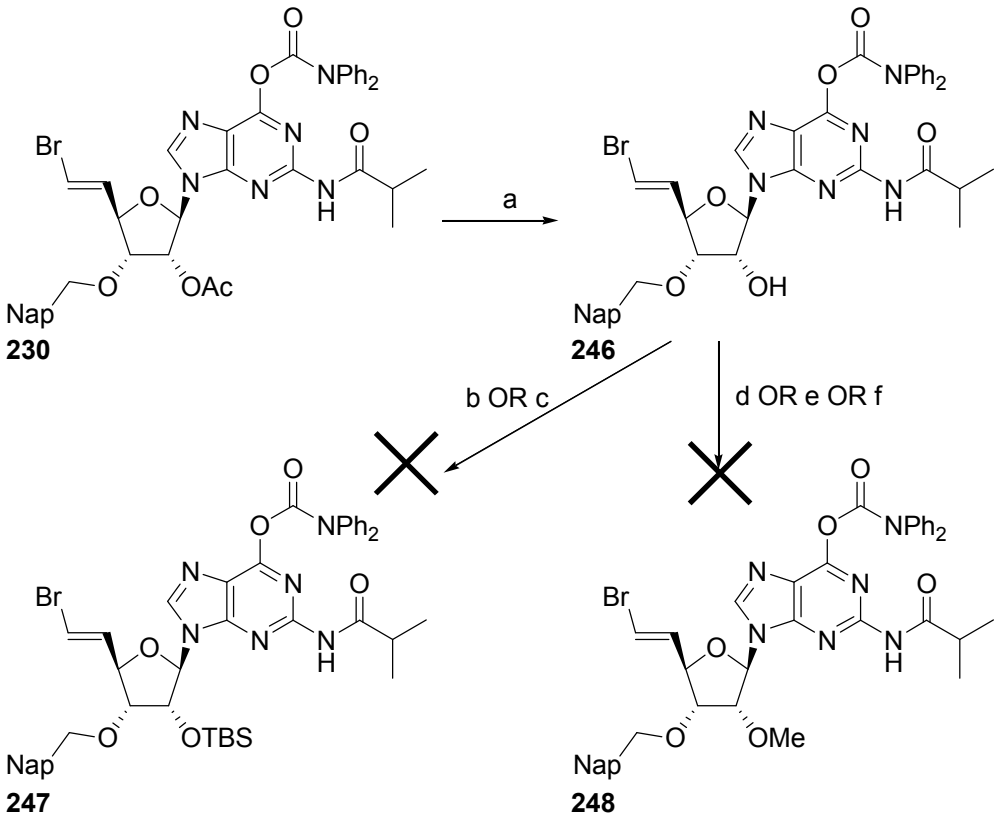
then the 2'-OH methylated to form uridine **245** using the same reagents and conditions as used for the adenosine series (Scheme 86) although this was not further pursued.



Scheme 86. Reagents & conditions: a. EtOH, 2N NaOH, r.t. (74%), b. NaH, MeI, THF, 4 ^{o}C (73%).

2.3.5.4 Guanosine nucleosides

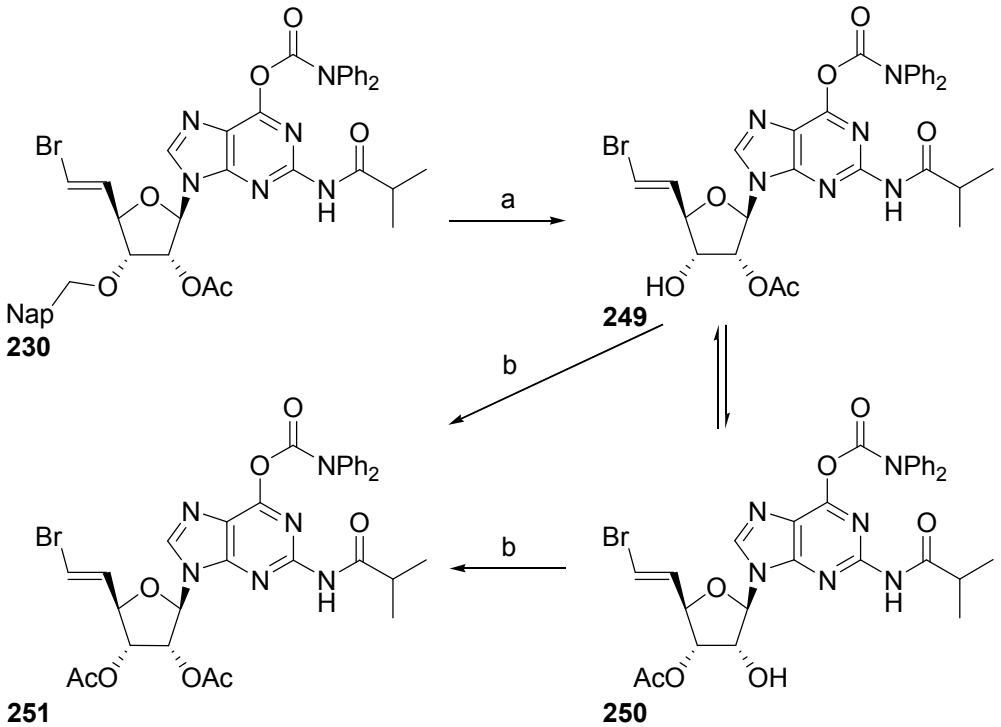
With the guanosine vinyl bromide **230** formed, the manipulation of protecting groups at the 2'-position was attempted. The acetate was removed using a similar method to that of the adenosine and cytidine (Scheme 87). Deprotection of the 2'-OAc of guanosine **230** was achieved using 2M NaOH in a 5:4:1 v/v mixture of THF:MeOH:H_2O at 0 ^{o}C for ten minutes, obtaining the 2'-OH guanosine **246** in a 98% yield.[236] Unfortunately it was not possible to form the 2'-OTBS ether **247** using the standard TBSCl or TBSOTf procedures.



Scheme 87. Reagents & conditions: a. 2N NaOH, 5:4:1 THF-MeOH-H_2O, 0 ^oC (98%); b. Imidazole (2.4 eq.), TBSCl (1.2 eq.), DMF, r.t.; c. TBSOTf (1.25 eq.), pyridine (1.75 eq.), DMF; d. NaH (5.0 eq.), MeI (3.0 eq.), THF, 5 ^oC; e. $MeO^+BF_4^-$ (1.0 eq.), proton sponge (1.17 eq.), CH_2Cl_2; f. DBU (2.5 eq.), MeI (5.0 eq.), MeCN.

Similarly, attempts to methylate the 2'-postion to form guanosine **248** using the previously used methyl iodide and sodium hydride conditions were also unsuccessful. Using the Meerwein's salt as an alternative electrophile[237] and the use of MeI and DBU (a non-nucleophilic base) were also unsuccessful at alkylating the 2'-OH of guanosine **246** (Scheme 87). Due to time limitations, a solution to this problem has not currently been identified.

On a more encouraging note, it was possible to oxidatively remove the 2-methylnaphthyl protecting group of the vinyl bromide guanosine **230** using DDQ (3.0 eq.) in 4:1 CH_2Cl_2:MeOH v/v at reflux over 5 hours (Scheme 88). Unfortunately, due to the acidic nature of the reaction media, a degree of acid mediated acyl migration was observed, leading to a 3:2 mixture of inseparable 2'-OAc **249** and 3'-OAc **250** products in a combined yield of 93%. Acylation of this mixture of isomers using Ac_2O (1.5 eq.), Et_3N (2.5 eq.) and DMAP (0.2 eq.) in CH_2Cl_2 generated the *bis*-acetate **251** in a 55% yield.



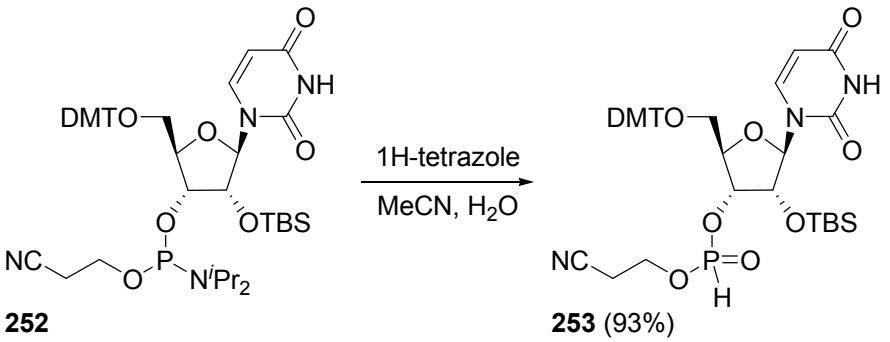
Scheme 88. Reagents & conditions: a. DDQ, 4:1 CH_2Cl_2:MeOH, reflux (93%); b. Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t. (55%).

2.4 Synthesis of *H*-Phosphonates

Previous work in the related DNA series has shown that the *H*-phosphonates could be accessed in a single step from the

commercially available phosphoramidites.[129,158] Tetrazoles are the most commonly used reagents for this process, being mildly acidic (*1H*-tetrazole, pK_a 4.9). These serve as good activating agents without interfering with the dimethoxytrityl protecting group (routinely cleaved from nucleosides by 80% AcOH in about 15 minutes).

Hydrolysis of phosphoramidite **252** using 2 equivalents of 1*H*-tetrazole in aqueous acetonitrile at room temperature obtained the uridine *H*-phosphonate **253** as a 3:2 mixture of diastereoisomers in 93% yield. The diastereomeric mixture (referred to as A and B, with δ_P 8.26 and 7.61 ppm respectively) was used as attained, without further purification or separation of the diastereoisomers (Scheme 89). This is advantageous since chromatography results in significantly reduced yields.



252 **253** (93%)

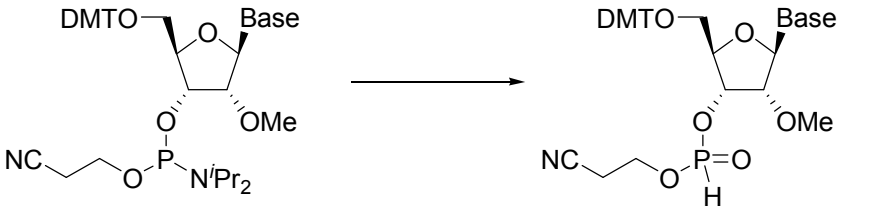
Scheme 89. Synthesis of the uridine *H*-phosphonate.

During the later stage of this research, we were unable to obtain 1*H*-tetrazole in its solid form (subsequently it was only available as

an acetonitrile solution). At this stage, crystalline 5-methyl-1*H*-tetrazole was used as an alternative.

When this reaction was repeated, different ratios of the two *H*-phosphonate diastereoisomers were observed. The length of reaction time does not have a correlation with the differing ratios. It can be speculated that reaction microconditions within the reaction vessel played a part. The 1*H*-tetrazole and 5-methyl 1*H*-tetrazole gave comparable yields and ratios, thus indicating that the additional methyl group did not have any effect on the reaction.

Since our aim was to apply the vinylphosphonate modification to a variety of systems, the 2'-OMe uridine *H*-phosphonate **256** and the purine *N*-6-benzoyl adenosine *H*-phosphonate **257** were also prepared in excellent yields from the respective phosphoramidites **254** and **255** (Scheme 90).



254 Base = U
255 Base = A(Bz)

256 Base = U (103%, 1:1)
257 Base = A(Bz) (99%, 1:1)

Scheme 90. Reagents & conditions: 5-Methyl 1*H*-tetrazole, MeCN, H_2O, r.t.

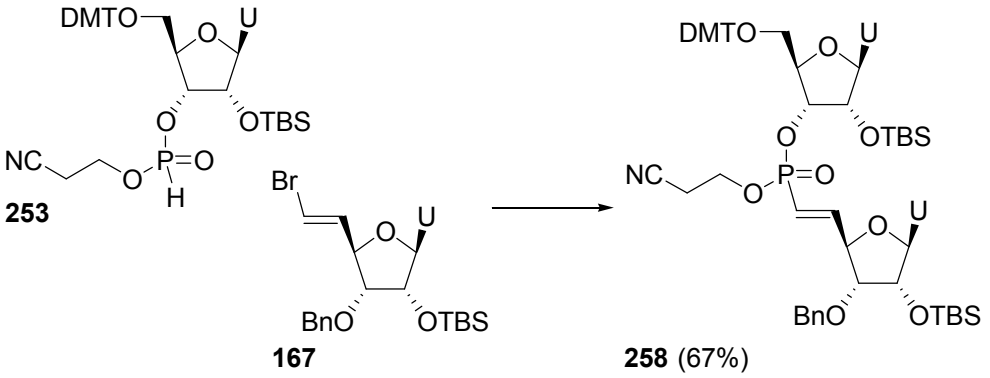
2.5 Palladium(0) Cross-Couplings

Due to the base sensitive nature of the cyanoethyl group it is necessary to find an alternative HBr scavenger to the traditionally used amine base. Propylene oxide has been found to be a suitable

alternative to this; however, its volatile nature means that it is necessary to carry out the reaction in a sealed tube. The reaction vessel used was either a small reactivial or a carousel tube from the Radleys carousel®.

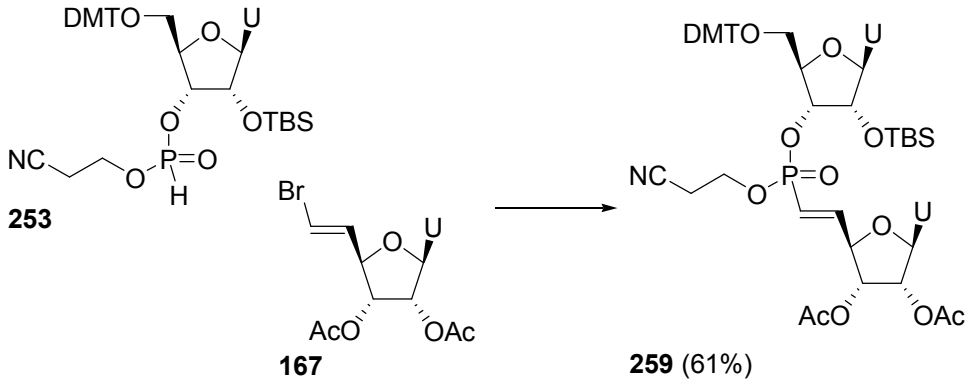
After completion of the first generation synthesis, the uridine vinyl bromide was used to test the Pd(0) cross-coupling reaction since this had previously not been carried out using RNA nucleosides. Using the methodology previously developed in the group for the cross-coupling reactions, $Pd(OAc)_2$ was used to generate the active catalytic Pd(0) species *in situ*.[155,238,157]

The *H*-phosphonate **253** was coupled to the first generation uridine vinyl bromide **167** using the standard coupling procedures optimised during work on the DNA series (Scheme 91).[129,154] The *H*-phosphonate was present in excess (1.3 eq.), along with $Pd(OAc)_2$ (0.2 eq.), 1,1'-*bis*(diphenylphosphino)ferrocene (0.4 eq.) and propylene oxide (20 eq.) in THF (scheme 46).[155,158,239] We were pleased to be able to form the U*U dimer **258** as a 2:1 mixture of diastereoisomers in a pleasing 67% yield. Purification by column chromatography and reverse phase HPLC was unsuccessful at removing a minor impurity of the oxidised 1,1'-*bis*(diphenylphosphino)ferrocene ligand [$dppf(O)_2$] and separating the diastereoisomers. At this stage the reaction was not further optimised.



Scheme 91. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.

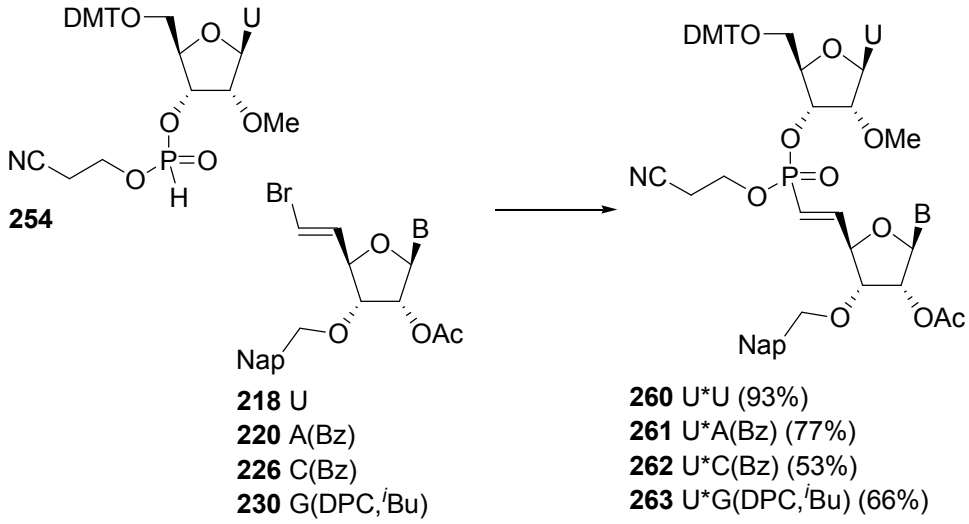
A proof of concept study was also obtained by carrying out the Pd(0) cross-coupling of 2'-OTBS *H*-phosphonate **253** and the *bis*-acetate uridine vinyl bromide **211** first obtained in the second generation synthesis prior to the final route being developed. Once again this gave pleasing results of 61% yield of the U*U dimer **259** (Scheme 92).



Scheme 92. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.

Initial investigations of the Pd(0) coupling with the second generation RNA vinyl bromides the Pd(0) couplings were carried out using the vinyl bromides products from the nucleoside base addition. Using the standard conditions the set of the four different vinyl bromides were coupled with the 2'-OMe uridine *H*-phosphonate

254 (1.3 eq.). The pleasing results obtained the four different vinylphosphonate dimers, U*U **260**, U*A$^{(Bz)}$ **261**, U*C$^{(Bz)}$ **262** and U*G$^{(DPC,iBu)}$ **263** in yields of 93%, 77%, 53% and 66% respectively (Scheme 38).

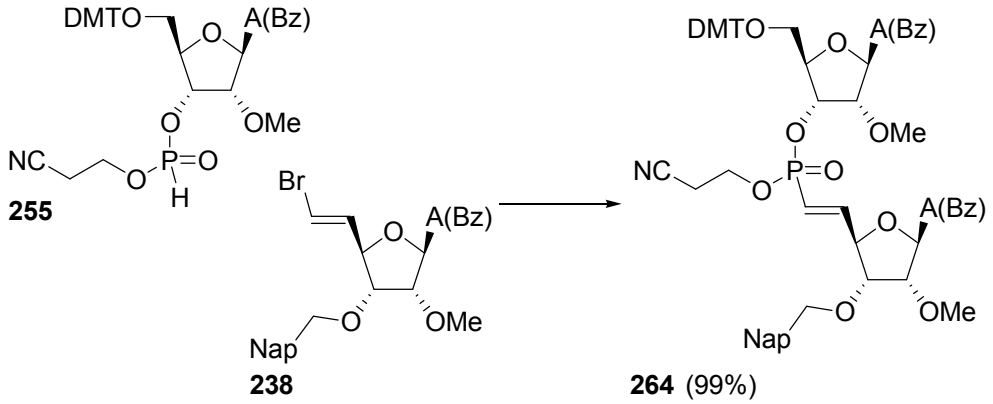


Scheme 93. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.

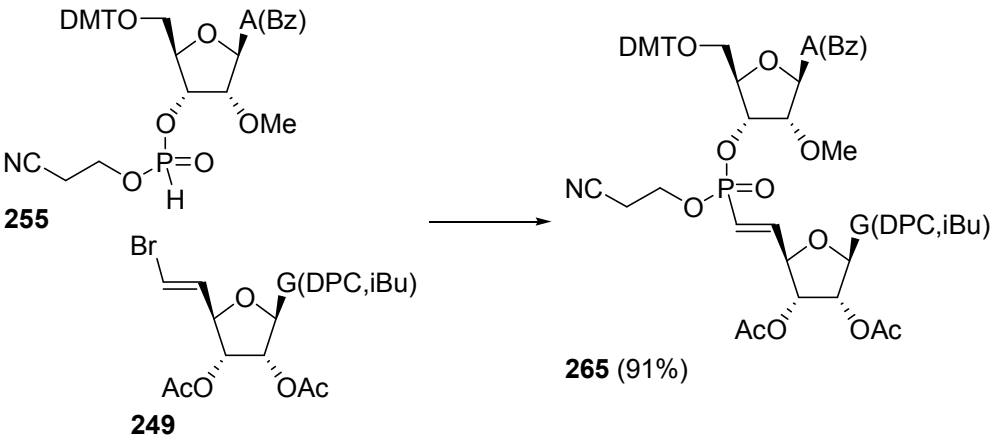
The results with the U*G$^{(DPC,iBu)}$ dimer **263** were particularly pleasing since this is the first time we were able to achieve a complete reaction without having to use sub-stoichiometric quantity of the palladium catalyst.[129] Bertram found that even after 24 hours the coupling of a thymidine *H*-phosphonate **79** with the deoxyribonucleotide G vinyl bromide failed to go to completion. After 7 hours reaction time, the formation of the T*G dimer (67% borsm) was observed, however, 46% of the unreacted vinyl bromide starting material was recovered.[129] In this case the only the amine of the guanine was protected and not the carbonyl group.

Until this stage, the Pd(0) cross-coupling reactions have focussed on pyrimidine dimers or mixed purine-pyrimidine dimers. With the

success of forming the U*A$^{(Bz)}$ **261** and U*G$^{(DPC,iBu)}$ **263** dimers in such good yields it was desirable to investigate purine-purine dimers. The adenosine **238** and guanosine vinyl bromides **249**, these were coupled to the adenosine *H*-phosphonate **255** in pleasing yields to obtain the A$^{(Bz)}$*A$^{(Bz)}$ **264** and A$^{(Bz)}$*G$^{(DPC,iBu)}$ **265** dimers in excellent 99% and 91% yields respectively (Scheme 94, Scheme 95).



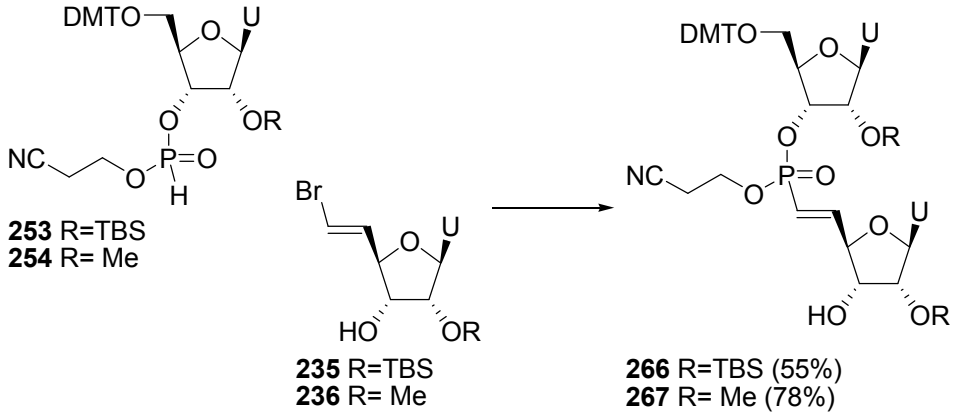
Scheme 94. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.



Scheme 95. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.

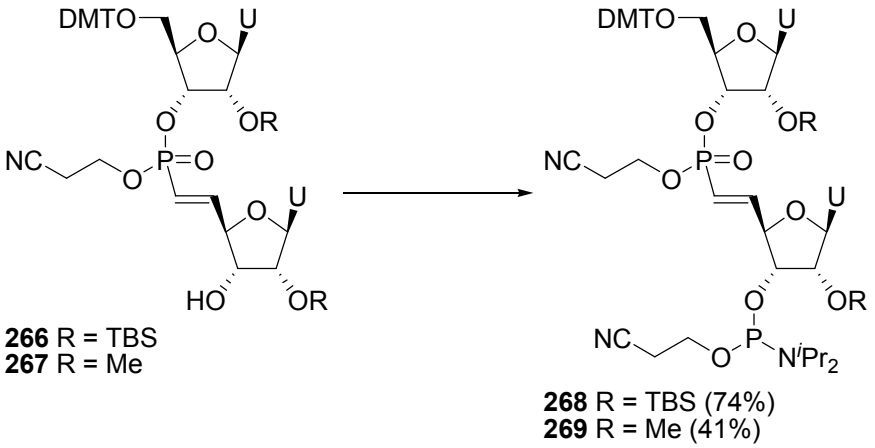
For the synthesis of the RNA oligomers, the 3'-OH U*U dimers with both the 2'-OMe and the 3'-OTBS groups were also synthesised. The two dimers U*U (2'-OTBS) **266** and U*U (2'-OMe) **267** were

obtained in 55% and 78% yields respectively as mixtures of diastereoisomers (Scheme 96).



Scheme 96. Reagents & conditions: $Pd(OAc)_2$, dppf, propylene oxide, THF.

To utilise the vinylphosphonate-linked dinucleotides in solid-phase oligonucleotide synthesis, it was necessary to elaborate the 3'-OH position of the U*U dimers as the phosphoramidite. This was achieved using 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.1 eq.), DIPEA (4 eq.) and DMAP (0.2 eq.) in CH_2Cl_2 in the presence of freshly activated 3Å molecular sieves and under a protective atmosphere (Scheme 97). The 2'-OTBS phosphoramidite dimer **268** was obtained in a 74% yield and the 2'-OMe phosphoramidite dimer **269** in a 41% yield from the corresponding 3'-OH U*U dimers **266** and **267** respectively (Scheme 97).



Scheme 97. Reagents & conditions: 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, DIPEA, DMAP, CH_2Cl_2.

With limited material and time constraints this reaction has not yet been optimised. Due to the two chiral phosphorus centres, these phosphoramidites were obtained as a mixture of the four possible diastereoisomers. The quality of the phosphoramidite reagent was key to the success of this reaction and was distilled under reduced pressure prior to use, to minimise the contamination with the decomposition product *H*-phosphonate (usually observed as an insoluble white solid). Unfortunately, despite investigating several different solvent systems the phosphoramidites were found to be co-polar with the *H*-phosphonate byproduct generated.

3. Conclusions and Future Work

3.1 Conclusions

Based on the aims set out at the start of this research, we have been very successful in developing a synthesis of the generic vinyl bromide from α-D-glucose. The desired *E*-vinyl bromide was obtained in stereochemically pure form and gave direct access to the four different vinyl bromide nucleosides as single stereoisomers. This is the first time that the four vinyl bromide nucleosides have been obtained as single stereoisomers and it is the first time that RNA nucleosides have been synthesised.

After obtaining the four different vinyl bromide nucleosides, the functionality of the 2'-positions were modified to be analogous to the 2'-blocking groups of the phosphoramidites used in solid phase oligonucleotide synthesis. Deprotection of the 2-methylnaphthyl protecting group at the 3'-position was also achieved for the pyrimidine nucleosides, although, regrettably due to time constraints this was not achieved in the purine nucleosides.

The palladium-catalysed cross-coupling reaction was successfully carried out between the vinyl bromide nucleosides of all four different bases and the uridine and adenosine *H*-phosphonates. These provide the first examples of vinylphosphonate-linked RNA dinucleotides and the first examples of purine-purine vinylphosphonate-linked dinucleotides. In addition to this, this was

the first time that the guanosine nucleoside had been successfully applied to the palladium-catalysed cross-coupling.

3.2 Future Work

After obtaining the vinylphosphonate-linked dinucleotide 3'-phosphoramidites, these could be incorporated into RNA oligonucleotides. This has previously been achieved with the vinylphosphonate-linked DNA phosphoramidites by Abbas and Bertram.[129,154]

With two interesting targets we attempted the synthesis of the oligonucleotides using an Applied Biosystems RNA/DNA synthesiser (Model 594). Standard RNA synthesis protocols were used apart from the coupling time on the occasions that the vinylphosphonate-linked U*U phosphoramidite was added. Using the same strategy as when vinylphosphonate-linked DNA oligomers were synthesised, the coupling time was increased by the addition of a 900 second waiting period after the solid support column had been flushed with the vinylphosphonate phosphoramidite and tetrazole solution (Figure 18). Although the phosphoramidites synthesised are obtained as mixtures of diastereoisomers the oligomers undergo a global deprotection at the end of the synthesis which removes the cyanoethyl protecting group and leaves the phosphorus centres achiral.

Unfortunately, the oligomer synthesis was not successful and there was poor coupling of the vinylphosphonate-linked dinucleotide. The conditions used were not optimised to the synthesis of RNA oligomers; typically, RNA oligonucleotide synthesis requires longer coupling times than DNA synthesis. Due to lack of time and material, it was unfortunate that we were not able to optimise the oligomer synthesis procedures; however this is a process that can be developed in the future.

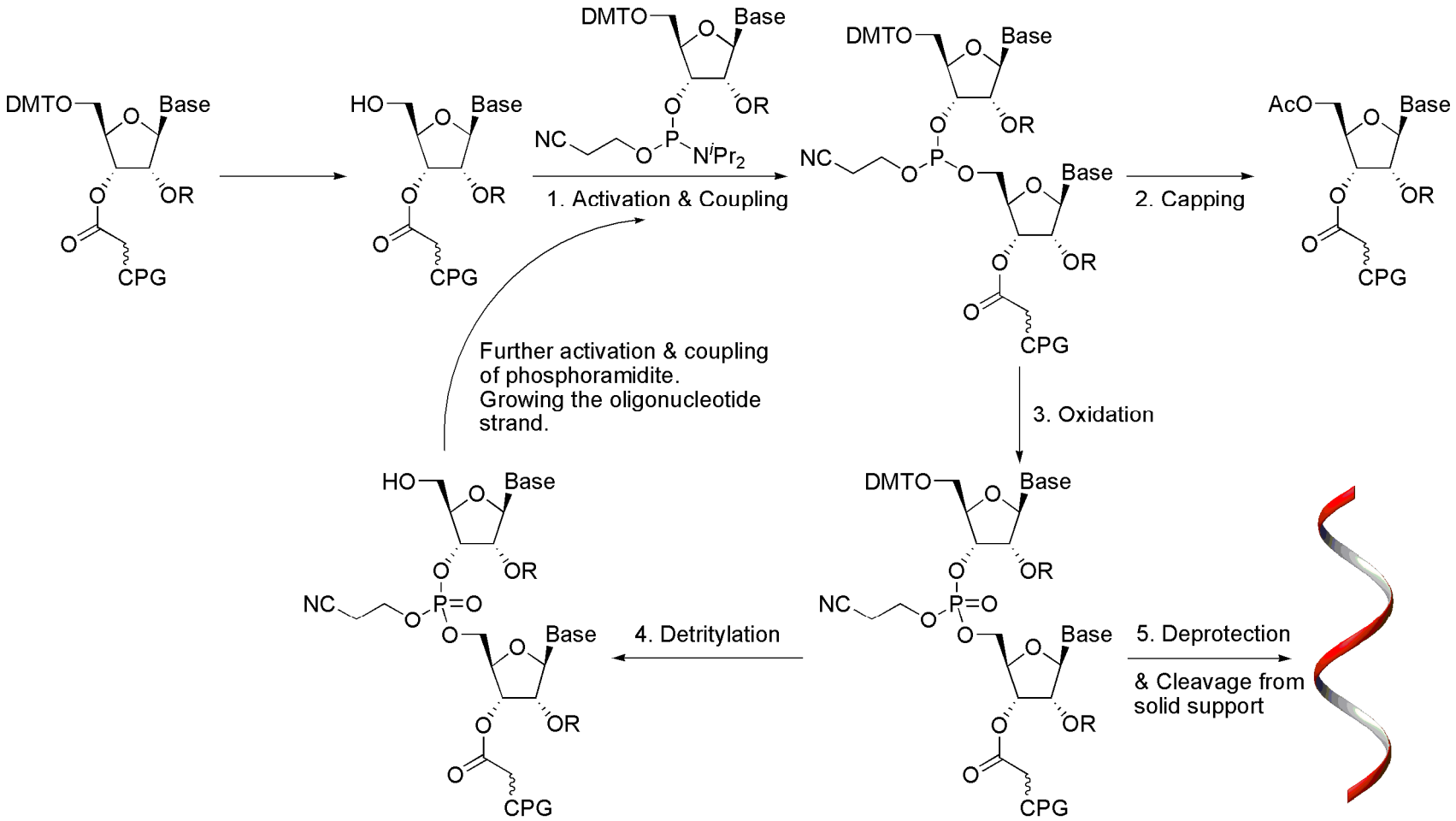


Figure 18. Synthesis cycle for the automated synthesis of oligonucleotides.

- EXPERIMENTAL -

4. Experimental

4.1 General Considerations

The starting materials used in the syntheses were obtained from commercial suppliers and were used without further purification. All reactions were carried out in oven dried glassware and were carried out under a protective argon atmosphere at room temperature unless otherwise stated. Dry Et_2O was obtained from solvent purification towers containing activated alumina. Early on in this project, the dry THF used was also obtained from towers containing activated alumina, however, in the second generation synthesis, the THF drying process was changed to using a still and freshly distilling the THF over potassium and using benzophenone.[240] Dry methanol was distilled over Mg/I_2[241] and CH_2Cl_2 over CaH_2 according to literature procedures.[242] Thin layer chromatography (tlc) was carried out using Merck silica gel precoated glass plates as the stationary phase. The tlc plate was then visualized under a UV lamp then stained with either basic potassium permanganate solution or acidic vanillin solution. The flash column chromatography was carried out using Merck silica gel 60, 35-70 μm as the stationary phase according to the procedure of Still *et al*.[243] The solvents used were all analytical grade reagents. The term 'petrol' refers to petroleum ether (40-60 ^oC fraction) and 'ether' refers to diethyl ether solvent. All water used was deionised water.

Microanalysis data was obtained using an Exeter Analytical CE-440 elemental analyzer. Infra-red spectra were obtained using either a Perkin Elmer 1600 FTIR spectrometer, a Bruker Tensor 27 or a Nicolet Avatar 360 FTIR. Spectra were acquired as dilute sample solutions in $CHCl_3$ or as thin films on a NaCl disc, or solid phase FT-IR (Nicolet Avatar 360 FTIR). Mass spectra were obtained using a Micromass AutoSpec or Micromass LCT spectrometers using electrospray (ES), fast atom bombardment (FAB), electron ionisation (EI) or chemical ionisation (CI). Optical rotations were measured using a JASCO Polarimeter DIP-370 (Na lamp, λ 589 nm) using a path length of 5 cm or 10 cm and concentrations are expressed in g/100 mL. In some instances, where a very low optical rotation was observed using the Na lamp an Optical Activity AA-100 Dual Wavelength polarimeter was used with a mercury lamp (λ 546 nm) and a 1 cm or 2 cm path length cell. Melting points were measured using a Gallenkamp variable heater melting point apparatus.

NMR spectra were obtained at 298 K unless otherwise stated at the stated frequency using a Bruker DRX500, Bruker AV400, Bruker AM400, Bruker DMX300 or a JEOL EX270 spectrometer. The samples were acquired using dilute solutions in $CDCl_3$ unless otherwise stated. The samples were referenced to a residual solvent peak ($CHCl_3$ δ_H 7.27 ppm, δ_C 77.1 ppm, d_4-MeOD δ_H 3.35 ppm, δ_C 49.0 ppm). The ^{31}P NMR spectra are recorded with H_3PO_4 as an external standard. All coupling constants are reported in

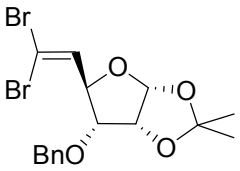
Hertz (Hz), and the multiplicities of the signals labelled are abbreviated as singlet (s), doublet (d), double doublet (dd), dt (doublet of triplets) and t (triplet), br (broad), m (multiplet) and sometimes apparent (app) or obscured (obs). The protons of the furanose ring of the sugars are labelled 1-*H*, 2-*H*, 3-*H etc.* In the case of the nucleosides, the nomenclature used for describing the protons of the furanose ring uses a superscript prime *i.e.* 1'-*H*, 2'-*H*, 3'-*H etc.* to distinguish them from the protons of the heterocycle nucleobases (5-*H*, 6-*H etc.*). The nucleoside bases of the vinylphosphonate dimers are numbered sequentially in the 3'-O $\rightarrow$ 5'-C direction. Assignments were made using 2-dimensional NMR spectroscopy techniques of ^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC. In the cases of the vinylphosphonate-linked dinucleotides, ^{1}H TOCSY was also used.

The phosphoramidite reagents were purchased from Link Technologies and were used without further purification.

4.2 Synthesis of the Generic Vinyl Bromide

4.2.1 First Generation Synthesis

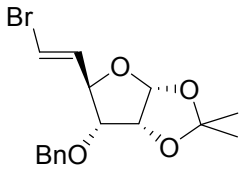
6-Dibromo-(5-deoxy-5-methylidene)-3-*O*-benzyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 162



Triphenyl phosphine (11.8 g, 45.0 mmol) and carbon tetrabromide (7.46 g, 22.5 mmol) were combined in CH_2Cl_2 (25 mL) at 0 °C for 30 minutes. The resulting solution was then transferred *via* canula into a flask containing the 3-OBn 5-aldehyde (3.13 g, 11.3 mmol) eluting with an additional portion of CH_2Cl_2 (25 mL). Stirring was maintained at 0 °C for 6 hours then saturated aqueous NH_4Cl solution (100 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL) then the combined organic phases were washed with brine (175 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the crude product was performed using column chromatography (2:1 Petrol/Et_2O eluant) to produce the dibromo olefin **162** (3.37 g, 69%) as a colourless oil. R_f 0.36 (2:1 Petrol/Et_2O); $[\alpha]_D^{29}$ +4.63 (*c* 0.73, $CHCl_3$); found C, 44.42%; H, 4.14% ($C_{16}H_{18}Br_2O_4$ requires C, 44.27%; H, 4.14%); ν_{max}/cm^{-1} ($CHCl_3$) 2985 (C-H), 2934 (C-H), 2872 (C-H), 1629 (C=C), 1498 (C=C), 1455 (C=C), 1384, 1376; δ_H (400 MHz, $CDCl_3$) 7.42-7.30 (5H, m, Ar-*H*), 6.30 (1H, d, *J* 8.6, 5-*H*), 5.70 (1H, d, *J* 3.6, 1-*H*), 4.77 (1H, app d, *J* ~8.6, 4-*H*), 4.75 (1H, d, *J* 12.5, C*H*H), 4.65 (1H, d, *J* 12.5, C*H*H), 4.57 (1H, dd, *J* 4.2 and 3.6, 2-*H*), 3.60 (1H, dd, *J* 8.9 and 4.2, 3-*H*), 1.56 (3H, s, CH_3), 1.37 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 148.6 (6-*C*Br_2), 137.3 (Ar-*C*), 135.2 (5-*C*H),

128.6 (2 x Ar-*C*H), 128.2 (Ar-*C*H), 127.9 (2 x Ar-*C*H), 113.5 (*C*(CH_3)$_2$), 103.8 (1-*C*H), 80.9 (3-*C*H), 78.1 (4-*C*H), 77.6 (2-*C*H), 72.3 (*C*H_2), 26.9 (CH_3), 26.6 (CH_3); *m/z* (ES+) 454.9466 (37) (M+Na, $C_{16}H_{18}{}^{79}Br^{79}BrO_4Na^+$ requires 454.9470), 456.9450 (100) (M+Na, $C_{16}H_{18}{}^{79}Br^{81}BrO_4Na^+$ requires 456.9449), 458.9453 (30) (M+Na, $C_{16}H_{18}{}^{81}Br^{81}BrO_4Na^+$ requires 458.9429).

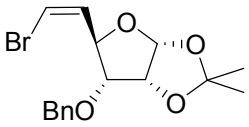
6-*E*-bromo-(5-deoxy-5-methylidene)-3-*O*-benzyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 163



Dimethyl phosphite (7.80 mL, 85.3 mmol) and Et_3N (5.90 mL, 42.6 mmol) were added to a stirred solution of dibromo olefin **162** (9.26 g, 21.3 mmol) in dry DMF (110 mL). The reaction mixture was heated at 110 ^oC for 17 hours then cooled, diluted with water (200 mL) and extracted with Et_2O (4 x 120 mL). The combined organics were washed with water (150 mL) then brine (150 mL), dried ($MgSO_4$) and evaporated to give the crude products as a 3:1 mixture of *E*- and *Z*- isomers respectively. Column chromatography (2:1 Petrol/Et_2O eluant) isolated the desired *trans*-vinyl bromide **163** (3.40 g, 45%) as a colourless oil. R_f 0.42 (2:1 Petrol:Et_2O); $[\alpha]_D^{27}$ +41.1 (*c* 0.42 in $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2985 (C-H), 2936 (C-H), 2899 (C-H), 2872 (w), 1627 (C=C), 1455 (C=C), 1384, 1376; δ_H (400 MHz, $CDCl_3$) 7.40-7.32 (5H, m, Ar*H*), 6.49 (1H, dd, *J* 13.7, 1.0, 6-*H*), 6.15 (1H, dd, *J* 13.7, 6.8, 5-*H*), 5.73 (1H, d, *J* 3.9, 1-*H*), 4.76 (1H, d, *J* 12.2, C*H*H), 4.60 (1H, d, *J* 12.2, CH*H*), 4.57 (1H, dd, *J* 4.1 and 3.9, 2-*H*), 4.46 (1H, ddd, *J*

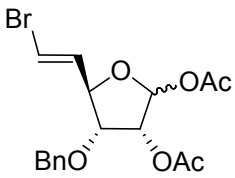
8.6, 6.8, 1.0, 4-*H*), 3.52 (1H, dd, *J* 8.6, 4.1, 3-*H*); δ_C (100 MHz, $CDCl_3$) 137.1 (Ar-*C*), 134.1 (5-*C*H), 128.6 (2 x Ar-*C*H), 128.2 (Ar-*C*H), 128.1 (Ar-*C*H), 113.2 (*C*$(CH_3)_2$), 110.0 (6-*C*H), 103.7 (1-*C*H), 81.4 (3-*C*H), 78.2 (4-*C*H), 77.2 (2-*C*H), 72.4 (CH_2), 26.7 (*C*H_3), 26.4 (*C*H_3); *m/z* (ES+) 377.0365 (M+Na, $C_{16}H_{19}{}^{79}BrO_4Na^+$ requires 377.0364), 379.0362 (M+Na, $C_{16}H_{19}{}^{81}BrO_4Na^+$ requires 379.0344).

6-*Z*-bromo-(5-deoxy-5-methylidene)-3-*O*-benzyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 164



From the above procedure, further elution obtained *cis*-vinyl bromide **164** as a colourless oil, (484 mg, 18%). R_f 0.26 (2:1 Petrol:Et_2O); $[\alpha]_D{}^{27}$ +27.3 (*c* 0.95 in $CHCl_3$); found C, 54.03%; H, 5.26% ($C_{16}H_{19}BrO_4$ requires C, 54.08%; H, 5.26%); ν_{max}/cm^{-1} (CHCl3) 2985 (C-H), 2964 (C-H), 2935 (C-H), 2871 (C-H), 1630 (C=C), 1600 (C=C), 1495 (C=C), 1458 (C=C), 1384, 1375; δ_H (400 MHz, $CDCl_3$) 7.38-7.29 (1H, m, Ar-*H*), 6.49 (1H, dd, *J* 7.4, 0.9, 6-*H*), 6.05 (1H, dd, *J* 8.5, 7.4, 5-*H*), 5.74 (1H, d, *J* 3.9, 1-*H*), 5.04 (1H, app t, *J* ~8.5, 4-*H*), 4.73 (1H, d, *J* 12.5, C*H*H), 4.66 (1H, d, *J* 12.5, CH*H*,), 4.58 (1H, app t, *J* ~3.9, 2-*H*), 3.61 (1H, dd, *J* 8.6, 3.9, 3-*H*), 1.67 (3H, s, CH_3), 1.38 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 137.5 (Ar-*C*), 131.9 (5-*C*H), 128.5 (2 x Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (2 x Ar-*C*H), 113.4 (*C*$(CH_3)_2$), 113.1 (6-*C*H), 103.8 (1-*C*H), 81.3 (3-*C*H), 77.9 (2-*C*H), 75.8 (4-*C*H), 72.2 (CH_2), 26.9 (*C*H_3), 26.6 (*C*H_3); *m/z* (ES+) 377.0355 (M+Na, $C_{16}H_{19}{}^{79}BrO_4Na^+$ requires 377.0364), 379.0366 (M+Na, $C_{16}H_{19}{}^{81}BrO_4Na^+$ requires 377.0364).

6-*E*-bromo-(5-deoxy-5-methylidene)-3-*O*-benzyloxy-1,2-*bis*-acetate-α/β-D-ribofuranose 165α/β

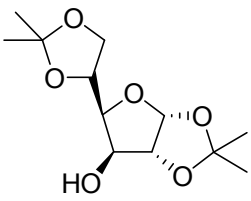


Vinyl bromide **163** (1.23 g, 3.47 mmol) was stirred in 60% v/v AcOH (55 mL) at reflux for 4 hours. The solvent was evaporated and the acetic acid was co-evaporated with toluene (2 x 30 mL). Dry pyridine (10 mL) and acetic anhydride (10 mL) were added to the residue which was then stirred at room temperature for 23 hours. H_2O (40 mL) was added and the aqueous layer extracted with Et_2O (3 x 30 mL). The combined organics were washed with brine (2 x 25 mL), $CuSO_4$ (20% w/v) solution (20 mL), dried ($MgSO_4$) and reduced *in vacuo*. The residue was co-evaporated with toluene (2 x 10 mL) to obtain *bis*-acetate **165** in 9:1 mixture of diastereoisomers (1.17 g, 85%) as a foam. R_f 0.42 and 0.21 (1:1 Petrol:Et_2O); for the mixture of anomers: found C, 51.40%; H, 4.73% ($C_{17}H_{19}BrO_6$ requires C, 51.14%; H, 4.80%); Major β isomer: R_f 0.42 (1:1 Petrol:Et_2O); $[\alpha]_D^{27}$ +14.4 (*c* 1.31, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2874 (C-H), 1746 (C=O), 1626 (C=C), 1455 (C-H), 1372; δ_H (400 MHz, $CDCl_3$) 7.40-7.29 (5H, m, Ar-H), 6.42 (1H, dd, *J* 13.6 and 1.0, 6-*H*), 6.16 (1H, dd, *J* 13.6 and 7.2, 5-*H*), 6.14 (1H, app s, 1-*H*), 5.30 (1H, app d, *J* ~4.4, 4-*H*), 4.64 (1H, d, *J* 11.6, C*H*H), 4.49 (1H, d, *J* 11.6, CH*H*), 4.46 (1H, app t, *J* 7.8, 2-*H*), 4.02 (1H, dd, *J* 7.8 and 4.4, 3-*H*), 2.15 (3H, s, 2-COCH_3), 2.08 (3H, s, 1-COCH_3)); δ_C (100 MHz, $CDCl_3$) 169.9 (2-*C*O), 169.1 (1-*C*O), 137.0 (Ar-*C*), 135.5 (5-*C*H), 128.7 (2 x Ar-*C*H), 128.3 (Ar-*C*H), 128.1 (2 x Ar-*C*H), 110.1 (6-*C*H), 98.5 (1-*C*H), 82.0 (2-*C*H),

80.2 (3-*C*H), 73.5 (*C*H_2), 73.4 (4-*C*H), 21.2 (2-CO*C*H_3), 20.9 (1-CO*C*H_3). Minor α isomer: R_f 0.21 (1:1 Petrol:Et_2O); δ_H (270 MHz, $CDCl_3$) 7.37-7.30 (5H, m, Ar-H), 6.38 (1H, d, *J* 4.7, 1-*H*), 6.37 (1H, dd, *J* 13.6 and 1.2, 6-*H*), 6.04 (1H, dd, *J* 13.6 and 6.8, 5-*H*), 5.19 (1H, dd, *J* 6.3 and 4.7, 2-*H*), 4.67 (1H, d, *J* 11.9, C*H*H), 6.49 (1H, d, *J* 11.9, CH*H*), 4.54 (1H, ddd, *J* 6.8, 5.7 and 1.2, 4-*H*), 3.88 (1H, dd, *J* 6.3 and 5.7, 3-*H*), 2.15 (3H, s, COC*H*$_3$), 2.14 (3H, s, COC*H*$_3$); *m/z* (ES+) for the mixture of anomers: 421.0279 (M+Na, $C_{17}H_{19}{}^{79}BrO_6Na^+$ requires 421.0263), 423.0237 (M+Na, $C_{17}H_{19}{}^{79}BrO_6Na^+$ requires 423.0242).

4.2.2 Second Generation Synthesis

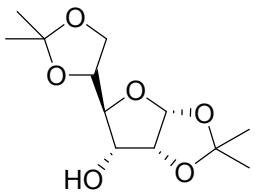
1,2:5,6-Diisopropylidene-α-D-glucofuranose 172



Following a modified literature procedure,[170] iodine (7.16 g, 56.4 mmol), was added to a stirring suspension of α-D-glucose **173** (50.8 g, 282 mmol) in acetone (2.5 L). The reaction mixture was then heated at reflux for 4 ½ hours then cooled, filtered and the filtrate quenched with saturated Na_2SO_3 solution (750 mL). The acetone was removed *in vacuo* and the remaining solution was extracted with Et_2O (3 x 500 mL). The combined organics were dried (Na_2SO_4) then concentrated *in vacuo* and the pale yellow solid was recrystallised from petrol to a obtain glucofuranose **172** as a white solid 53.1 g (72%). m.p. 108–110 °C (Lit. 108-109 °C, aq. MeOH)[244]; R_f 0.46 (4:1 Et_2O:Petrol); $[\alpha]_D^{23}$ -10.3 (*c* 0.73, $CHCl_3$), (Lit. $[\alpha]_D$ -13.51, $CHCl_3$);[170] found C, 55.38%; H, 7.69% ($C_{12}H_{20}O_6$

requires C 55.37%, H 7.74%); ν_{max}/cm^{-1} ($CHCl_3$) 3607 (O-H), 3474 (O-H), 2988 (C-H), 2938 (C-H), 2892 (C-H), 1455 (C-H), 1384, 1374; δ_H (400 MHz, $CDCl_3$) 5.95 (1H, d, *J* 3.6, 1-*H*), 4.54 (1H, app d, *J* ~3.6, 2-*H*), 4.35 (2H, m, 3-*H* and 5-*H*), 4.18 (1H, dd, *J* 8.8 and 5.7, 6-*H*a), 4.08 (1H, dd, *J* 7.6 and 3.2, 4-*H*), 3.99 (1H, dd, *J* 8.8 and 5.7, 6-*H*b), 2.55 (1H, d, *J* 4.0, O*H*), 1.51 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.33 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 111.8 (*C*), 109.7 (*C*), 105.3 (1-*C*H), 85.1 (2-*C*H), 81.1 (4-*C*H), 75.3 (3-*C*H), 73.5 (5-*C*H), 67.7 (CH_2), 26.9 (*C*H_3), 26.8 (*C*H_3), 26.2 (*C*H_3), 25.1 (*C*H_3); *m/z* (ES+) 261.1333 (M+H, $C_{12}H_{21}O_6^+$ requires 261.1338), 283.1153 (M+Na, $C_{12}H_{20}O_6Na^+$ requires 283.1152).

1,2:5,6-Diisopropylidene-α-D-allofuranose 174

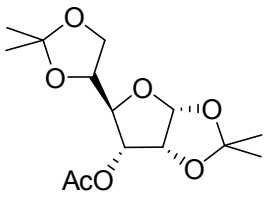


Using a modified procedure of Saito *et al.*[176] PDC (908 mg, 2.41 mmol) and Celite® (3.10 g) were added to a stirred solution of glucofuranose **172** (9.37 g, 36.0 mmol) in CH_2Cl_2 (70 mL) and stirred at room temperature. After ten minutes, Ac_2O (11.2 mL, 11.9 mmol) was added and the reaction mixture was heated at reflux for 3 hours. The solvent was removed *in vacuo* to obtain a crude residue. Water (5 mL) was added and the acetic acid was co-evaporated with toluene (3 x 30 mL). The residue was then taken up in EtOAc (20 mL) and filtered through a pad of silica and eluted with EtOAc/CH_2Cl_2 (9:1 v/v) (3 x 100 mL). The combined filtrates were

reduced to a crude white solid of ketone (10.0 g, quantitative yield), R_f 0.72 and 0.31 (Et_2O).

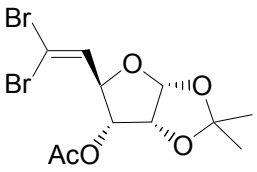
The crude ketone (10.0 g, 36.0 mmol) was taken up in anhydrous Et_2O (195 mL) and stirred at 0 °C. $NaBH_4$ (1.76 g, 46.6 mmol) was added and after a further 10 minutes MeOH (50 mL) was added and the reaction mixture was stirred for 17 hours (0 °C → r.t.). The reaction mixture was quenched with H_2O (100 mL) and the aqueous layer was extracted with Et_2O (3 x 80 mL). The combined organics were dried ($MgSO_4$), filtered and reduced to obtain allofuranose **174** a white solid (7.92 g, 84% over 2 steps). m.p. 76–78 °C (Lit. 75-76 °C, petrol)[179]; R_f (3:2 Et_2O:Petrol) 0.19; $[\alpha]_D^{23}$ +41.9 (*c* 0.73, $CHCl_3$) (Lit. $[\alpha]_D^{22}$ +38.0, $CHCl_3$);[179] ν_{max}/cm^{-1} ($CHCl_3$) 3556 (O-H), 2988 (C-H), 2937 (C-H), 2896 (C-H), 1455 (C-H), 1384, 1375; δ_H (400 MHz, $CDCl_3$) 5.82 (1H, d, *J* 3.8, 1-*H*), 4.62 (1H, dd, *J* 5.2 and 3.8, 2-*H*), 4.31 (1H, app d, *J* 6.6 and 4.7, 4-*H*), 4.09 (1H, dd, *J* 8.5 and 6.6, 6-*H*a), 4.04 (1H, dd, *J* 8.4, 6.6 and 5.2, 3-*H*), 4.02 (1H, dd, *J* 8.5 and 6.6, 6-*H*b), 3.82 (1H, dd, *J* 8.5 and 4.7, 5-*H*), 2.55 (1H, d, *J* 8.4, O*H*), 1.59 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.38 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 112.9 (1,2-*C*$(CH_3)_2$), 109.9 (5,6-*C*$(CH_3)_2$), 104.0 (1-*C*H), 79.8 (4-*C*H), 79.0 (2-*C*H), 75.7 (5-*C*H), 72.6 (3-*C*H), 66.0 (6-CH_2), 26.7 (*C*H_3), 26.6 (*C*H_3), 26.4 (*C*H_3) and 25.4 (*C*H_3); *m/z* (ES+) 283.1146 (M+Na, $C_{12}H_{21}O_6Na^+$ requires 283.1152).

3-*O*-Acetyl-1,2:5,6-diisopropylidene-α-D-allofuranose 190



Neat Et_3N (8.30 mL, 59.4 mmol), Ac_2O (2.90 mL, 30.9 mmol) and DMAP (242 mg, 1.98 mmol) were added sequentially to a stirring solution of allofuranose **174** (5.15 g, 19.8 mmol) in CH_2Cl_2 (100 mL) at r.t. Stirring was maintained under N_2 (g) atmosphere for 19 hours then the reaction mixture was quenched with saturated aqueous NH_4Cl solution (80 mL). Phases were separated and the aqueous phase was re-extracted with Et_2O (3 x 50 mL). The combined organics were washed with brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo* to crystalline white solid of acetate **190** (5.67 g, 95%). m.p. 73-75 ^oC; R_f 0.13 (2:1 Petrol:Et_2O); $[\alpha]_D^{23}$ +94.9 (*c* 1.02, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2986 (C-H), 2937 (C-H), 2892 (C-H), 1742 (C=O), 1455 (C-H, C-O), 1385, 1374; δ_H (400 MHz, $CDCl_3$) 5.82 (1H, d, *J* 3.8, 1-*H*), 4.88 (1H, dd, *J* 8.6 and 5.0, 3-*H*), 4.82 (1H, dd, *J* 5.0 and 3.8, 2-*H*), 4.30 (1H, ddd, *J* 6.9, 5.6 and 4.3, 5-*H*), 4.16 (1H, dd, *J* 8.6 and 4.3, 4-*H*), 4.07 (1H, dd, *J* 8.6 and 6.9, 6-*H*a), 3.91 (1H, dd, *J* 8.6 and 5.6, 6-*H*b), 2.13 (3H, s, $COCH_3$), 1.56 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.35 (3H, s, CH_3) and 1.34 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 170.0 (*C*OCH_3), 113.1 (*C*), 110.0 (*C*), 104.1 (1-*C*H), 77.7 (2-*C*H), 77.5 4-*C*H), 75.0 (5-*C*H), 72.6 (3-*C*H), 65.6 (6-CH_2), 26.7 (*C*H_3), 26.6 (*C*H_3), 26.2 (*C*H_3), 25.0 (*C*H_3), 20.7 (CO*C*H_3); *m/z* (ES+) 303.1443 (M+H, $C_{14}H_{23}O_7$ requires 303.1438), 325.1260 (M+Na, $C_{14}H_{22}O_7Na^+$ requires 325.1258).

6-Dibromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 195



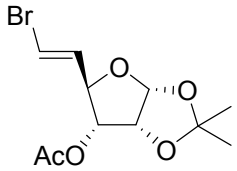
Method 1. Periodic acid (1.34 g, 5.87 mmol) was added to a stirring solution of allofuranose **190** in dry THF (9.5 mL). Stirring was maintained for 6 hours then $NaIO_4$ (1.17 g, 2.35 mmol) was added. After a further 18 hours the reaction mixture was filtered and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (10 mL) and was washed with $Na_2S_2O_3$ solution (10 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to a pale yellow oil aldehyde **192** (1.06 g).

Solid CBr_4 (3.05 g, 9.21 mmol) was added to a stirred solution of Ph_3P (4.83 g, 18.4 mmol) in dry CH_2Cl_2 (9 mL) at 0 ^oC under an argon atmosphere. Stirring was maintained for 1 hr then a solution of the crude aldehyde **192** (1.06 g) in dry CH_2Cl_2 (9 mL) was added. The reaction mixture was stirred for 18 hours gradually warming to r.t. and was then quenched with water (25 mL), separated and the aqueous phase re-extracted with Et_2O (3 x 10 mL). Combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$) and reduced *in vacuo* to crude yellow solid (4.50 g). Purification by column chromatography (2:1 petrol/Et_2O) gave dibromo olefin **195** as a colourless oil (662 mg, 37% over 2 steps).

Method 2. A solution of KHMDS (26.3 mL, 0.5 M in toluene, 13.8 mmol) was added to a stirried solution of $Ph_3P^+CHBr_2.Br^-$ (6.71 g, 13.0 mmol) in dry CH_2Cl_2 (7 mL) at -10 ^oC over 75 minutes. A

solution of crude aldehyde **92** (1.63 g, 7.08 mmol) in dry CH_2Cl_2 (25 mL) was then added dropwise over 15 minutes. The reaction mixture was gradually warmed to r.t. over 3 hours then quenched with water (30 mL). The phases were separated and the aqueous layer was re-extracted with Et_2O (3 x 15 mL). Combined organic portions were dried ($MgSO_4$), filtered and reduced *in vacuo* to a brown residue. Purification by column chromatography (2:1 Petrol:Et_2O) produced dibromo olefin **195** as a pale orange oil (1.05 g, 38% over 2 steps). R_f 0.25 (2:1 Pet/Et_2O); $[\alpha]_D^{29}$ +27.9 (*c* 0.22, $CHCl_3$); found C, 34.37%; H, 3.59% ($C_{11}H_{14}Br_2O_5$ requires C, 34.22%; H, 3.66%); ν_{max}/cm^{-1} ($CHCl_3$) 3025 (C-H), 1743 (C=O), 1602 (C=C), 1518 (C=C), 1424, 1376; δ_H (400 MHz, $CDCl_3$) 6.44 (1H, dd, *J* 8.3, 5-H), 5.82 (1H, dd, *J* 3.7, 1-H), 4.81 (1H, dd, *J* 9.2 and 8.3, 4-H), 4.79 (1H, dd, *J* 4.5 and 3.7, 2-H), 4.68 (1H, dd, *J* 9.2 and 4.5, 3-H), 2.17 (3H, s, $COCH_3$), 1.61 (3H, s, CH_3), 1.36 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 170.2 (*C*O), 134.2 (5-*C*H), 113.6 ($C(CH_3)_2$), 104.0 (1-*C*H), 95.8 (6-*C*Br_2), 77.1 (2-*C*H), 77.0 (4-*C*H), 75.4 (3-*C*H), 26.7 (CH_3), 26.4 (CH_3), 20.7 ($COCH_3$).

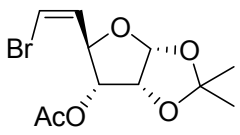
6-*E*-bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 205



Dimethylphosphite (102 μL, 1.11 mmol) and diisopropylamine (78 μL, 554 μmol) were added to a stirred solution of dibromo olefin **195** (107 mg, 277 μmol) in dry DMF (1.38 mL) at r.t. under an Argon

atmosphere. Stirring was maintained for 16 hours then the reaction mixture was quenched with H_2O (2 mL) and extracted with Et_2O (2 x 2 mL). The combined organics were washed with brine (2 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to a 3:1 mixture of *E*- and *Z*-isomers **205** and **206** respectively. Purification by column chromatography yielded the *trans*-vinyl bromide **205** as a white solid (34 mg, 40%). m.p. 68-69 °C; R_f 0.28 (3:1 Petrol:Et_2O); $[\alpha]_D^{22}$ +80.0 (*c* 1.40, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2984 (C-H), 2939 (C-H), 2903 (C-H), 1742 (C=O), 1628 (C=C), 1376; δ_H (400 MHz, $CDCl_3$) 6.51 (1H, dd, *J* 13.7 and 0.8, 6-*H*), 6.19 (1H, dd, *J* 13.7 and 6.4, 5-*H*), 5.81 (1H, d, *J* 3.8, 1-*H*), 4.81 (1H, dd, *J* 4.0 and 3.8, 2-*H*), 4.51 (1H, dd, *J* 9.0 and 4.0, 3-*H*), 4.47 (1H, ddd, *J* 9.0, 6.4 and 0.8, 4-*H*), 2.13 (3H, s, $COCH_3$), 1.55 (3H, s, CH_3), 1.32 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 170.2 ($COCH_3$), 133.2 (5-*C*H), 113.2 ($C(CH_3)_2$), 110.9 (6-*C*H) 103.9 (1-*C*H), 77.3 (2-*C*H), 77.0 (4-*C*H), 75.7 (3-*C*H), 26.6 (CH_3), 26.5 (CH_3), 20.7 ($COCH_3$); *m/z* (ES+) 328.9980 (M+Na, $C_{11}H_{15}{}^{79}BrO_5Na^+$ requires 329.0001), 331.0010 (M+Na, $C_{11}H_{15}{}^{81}BrO_5Na^+$ requires 330.9980).

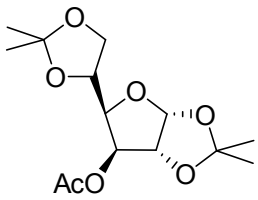
6-*Z*-bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-ribofuranose 206



In the above procedure, further elution during chromatography led to isolation of *cis*-vinyl bromide **206** as a colourless oil (5 mg, 6%). R_f 0.18 (3:1 Petrol:Et_2O); $[\alpha]_D^{29}$ +10.3 (*c* 0.40, $CHCl_3$); ν_{max}/cm^{-1}

($CHCl_3$) 2975 (C-H), 2937 (C-H), 1742 (C=O), 1632 (C=C), 1454, 1376; δ_H (400 MHz, $CDCl_3$) 6.48 (1H, dd, *J* 7.4 and 1.0, 6-*H*), 6.14 (1H, dd, *J* 8.2 and 7.4, 5-*H*), 5.85 (1H, d, *J* 3.7, 1-*H*), 5.08 (1H, ddd, *J* 9.2, 8.2 and 1.0, 4-*H*), 4.79 (1H, dd, *J* 4.6 and 3.7, 2-*H*), 4.73 (1H, dd, *J* 9.2 and 4.6, 3-*H*), 2.15 (3H, s, COCH_3), 1.64 (3H, s, C(CH_3)), 1.37 (3H, s, C(CH_3)); δ_C (100 MHz, $CDCl_3$) 170.5 (*C*O), 131.1 (5-*C*H), 113.6 (*C*(CH_3)), 113.2 (6-*C*H), 104.2 (1-*C*H), 77.4 (2-*C*H), 75.7 (3-*C*H), 75.0 (4-*C*H), 26.8 (*C*H_3), 26.7 (*C*H_3), 20.8 (CO*C*H_3); *m/z* (ES+) 329.0020 (M+Na, $C_{11}H_{15}{}^{79}BrO_5Na^+$ requires 329.0001), 330.9987 (M+Na, $C_{11}H_{15}{}^{81}BrO_5Na^+$ requires 330.9980).

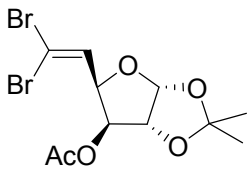
3-*O*-Acetyl-1,2:5,6-diisopropylidene-α-D-allofuranose 193



Neat Et_3N (45.5 mL, 327 mmol), DMAP (1.33 g, 10.9 mmol) and Ac_2O (15.4 mL, 164 mmol) were successively added to a stirred solution of glucofuranose **172** 28.5 g, 109 mmol) in CH_2Cl_2 (550 mL). Stirring at r.t. was maintained for 3 hours until tlc indicated no residual starting material. The reaction was quenched with saturated NH_4Cl solution (200 mL), and stirred for 5 minutes then separated and the aqueous phase re-extracted with Et_2O (2 x 100 mL). The combined organics were washed with brine (200 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to a pale brown solid. The acetate **193** was obtained as pale yellow crystals 31.8 g (96%) following recrystallisation from petrol. m.p. 60-62 °C (lit. 62.0-62.5 °C, aq. MeOH);[194] R_f 0.65 (4:1 Et_2O:Petrol); $[\alpha]_D{}^{25}$ -31.1 (*c* 0.41, $CHCl_3$), (Lit. $[\alpha]_D{}^{22}$ -27.9, $CHCl_3$);[194] ν_{max}/cm^{-1} ($CHCl_3$)

2988 (C-H), 2938 (C-H), 2903 (C-H), 1746 (C=O), 1455, 1374; δ_H (400 MHz, $CDCl_3$) 5.86 (1H, d, *J* 3.7, 1-*H*), 5.24 (1H, app d, *J* ~2.4, 3-*H*), 4.48 (1H, app d, *J* ~3.7, 2-*H*), 4.21 (2H, m, 4-*H* and 5-*H*), 4.06 (1H, dd, *J* 8.4 and 5.4, 6-*H*a), 4.01 (1H, dd, *J* 5.4 and 3.6, 6-*H*b), 2.09 (3H, s, $COCH_3$), 1.50 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.31 (3H, s, CH_3) and 1.29 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 169.6 ($COCH_3$), 112.3 (*C*) 109.3 (*C*), 105.1 (1-*C*H), 83.4 (2-*C*H), 79.7 (5-*C*H), 76.2 (3-*C*H), 72.5 (4-*C*H), 67.2 (6-CH_2), 26.9 (CH_3), 26.8 (CH_3), 26.2 (CH_3), 25.3 (CH_3) and 20.9 ($COCH_3$); *m/z* (ES+) 303.1446 (M+H, $C_{15}H_{23}O_7^+$ requires 303.1438), 325.1261 (M+Na, $C_{15}H_{22}O_7Na^+$ requires 325.1328).

6-Dibromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-xylofuranose 196

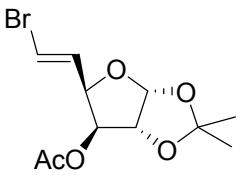


Periodic acid (9.25 g, 40.6 mmol) was added to a stirred solution of acetate **193** (9.82 g, 32.5 mmol) in 1:1 v/v dry EtOAc:THF (65 mL) (EtOAc dried with $CaCl_2$, THF with Na wire). Stirring at r.t. was maintained for 5 hours then the reaction mixture was filtered under vacuum. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (3 x 60 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to obtain the crude aldehyde **194** as a yellow syrup (5.60 g).

Solid Ph_3P (29.5 g, 93.7 mmol) was added to a stirring solution of CBr_4 (16.1 g, 48.6 mmol) in dry CH_2Cl_2 at 0°C under an Argon atmosphere and the resulting solution was stirred for 80 mins. To this mixture was then added a solution of crude aldehyde **194**

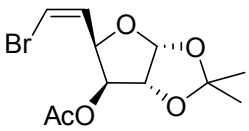
(5.60g, ~24.3 mmol crude material) in dry CH_2Cl_2 (49 mL). Stirring was maintained for a further 17 ½ hours (0 °C to r.t.) and the reaction mixture was then quenched with saturated NH_4Cl solution (100 mL). The phases were separated and the aqueous phase was re-extracted with Et_2O (3 x 50 mL). The combined organics were washed with brine (2 x 100 mL), dried ($MgSO_4$) then concentrated *in vacuo* to a brown residue (42.7 g). Following purification by column chromatography (2:1 Petrol:Et_2O) the dibromo olefin **196** was obtained as a white solid (5.55 g, 59%). R_f 0.34 (2:1 Petrol:Et_2O); m.p. 118-120 °C; $[\alpha]_D^{25}$ -48.7 (*c* 0.42, $CHCl_3$); found C, 34.62%; H, 3.67% ($C_{11}H_{14}Br_2O_5$ requires C, 34.22%; H, 3.66%); ν_{max}/cm^{-1} ($CHCl_3$) 2978 (C-H), 2936 (C-H), 2875 (C-H), 1747 (C=O), 1633 (C=C), 1455, 1376; δ_H (400 MHz, $CDCl_3$) 6.50 (1H, d, *J* 7.6, 5-*H*), 5.93 (1H, d, *J* 3.7, 1-*H*), 5.31 (1H, dd, *J* 3.7 and 3.1, 3-*H*), 4.92 (1H, dd, *J* 7.6 and 3.1, 4-*H*), 4.54 (1H, d, *J* 3.7, 2-*H*), 2.12 (3H, s, COCH_3), 1.55 (3H, s, CH_3), 1.33 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 169.5 (*C*O), 131.8 (5-*C*H), 112.5 (6-*C*Br_2), 104.5 (1-*C*H), 94.4 (*C*$(CH_3)_2$), 83.3 (3-*C*H), 79.3 (2-*C*H), 77.2 (4-*C*H), 26.8 (C(*C*H_3)), 26.3 (C(*C*H_3)), 20.7 (CO*C*H_3); *m/z* (EI+) 383.9234 (M^+, $C_{11}H_{14}{}^{79}Br^{79}BrO_5^+$ requires 383.9208), 385.9193 (M^+, $C_{11}H_{14}{}^{79}Br^{81}BrO_5^+$ requires 385.9188), 387.9154 (M^+,$C_{11}H_{14}{}^{81}Br^{81}BrO_5^+$ requires 397.9167).

6-*E*-Bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-xylofuranose 207



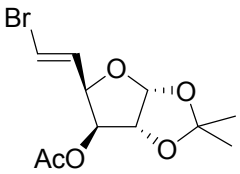
Neat $(MeO)_2P(O)H$ (1.60 mL, 17.4 mmol) and $^{i}Pr_2NH$ (2.45 mL, 17.4 mmol) were added sequentially to a stirring solution of dibromo olefin **196** (3.37 g, 8.73 mmol) in dry DMF (45 mL) and the resulting solution was stirred at r.t. for 22 hours. The reaction was then quenched with water (50 mL) and extracted with Et_2O (3 x 30 mL). The combined organics were washed with brine (30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to obtain a mixture of *E*- and *Z*-isomers as a pale yellow oil (4.10 g, *E:Z* 65:35). Separation of the *E* and *Z* isomers using column chromatography (2:1 Petrol: Et_2O) gave the *trans*-vinyl bromide **207** (1.97 g, 74%) as a colourless oil. R_f 0.55 (2:1 Petrol: Et_2O) $[\alpha]_D^{29}$ -17.5 (*c* 0.83, $CHCl_3$); found C, 42.85%; H, 4.82% ($C_{11}H_{15}BrO_5$ requires C, 43.02%; H, 4.92%); ν_{max}/cm^{-1} (thin film) 2989 (C-H), 2940 (C-H), 1746 (C=O), 1627 (C=C), 1611 (C=C), 1454, 1375; δ_H (270 MHz, $CDCl_3$) 6.51 (1H, dd, *J* 13.7 and 1.3, 6-*H*); 6.15 (1H, dd, *J* 13.7 and 6.1, 5-*H*), 5.93 (1H, d, *J* 3.7, 1-*H*), 5.20 (1H, dd, *J* 3.7 and 3.0, 3-*H*), 4.73 (1H, ddd, *J* 6.1, 3.0 and 1.3, 4-*H*), 4.56 (1H, dd, *J* 3.7 and 3.0, 2-*H*), 2.08 (3H, s, $COCH_3$), 1.52 (3H, s, CH_3), 1.32 (3H, s, CH_3); δ_C (68 MHz, $CDCl_3$) 169.7 ($COCH_3$), 130.1 (5-*C*H), 112.3 ($C(CH_3)_2$), 110.8 (6-*C*H), 104.5 (1-*C*H), 83.4 (2-*C*H), 79.2 (4-*C*H), 76.8 (3-*C*H), 26.8 (CH_3), 26.2 (CH_3), 20.8 ($COCH_3$).

6-*Z*-Bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-xylofuranose 208



In the above procedure, further elution during chromatography isolated *cis*-vinyl bromide **208** as a colourless solid (614 mg, 24%). R_f 0.43 (2:1 Petrol: Et_2O); m.p. 74-75 °C; $[\alpha]_D^{26}$ -106 (*c* 1.02, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2984 (C-H), 2938 (C-H), 1747 (C=O), 1631 (C=C), 1455, 1384, 1375; δ_H (270 MHz, $CDCl_3$) 6.42 (1H, dd, *J* 7.4 and 1.3, 6-*H*), 6.20 (1H, dd, *J* 7.4 and 7.2, 5-*H*), 5.95 (1H, d, *J* 3.7, 1-*H*), 5.34 (1H, d, *J* 3.2 and 3.0, 3-*H*), 5.16 (1H, ddd, *J* 7.2, 3.2 and 1.3, 4-*H*), 4.55 (1H, dd, *J* 3.7 and 3.2, 2-*H*), 2.07 (3H, s, $COCH_3$), 1.52 (3H, s, CH_3), 1.33 (3H, s, CH_3); δ_C (68 MHz, $CDCl_3$) 169.6 ($COCH_3$), 128.8 (5-*C*H), 112.4 ($C(CH_3)_2$), 111.8 (6-*C*H), 104.7 (1-*C*H), 83.6 (2-*C*H), 77.5 (4-*C*H), 77.0 (3-*C*H), 26.8 (CH_3), 26.4 (CH_3), 20.8 ($COCH_3$); *m/z* (ES+) 329.0003 (M+Na, $C_{11}H_{15}{}^{79}BrO_5Na^+$ requires 329.0001), 330.9975 (M+Na, $C_{11}H_{15}{}^{81}BrO_5Na^+$ requires 330.9980).

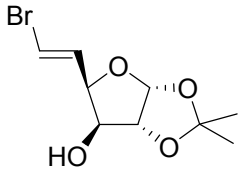
6-*E*-Bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2-*O*-isopropylidene-α-D-xylofuranose 207



Isomerism of *Z*-alkene 208. A solution of *cis*-vinyl bromide **208** (2.95 g, 9.60 mmol) in CCl_4 (100 mL) was degassed using a steady stream of argon for 30 mins. Neat AIBN (158 mg, 960 μmol) and NBS (1.70 g, 9.60 mmol) were then added and the reaction mixture was heated to 80 °C for 2 hours. The solution was then cooled, filtered through a sinter, eluting with further CCl_4 (50 mL). The combined

filtrates were washed sequentially with H_2O (50 mL), saturated $NaHCO_3$ solution (50 mL) and brine (100 mL) then dried ($MgSO_4$), filtered and concentrated *in vacuo* to a yellow oil (*E:Z* ratio 11:7). The isomers were separated by column chromatography (3:1 Petrol:Et_2O) to obtain *E*-vinyl bromide **207** as a colourless oil (1.63 g, 55 %) and *Z*-vinyl bromide **208** as a white solid (37%). Combined yield: 2.73 g (93%). Spectroscopic properties of the product were identical to alkenes obtained from the Wittig reaction.

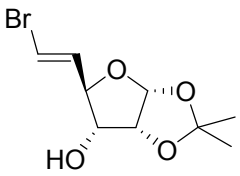
6-*E*-Bromo-(5-deoxy-5-methylidene)-1,2-*O*-isopropylidene-α-D-xylofuranose 209



Solid K_2CO_3 (420 mg, 3.04 mmol) was added to a stirred solution of acetate **207** (1.87g, 6.09 mmol) in dry MeOH (60 mL) and stirring was maintained for 14 h. The solvent was evaporated *in vacuo* and the residue extracted with CH_2Cl_2 (30 mL), dried ($MgSO_4$) and the extracts concentrated to give the alcohol **209** (1.44 g, 87%) as a white solid. R_f 0.18 (2:1 Petrol: Et_2O); m.p. 119-122 °C; $[\alpha]_D^{29}$ -69.7 (*c* 0.66, $CHCl_3$); found C, 40.61%; H, 4.89% ($C_9H_{13}BrO_4$ requires C, 40.78%; H, 4.94%); ν_{max}/cm^{-1} (solution, $CHCl_3$) 3610 (O-H), 3578 (O-H), 2982 (C-H), 2937 (C-H), 2882 (C-H), 1626 (C=C), 1454, 1385, 1376; δ_H (400 MHz, $CDCl_3$) 6.59 (1H, dd, *J* 13.7 and 1.5, 6-*H*), 6.29 (1H, dd, *J* 13.7 and 5.7, 5-*H*), 5.96 (1H, d, *J* 3.7, 1-*H*), 4.68 (1H, ddd, *J* 5.7, 2.6 and 1.5, 4-*H*), 4.57 (1H, d, *J* 3.7 and 3.2, 2-*H*), 4.15 (1H, app t, *J* 4.6, 3.2 and 2.6 3-*H*), 1.67 (1H, d, *J* 4.6, O*H*), 1.51 (3H, s, CH_3), 1.33 (3H, s, CH_3); δ_C

(100 MHz, $CDCl_3$) 130.5 (5-*C*H), 112.0 (*C*$(CH_3)_2$); 111.0 (6-*C*H), 104.5 (1-*C*H), 84.8 (2-*C*H), 80.4 (4-*C*H), 75.7 (3-*C*H), 26.7 (*C*H_3), 26.1 (*C*H_3).

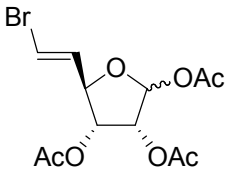
6-*E*-Bromo-(5-deoxy-5-methylidene)-1,2-*O*-isopropylidene-α-D-ribofuranose 210



Dess Martin periodinane[160,161] (4.61 g, 10.9 mmol) was added to a stirred solution of alcohol **209** (1.44 g, 5.43 mmol) in dry CH_2Cl_2 (20 mL) at r.t. under an argon atmosphere and stirring was maintained for 5 ¼ h then the reaction mixture was quenched with $Na_2S_2O_3$ solution (10 mL) and saturated $NaHCO_3$ (10 mL). After a further 15 minutes of stirring, the emulsion was extracted with Et_2O (3 x 15 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to the ketone (1.69 g) as a white solid. The crude ketone was dissolved in dry CH_2Cl_2 (25 mL) and dry MeOH (7.0 mL) and cooled to 0 °C under an argon atmosphere. Solid $NaBH_4$ (1.31 g, 4.98 mmol) was added and the resulting solution was stirred for 19 h before quenching with water (25 mL). The aqueous phase was separated and re-extracted with Et_2O (3 x 20 mL). The combined organics were washed with brine (2 x 20 mL), dried ($MgSO_4$) then concentrated to alcohol **210** (1.04 g, 79% over 2 steps) as a crystalline white solid. R_f 0.48 (1:3 Petrol: Et_2O). m.p. 118-121 °C; $[\alpha]_D^{28}$ +39.5 (*c* 0.26, $CHCl_3$); found C, 40.71%; H, 4.91% ($C_9H_{13}BrO_4$ requires C, 40.78%; H, 43.94); ν_{max}/cm^{-1} ($CHCl_3$) 2985 (O-H), 2939 (O-H), 2872 (C-H), 1629 (C=C), 1456,

1396, 1385, 1377; δ_H (400 MHz, $CDCl_3$) 6.51 (1H, dd, *J* 13.7 and 1.1, 6-*H*), 6.28 (1H, dd, *J* 13.7 and 6.3, 5-*H*), 5.83 (1H, d, *J* 4.0, 1-*H*), 4.59 (1H, dd, *J* 5.0 and 4.0, 2-*H*), 4.15 (1H, ddd, *J* 8.5, 6.3 and 1.1, 4-*H*), 3.75 (1H, app dd, *J* ~8.5 and ~5.0, 3-*H*), 2.39 (1H, br d, *J* 9.8, O*H*), 1.58 (3H, s, CH_3), 1.38 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 133.6 (5-*C*H), 112.9 (*C*$(CH_3)_2$), 109.7 (6-*C*H), 103.7 (1-*C*H), 80.2 (4-*C*H), 78.2 (2-*C*H), 75.7 (3-*C*H), 26.5 (*C*H_3), 26.4 (*C*H_3).

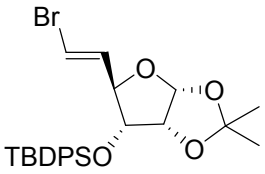
6-*E*-Bromo-(5-deoxy-5-methylidene)-3-*O*-acetyloxy-1,2,3-*tri*-*O*-acetyl-α/β-D-ribofuranose 186α/β



A solution of alcohol **210** 1.02 g, 3.85 mmol) in 70% (v/v) aqueous AcOH (64 mL) was heated at reflux for 3 h then the solvent was removed by co-evaporation with toluene (2 x 30 mL). The residue was dissolved in dry pyridine (10 mL) then Ac_2O (10.9 mL) was added the resulting solution was stirred under an argon atmosphere at r.t. for 3 h. The solvent was removed by co-evaporation with toluene (3 x 25 mL) to orange oil (1.48 g, 3:1 mixture of anomers). Purification by column chromatography yielded *tri*-acetate **186** (1.11 g, 82%) as a colourless oil containing an unseparated mixture of anomers. As a mixture of anomers: $[\alpha]_D^{29}$ +5.1 (*c* 0.59, $CHCl_3$); as a mixture of anomers: found C, 41.04%; H, 4.28% ($C_{12}H_{15}BrO_7$ requires C, 41.04%; H, 4.31%); ν_{max}/cm^{-1} ($CHCl_3$) 2926 (C-H), 1751 (C=O), 1626 (C=C), 1451 (C-H, C-O), 1372 (C-H, C-O). **Major anomer:** R_f 0.30 (1:1 Pentane:Et_2O); δ_H

(400 MHz, $CDCl_3$) 6.47 (1H, dd, *J* 13.6 and 1.1, 6-*H*), 6.25 (1H, dd, *J* 13.6 and 7.0, 5-*H*), 6.16 (1H, d, *J* 1.0, 1-*H*), 5.33 (1H, dd, *J* 4.7 and 1.0, 2-*H*), 5.24 (1H, dd, *J* 7.0 and 4.7, 3-*H*), 4.57 (1H, app t, *J* ~7.0 and 1.1, 4-*H*), 2.13 (3H, s, COCH_3), 2.12 (3H, s, COCH_3), 2.09 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 169.7 (2-*C*O), 169.4 (1-*C*O), 169.1 (3-*C*O), 134.3 (5-*C*H), 110.8 (6-*C*H), 98.0 (1-*C*H), 81.3 (4-*C*H), 73.9 (2-*C*H), 73.3 (3-*C*H), 21.0 (3-CO*C*H_3), 20.5 (1-CO*C*H_3 and 2-CO*C*H_3). **Minor anomer:** R_f 0.18 (1:1 Pentane:Et_2O); δ_H (400 MHz, $CDCl_3$) 6.50 (1H, dd, *J* 13.6 and 1.4, 6-*H*), 6.42 (1H, d, *J* 4.6, 1-*H*), 6.27 (1H, dd, *J* 13.6 and 5.7, 5-*H*), 5.22 (1H, dd, *J* 6.6 and 4.6, 2-*H*), 5.08 (1H, dd, *J* 6.6 and 3.7, 3-*H*), 4.63 (1H, ddd, *J* 5.7, 3.7 and 1.4, 4-*H*), 2.12 (3H, s, COCH_3), 2.10 (3H, s, COCH_3), 2.07 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 170.0 (2-*C*O), 169.5 (1-*C*O), 169.3 (3-*C*O), 132.9 (5-*C*H), 110.4 (6-*C*H), 93.6 (1-*C*H), 82.8 (4-*C*H), 72.0 (3-*C*H), 69.3 (2-*C*H), 20.9 (3-CO*C*H_3), 20.5 (1-CO*C*H_3) 20.2 (2-CO*C*H_3); as a mixture of anomers: *m/z* (ES+) 372.9897 (M+Na, $C_{12}H_{15}{}^{79}BrO_7Na^+$ requires 372.9899), 374.9877 (M+Na, $C_{12}H_{15}{}^{81}BrO_7Na^+$ requires 374.9878).

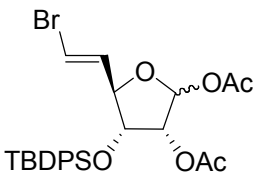
6-*E*-Bromo-(5-deoxy-5-methylidene)-3-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-α-D-xylofuranose 270



Imidazole (740 mg, 10.8 mmol) then TBDPSCl (1.41 mL, 5.43 mmol) were added to a stirred solution of alcohol **210** (1.20 g, 4.52 mmol) in dry DMF (15 mL) under argon. The reaction mixture was stirred for 23 ½ hours then quenched with water (15 mL) and extracted

with Et_2O (3 x 15 mL). The combined organics were washed with brine (15 mL), dried (Na_2SO_4) the concentrated *in vacuo* to a yellow solid. Following purification by column chromatography (4:1 Petrol:Et_2O), silyl ether **270** (2.27 g, 99%) was obtained as a pale yellow syrup. R_f 0.63 (1:1 Petrol:Et_2O); $[\alpha]_D^{17}$ -7.75 (*c* 0.52, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2961 (C-H), 2932 (C-H), 2896 (C-H), 2859 (C-H), 1627 (C=C), 1463, 1384, 1375; δ_H (400 MHz, $CDCl_3$) 7.50-7.36 (10H, m, Ar*H*), 6.48 (1H, dd, *J* 13.6 and 0.9, 6-*H*), 6.00 (1H, dd, *J* 13.6 and 7.5, 5-*H*), 5.54 (1H, d, *J* 3.7, 1-*H*), 4.42 (1H, ddd, *J* 8.7, 7.5 and 0.9, 4-*H*), 4.08 (1H, dd, *J* 4.4 and 3.7, 2-*H*), 3.62 (1H, dd, *J* 8.7 and 4.4, 3-*H*), 1.61 (3H, s, CH_3), 1.29 (3H, s, CH_3), 1.10 (6H, s, 2 x CH_3), 1.09 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 136.1 (Ar-*C*H), 136.0 (Ar-*C*H), 134.9 (5-*C*H), 134.7 (Ar-*C*H), 133.4 (Ar-*C*), 132.7 (Ar-*C*), 130.1 (Ar-*C*H), 130.0 (Ar-*C*H), 129.7 (Ar-*C*H), 127.9 (Ar-*C*H), 127.8 (Ar-*C*H), 127.7 (Ar-*C*H), 127.6 (Ar-*C*H), 112.7 ($C(CH_3)_2$), 110.3 (6-*C*H), 103.6 (1-*C*H), 79.9 (4-*C*H), 78.6 (2-*C*H), 76.8 (3-*C*H), 26.8 ($C(CH_3)$), 26.6 ($C(CH_3)$), 26.4 ($C(CH_3)$), 19.3 (Si*C*); *m/z* (ES+) 525.1073 (M+Na, $C_{25}H_{31}{}^{79}BrNaO_4Si^+$ requires 525.1067), 527.1049 (M+Na, $C_{25}H_{31}{}^{81}BrNaO_4Si^+$ requires 527.1052).

6-*E*-Bromo-(5-deoxy-5-methylidene)-3-(*tert*-butyldiphenyl-silyl)-1,2 -*bis*-*O*-acetyl-α/β-D-ribofuranose 212

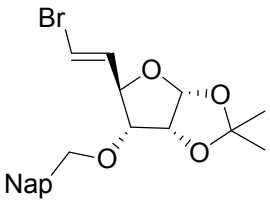


A solution of **270** (925 mg, 1.84 mmol) in 70% AcOH aq. v/v (30 mL) was heated at reflux for 2 ½ hours. The reaction mixture was then

cooled to r.t. and co-evaporated with toluene (3 x 25 mL). The residue was then dissolved in dry pyridine (50 mL), Ac_2O (5.20 mL) added and the reaction mixture was stirred at r.t. for 15 hours. The reaction mixture was then concentrated *in vacuo*, co-evaporating with toluene (5 x 10 mL) to yellow oil (4:1 mixture of anomers). The residue was purified by column chromatography (3:1 Petrol:Et_2O) to obtain a 4:1 mixture of anomers the *bis*-acetate **212** which were not separated as a pale yellow oil (857 mg, 86%). R_f 0.35 and 0.32 (3:1 Petrol:Et_2O); as a 4:1 mixture of anomers: $[\alpha]_D^{21}$ -12.5 (*c* 1.33, $CHCl_3$); as a mixture of anomers: ν_{max}/cm^{-1} ($CHCl_3$) 2957 (C-H), 2932 (C-H), 2895 (C-H), 2859 (C-H), 1746 (C=O), 1626 (C=C), 1462, 1372; **Major isomer:** δ_H (400 MHz, $CDCl_3$) 7.76-7.72 (2H, m, Ar-*H*), 7.76-7.65 (4H, m, Ar-*H*), 7.53-7.35 (6H, m, Ar-*H*), 6.23 (1H, dd, *J* 13.5 and 0.8, 6-*H*), 6.04 (1H, d, *J* 0.7, 1-*H*), 5.75 (1H, dd, *J* 13.5 and 5.6, 5-*H*), 4.86 (1H, dd, *J* 4.6 and 0.7, 2-*H*), 4.46 (1H, ddd, *J* 6.8, 5.6 and 0.8, 4-*H*), 4.30 (1H, dd, *J* 6.8 and 4.6, 3-*H*), 2.16 (3H, s, COCH_3), 2.00 (3H, s, COCH_3) 1.10 (9H, s, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$); 169.7 (*C*O), 169.1 (*C*O), 135.9 (Ar-*C*H), 135.8 (2 x Ar-*C*H), 135.2 (5-*C*H), 132.6 (Ar-*C*), 132.3 (Ar-*C*), 130.4 (Ar-*C*H), 129.7 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*H), 127.7 (Ar-*C*H), 110.4 (6-*C*H), 97.8 (1-*C*H), 84.0 (4-*C*H), 75.3 (2-*C*H), 74.6 (3-*C*H), 34.2, 26.9 (C*C*H_3), 26.8 (C*C*H_3), 26.6 (C*C*H_3), 21.1 (CO*C*H_3), 21.0 (CO*C*H_3), 19.2 (Si*C*(CH_3)); **Minor isomer:** δ_H (400 MHz, $CDCl_3$) 7.76-7.72 (2H, m, Ar-*H*), 7.76-7.65 (4H, m, Ar-*H*), 7.53-7.35 (6H, m, Ar-*H*), 6.37 (1H, d, *J* 4.5, 1-*H*), 6.07 (1H, dd, *J* 13.6 and 1.3, 6-*H*), 5.68 (1H,

dd, *J* 13.6 and 6.6, 5-*H*), 4.91 (1H, dd, *J* 5.6 and 4.5, 2-*H*), 4.53 (1H, ddd, *J* 6.6, 3.5 and 1.3, 4-*H*), 4.20 (1H, dd, *J* 5.6 and 3.5, 3-*H*), 2.17 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 1.13 (9H, s, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 169.7 (*C*O), 169.1 (*C*O), 135.9 (Ar-*C*H), 135.8 (Ar-*C*H), 135.3 (Ar-*C*H), 132.6 (Ar-*C*H), 132.3 (Ar-*C*H), 128.0 (2 x Ar-*C*H), 134.8 (5-*C*H), 133.6 (Ar-*C*), 133.0 (Ar-*C*), 127.9 (Ar-*C*H), 109.9 (6-*C*H), 93.9 (1-*C*H), 85.7 (4-*C*H), 73.4 (3-*C*H), 71.2 (2-*C*H), 26.9 (C*C*H_3), 26.8 (C*C*H_3), 26.6 (C*C*H_3), 20.8 (CO*C*H_3), 20.5 (CO*C*H_3), 19.0 (Si*C*(CH_3)); *m/z* (ES+) 569.0941 (M+Na, $C_{26}H_{31}{}^{79}BrNaO_6Si^+$ requires 569.0965), 571.0915 (M+Na, $C_{26}H_{31}{}^{81}BrNaO_6Si^+$ requires 571.0951).

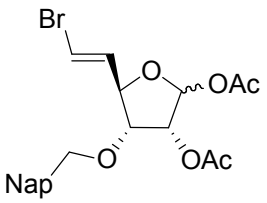
6-*E*-Bromo-(5-deoxy-5-methylidene)-3-(2-methylnaphthyl)-1,2-*O*-isopropylidene-α-D-xylofuranose 160



Solid 2-(Bromomethyl)naphthalene (1.05 g, 4.75 mmol), KOH (400 mg, 7.13 mmol) and 18-crown-6 (42 mg, 158 μmol) were added to a stirring solution of **210** (1.05 g, 3.96 mmol) in dry THF (7.9 mL) at r.t. under an argon atmosphere. Stirring was maintained for 16 hours, then water (10 mL) was added and the mixture was extracted with Et_2O (3 x 6 mL). The combined organics were washed with brine (10 mL), dried (Na_2SO_4), and concentrated to yellow syrup. Purification by column chromatography (3:1 Petrol:Et_2O) yielded the naphthyl ether **160** (1.57 g, 98%) as a colourless oil. R_f 0.30 (3:1 Petrol:Et_2O); $[\alpha]_D^{23}$ +10.6 (*c* 1.23, $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2986 (C-H), 2936 (C-H), 2899 (C-H),

2973 (C-H), 1627 (C=C), 1497 (C=C), 1455, 1381, 1372; δ_H (400 MHz, $CDCl_3$) 7.89-7.85 (3H, m, Ar-*H*), 7.82 (1H, s, 1-Ar-*H*), 7.52-7.48 (3H, m, Ar-*H*), 6.52 (1H, dd, *J* 13.7 and 1.1), 6.18 (1H, dd, *J* 13.7 and 6.8, 5-*H*), 5.72 (1H, d, *J* 3.7, 1-*H*), 4.93 (1H, d, *J* 12.4, C*H*H), 4.78 (1H, *J* 12.4, CH*H*), 4.58 (1H, dd, *J* 4.2 and 3.7, 2-*H*), 4.50 (1H, ddd, *J* 8.9, 6.8 and 1.1, 4-*H*), 3.56 (1H, dd, *J* 8.9 and 4.2, 3-*H*), 1.63 (3H, s, CH_3), 1.37 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 134.9 (2-Ar-*C*), 134.5 (5-*C*H), 133.5 (Ar-*C*), 133.4 (Ar-*C*), 128.8 (Ar-*C*H), 128.3 (Ar-*C*H), 128.1 (Ar-*C*H), 127.3 (1-Ar-*C*H), 126.6 (Ar-*C*H), 126.5 (Ar-*C*H), 126.1 (Ar-*C*H), 113.5 (*C*$(CH_3)_2$), 110.4 (6-*C*H), 104.1 (1-*C*H), 81.6 (3-*C*H), 78.5 (4-*C*H), 77.6 (2-*C*H), 72.0 (CH_2), 27.1 (*C*H_3), 26.8 (*C*H_3); *m/z* (ES+) 427.0502 (M+Na, $C_{20}H_{21}{}^{79}BrNaO_4^+$ requires 427.0521), 429.0483 (M+Na, $C_{20}H_{21}{}^{81}BrNaO_4^+$ requires 429.0500.

6-*E*-Bromo-(5-deoxy-5-methylidene)-3-(2-methylnaphthyl)-1,2-*bis*-*O*-acetyl-α/β-D-ribofuranose 218



A solution of acetonide **160** (1.51 g, 3.73 mmol) in 70% v/v AcOH (60 mL) was heated at reflux for 4 ½ hours then cooled and co-evaporated with toluene (3 x 25 mL). The resulting residue was dissolved in dry pyridine (12.2 mL, 123 mmol) and stirred at r.t. under an argon atmosphere. Neat Ac_2O (10.6 mL, 112 mmol) was added and stirring maintained for 16 hours. The reaction mixture was then diluted with water (15 mL) and extracted with Et_2O (3 x 15 mL). The combined organics were washed sequentially with

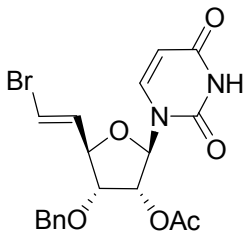
aqueous $CuSO_4$ (20% w/v) solution (2 x 15 mL), brine (2 x 15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography gave *bis*-acetate **218** (1.42 g, 85%) as a colourless oil as a 9:1 mixture of anomers. **Major anomer:** R_f 0.20 (3:1 Petrol:Et_2O); $[\alpha]_D^{29}$ +34.5 (*c* 0.95, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2977 (C-H), 2923 (C-H), 2875 (C-H), 1746 (C=O), 1626 (C=C), 1455, 1372; δ_H (400 MHz, $CDCl_3$) 7.88-7.85 (3H, m, Ar-*H*), 7.66 (1H, s, 1-Ar-*H*), 7.52-7.50 (2H, m, Ar-*H*), 7.41 (1H, dd, *J* 8.4 and 1.4, Ar-*H*), 6.46 (1H, dd, *J* 13.6 and 0.7, 6-*H*), 6.18 (1H, dd, *J* 13.6 and 7.4, 5-*H*), 6.16 (1H, app s, 1-*H*), 5.35 (1H, app d, *J* ~4.4, 2-*H*), 4.82 (1H, d, *J* 12.0, C*H*H), 4.66 (1H, d, *J* 12.0, CH*H*), 4.51 (1H, dd, *J* 7.9, 7.4 and 0.7, 4-*H*), 4.07 (1H, dd *J* 7.9 and 4.4, 3-*H*), 2.17 (3H, s, 2-COC*H*$_3$), 2.07 (3H, s, COC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 169.9 (2-*C*O), 169.0 (1-*C*O), 135.4 (5-*C*H), 134.5 (Ar-*C*), 133.2 (2-Ar-*C*), 128.5 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*), 127.8 (Ar-*C*H), 126.8 (Ar-*C*H), 126.4 (Ar-*C*H), 126.3 (Ar-*C*H), 125.6 (Ar-*C*H), 110.2 (6-*C*H), 98.4 (1-*C*H), 81.9 (4-*C*H), 79.9 (3-*C*H), 73.3 (2-*C*H), 73.3 (CH_2), 21.1 (1-COC*H*$_3$), 20.8 (2-COC*H*$_3$); **Minor anomer:** R_f (3:1 Petrol:Et_2O) 0.11; $[\alpha]_D^{29}$ +59.4 (*c* 0.94, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2985 (C-H), 2923 (C-H), 2873 (C-H), 1747 (C=O), 1627 (C=C), 1602 (C=C), 1455, 1374; δ_H (400 MHz, $CDCl_3$) 7.88 -7.83 (3H, m, Ar-*H*), 7.76 (1H, s, 1-Ar-*H*), 7.52-7.49 (2H, m, Ar-*H*), 7.43 (1H, dd, *J* 8.4 and 1.6, Ar-*H*), 6.38 (1H, dd, *J* 13.6 and 1.1, 6-*H*), 6.39 (1H, d, *J* 4.6, 1-*H*), 6.06 (1H, dd, *J* 13.6 and 6.8, 5-*H*), 5.23 (1H, dd, *J* 6.2 and 4.6, 2-*H*), 4.82 (1H, d, *J* 12.1, C*H*H), 4.67 (1H, d, *J* 12.1, CH*H*), 4.58 (1H, ddd, *J* 6.8, 6.3

and 1.1, 4-*H*), 3.93 (1H, app dd, *J* 6.3 and 6.2, 3-*H*), 2.16 (3H, s, COC*H*$_3$), 2.15 (3H, s, COC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 170.0 (*C*O), 169.7 (*C*O), 135.5 (5-*C*H), 134.6 (Ar-*C*), 133.2 (Ar-*C*), 128.5 (Ar-*C*H), 127.9 (Ar-*C*H), 127.8 (Ar-*C*H), 126.8 (Ar-*C*H), 126.4 (Ar-*C*H), 126.3 (Ar-*C*H), 125.7 (Ar-*C*H), 110.6 (6-*C*H), 94.2 (1-*C*H), 82.7 (4-*C*H), 78.5 (3-*C*H), 73.5 (CH_2), 70.3 (2-*C*H), 21.1 (1-COCH_3), 20.6 (2-COCH_3); as a mixture of anomers: *m/z* (ES+) 471.0419 (M+Na, $C_{21}H_{21}{}^{79}BrNaO_6^+$ requires 471.0414), 473.0390 (M+Na, $C_{21}H_{21}{}^{81}BrNaO_6^+$ requires 473.0399).

4.3 Vinyl Bromide Nucleosides

4.3.1 First Generation Nucleosides

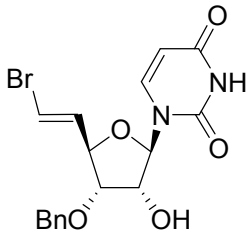
6'-*E*-bromo-(5'-deoxy-5'-methylidene)-3'-*O*-benzyloxy-2'-acetoxy uridine 166



Neat BSA (2.17 mL, 8.79 mmol) was added to a stirred suspension of the mixture of anomers of *bis*-acetate **165** (1.17 g, 2.93 mmol) and uracil (985 mg, 8.79 mmol) in dry MeCN (50 mL). The reaction mixture was heated at 65 °C for 90 minutes then TMSOTf (670 μL, 4.34 mmol) was added. Stirring was maintained for a further 16 hours, the mixture was then cooled, diluted with CH_2Cl_2 (30 mL) and extracted with saturated $NaHCO_3$ (20 mL). The organic portion was separated and dried ($MgSO_4$) and evaporated to a crude oil. Flash column chromatography (3:2 EtOAc/Petrol) yielded uridine **166** as a white solid (1.16 g, 85%). R_f 0.24 (3:2 EtOAc:Petrol); m.p. 76-78 °C; $[\alpha]_D^{27}$ +71.9 (*c* 0.52 in $CHCl_3$);

found C, 50.28%; H, 4.14%; N, 5.94%; ($C_{19}H_{19}BrN_2O_6$ requires C 50.57%, H 4.14%, N 5.94%); ν_{max}/cm^{-1} ($CHCl_3$) 3694 (N-H), 3399 (N-H), 1744 (C=O), 1722 (C=O), 1716 (C=O), 1697 (C=O), 1629 (N-H), 1603 (N-H), 1455 (C=C), 1375; δ_H (400 MHz, $CDCl_3$) 9.28 (1H, br s, N*H*), 7.39-7.29 (5H, m, Ar*H*), 7.18 (1H, d, *J* 8.1, 6-*H*), 6.49 (1H, dd, *J* 13.6 and 0.9, 6'-*H*), 6.22 (1H, dd, *J* 13.6 and 7.1, 5'-*H*), 5.77 (1H, dd, *J* 8.1 and 2.1, 5-*H*), 5.68 (1H, d, *J* 2.7, 1'-*H*), 5.45 (1H, dd, *J* 5.8 and 2.7, 2'-*H*), 4.62 (1H, d, *J* 11.5, C*H*H), 4.49 (1H, d, *J* 11.5, CH*H*), 4.38 (1H, ddd, *J* 7.1, 1.6 and 1.0, 4'-*H*), 4.13 (1H, dd, *J* 5.8 and 1.6, 3'-*H*), 2.14 (3H, s, $COCH_3$); δ_C (100 MHz, $CDCl_3$) 170.0 (2'-*C*OCH_3), 163.6 (2-*C*O), 149.9 (4-*C*O), 141.1 (6-*C*H), 136.9 (Ar-*C*), 133.5 (5'-*C*H), 128.8 (2 x Ar-*C*H), 128.5 (Ar-*C*H), 128.3 (2 x Ar-*C*H), 111.4 (6'-*C*H), 103.0 (5-*C*H), 91.6 (1'-*C*H), 81.9 (4'-*C*H), 79.1 (3'-*C*H), 73.9 (CH_2), 73.6 (2'-*C*H), 20.9 (2'-CO*C*H_3); *m/z* (ES+) 473.0345 (M+Na, $C_{19}H_{19}{}^{79}BrN_2O_6Na^+$ requires 473.0324), 475.0292 (M+Na, $C_{19}H_{19}{}^{81}BrN_2O_6Na^+$ requires 475.0304).

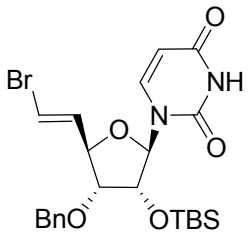
6-*E*-bromo-(5-deoxy-5-methylidene)-3-*O*-benzyloxy uridine 271



Solid K_2CO_3 (1.12 g, 2.40 mmol) was added to a stirred solution of uridine **166** (347 mg, 2.52 mmol) in MeOH (24 mL) at r.t. Stirring was maintained for 90 minutes then MeOH was removed by evaporation and the residue was taken up in CH_2Cl_2 (10 mL) and washed with water (10 mL). The organic portion was

dried (Na_2SO_4) and evaporated to the crude alcohol **271** as white foam (930 mg, 95%). The alcohol **217** was used directly without further purification. R_f 0.10 (neat Et_2O); m.p. 76-78 °C; $[\alpha]_D^{31}$ -66.0 (*c* 0.67, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3390 (O-H), 2925 (C-H), 1727 (C=O), 1698 (C=O), 1634 (N-H, C=C), 1611 (C-N, N-H), 1455 (C=C), 1381, 1093 (O-H); δ_H (270 MHz, $CDCl_3$) 9.97 (1H, br s, N*H*), 7.38-7.31 (5H, m, Ar-*H*), 7.24 (1H, d, *J* 8.0, 6-C*H*), 6.46 (1H, dd, *J* 13.6 and 0.8, 6'-*H*), 6.24 (1H, dd, *J* 13.6 and 6.6, 5'-*H*), 5.74 (1H, d, *J* 8.0, 5-C*H*), 5.67 (1H, d, *J* 3.2, 1'-*H*), 4.72 (1H, d, *J* 11.8, C*H*H), 4.63 (1H, d, *J* 11.8, CH*H*), 4.45 (1H, app t, *J* 6.9 and 6.6, 4'-*H*), 4.38 (1H, ddd, *J* 6.5, 4.7 and 3.2, 2'-*H*), 4.05 (1H, dd, *J* 6.9 and 6.5, 3'-*H*), 3.78 (1H, d, *J* 4.7, 2'-O*H*); δ_C (100 MHz, d_4-MeOD) 178.6 (4-*C*O), 161.3 (2-*C*O), 141.5 (6-*C*H), 140.0 (Ar-*C*), 137.3 (5'-*C*H), 130.3 (2 x Ar-*C*H), 130.1 (2 x Ar-*C*H), 129.8 (Ar-*C*H), 111.9 (6'-*C*H), 104.6 (5-*C*H), 94.0 (1'-*C*H), 83.5 (4'-*C*H), 82.4 (3'-*C*H), 74.9 (2'-*C*H), 74.4 (CH_2); *m/z* (ES+) 431.0182 (M+Na, $C_{17}H_{17}{}^{79}BrN_2O_5Na^+$ requires 431.0219), 433.0188 (M+Na, $C_{17}H_{17}{}^{81}BrN_2O_5Na^+$ requires 433.0198).

***E*-6'-Bromo-(5'-deoxy-5'-methylidene)-3'-*O*-benzyl-2'-(*tert*-butyldimethylsilyl)uridine 167**



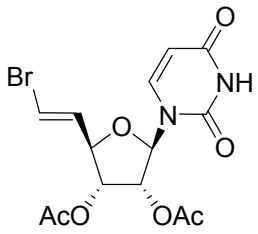
Imidazole (72 mg) and TBSCl (83 mg) were added to a stirred solution of alcohol **271** (180 mg) in dry DMF (1.7 mL) at r.t. under an argon atmosphere for 18 hours. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (5 mL) and

was extracted with Et_2O (3 x 3 mL). The ethereal extracts were washed with water (5 mL), dried (Na_2SO_4) and evaporated to a crude white solid. Uridine **167** was triturated in Et_2O, filtered and dried to a white powder (146 mg, 66%). R_f 0.52 (3:2 EtOAc:Petrol); m.p. 128-130 °C; $[\alpha]_D^{29}$ +91.1 (*c* 0.25, $CHCl_3$); found C, 52.55%; H, 5.88%; N, 5.21% ($C_{23}H_{31}BrN_2O_5Si$ requires C 52.77%, H 5.97%, N 5.35%); ν_{max}/cm^{-1} ($CHCl_3$) 3390 (N-H), 2954 (C-H), 2931 (C-H), 2859 (C-H), 1694 (C=O), 1627 (N-H, C=C), 1455 (C=C), 1390, 1362; δ_H (400 MHz, $CDCl_3$) 7.40-7.30 (5H, m, Ar-C*H*), 7.27 (1H, d, *J* 8.1, 6-*H*), 6.53 (1H, dd, *J* 13.6, 1.0, 6'-*H*), 6.27 (1H, dd, *J* 13.6, 7.2, 5'-*H*), 5.76 (1H, d, *J* 8.1, 5-*H*), 5.67 (1H, d, *J* 2.7, 1'-*H*), 4.72 (1H, d, *J* 11.8, C*H*H), 4.60 (1H, ddd, *J* 7.2, 7.0 and 1.0, 4'-*H*), 4.48 (1H, d, *J* 11.8, CH*H*), 4.43 (1H, dd, *J* 4.4, 2.7, 2'-*H*), 3.65 (1H, dd, *J* 7.0, 4.4, 3'-*H*), 0.92 (9H, s, C(CH_3)$_3$), 0.15 (3H, s, Si(CH_3)), 0.12 (3H, s, Si(CH_3)); δ_C (100 MHz, $CDCl_3$) 163.2 (4-*C*O), 149.9 (2-*C*O), 139.8 (6-*C*H), 138.1 (Ar-*C*), 137.0 (5'-*C*H), 128.6 (2 x Ar-*C*H), 128.2 (Ar-*C*H), 127.7 (2 x Ar-*C*H), 111.2 (6'-*C*H), 102.4 (5-*C*H), 92.6 (1'-*C*H), 81.6 (4'-*C*H), 80.1 (3'-*C*H), 73.7 (2'-*C*H), 72.7 (CH_2), 25.66 (C(*C*H_3)$_3$), 18.1 (Si*C*), -4.7 (Si*C*H_3), -5.0 (Si*C*H_3); *m/z* (ES+) 523.1267 (M+H, $C_{23}H_{32}{}^{79}BrN_2O_5Si^+$ requires 523.1264), 525.1178 (M+H, $C_{23}H_{32}{}^{81}BrN_2O_5Si^+$ requires 525.1243), 545.1079 (M+Na, $C_{23}H_{31}{}^{79}BrN_2O_5SiNa^+$ requires 545.1083), 547.0923 (M+Na, $C_{23}H_{31}{}^{81}BrN_2O_5SiNa^+$ requires 547.1063).

4.3.2 Second Generation Nucleosides

4.3.2.1 Vinyl Bromide Uridines

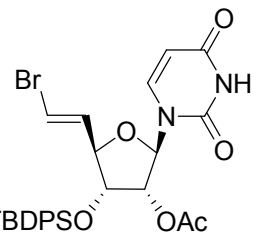
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-*O*-acetyl-2'-*O*-acetyl uridine 211



Uracil (747 mg, 6.66 mmol) was added to a stirring solution of triacetate **186** (780 mg, 2.22 mmol) in dry MeCN (37 mL) at r.t. under an argon atmosphere. Neat BSA (1.65 mL, 6.66 mmol) was added and the reaction mixture was heated at 60-65 ^{o}C for 40 minutes then TMSOTf (508 μL, 3.29 mmol) was added and stirring was maintained for 3 ½ h. The reaction mixture was cooled to r.t. then diluted with CH_2Cl_2 (40 mL) then washed with $NaHCO_3$ solution (20 mL). The organic phase was dried (Na_2SO_4) then concentrated *in vacuo* to a yellow glass (1.21 g). Uridine **211** was obtained as a colourless syrupy foam (892 mg, 99%) following column chromatography (3:2 EtOAc:Petrol). R_f 0.16 (3:2 EtOAc:Petrol). $[\alpha]_D^{31}$ +4.05 (*c* 0.59, $CHCl_3$); found C, 40.92; H, 3.65; N 6.66% ($C_{15}H_{16}N_2BrO_6$ requires C, 41.71; H, 3.75; N, 6.95%); ν_{max}/cm^{-1} ($CHCl_3$) 3610 (N-H), 3385 (N-H), 2923 (C-H), 2851 (C-H), 1749 (C=O), 1723 (C=O), 1698 (C=O), 1631 (N-H, C=C), 1602 (C=C), 1456, 1376; δ_H (400 MHz, $CDCl_3$) 9.27 (1H, br s, N*H*), 7.23 (1H, d, *J* 8.1, 6-*H*), 6.57 (1H, dd, *J* 13.7 and 1.1, 6'-*H*), 6.36 (1H, dd, *J* 13.7 and 6.9, 5'-*H*), 5.85 (1H, d, *J* 4.5, 1'-*H*), 5.82 (1H, dd, *J* 8.1 and 2.0, 5-*H*), 5.41 (1H, dd, *J* 6.0 and 4.5, 2'-*H*), 5.26 (1H, app t, *J* 6.0, 3'-*H*), 4.51 (1H, ddd, *J* 6.9, 6.0 and 1.1, 4'-*H*), 2.13 (3H, s, COC*H*$_3$), 2.12 (3H, s, COC*H*$_3$); δ_C (100 MHz,

$CDCl_3$) 169.7 (2'-*C*O), 169.6 (3'-*C*O), 162.9 (4-*C*O), 150.1 (2-*C*O), 140.3 (6-*C*H), 132.7 (5'-*C*H), 112.0 (6'-*C*H), 103.5 (5-*C*H), 89.4 (1'-*C*H), 81.4 (4'-*C*H), 72.7 (2'-*C*H), 72.6 (3'-*C*H) 20.6 (2'-CO*C*H_3), 20.5 (3'-CO*C*H_3); *m/z* (ES+) 424.9980 (M+Na, $C_{15}H_{16}N_2{}^{79}BrO_6Na^+$ requires 424.9955), 426.9961 (M+Na, $C_{15}H_{16}N_2{}^{81}BrO_6Na^+$ requires 426.9940).

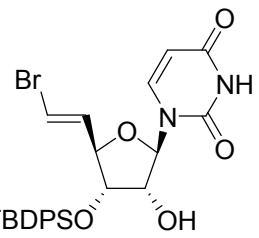
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(*tert*-butyldiphenyl-silyl)-2'-*O*-acetyl uridine 213



Uracil (491 mg, 4.38 mmol) then BSA (1.08 mL, 4.38 mmol) were added to a stirred solution of *bis*-actate **212** (800 mg, 1.46 mmol) in dry MeCN (25 mL) under an argon atmosphere. The resulting suspension was heated at 65 °C for 1 ½ hours then TMSOTf (0.33 mL) was added and stirring maintained for a further 19 hours. The reaction mixture was cooled to r.t., diluted with CH_2Cl_2 (25 mL) and washed with saturated $NaHCO_3$ solution (25 mL) then brine (25 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to white syrupy foam **213** (732 mg, 84%). R_f 0.38 (8:1 Et_2O:Petrol); $[\alpha]_D^{20}$ +5.76 (*c* 0.63, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3389 (N-H), 2960 (C-H), 2933 (C-H), 2898 (C-H), 2860 (C-H), 1743 (C=O), 1722 (C=O), 1697 (C=O), 1633 (N-H, C=C), 1456, 1375; δ_H (400 MHz, $CDCl_3$) 7.67-7.62 (4H, m, Ar-*H*), 7.49-7.38 (6H, m, Ar-*H*), 7.02 (1H, d, *J* 8.0, 6-C*H*), 6.19 (1H, dd, *J* 13.6 and 0.5, 6'-*H*), 5.89 (1H, dd, *J* 13.6 and 7.3, 5'-*H*), 5.79 (1H, d, *J* 4.4, 1'-*H*), 5.71 (1H, d, *J* 8.0, 5-C*H*), 4.89 (1H, app

ddd, *J* 4.4, ~2.6 and ~2.1, 2'-*H*), 4.34 (2H, m, 3'-*H* and 4'-*H*), 2.07 (3H, s, $COCH_3$), 1.11 (9H, s, $C(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) 170.1 ($COCH_3$), 162.9 (4-*C*O), 149.8 (2-*C*O), 140.5 (6-*C*H), 135.9 (2 x Ar-*C*H), 135.8 (2 x Ar-*C*H), 133.4 (5'-*C*H), 132.6 (Ar-*C*), 132.4 (Ar-*C*), 130.5 (Ar-*C*H), 130.4 (Ar-*C*H), 128.1 (2 x Ar-*C*H), 128.0 (2 x Ar-*C*H), 111.6 (6'-*C*H), 103.1 (5-*C*H), 89.9 (1'-*C*H), 84.2 (4'-*C*H), 77.3 (3'-*C*H), 74.0 (2'-*C*H), 26.8 (3 x $SiC(CH_3)_3$), 20.8 (2'-$COCH_3$) 19.3 ($SiC(CH_3)_3$); *m/z* (ES+) 599.1195 (M+H, $C_{28}H_{32}{}^{79}BrN_2O_6Si^+$ requires 599.1213), 601.1163 (M+H, $C_{28}H_{32}{}^{81}BrN_2O_6Si^+$ requires 601.1193).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(*tert*-butyldiphenylsilyl) uridine 214



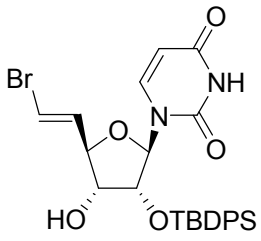
Method A. Solid K_2CO_3 (8 mg, 60 μmol) was added to a stirred solution of **213** (72 mg, 120 μmol) in MeOH (1.2 mL). The reaction mixture was stirred at r.t. for 21 hours then neutralised with 1N HCl, diluted with CH_2Cl_2 then concentrated *in vacuo* to give a 1:1 mixture of regioisomers **214** and **215**. Purification by column chromatography (98:2 $CHCl_3$:MeOH) was then used to separate the two isomers which re-equilibrated to a 7:3 ratio of 2'-OH and 3'-OH isomers **214** and **215** respectively. The alcohol **214** co-elutes with the residual starting material acetate **213** if any is present.

Method B: To a solution of acetate **213** (200 mg, 333 μmol) in dry methanol (1.60 mL) was added NaOMe (17.5 mg, 324 μmol). The

resulting suspension was stirred at r.t. for 6 hours and was then neutralised by the addition of saturated NH_4Cl solution (1 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 x 1 mL) and the The combined organics were then washed with brine (1 mL), dried (Na_2SO_4), filtered and evaporated to a white solid (171 mg) of a 1:1:1 ratio of acetate **213** starting material, and the 2'-OH **214** and 3'-OH **215** products. Purification by column chromatography (98:2 $CHCl_3$:MeOH) allowed partial separation of products. Alcohol **214**, the major isomer, was obtained as a 1:1 mixture of **214** and starting material **213** (79 mg, **29** 20%, **30** 20% (26% borsm) as a white solid), m.p. 83-85 ^oC. R_f 0.50 (9:1 $CHCl_3$:MeOH); Mixture of isomers $[\alpha]_D^{23}$ +23.9 (*c* 0.54, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3386 (N-H), 2949 (C-H), 2932 (C-H), 2859 (C-H), 1745 (C=O), 1718 (C=O), 1697 (C=O), 1631 (C=C), 1457, 1388, 1365, 1349; δ_H (400 MHz, $CDCl_3$) 9.27 (1H, br s, N*H*), 7.10 (1H, d, *J* 8.1, 6-C*H*), 6.11 (1H, d, *J* 13.6, 6'-*H*), 5.74 (1H, dd, *J* 13.6 and 7.8, 5'-*H*), 5.70 (2H, m, 1'-*H* and 5-C*H*), 4.29 (1H, dd, *J* 7.8 and 5.5, 4'-*H*), 4.22 (1H, dd, *J* 5.5 and 5.3, 3'-*H*), 4.03 (1H, ddd, *J* 5.3, 4.5 and 3.9, 2'-*H*), 3.24 (1H, br s, 2'-O*H*), 1.14 (9H, s, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 163.1 (4-*C*O), 150.1 (2-*C*O), 140.6 (6-*C*H), 136.0 (Ar-*C*H), 135.9 (Ar-*C*H), 135.7 (Ar-*C*H), 133.3 (5'-*C*H), 132.1 (Ar-C), 131.8 (Ar-*C*), 130.8 (Ar-*C*H), 130.7 (Ar-*C*H), 130.6 (Ar-*C*H), 128.3 (2 x Ar-*C*H), 128.2 (2 x Ar-*C*H), 111.4 (6'-*C*H), 102.8 (5-*C*H), 92.1 (1'-*C*H), 83.9 (4'-*C*H), 76.5 (3'-*C*H), 75.0 (2'-*C*H), 27.0 (3 x SiC(CH_3)), 19.2 (Si*C*(CH_3)$_3$); *m/z* (ES+) 579.0926 (M+Na,

$C_{26}H_{29}{}^{79}BrN_2NaO_5Si^+$ requires 579.0921), 581.0894 (M+Na, $C_{26}H_{29}{}^{81}BrN_2NaO_5Si^+$ requires 581.0906).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-(*tert*-butyldiphenylsilyl) uridine 215

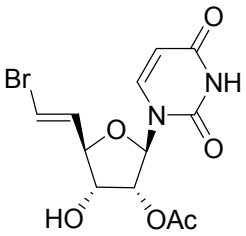


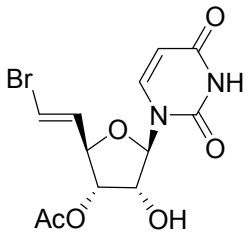
Method B: Using the above method B, alcohol **215**, the minor isomer was obtained as white solid (36.4 mg, 20%), m.p. 100-102 °C. R_f 0.59 (9:1 $CHCl_3$:MeOH); $[\alpha]_D{}^{25}$ 32.4 (*c* 0.98, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3538 (N-H), 3390 (N-H), 2953 (C-H), 2932 (C-H), 2887 (C-H), 2860 (C-H), 1721 (C=O), 1697 (C=O), 1632 (C=C), 1456, 1391, 1382, 1364; δ_H (400 MHz, $CDCl_3$) 9.05 (1H, br s, N*H*), 7.74-7.35 (10H, m, 10 x Ar*H*), 6.46 (1H, dd, *J* 13.7 and 1.0, 6'-*H*), 6.40 (1H, d, *J* 8.0, 6-C*H*), 6.29 (1H, dd, *J* 13.7 and 6.0, 5'-*H*), 5.48 (1H, d, *J* 4.4, 1'-*H*), 5.38 (1H, dd, *J* 8.0 and 2.1, 5-C*H*), 4.55 (1H, dd, *J* 5.8 and 4.4, 2'-*H*), 4.34 (1H, ddd, *J* 6.0, 5.9, and 1.0, 4'-*H*), 4.15 (1H, dd *J* 6.2, 5.9 and 5.8, 3'-*H*), 2.99 (1H, d, *J* 6.2, 3'-O*H*), 1.12 (9H, s, C(CH_3)$_3$); δ_C (125 MHz, $CDCl_3$) 162.4 (4-*C*O), 149.2 (2-*C*O), 141.0 (6-*C*H), 135.8 (2 x Ar-*C*H), 135.6 (2 x Ar-*C*H), 133.9 (5'-*C*H), 131.9 (Ar-*C*), 131.5 (Ar-*C*), 130.8 (Ar-*C*H), 130.7 (Ar-*C*H), 129.7 (Ar-*C*H), 128.4 (Ar-*C*H), 128.3 (Ar-*C*H), 127.8 (Ar-*C*H), 127.7 (Ar-*C*H), 110.4 (6'-*C*H), 102.7 (5-*C*H), 92.7 (1'-*C*H), 84.2 (4'-*C*H), 74.0 (3'-*C*H), 73.6 (2'-*C*H), 27.0 (3 x SiC(*C*H_3)), 19.3 (Si*C*(CH_3)$_3$); *m/z* (ES+) 557.1079 (M+H, $C_{26}H_{30}{}^{79}BrN_2O_5Si^+$ requires 557.1107), 579.0911 (M+Na,

$C_{26}H_{29}{}^{79}BrN_2NaO_5Si^+$ requires 579.0921), 581.0905 (M+Na, $C_{26}H_{29}{}^{81}BrN_2NaO_5Si^+$ requires 581.0906).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-*O*-acetyl uridine 216

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-*O*-acetyl uridine 217





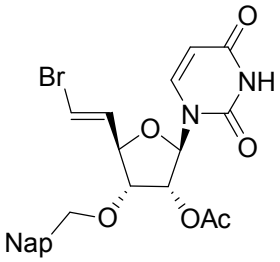
Method 1. Glacial AcOH (21 μL, 368 μmol) and 1M TBAF in THF (184 μL, 184 μmol) were added to a stirred solution of **213** (92 mg, 153 μmol) in dry THF (770 μL). The reaction mixture was stirred under an argon atmosphere for 1 ¼ hour and was then quenched with water (1 mL) then extracted with Et_2O (3 x 1 mL). The combined organics were washed with brine (1 mL), dried (Na_2SO_4) and evaporated to yellow oil which was purified by column chromatography (9:1 $CHCl_3$:Petrol → 18:1:1 $CHCl_3$:Petrol:MeOH) to a yellow syrup containing an inseparable 2:1 mixture of **216** and **217** respectively (30.6 mg, 55%).

Method 2: Glacial AcOH (18.5 μL, 320 μmol) and 1M TBAF in THF (160 μL, 160 μmol) were added to a stirred solution of **213** (80 mg, 133 μmol) in dry THF (650 μL) under an argon atmsphere. Stirring at r.t. was maintained for 1 ½ hours then SiO_2 was added and the reaction mixture evaporated and purified by column chromatography (9:1 $CHCl_3$:Petrol then 18:1:1

$CHCl_3$:Petrol:MeOH). Acetates **216** and **217** were obtained in a 2:1 ratio respectively as a white solid (45.4 mg, 94%), as a mixture of isomers: m.p. 80-82 °C. R_f 0.05 (8:1 Et_2O:Petrol); as a mixture of isomers: $[\alpha]_D^{23}$ +31.8 (*c* 0.36, $CHCl_3$); as a mixture of isomers: ν_{max}/cm^{-1} ($CHCl_3$) 3588 (N-H), 3389 (N-H), 2938 (C-O), 1742 (C=O), 1716 (C=O), 1697 (C=O), 1628 (C=C), 1456, 1377; **Major Isomer** (3'-OAc, **217**): δ_H (400 MHz, $CDCl_3$) 10.22 (1H, br s, N*H*), 7.38 (1H, d, *J* 8.1, 6-*H*), 6.58 (1H, dd, *J* 13.7 and 0.9, 6'-*H*), 6.34 (1H, dd, *J* 13.7 and 7.1, 5'-*H*), 5.82(1H, d, *J* 4.4, 1'-*H*), 5.80 (1H, d, *J* 8.1, 5-*H*), 4.93 (1H, dd, *J* 5.7 and 5.3, 3'-*H*), 4.60 (1H, ddd, *J* 7.1, 5.7 and 0.9, 4'-*H*), 4.52 (1H, app dd, *J* ~5.7 and ~4.4, 2'-*H*), 2.17 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 170.6 (*C*OCH_3), 163.9 (N*C*O), 151.0 (CH*C*O), 140.0 (6-*C*H), 133.9 (5'-*H*), 111.8 (6'-*C*H), 103.1 (5-*C*H), 91.1 (1'-*C*H), 81.3 (4'-*C*H), 74.5 (3'-*C*H), 72.7 (2'-*C*H), 20.8 (CO*C*H_3); **Minor Isomer** (2'-OAc, **216**): δ_H (400 MHz, $CDCl_3$) 9.94 (1H, br s, N*H*), 7.29 (1H, d, *J* 8.1, 6-C*H*), 6.56 (1H, dd, *J* 13.7 and 0.8, 6'-*H*), 6.37 (1H, dd, *J* 13.7 and 7.7, 5'-*H*), 5.78 (1H, d, *J* 8.1, 5-C*H*), 5.72 (1H, d, *J* 2.8, 1'-*H*), 5.35 (1H, dd, *J* 5.7 and 2.8, 2'-*H*), 4.39 (1H, app dd, *J* ~7.2 and ~5.7, 3'-*H*), 4.33 (1H, ddd, *J* 7.7, 7.2 and 0.8, 4'-*H*), 2.16 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 170.8 (*C*OCH_3), 163.9 (4-*C*O), 150.3 (2-*C*O), 141.3 (6-*C*H), 133.6 (5'-*C*H), 111.3 (6'-*C*H), 103.0 (5-C*H*), 90.6 (1'-*C*H), 83.0 (4'-*C*H), 75.3 (2'-*C*H), 72.4 (3'-*C*H), 20.8 (CO*C*H_3); as a mixture of isomers: *m/z* (ES+) 361.0035 (M+H, $C_{12}H_{14}{}^{79}BrN_2O_6^+$ requires 361.0035), 363.0020 (M+H, $C_{12}H_{14}{}^{81}BrN_2O_6^+$ requires 363.0015), 382.9854 (M+Na,

$C_{12}H_{13}{}^{79}BrN_2O_6Na^+$ requires 382.9848), 384.9834 (M+Na, $C_{12}H_{13}{}^{81}BrN_2O_6Na^+$ requires 384.9834.

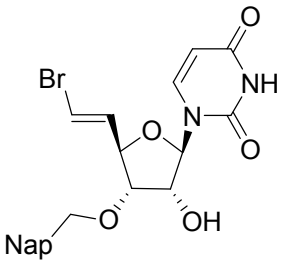
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-2'-*O*-acetyl uridine 219



Uracil (920 mg, 8.21 mmol) and BSA (2.03 mL, 8.21 mmol) were added to a stirred solution of *bis*-acetate **218** (1.23 g, 2.74 mmol) in dry MeCN (45 mL). The resulting suspension was heated to 65 °C and maintained at this temperature with stirring for 17 hours. The reaction mixture was then cooled to r.t., diluted with CH_2Cl_2 (40 mL) and quenched with a saturated solution of $NaHCO_3$ (45 mL). The organic phase was separated, washed with brine (45 mL), dried (Na_2SO_4) then filtered and concentrated to a yellow foam. Purification by column chromatography (9:1:1 $CHCl_3$:MeOH:Petrol) produced **219** as a white foam (1.10 g, 83%). m.p. 84-86 °C, R_f 0.24 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{25}$ +84.1 (*c* 1.36, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3387 (N-H), 3182 (N-H), 3118 (C-H), 2933 (C-H), 2879 (C-H), 1744 (C=O), 1723 (C=O), 1713 (C=O), 1694 (C=O), 1631 (C=C), 1455, 1375; δ_H (400 MHz, $CDCl_3$) 8.74 (1H, br s, NH), 7.87-7.84 (3H, m, Ar-H), 7.75 (1H, s, 1-Ar-*H*), 7.52-7.49 (2H, m, Ar-*H*), 7.40 (1H, dd, *J* 8.5 and 1.6, Ar-*H*), 7.16 (1H, d, *J* 8.1, 6-*H*), 6.51 (1H, dd, *J* 13.7 and 0.9, 6'-*H*), 6.23 (1H, dd, *J* 13.7 and 7.3, 5'-*H*), 5.75 (1H, d, *J* 8.1 and 2.2, 5-*H*), 5.71 (1H, d, *J* 2.7, 1'-*H*), 5.48 (1H, dd, *J* 5.8 and 2.7, 2'-*H*), 4.79 (1H, d, *J* 11.7, C*H*H), 4.65 (1H, d, *J*

11.7, CH*H*), 4.42 (1H, ddd, *J* 7.7, 7.3 and 0.9, 4'-*H*), 4.15 (1H, dd, *J* 7.7 and 5.8, 3'-*H*), 2.16 (3H, s, 2'-COC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*OCH$_3$), 162.7 (4-*C*O), 140.8 (6-*C*H), 134.3 (Ar-*C*), 133.4 (5'-*C*H), 133.3 (Ar-*C*), 128.5 (Ar-*C*), 128.1 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (1-Ar-*C*H), 126.5 (Ar-*C*), 126.4 (Ar-*C*), 125.8 (Ar-*C*), 111.5 (6'-*C*H), 103.1 (5-*C*H), 91.4 (1'-*C*H), 82.0 (4'-*C*H), 79.0 (3'-*C*H), 73.9 (CH_2), 73.6 (2'-*C*H), 20.8 (CO*C*H$_3$); *m/z* (ES+) 523.0521 (M+Na, $C_{23}H_{21}{}^{79}BrN_2NaO_6{}^+$ requires 523.0475), 525.0498 (M+Na, $C_{23}H_{21}{}^{81}BrN_2NaO_6{}^+$ requires 525.0460).

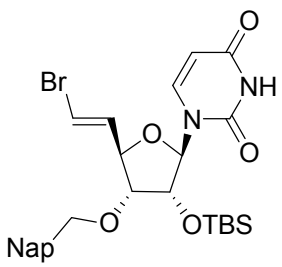
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl) uridine 231



Solid K_2CO_3 (281 mg, 2.03 mmol) was added to a stirred solution of **219** in MeOH (8 mL) and stirring was maintained at r.t. for 4 hours. The reaction mixture was then quenched with concentrated HCl (168 μL) and stirred for a further 10 minutes then concentrated *in vacuo* to a white solid. Purification by column chromatography (195:5 CH_2Cl_2:MeOH) produced **231** white solid (856 mg, 92%); m.p. 85-86 °C. R_f 0.42 (95:5 CH_2Cl_2:MeOH); $[\alpha]_D{}^{27}$ +54.8 (*c* 1.20, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3696 (O-H), 3689 (O-H), 3532 (N-H), 3388 (N-H), 2974 (C-H), 2874 (C-H), 1713 (C=O), 1694 (C=O), 1627 (C=C), 1603 (C=C), 1456, 1385, 1363, 1322; δ_H (400 MHz, $CDCl_3$) 9.08 (1H, br s, N*H*), 7.89-7.84 (3H, m, Ar-*H*), 7.79 (1H, s, Ar-*H*), 7.54-7.50 (2H, m, Ar-*H*), 7.45 (1H, dd, *J* 8.4 and 2.0, Ar-*H*), 7.20 (1H, d, *J* 8.0, 6-*H*), 6.45 (1H, dd, *J* 13.6

and 1.0, 6'-*H*), 6.24 (1H, dd, *J* 13.6 and 7.2, 5'-*H*), 5.72 (1H, dd, *J* 8.0 and 3.2, 5-*H*), 5.61 (1H, d, *J* 3.2, 1'-*H*), 4.86 (1H, d, *J* 12.0, C*H*H), 4.80 (1H, d, *J* 12.0, CH*H*), 4.44 (1H, ddd, *J* 7.2, 6.4 and 1.0, 4'-*H*), 4.36 (1H, ddd, *J* 6.0, 4.4 and 3.2, 2'-*H*), 4.13 (1H, dd *J* 6.4 and 6.0, 3'-*H*), 3.37 (1H, d, *J* 4.4, 2'-O*H*); δ_C (100 MHz, $CDCl_3$) 163.0 (4-*C*O), 150.2 (2-*C*O), 141.2 (6-*C*H), 134.0 (Ar-*C*), 133.9 (5'-*C*H), 133.4 (Ar-*C*), 128.8 (Ar-*C*H), 128.1 (Ar-*C*H), 127.9 (Ar-*C*H), 127.3 (Ar-*C*H), 126.6 (Ar-*C*H), 126.6 (Ar-*C*), 125.7 (Ar-*C*H), 125.6 (Ar-*C*H), 111.2 (6'-*C*H), 102.8 (5-*C*H), 93.7 (1'-*C*H), 82.2 (4'-*C*H), 80.2 (3'-*C*H), 73.6 (*C*H_2), 73.0 (2'-*C*H); *m/z* (ES+) 359.0547 (M+H, $C_{21}H_{20}{}^{79}BrN_2O_5^+$ requires 359.0556), 361.0522 (M+H, $C_{21}H_{20}{}^{81}BrN_2O_5^+$ requires 361.0535), 481.0370 (M+Na, $C_{21}H_{19}{}^{79}BrN_2O_5Na^+$ requires 481.0370), 483.0357 (M+Na, $C_{21}H_{19}{}^{81}BrN_2O_5Na^+$ requires 483.0355).

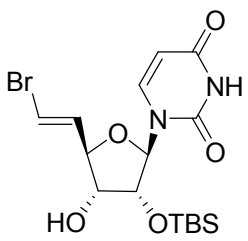
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-(*tert*-butyldimethylsilyl)-3'-(2-methylnaphthyl) uridine 232



Imidazole (445 mg, 6.53 mmol) then TBSCl (492 mg, 3.26 mmol) were added to a stirred solution of **231** (1.00 g, 2.18 mmol) in dry DMF (11 mL). Stirring was maintained under argon at r.t. for 26 hours then H_2O (15 mL) was added and the reaction mixture was extracted with $CHCl_3$ (3 x 10 mL). The combined organics were washed with brine (2 x 10 mL), dried (Na_2SO_4), filtered and evaporated to a pale yellow foam. The TBS ether **232** was obtained by trituration with pentane as a white solid

(1.23 g, 98%) m.p. 74-76 °C. R_f 0.16 (95:5 $CHCl_3$:MeOH); $[\alpha]_D^{25}$ +90.7 (*c* 0.73, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3390 (N-H), 2953 (C-H), 2930 (C-H), 2886 (C-H), 2858 (C-H), 1711 (C=O), 1694 (C=O), 1626 (C=C), 1603 (C=C), 1456, 1390, 1362; CHN: found C, 44.50%; H, 5.65%; N 6.07% ($C_{16}H_{25}BrN_2O_5Si$ requires C, 44.34%; H, 5.81%; N, 6.46%); δ_H (400 MHz, $CDCl_3$) 9.20 (1H, br s, NH), 7.87-7.83 (3H, m, Ar-*H*), 7.76 (1H, s, 1-Ar-*H*), 7.52-7.49 (2H, m, Ar-*H*), 7.44-7.41 (1H, dd, *J* 8.5 and 1.5, Ar-H), 7.26 (1H, d, *J* 8.2, 6-*H*), 6.56 (1H, dd, *J* 13.6 and 0.9, 6'-C*H*), 6.28 (1H, dd, *J* 13.6 and 7.3, 5'-*H*), 5.74 (1H, dd, *J* 8.2 and 2.0, 5-*H*), 5.68 (1H, d, *J* 2.4, 1'-*H*), 4.89 (1H, d, *J* 12.1, C*H*H), 4.65 (1H, m, *J* 7.3, 7.2 and 0.9, 4'-*H*), 4.64 (1H, d, *J* 12.1, CH*H*), 4.48 (1H, dd, *J* 4.4 and 2.4, 2'-*H*), 3.71 (1H, dd, 7.2 and 4.4, 3'-*H*), 0.94 (9H, s, C(C*H*$_3$)$_3$), 0.18 (3H, s, C*H*$_3$), and 0.16 (3H, s, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 163.2 (4-*C*O), 149.9 (2-*C*O), 139.8 (6-*C*H), 134.6 (Ar-*C*), 134.1 (5'-*C*H), 133.2 (Ar-*C*), 133.1 (Ar-*C*), 128.5 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*H), 126.5 (Ar-*C*H), 126.4 (Ar-*C*H), 126.3 (Ar-*C*H), 125.5 (Ar-*C*H), 111.4 (6'-*C*H), 102.5 (5-*C*H), 92.8 (1'-*C*H), 81.6 (4'-*C*H), 80.2 (3'-*C*H), 73.8 (2'-*C*H), 72.8 (CH_2), 25.7 (SiC(*C*H$_3$)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), -4.6 (Si(*C*H$_3$)), -4.9 (Si(*C*H$_3$)); *m/z* (ES+) 573.1400 (M+H, $C_{27}H_{34}{}^{79}BrN_2O_5Si^+$ requires 573.1420), 575.1387 (M+H, $C_{27}H_{34}{}^{81}BrN_2O_5Si^+$ requires 575.1400), 595.1214 (M+Na, $C_{27}H_{33}{}^{79}BrN_2O_5SiNa^+$ requires 595.1234), 597.1198 (M+Na, $C_{27}H_{33}{}^{81}BrN_2O_5SiNa^+$ requires 597.1219).

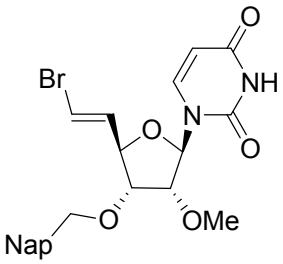
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-(*tert*-butyldimethylsilyl) uridine 235



Neat DDQ (715 mg, 3.15 mmol) was added to a stirred solution of **232** (602 mg, 1.05 mmol) in 4:1 v/v CH_2Cl_2:MeOH (10.5 mL) under an argon atmosphere. The solution was heated to reflux for 22 hours then the reaction mixture was quenched with saturated solution of $NaHCO_3$ (10 mL). The phases were separated and the aqueous phase was re-extracted with CH_2Cl_2 (3 x 10 mL). The combined organics were washed with brine (2 x 10 mL), dried (Na_2SO_4), filtered and evaporated to an orange solid. Column chromatography (95:5 $CHCl_3$:MeOH) followed by recrystallisation ($CHCl_3$) produced uridine **235** as a white solid (437 mg, 96%); m.p. 195-196°C. R_f 0.38 (95:5 CH_2Cl_2:MeOH); $[\alpha]_D^{23}$ +54.3 (*c* 0.73, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3538 (O-H, N-H), 3387 (N-H), 3178 (N-H), 2954 (C-H), 2931 (C-H), 2897 (C-H), 2885 (C-H), 2859 (C-H), 1713 (C=O), 1694 (C=O), 1626 (C=O), 1455, 1390, 1363; δ_H (400 MHz, $CDCl_3$) 8.81 (1H, br s, N*H*), 7.24 (1H, d, *J* 8.1, 6-*H*), 6.57 (1H, dd, *J* 13.7 and 1.1, 6'-*H*), 6.38 (1H, dd, *J* 13.7 and 6.6, 5'-*H*), 5.80 (1H, dd, *J* 8.1 and 2.8, 5-*H*), 5.69 (1H, d, *J* 2.8, 1'-*H*), 4.35-4.32 (2H, m, 2'-*H* and 4'-*H*), 3.94 (1H, ddd, *J* 8.1, 6.6 and 4.8, 3'-*H*), 2.59 (1H, d, *J* 8.1, 3'-O*H*), 0.94 (9H, s, C(CH_3)$_3$), 0.18 (3H, s, SiCH_3), 0.15 (3H, s, SiCH_3); δ_C (100 MHz, $CDCl_3$) 162.8 (4-*C*O), 149.8 (2-*C*O), 139.9 (6-*C*H), 133.7 (5'-*C*H), 110.7 (6'-*C*H), 103.0 (5-*C*H), 91.8 (1'-*C*H), 83.6 (4'-*C*H), 75.0 (2'-*C*H), 73.6 (3'-*C*H), 25.7 (SiC(*C*H_3)$_3$), 18.1 (Si*C*(CH$_3$)$_3$), -4.5 (Si*C*H$_3$), -

5.1 (Si*C*H_3); *m/z* (ES+) 433.0793 (M+H, $C_{16}H_{26}{}^{79}BrN_2O_5Si^+$ requires 433.0794), 435.0769 (M+H, $C_{16}H_{26}{}^{81}BrN_2O_5Si^+$ requires 435.0774), 455.0603 (M+Na, $C_{16}H_{25}{}^{79}BrN_2O_5SiNa^+$ requires 455.0608), 457.0584 (M+Na, $C_{16}H_{25}{}^{81}BrN_2O_5SiNa^+$ requires 457.0593).

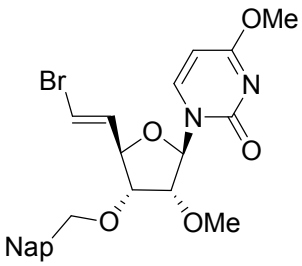
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-*O*-methyl-3'-(2-methylnaphthyl) uridine 233



A solution of alcohol **231** (670 mg, 1.46 mmol) in dry THF (14.6 mL) under an argon atmosphere was cooled to 0 °C. Solid NaH (60% w/w dispersion on mineral oil, 175 mg, 4.38 mmol) was added and stirring was maintained for 30 minutes then MeI (454 μL, 7.29 mmol) was added. Stirring was maintained at 4 °C for a further 21 hours then ice cold H_2O (6 mL) was added followed by EtOAc (20 mL). The organic phase was separated then washed with saturated $NaHCO_3$ solution (3 x 6 mL), dried (Na_2SO_4) then filtered and concentrated *in vacuo* to a pale yellow foam (630 mg). Purification by column chromatography (98:2 CH_2Cl_2:MeOH) enabled separation of the *mono*- and *bis*-methylated compounds. The mono-methylated uridine, **233** was obtained as a white foam (406 mg, 59%), m.p. 83-84 °C. R_f 0.10 (3:1 Et_2O:Petrol); $[\alpha]_D{}^{22}$ +66.5 (*c* 0.24, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3389 (N-H), 3003 (C-H), 1714 (C=O), 1694 (C=O), 1627 (C=C), 1454, 1361; δ_H (400 MHz, $CDCl_3$) 8.93 (1H, br s, N*H*), 7.88-7.84 (3H, m, Ar-*H*), 7.78 (1H, s, Ar-*H*), 7.52-7.50 (2H, m, Ar-*H*), 7.46 (1H, dd, *J* 8.4 and 1.6, Ar-

H), 7.22 (1H, d, *J* 8.1, 6-*H*), 6.55 (1H, dd, *J* 13.6 and 0.9, 6'-*H*), 6.28 (1H, dd, *J* 13.6 and 7.0, 5'-*H*), 5.81 (1H, d, *J* 2.0, 1'-*H*), 5.74 (1H, d, *J* 8.1, 5-*H*), 4.84 (1H, d, *J* 12.2, C*H*H), 4.74 (1H, d, *J* 12.2, CH*H*), 4.59 (1H, ddd, *J* 7.8, 7.0 and 0.9, 4'-*H*), 3.91 (1H, dd, *J* 4.8 and 2.0, 2'-*H*), 3.81 (1H, dd, *J* 7.8 and 4.8, 3'-*H*), 3.56 (3H, s, OC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 162.9 (4-*C*O), 149.8 (2-*C*O), 139.7 (6-*C*H), 134.3 (Ar-*C*), 133.8 (5'-*C*H), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 128.6 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*H), 126.9 (Ar-*C*H), 126.5 (Ar-*C*H), 126.4 (Ar-*C*H), 125.7 (Ar-*C*H), 111.4 (6'-*C*H), 102.8 (5-*C*H), 90.3 (1'-*C*H), 81.5 (4'-*C*H), 81.4 (2'-*C*H), 79.3 (3'-*C*H), 73.1 (*C*H$_2$), 58.8 (C*H*$_3$); *m/z* (ES+) 473.0720 (M+H, $C_{22}H_{22}{}^{79}BrN_2O_5^+$ requires 473.0712), 475.0708 (M+H, $C_{22}H_{22}{}^{81}BrN_2O_5^+$ requires 475.0692), 495.0537 (M+Na, $C_{22}H_{21}{}^{79}BrN_2NaO_5^+$ requires 495.0532), 497.0524 (M+Na, $C_{22}H_{21}{}^{81}BrN_2NaO_5^+$ requires 497.0511).

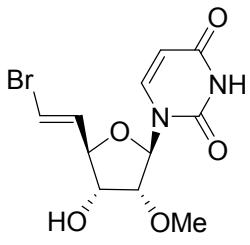
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-*O*-methyl-3'-(2-methylnaphthyl) 5-methyluridine 234



From the above procedure, further elution obtained the *bis*-methylated uridine **234** (137 mg, 20%) as a white foam; m.p. 54-56 °C. R_f 0.22 (3:1 Et_2O:Petrol); $[\alpha]_D{}^{22}$ +74.2 (*c* 0.79, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2995 (C-H), 2930 (C-H), 2872 (C-H), 1709 (C=O), 1666 (C=O), 1628 (C=C), 1461, 1372; δ_H (400 MHz, $CDCl_3$) 7.88-7.83 (3H, m, Ar-*H*), 7.78 (1H, s, Ar-*H*), 7.52-7.50 (2H, m, Ar-*H*), 7.46 (1H, dd, *J* 8.4 and 1.5, Ar-*H*), 7.20

(1H, d, *J* 8.1, 6-*H*), 6.56 (1H, dd, *J* 13.7 and 1.0, 6'-*H*), 6.29 (1H, dd, *J* 13.7 and 7.2, 5'-*H*), 5.82 (1H, d, *J* 1.5, 1'-*H*), 5.79 (1H, d, *J* 8.1, 5-*H*), 4.84 (1H, d, *J* 12.1, C*H*H), 4.74 (1H, d, *J* 12.1, CH*H*), 4.60 (1H, ddd, *J* 8.1, 7.2 and 1.0, 4'-*H*), 3.88 (1H, dd, *J* 5.0 and 1.5, 2'-*H*), 3.80 (1H, dd, *J* 8.1 and 5.0, 3'-*H*), 3.59 (3H, s, COCH_3), 3.32 (3H, s, 2'-COCH_3); δ_C (100 MHz, $CDCl_3$) 162.5 (4-*C*), 150.7 (2-*C*), 137.1 (*6*-*C*H), 134.4 (Ar-*C*), 133.8 (5'-*C*H), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 128.6 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*H), 126.9 (Ar-*C*H), 126.5 (Ar-*C*H), 126.4 (Ar-*C*H), 125.7 (Ar-*C*H), 111.5 (6'-*C*H), 102.1 (5-*C*H), 90.8 (1'-*C*H), 81.5 (4'-*C*H), 81.4 (2'-*C*H), 79.4 (3'-*C*H), 73.2 (CH_2), 58.8 (CO*C*H_3), 27.6 (2'-O*C*H_3); *m/z* (ES+) 487.0877 (M+H, $C_{23}H_{24}{}^{79}BrN_2O_5^+$ requires 487.0869), 489.0858 (M+H, $C_{23}H_{24}{}^{81}BrN_2O_5^+$ requires 489.0848), 509.0693 (M+Na, $C_{23}H_{23}{}^{79}BrN_2O_5Na^+$, requires 509.0688), 511.0676 (M+Na, $C_{23}H_{23}{}^{81}BrN_2O_5Na^+$, requires 511.0668).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-*O*-methyl uridine 236

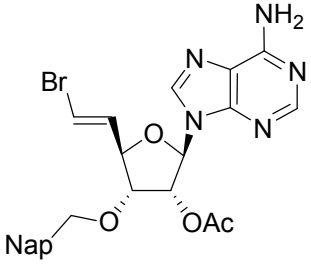


Neat DDQ (309 mg, 1.36 mmol) was added to a stirring solution of uridine **233** (215 mg, 454 mmol) in 4:1 v/v dry CH_2Cl_2:MeOH (4.5 mL) under an argon atmosphere. The solution was then heated at reflux for 10 hours then cooled to r.t. and quenched with saturated $NaHCO_3$ solution (4 mL) and then extracted with EtOAc (4 x 4 mL). The combined organic extracts were washed with brine (4 mL), dried (Na_2SO_4), filtered and concentrated *in*

vacuo to a red residue. Purification by column chromatography (195:5 CH_2Cl_2:MeOH) followed by recrystallisation (1:1 v/v toluene:$CHCl_3$) produced uridine **236** as off-white crystalline needles (123 mg, 81%); m.p. 141-143 °C. R_f 0.25 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{27}$ +106 (*c* 0.62, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3534 (O-H, N-H), 3389 (O-H, N-H), 2940 (C-H), 2841 (C-H), 1714 (C=O), 1694 (C=O), 1628 (C=C), 1456, 1392, 1362; δ_H (400 MHz, d_4-MeOD) 7.58 (1H, d, *J* 8.5, 6-*H*), 6.65 (1H, dd, *J* 13.5 and 1.0, 6'-*H*), 6.44 (1H, dd, *J* 13.5 and 7.5, 5'-*H*), 5.83 (1H, d, *J* 2.5, 1'-*H*), 5.72 (1H, d, *J* 8.5, 5-*H*), 4.29 (1H, ddd, *J* 7.5, 7.5 and 1.0, 4'-*H*), 4.05 (1H, dd, *J* 7.5 and 5.5, 3'-*H*), 3.87 (1H, dd, *J* 5.5 and 2.5, 2'-*H*), 3.52 (3H, s, C*H*$_3$); δ_C (126 MHz, d_4-MeOD) 166.9 (4-*C*O), 152.7 (2-*C*O), 143.0 (6-*C*H), 136.8 (5'-*C*H), 112.2 (6'-*C*H), 103.8 (5-*C*H), 91.1 (1'-*C*H), 85.3 (4'-*C*H), 84.8 (2'-*C*H), 74.8 (3'-*C*H), 49.1 (*C*H$_3$); *m/z* (ES+) 333.0087 (M+H, $C_{11}H_{14}{}^{79}BrN_2O_5^+$ requires 333.0086), 335.0071 (M+H, $C_{11}H_{14}{}^{81}BrN_2O_5^+$ requires 335.0066), 354.9902 (M+Na, $C_{11}H_{13}{}^{79}BrN_2O_5Na^+$ requires 354.9900), 356.9885 (M+Na, $C_{11}H_{13}{}^{81}BrN_2O_5Na^+$ requires 356.9885).

4.3.2.2 Vinyl Bromide Adenosines

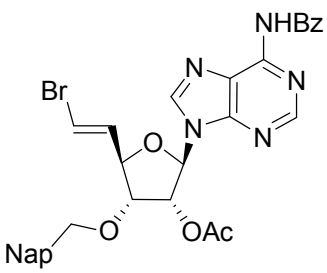
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-2'-*O*-acetyl adenosine 220



To a stirred suspension of bis-acetate **210** (170 mg, 378 μmol) and adenine (77 mg, 567 μmol) in dry acetonitrile (2.5 mL) at r.t. under an argon atmosphere was added

$SnCl_4$ (111 μL, 946 μmol). After 15 hours, the reaction mixture was concentrated *in vacuo* and the residue then partitioned between saturated $NaHCO_3$ solution (20 mL) and $CHCl_3$ (20 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to a pale yellow solid (107 mg). Purification by column chromatography (98:2 $CHCl_3$:MeOH) obtained adenosine **220** as a white solid (48.8 mg, 25%), m.p. 69-71 °C. R_f 0.27 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{22}$ +29.7 (*c* 0.70, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3686 (N-H), 3524 (N-H), 3413 (N-H), 2985 (C-H), 1748 (C=O), 1631 (C=O), 1588 (C=C), 1470 (C=C), 1372, 1329, 1295; δ_H (400 MHz, $CDCl_3$) 8.22 (1H, s, 2-*H*), 7.88-7.80 (5H, m, 8-*H* and 4 x Ar-*H*), 7.52-7.50 (2H, m, Ar-*H*), 7.45 (1H, dd, *J* 8.4 and 1.6, Ar-*H*), 6.49 (1H, dd, *J* 13.7 and 0.8, 6'-*H*), 6.29 (1H, dd, *J* 13.7 and 7.4, 5'-*H*), 6.04 (1H, d, *J* 2.8, 1'-*H*), 5.99 (2H, br s, N*H*$_2$), 5.96 (1H, dd, *J* 5.3 and 2.8, 2'-*H*), 4.84 (1H, d, *J* 11.8, C*H*H), 4.74 (1H, dd, *J* 7.3 and 5.3, 3'-*H*), 4.73 (1H, d, *J* 11.8, CH*H*), 4.53 (1H, ddd, *J* 7.4, 7.3 and 0.8, 4'-*H*), 2.17 (3H, s, COC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 169.9 (*C*OCH$_3$), 155.7 (6-*C*), 153.2 (2-*C*H), 149.3 (4-*C*), 139.7 (8-*C*H), 134.4 (Ar-*C*(CH_2)), 133.9 (5'-*C*H), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 128.5 (Ar-*C*H), 128.0 (Ar-*C*H), 127.8 (Ar-*C*H), 127.3 (Ar-*C*H), 126.4 (Ar-*C*H), 126.3 (Ar-*C*H), 125.9 (Ar-*C*H), 120.4 (5-*C*), 111.2 (6'-*C*H), 88.0 (1'-*C*H), 82.2 (4'-*C*H), 79.0 (3'-*C*H), 73.7 (CH_2), 73.6 (2'-*C*H), 20.8 (CO*C*H$_3$); *m/z* (ES+) 524.0928 (M+H, $C_{24}H_{23}{}^{79}BrN_5O_4^+$ requires 524.0928), 526.0920 (M+H, $C_{24}H_{23}{}^{81}BrN_5O_4^+$ requires 524.0928).

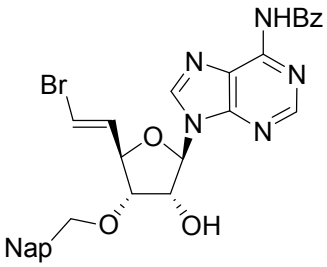
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-2'-*O*-acetyl *N*-benzoyladenosine 221



Benzoyl adenine (998 mg, 4.17 mmol) and BSA (1.24 mL, 5.01 mmol) were added to a stirring solution of bis-acetate **218** (750 mg, 1.67 mmol) in dry toluene (16.7 mL) at r.t. under an argon atmosphere. The reaction mixture was then heated to 80 °C for 3 hours then TMSOTf (380 μL, 2.47 mmol) was added. Stirring at 80 °C maintained for a further 3 ½ hours then the reaction mixture was cooled to r.t., diluted with EtOAc (20 mL), washed with a saturated $NaHCO_3$ solution (25 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to a pale yellow foam. Purification by column chromatography produced benzoyladenosine **221** as a white foam (995 mg, 94%), m.p. 96-97 °C. R_f 0.45 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +17.6 (*c* 1.51, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3405 (N-H), 2992 (C-H), 2933 (C-H), 2882 (C-H), 1748 (C=O), 1710 (C=O), 1613 (C=C), 1588 (C=C), 1457, 1438, 1372, 1328; δ_H (400 MHz, $CDCl_3$) 9.01 (1H, br s, N*H*), 8.70 (1H, s, 2-C*H*), 8.03-8.01 (3H, m, 2 x Ar-*C*H and 8-C*H*), 7.89-7.85 (3H, m, 3 x Ar-C*H*), 7.81 (1H, s, Ar-*H*), 7.61 (1H, d, *J* 7.0, Ar-C*H*), 7.55-7.51 (4H, m, Ar-C*H*), 7.46 (1H, dd, *J* 7.0 and 1.2, Ar-C*H*), 6.49 (1H, d, *J* 13.7, 6'-*H*), 6.28 (1H, dd, *J* 13.7 and 7.4, 5'-*H*), 6.10 (1H, d, *J* 2.8, 1'-*H*), 5.96 (1H, dd, *J* 5.3 and 2.8, 2'-*H*), 4.86 (1H, d, *J* 11.5, C*H*H), 4.75 (1H, d, *J* 11.5, CH*H*), 4.72 (1H, dd, *J* 7.1 and 5.3, 3'-*H*), 4.55 (1H, dd, *J* 7.4 and 7.1, 4'-*H*), 2.18 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 169.9 (*C*OCH_3), 164.6 (N*C*OPh), 152.9 (2-*C*H), 151.2 (6-*C*),

149.8 (4-*C*H), 142.1 (8-*C*H), 134.3 (Ar-*C*), 133.7 (5'-*C*H), 133.6 (Ar-*C*), 133.2 (Ar-*C*), 133.1 (NCO-Ar-*C*), 132.9 (Ar-*C*H), 129.0 (2 x Ar-*C*H), 128.6 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (2 x Ar-*C*H), 127.8 (Ar-*C*H), 127.4 (Ar-*C*H), 126.5 (Ar-*C*H), 126.4 (Ar-*C*H), 125.9 (Ar-*C*H), 123.8 (5-*C*), 111.4 (6'-*C*H), 88.2 (1'-*C*H), 82.4 (4'-*C*H), 78.9 (3'-*C*H), 73.8 (CH_2), 73.6 (2'-*C*H), 20.8 ($CO\mathit{C}H_3$); *m/z* (ES+) 628.1128 (M+H, $C_{31}H_{27}{}^{79}BrN_5O_5$ requires 628.1190), 630.1154 (M+H, $C_{31}H_{27}{}^{81}BrN_5O_5$ requires 630.1175), 650.0984 (M+Na, $C_{31}H_{26}{}^{79}BrN_5O_5Na^+$ requires 650.1005), 652.0982 (M+Na, $C_{31}H_{26}{}^{81}BrN_5O_5Na^+$ requires 652.0995).

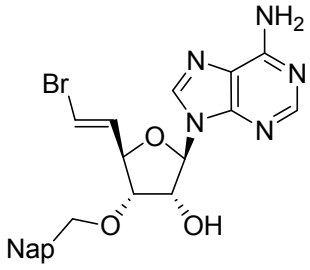
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-*N*-benzoyladenosine 237



A 1:1 v/v solution of EtOH-2N NaOH (22.5 mL) was added to a stirred solution of acetate **221** (1.70 g, 2.70 mmol). The reaction mixture was stirred for 10 minutes at r.t. and was then neutralised with saturated NH_4Cl solution (40 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The combined organics were washed with brine (25 mL), dried (Na_2SO_4), filtered and evaporarted *in vacuo* to a pale brown oil (2.20 g). Purification by column chromatography (97:3 CH_2Cl_2:MeOH) obtained alcohol **237** as a pale yellow foam (1.17 g, 74%), m.p. 100-102 °C. R_f 0.21 (97:3 CH_2Cl_2:MeOH); $[\alpha]_D{}^{23}$ -16.3 (*c* 0.65, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3528 (O-H), 3399 (N-H), 2991 (C-H), 2933 (C-H), 2872 (C-H), 1709 (C=O), 1613 (C=C), 1586 (C=C), 1483, 1455, 1400,

1359, 1327; δ_H (500 MHz, $CDCl_3$) 9.05 (1H, br s, N*H*), 8.71 (1H, s, 2-C*H*), 8.02-8.01 (2H, m, Ar-*H*), 7.99 (1H, s, 8-C*H*), 7.90 (1H, d, *J* 8.0, Ar-*H*), 7.88-7.85 (2H, m, Ar-*H*), 7.84 (1H, s, Ar-*H*), 7.62-7.59 (1H, m, Ar-*H*), 7.54-7.49 (5H, m, Ar-*H*), 6.46 (1H, dd, *J* 14.0 and 1.0, 6'-*H*), 6.31 (1H, dd, *J* 14.0 and 7.0, 5'-*H*), 5.95 (1H, d, *J* 3.5, 1'-*H*), 4.93 (1H, d, *J* 11.3, C*H*H), 4.90 (1H, d, *J* 11.3, CH*H*), 4.83 (1H, dd, *J* 4.5 and 3.5, 2'-*H*), 4.58 (1H, ddd, *J* 7.0, 5.5 and 1.0, 4'-*H*), 4.54 (1H, dd, *J* 5.5 and 4.5, 3'-*H*), 3.72 (1H, br s, O*H*); δ_C (126 MHz, $CDCl_3$) 164.7 (N*C*OPh), 152.7 (2-*C*H), 151.2 (6-*C*), 149.7 (4-*C*), 142.2 (8-*C*H), 134.0 (5'-*C*H), 133.9 (Ar-*C*H), 133.6 (Ar-*C*), 133.5 (Ar-*C*), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 132.9 (Ar-*C*H), 128.9 (2 x Ar-*C*H), 128.8 (Ar-*C*H), 128.7 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (Ar-*C*H), 127.8 (Ar-*C*H), 127.5 (Ar-*C*H), 126.6 (Ar-*C*H), 125.8 (Ar-*C*H), 123.6 (5-*C*H), 110.9 (6'-*C*H), 90.2 (1'-*C*H), 82.5 (4'-*C*H), 80.5 (3'-*C*H), 73.6 (CH_2), 73.1 (2'-*C*H); *m/z* (ES+) 586.1076 (M+H, $C_{29}H_{25}{}^{79}BrN_5O_4^+$ requires 586.1084), 588.1078 (M+H, $C_{29}H_{25}{}^{81}BrN_5O_4^+$ requires 588.1069), 608.0903 (M+Na, $C_{29}H_{24}{}^{79}BrN_5O_4Na^+$ requires 608.0909), 610.0887 (M+Na, $C_{29}H_{24}{}^{79}BrN_5O_4Na^+$ requires 610.0889).

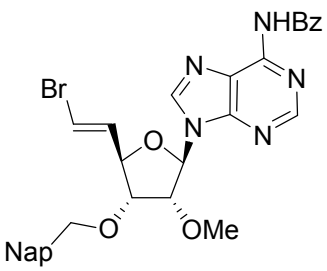
6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl) adenosine 238



In the above procedure, alcohol **238** was obtained as a colourless glass (20 mg, 1%), m.p. 90-92 °C. R_f 0.13 (97:3 CH_2Cl_2:MeOH); $[\alpha]_D{}^{27}$ -2.56 (*c* 0.56, $CHCl_3$);

ν_{max}/cm^{-1} ($CHCl_3$) 3697 (O-H), 3632 (O-H), 3535 (O-H, N-H), 3413 (N-H), 2980 (C-H), 2944 (C-H), 2841 (C-H), 1631 (N-H, C=C), 1587 (N-H, C=C), 1469 (C-H, C=C), 1369, 1339; δ_H (500 MHz, $CDCl_3$) 8.25 (1H, s, 2-*C*H), 7.89-7.80 (4H, m, Ar-*H*), 7.83 (1H, s, 8-C*H*), 7.53-7.49 (3H, m, Ar-*H*), 6.45 (1H, dd, *J* 13.5 and 1.0, 6'-*H*), 6.31 (1H, dd, *J* 13.5 and 7.0, 5'-*H*), 5.92 (1H, d, *J* 4.0, 1'-*H*), 5.90 (1H, br s, N*H*$_2$), 5.79 (1H, br s, N*H*$_2$), 4.94 (1H, d, *J* 12.0, C*H*H), 4.89 (1H, d, *J* 12.0, CH*H*), 4.82 (1H, dd, *J* 4.5 and 4.0, 2'-*H*), 4.60 (1H, ddd, *J* 7.0, 5.5 and 1.0, 4'-*H*), 4.48 (1H, dd, *J* 5.5 and 4.5, 3'-*H*), 4.39 (1H, br s, O*H*); δ_C (126 MHz, $CDCl_3$) 155.6 (*6-C*), 153.0 (2-*C*H), 149.9 (4-*C*), 139.8 (8-*C*H), 134.3 (5'-*C*H), 134.2 (Ar-*C*), 133.4 (Ar-*C*), 133.3 (Ar-*C*), 128.8 (Ar-C*H*), 128.0 (Ar-C*H*), 127.9 (Ar-C*H*), 127.5 (Ar-C*H*), 126.6 (Ar-C*H*), 126.5 (Ar-C*H*), 125.8 (Ar-C*H*), 120.4 (5-*C*), 110.7 (6'-*C*H), 90.3 (1'-*C*H), 82.8 (4'-*C*H), 80.6 (3'-*C*H), 73.6 (*C*H$_2$), 73.4 (2'-*C*H); *m/z* (ES+) 482.0820 (M+H, $C_{22}H_{23}{}^{79}BrN_5O_3{}^+$ requires 482.0822), 484.0813 (M+H, $C_{22}H_{23}{}^{81}BrN_5O_3{}^+$ requires 484.0807).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-2'-*O*-methyl *N*-benzoyladenosine 239



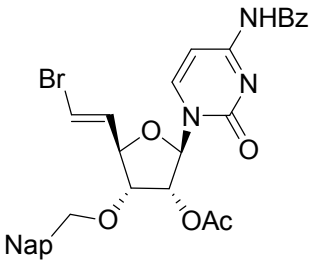
A stirred solution of alcohol **237** (1.10 g, 1.87 mmol) in dry THF (20 mL) under argon was cooled to 4 °C in a cold room. Solid NaH (60% w/w dispersion, 375 mg, 9.38 mmol) was added and followed by MeI (350 μL, 5.63 mmol). Stirring was maintained at 4 °C for 13 ½ hours then saturated

NH_4Cl solution (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered and evaporated to a yellow solid (1.27 g). Purification by column chromatography obtained the methylated adenosine **239** as a pale yellow foam (822 mg, 73%); R_f 0.19 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{26}$ +2.06 (*c* 1.03, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3664 (O-H), 3406 (N-H), 2992 (C-H), 2933 (C-H), 2885 (C-H), 2834 (C-H), 1744 (C=N), 1724 (C=N), 1673 (C=C), 1650 (C=C), 1614 (C=O), 1585 (C=C), 1499 (C-C), 1498 (C=C), 1484 (C=C), 1398 (C-O), 1356, 1329; δ_H (500 MHz, $CDCl_3$) 9.00 (1H, s, N*H*), 8.73 (1H, s, 2-C*H*), 8.05 (1H, s, 8-C*H*), 8.00 (2H, d, *J* 7.5, Ar-*H*), 7.89-7.81 (5H, m, Ar-*H*), 7.63-7.58 (1H, m, Ar-*H*), 7.54-7.49 (6H, m, Ar-*H*), 7.41-7.40 (1H, m, Ar-*H*), 6.52 (1H, dd, *J* 13.5 and 1.0, 6'-*H*), 6.38 (1H, dd, *J* 13.5 and 6.8, 5'-*H*), 6.11 (1H, d, *J* 3.7, 1'-*H*), 4.90 (1H, d, *J* 12.0, C*H*H), 4.86 (1H, d, *J* 12.0, CH*H*), 4.67 (1H, ddd, *J* 6.8, 6.2 and 1.0, 4'-*H*), 4.62 (1H, dd, *J* 5.0 and 3.7, 2'-*H*), 4.30 (1H, dd, *J* 6.2 and 5.0, 3'-*H*), 3.50 (3H, s, C*H*$_3$); δ_C (126 MHz, $CDCl_3$) 164.6 (*C*OPh), 152.8 (2-*C*H), 151.3 (6-*C*), 149.8 (4-*C*), 142.2 (8-*C*H), 134.5 (Ar-*C*), 134.2 (5'-*C*H), 134.0 (Ar-*C*), 133.2 (Ar-*C*), 133.1 (Ar-*C*), 132.9 (Ar-*C*H), 129.0 (2 x Ar-*C*H), 128.6 (Ar-*C*H), 128.0 (Ar-*C*H), 127.9 (2 x Ar-*C*H), 127.8 (Ar-*C*H), 127.2 (Ar-*C*H), 126.5 (Ar-*C*H), 126.4 (Ar-*C*H), 125.9 (Ar-*C*H), 124.0 (5-*C*), 110.9 (6'-*C*H), 88.2 (1'-*C*H), 82.3 (4'-*C*H), 80.9 (3'-*C*H), 79.4 (2'-*C*H), 73.2 (*C*H_2), 58.9 (*C*H_3); *m/z* (ES+) 600.1243 (M+H, $C_{30}H_{27}{}^{79}BrN_5O_4^+$ requires 600.1241), 602.1219 (M+H, $C_{30}H_{27}{}^{81}BrN_5O_4^+$ requires 602.1226), 622.1067

(M+Na, $C_{30}H_{26}{}^{79}BrN_5O_4Na^+$ requires 622.1060), 624.1060 (M+Na, $C_{30}H_{26}{}^{81}BrN_5O_4Na^+$ requires 624.1045).

4.3.2.3 Vinyl Bromide Cytidines

6′-*E*-Bromo-(5′-deoxy-5′-methylidene)-3′-(2-methylnaphthyl)-2′-*O*-acetyl *N*-benzoylcytidine 226

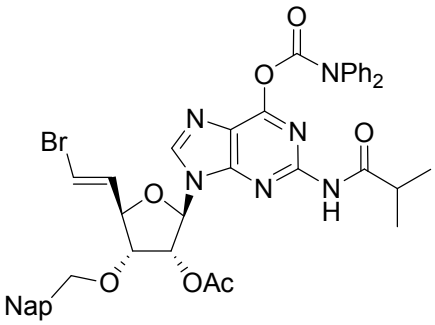


To a stirred solution of *bis*-acetate **218** (1.45 g, 3.23 mmol) in dry MeCN (15 mL) under an inert atmosphere was added BSA (2.00 mL, 8.07 mmol) and benzoyl cytosine **225** (729 mg, 3.39 mmol). The resulting suspension was heated to 70 °C for 90 minutes, the solution was then removed from heat, cooled for 5 minutes then TMSOTf was added (1.50 mL, 9.68 mmol). The reaction mixture was then heated at 70 °C for a further 75 minutes then cooled to r.t. The reaction mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$ solution (30 mL), dried ($NaSO_4$), filtered and evaporated to a brown foam. Purification by column chromatography (98:2 CH_2Cl_2:MeOH) followed by trituration with EtOAc produced the cytidine **226** as an off-white solid (593 mg, 30%); m.p. 93-94 °C; R_f 0.25 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +144 (*c* 0.85, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3404 (N-H), 2880 (C-H), 1746 (C=O), 1672 (C=O), 1628 (C=C), 1556, 1483, 1370; δ_H (500 MHz, DMSO) 11.33 (1H, N*H*), 8.16 (1H, d, *J* 7.0, 6-*H*), 8.01 (2H, d, *J* 7.9, Ar-*H*), 7.91 (3H, m, Ar-*H*), 7.80 (1H, s, Ar-*H*), 7.63 (1H, dd, *J* 7.3 and 6.9, Ar-*H*), 7.51 (4H, m, Ar-*H*), 7.40 (2H, m, Ar-*H* and 5-*H*), 6.96 (1H, d, *J* 13.3,

6'-*H*), 6.58 (1H, dd, *J* 13.3 and 8.5, 5'-*H*), 5.89 (1H, d, *J* 2.0, 1'-*H*), 5.61 (1H, dd, *J* 5.4 and 2.0, 2'-*H*), 4.72 (1H, d, *J* 12.1, C*H*H), 4.67 (1H, d, *J* 12.1, CH*H*), 4.54 (1H, dd, *J* 8.5 and 8.0, 4'-*H*), 4.27 (1H, dd, *J* 8.0 and 5.4, 3'-*H*), 2.14 (3H, s, COCH_3); δ_C (100 MHz, $CDCl_3$) 170.2 (*C*OPh), 147.2 (2-*C*), 136.3 (Ar-*C*), 135.5 (5'-*C*H), 133.8 (Ar-*C*), 133.7 (Ar-*C*), 133.6 (Ar-*C*), 133.5 (6-*C*H), 129.4 (2 x Ar-*C*H), 129.4 (2 x Ar-*C*H), 128.8 (Ar-*C*H), 128.7 (Ar-*C*H), 128.5 (Ar-*C*H), 127.2 (Ar-*C*H), 127.0 (Ar-*C*H), 126.9 (Ar-*C*H), 126.6 (Ar-*C*H), 113.6 (6'-*C*H), 97.5 (5-*C*H), 90.8 (1'-*C*H), 82.3 (4'-*C*H), 79.5 (3'-*C*H), 74.2 (2'-*C*H), 73.1 (*C*H_2), 21.6 (COCH_3); *m/z* (ES+) 604.1063 (M+H, $C_{30}H_{27}{}^{79}BrN_3O_6^+$ requires 604.1078), 606.1074 (M+H, $C_{30}H_{27}{}^{81}BrN_3O_6^+$ requires 606.1063).

4.3.2.4 Vinyl Bromide Guanosines

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl)-2'-*O*-acetyl 2-*N*-isobutyryl-6-*O*-diphenylcarbamoyl guanosine 230

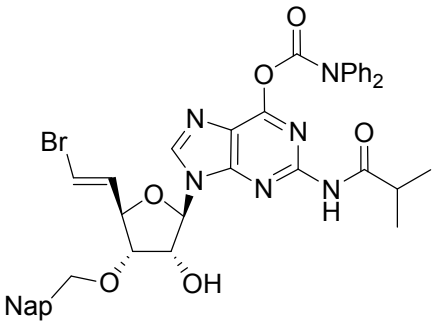


Neat BSA (742 mL, 3.00 μmol) and 2-*N*-isobutyryl-6-*O*-diphenylcarbamoyl guanosine **229** (1.04 g, 2.50 mmol) were added to a stirred solution of *bis*-acetate **218** (450 mg, 1.00 mmol) in dry toluene (10 mL). The reaction mixture was heated to 80 °C and maintained at this temperature for 90 minutes in which time the suspension became a clear solution. Neat TMSOTf (229 μL, 1.48 mmol) was added and stirring was

maintained for a further 2 ½ hours. The reaction mixture was then removed from the heat, cooled and diluted with EtOAc (15 mL) then washed sequentially with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic phase was separated, dried (Na_2SO_4), filtered and concentrated to a grey foam. Following purification by column chromatography obtained guanosine **230** (610 mg, 76%) as a white foam, m.p. 107-108 °C. R_f 0.53 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +59.4 (*c* 0.72, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3427 (N-H), 2974 (C-H), 1746 (C=O), 1622 (C=C), 1588, 1491, 1452, 1406, 1372, 1322, 1303; δ_H (400 MHz, $CDCl_3$) 8.01 (1H, br s, N*H*), 7.93 (1H, s, 8-C*H*), 7.85-7.82 (4H, m, Ar-*H*), 7.51-7.32 (12H, m, Ar-*H*), 7.26 (1H, m, Ar-*H*), 6.52 (1H, d, *J* 13.6, 6'-*H*), 6.48 (1H, dd, *J* 13.6 and 4.9, 5'-*H*), 5.95 (1H, d, *J* 2.4, 1'-*H*), 5.82 (1H, dd, *J* 5.5 and 2.4, 2'-*H*), 5.38 (1H, dd, *J* 7.3 and 5.5, 3'-*H*), 4.95 (1H, d, *J* 11.6, C*H*H), 4.87 (1H, d, *J* 11.6, CH*H*), 4.51 (1H, app ddd, *J* 7.3, 4.9 and ~2.1, 4'-*H*), 2.70 (1H, sept, *J* 6.9, C*H*$(CH_3)_2$), 2.12 (3H, s, 2'-COC*H*$_3$), 1.29 (3H, d, *J* 6.9, CH(C*H*$_3$)), 1.26 (3H, d, *J* 6.9, CH(C*H*$_3$)); δ_C (126 MHz, $CDCl_3$) 172.3 (*C*OCH$(CH_3)_2$), 170.0 (*C*OCH$_3$), 156.4 (6-*C*), 154.1 (2-*C*), 152.0 (4-*C* and *C*ONPh$_2$), 150.3 (2 x Ar-*C*), 143.2 (Ar-*C*H), 135.3 (Ar-*C*), 134.1 (5'-*C*H), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 129.3 (Ar-*C*H), 128.3 (2 x Ar-*C*H), 127.8 (2 x Ar-*C*H), 127.7 (2 x Ar-*C*H), 127.2 (2 x Ar-*C*H), 127.1 (2 x Ar-*C*H), 126.2 (2 x Ar-*C*H), 126.1 (2 x Ar-*C*H), 126.1 (2 x Ar-*C*H), 122.0 (5-*C*), 111.3 (6'-*C*H), 88.7 (1'-*C*H), 82.9 (4'-*C*H), 79.3 (3'-*C*H), 74.4 (2'-*C*H), 73.9 (CH_2), 36.6 (COC*H*$(CH_3)_2$), 20.8 (2'-*C*OCH$_3$), 19.5 (CH(*C*H$_3$)$_2$), 19.3 (CH(*C*H$_3$)$_2$);

m/z (ES+) 805.1966 (M+H, $C_{41}H_{38}{}^{79}BrN_6O_7^+$ requires 805.1985), 807.1970 (M+H, $C_{41}H_{38}{}^{81}BrN_6O_7^+$ requires 807.1965), 827.1769 (M+Na, $C_{41}H_{37}{}^{79}BrN_6NaO_7^+$ requires 827.1799), 829.1773 (M+Na, $C_{41}H_{37}{}^{81}BrN_6NaO_7^+$ requires 829.1784).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-(2-methylnaphthyl) 2-*N*-*iso*-butyryl-6-*O*-diphenylcarbamoyl guanosine 246

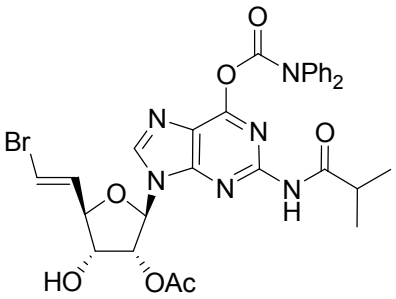


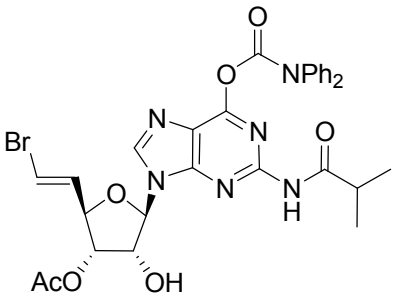
A stirred solution of **230** (200 mg, 248 μmol) in 5:4:1 v/v THF:MeOH:H_2O (14 mL) was cooled to 0 °C in an ice bath. A solution of NaOH (2N, 990 μL, 1.99 mmol) was added and stirring was maintained for 10 mins. Solid NH_4Cl (114 mg, 2.13 mmol) was added and stirring was continued for a further 30 mins. The solvent was removed *in vacuo* and the remaining residue was taken up in CH_2Cl_2 (10 mL) and filtered through a plug of silica, eluting with 5:1 v/v CH_2Cl_2:MeOH (10 mL). The filtrate was evaporated to give guanosine **246** (186 mg, 98%) as a white foam; m.p. 112-114 °C. R_f 0.40 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{26}$ +48.4 (*c* 0.43, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3425 (O-H), 3377 (N-H), 2974 (C-H), 2936 (C-H), 2877 (C-H), 1746 (C=O), 1717 (C=O), 1621 (C=O), 1588 (C=C, C-N, N-H), 1492 (C=C), 1452 (C=C), 1407, 1386, 1365, 1323, 1301; δ_H (500 MHz, $CDCl_3$) 8.11 (1H, br s, N*H*), 8.07 (1H, s, 8-C*H*), 7.87-7.83 (4H, m, Ar-*H*), 7.56 (1H, dd, *J* 8.5 and 1.5, Ar-*H*), 7.51-7.48 (2H, m, Ar-*H*), 7.47-7.42 (4H, m, Ar-*H*), 7.39-7.36 (4H, m, Ar-*H*), 7.26 (2H, m, Ar-*H*), 6.34 (1H, dd, *J* 13.5

and 0.5, 6'-*H*), 6.29 (1H, dd, *J* 13.5 and 6.8, 5'-*H*), 5.92 (1H, d, *J* 4.5, 1'-*H*), 5.16 (1H, d, *J* 11.5, C*H*H), 5.11 (1H, br s, 2'-O*H*), 4.98 (1H, d, *J* 11.5, CH*H*), 4.81 (1H, app ddd, *J* 4.7, 4.5 and ~1.5, 2'-*H*), 4.74 (1H, dd, *J* 4.7 and 4.0, 3'-*H*), 4.63 (1H, dd, *J* 6.8, 4.0 and 0.5, 4'-*H*), 2.61 (1H, dq, *J* 7.0 and 6.5, C*H*(CH_3)$_2$), 1.29 (3H, d, *J* 7.0, CH(C*H*$_3$)), 1.28 (3H, d, *J* 6.5, CH(C*H*$_3$)); δ_C (126 MHz, $CDCl_3$) 174.6 (*C*OCH(CH_3)$_2$), 156.4 (6-*C*), 153.8 (2-*C*), 151.6 (4-*C*), 150.4 (*C*ONPh_2), 143.3 (8-*C*H), 141.7 (2 x Ar-*C*), 135.3 (Ar-*C*), 134.4 (5'-*C*H), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 129.3 (5 x Ar-*C*H), 128.5 (2 x Ar-*C*H), 128.0 (2 x Ar-*C*H), 127.8 (2 x Ar-*C*H), 127.1 (2 x Ar-*C*H), 126.3 (2 x Ar-*C*H), 126.2 (2 x Ar-*C*H), 126.0 (2 x Ar-*C*H), 121.8 (5-*C*), 110.4 (6'-*C*H), 90.9 (1'-*C*H), 84.2 (4'-*C*H), 81.2 (3'-*C*H), 74.9 (2'-*C*H), 73.8 (CH_2), 37.1 (*C*H(CH_3)$_2$), 19.5 (CH(*C*H_3)$_2$), 19.4 (CH(*C*H_3)$_2$); *m/z* (ES+) 763.1825 (M+H, $C_{39}H_{36}{}^{79}BrN_6O_6^+$ requires 763.1874), 765.1844 (M+H, $C_{39}H_{36}{}^{81}BrN_6O_6^+$ requires 765.1859), 785.1670 (M+Na, $C_{39}H_{35}{}^{79}BrN_6NaO_6^+$ requires 785.1694), 787.1673 (M+Na, $C_{39}H_{35}{}^{81}BrN_6NaO_6^+$ requires 787.1679).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-2'-*O*-acetyl-2-*N*-*iso*-butyryl-6-*O*-diphenylcarbamoyl guanosine 249

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)-3'-*O*-acetyl-2-*N*-*iso*-butyryl-6-*O*-diphenylcarbamoyl guanosine 250

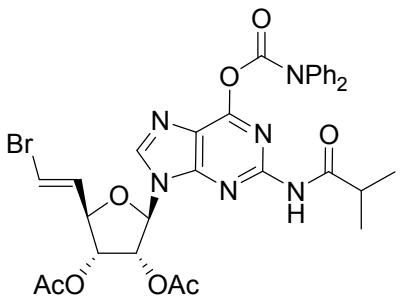




Solid DDQ (28 mg, 124 μmol) was added to a stirred solution of **246** (100 mg, 124 μmol) in 4:1 v/v CH_2Cl_2:MeOH (distilled) (2.5 mL) under an argon atmosphere. The reaction mixture was heated to a gentle reflux at 40 °C for 11 hours then cooled and quenched with saturated $NaHCO_3$ solution (2 mL). The aqueous phase was re-extracted with CH_2Cl_2 (3 x 2 mL) and then the combined organics were then washed with brine (2 mL), dried (Na_2SO_4) and evaporated to an off-white solid. Repeated trituration with warm hexane (3 x 1 mL) generated a 3:2 mixture of **249** and **250** isomers (77 mg, 93%) as an off-white solid. Column chromatography was unsuccessful at separating the individual components and the mixture was taken on without further purification. R_f 0.34, 0.29 (96:4 CH_2Cl_2:MeOH); as a mixture of isomers: $[\alpha]_D^{25}$ +31.1 (*c* 1.85, $CHCl_3$); as a mixture of isomers: ν_{max}/cm^{-1} ($CHCl_3$) 3424 (O-H), 3391 (N-H), 3304 (N-H), 2969 (C-H), 2935 (C-H), 2908 (C-H), 2832 (C-H), 1746 (C=O), 1716 (C=O), 1694 (C=N), 1622 (C=C), 1589 (C=C), 1491 (C=C), 1454, 1372, 1342, 1322, 1303; as a mixture of isomers: *m/z*

(ES+) 665.1372 (M+H, $C_{30}H_{30}{}^{79}BrN_6O_9^+$ requires 665.1359), 667.1366 (M+H, $C_{30}H_{30}{}^{81}BrN_6O_9^+$ requires 667.1339), 687.1154 (M+Na, $C_{30}H_{30}{}^{79}BrN_6O_9Na^+$ requires 687.1179), 689.1158 (M+Na, $C_{30}H_{30}{}^{81}BrN_6O_9Na^+$ requires 689.1148).

6'-*E*-Bromo-(5'-deoxy-5'-methylidene)- 2'-*O*-acetyl, 3'-*O*-acetyl-2-*N*-*iso*-butyryl-6-*O*-diphenylcarbamoyl guanosine 251

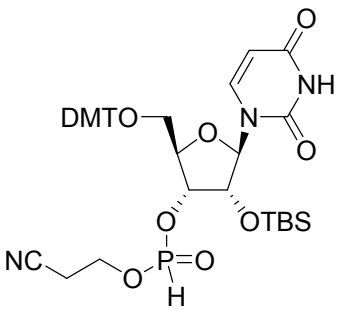


Dry Et_3N (100 μL, 715 μmol), DMAP (7 mg, 57.2 μmol) then Ac_2O (40 μL, 429 μmol) were added to a stirred solution of the mixture of acetates **49** and **50** (190 mg, 286 μmol) in dry CH_2Cl_2 (3 mL) at r.t. under an argon atmosphere. The solution was stirred for 20 hours and was then diluted with water (2 mL) and the aqueous phase re-extracted with CH_2Cl_2 (2 x 2 mL). The combined organics were washed with brine (2 mL), dried (Na_2SO_4) and concentrated to a brown residue. Purification by column chromatography obtained the guanosine *bis*-acetate **251** (110 mg, 55%) as a pale yellow foam. R_f 0.38 (97:3 CH_2Cl_2:MeOH); $[\alpha]_D^{22}$ +29.4 (*c* 0.70, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3672 (N-H), 3602 (N-H), 2974 (C-H), 2935 (C-H), 2876 (C-H), 1748 (C=O), 1697 (C=N), 1622 (C=C), 1590 (C=C), 1491 (C=C), 1453 (C=C), 1405, 1386, 1372; δ_H (500 MHz, $CDCl_3$) 8.08 (1H, br s, N*H*), 7.99 (1H, s, 8-C*H*), 7.86-7.82 (2H, m, Ar-*H*), 7.49-7.43 (4H, m, Ar-*H*), 7.38-7.35 (4H, m, Ar-*H*), 6.66 (1H, dd, *J* 13.7 and 7.0, 5'-*H*), 6.55 (1H, d, *J* 13.7,

6'-*H*), 6.04 (2H, m, 1'-*H* and 2'-*H*), 5.81 (1H, dd, *J* 4.7 and 4.6, 3'-*H*), 4.62 (1H, dd, *J* 7.0 and 4.6, 4'-*H*), 2.86 (1H, app sept, *J* 0.5, COC*H*), 2.16 (3H, s, COCH_3), 2.08 (3H, s, COCH_3), 1.28 (3H, d, *J* 1.5, CH(CH_3)$_2$), 1.27 (3H, d, *J* 1.5 CH(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 174.6 (*C*OCH(CH_3)$_2$), 169.6 (*C*OCH_3), 169.5 (*C*OCH_3), 156.5 (6-*C*), 154.4 (2-*C*), 152.3 (4-*C*), 150.3 (*C*ONPh_2), 142.8 (8-*C*H), 148.8 (Ar-*C*), 148.7 (Ar-*C*), 133.1 (5'-*C*H), 129.4 (Ar-*C*H), 129.31 (Ar-*C*H), 129.26 (2 x Ar-*C*H), 128.3 (Ar-*C*H), 128.0 (Ar-*C*H), 127.8 (Ar-*C*H), 127.1 (Ar-*C*H), 126.2 (Ar-*C*H), 126.1 (Ar-*C*H), 118.0 (5-*C*), 111.8 (6'-*C*H), 87.1 (1'-*C*H), 82.6 (4'-*C*H), 73.2 (3'-*C*H), 72.6 (2'-*C*H), 36.4 (*C*H(CH_3)$_2$), 20.6 (CO*C*H_3), 20.5 (CO*C*H_3), 19.4 (CH(*C*H_3)$_2$), 19.3 (CH(*C*H_3)$_2$); *m/z* (ES+) 707.1471 (M+H, $C_{32}H_{32}{}^{79}BrN_6O_8^+$ requires 707.1465), 709.1449 (M+H, $C_{32}H_{32}{}^{81}BrN_6O_8^+$ requires 709.1445), 729.1289 (M+Na, $C_{32}H_{31}{}^{79}BrN_6O_8Na^+$ requires 729.1284), 731.1258 (M+Na, $C_{32}H_{31}{}^{81}BrN_6O_8Na^+$ requires 731.1264).

4.4 *H*-Phosphonates

2'-*O*-(*tert*-butyldimethylsilyl)-3'-*O*-(2-cyanoethyl)-*H*-phosphonate-5'-*O*-(4,4'-dimethoxytrityl) uridine 253

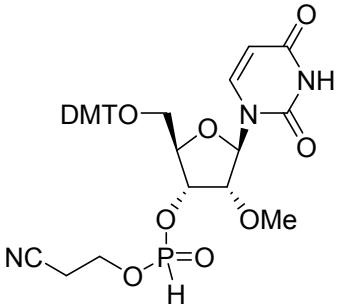


Solid 1*H*-tetrazole (28 mg, 546 μmol) was added to a stirred solution to phosphoramidite **252** (235 mg, 273 μmol) in dry MeCN (2.3 mL) at r.t. under an argon atmosphere. After 10 minutes water (0.4 mL) was added and the stirring maintained for 1 hour.

CH_2Cl_2 (20 mL) was added, the organic portion was washed with brine (20 mL), dried ($MgSO_4$) and evaporated under reduced pressure to obtain *H*-phosphonate **253** as a white foam (198 mg, 93%) as a 3:2 mixture of diastereoisomers. The *H*-phosphonate **253** was used in the Pd-coupling reaction without further purification. Data is reported for the 3:2 mixture of diastereoisomers which were not separated. R_f 0.36 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{32}$ +12.7 (*c* 0.51, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3391 (N-H), 2955 (C-H), 2932 (C-H), 2859 (C-H), 1716 (C=O), 1695 (C=O), 1636 (C=C), 1609 (C=C, N-H), 1582 (C=C), 1508, 1458, 1389, 1363; δ_H (400 MHz, $CDCl_3$) 8.32 (4H, br s, N*H* major and minor), 7.90 (1H, d, *J* 8.1, 6-*H* major), 7.80 (1H, d, *J* 8.1, 6-*H* minor), 7.38-20 (9H, m, Ar*H*), 7.13 (1H, d, *J* 740, P*H*), 6.92 (1H, d, *J* 724, P*H*), 6.87 (4H, dd, *J* 11.7 and 2.9, Ar*H*), 6.20 (0.5H, s, P*H*), 6.08 (1H, d, *J* 6.2, 1'-*H* major), 6.01 (0.5H, s, P*H*), 5.97 (1H, d, *J* 4.5, 1'-*H* minor), 5.31 (1H, d, *J* 8.1, 5-*H* major), 5.27 (1H, d, *J* 8.1, 5-*H* minor), 5.11 (1H, ddd, *J* 7.7, 4.4 and 2.5, 3'-*H* major), 4.99 (1H, app dt, *J* 9.7 and 4.7, 3'-*H* minor), 4.57 (1H, dd, *J* 5.9 and 4.7, 2'-*H* major), 4.51 (1H, t, *J* 4.5, 2'-*H* minor), 4.37-4.34 (1H, m, 4'-*H* minor), 4.32-4.14 (5H, m, 4'-major and C*H*$_2$CH$_2$CN major and minor), 3.82 (6H, s, 2 x Ar-OC*H*$_3$ minor), 3.81 (3H, s, Ar-OC*H*$_3$ major), 3.81 (3H, s, Ar-OC*H*$_3$ major), 3.66 (1H, dd, *J* 11.2 and 2.5, 5'-*Ha* minor), 3.60 (1H, dd, *J* 11.1 and 2.5, 5'-*Ha* major), 3.49 (1H, dd, *J* 11.1 and 2.0, 5'-*Hb* major), 3.46 (1H, dd, *J* 11.2 and 2.1, 5'-*Hb* minor), 2.74 (2H, ddd, *J* 11.0, 6.2 and 6.1, CH$_2$C*H*$_2$CN minor), 2.71 (1H, ddd, *J* 10.4, 5.9 and 5.1, CH$_2$C*H*$_2$CN

major), 2.67 (1H, ddd, *J* 10.4, 5.9 and 4.4, CH_2CH_2CN major), 0.91 (18H, s, $SiC(CH_3)_3$ major and minor), 0.19 (3H, s, $SiCH_3$ minor), 0.15 (3H, s, $SiCH_3$ major), 0.14 (3H, s, $SiCH_3$ major), 0.10 (3H, s, $SiCH_3$ minor); δ_C (100 MHz, $CDCl_3$) 162.7 (4-*C*), 162.5 (4-*C*O), 159.0 (4 x Ar-*C*OCH_3, major and minor), 150.2 (2-*C*O), 150.1 (2-*C*O), 144.1 (2 x Ar*C*, major and minor), 140.1 (6-*C*H), 139.9 (6-*C*H), 134.6 (2 x Ar*C*, major and minor), 134.5 (2 x Ar-*C*, major and minor), 130.3 (2 x Ar*C*H), 130.2 (2 x Ar*C*H), 130.1 (2 x Ar*C*H), 128.3 (4 x Ar*C*H), 127.6 (Ar*C*H), 127.5 (Ar*C*H), 116.3 (*C*N), 116.2 (*C*N), 113.5 (Ar*C*H), 113.4 (Ar*C*H), 102.8 (5-*C*H minor), 102.4 (5-*C*H major), 88.6 (*C*Ph(4-$OMePh)_2$), 87.7 (4'-*C*H, major), 87.6 (*C*Ph(4-$OMePh)_2$), 87.2 (4'-*C*H, minor), 82.1 (1'-*C*H minor), 81.5 (1'-*C*H major), 77.3 (3'-*C*H, major), 75.1 (2'-*C*H, minor), 75.0 (2'-*C*H, major), 74.8 (3'-*C*H, minor), 62.7 (CH_2CH_2CN), 61.5 (CH_2CH_2CN), 60.5 (CH_2CH_2CN), 60.4 (CH_2CH_2CN), 55.4 (2 x OCH_3), 25.7 ($SiC(CH_3)_3$), 25.6 ($SiC(CH_3)_3$), 20.0 ($SiC(CH_3)_3$), 18.0 ($SiC(CH_3)_3$), -4.8 (2 x $Si(CH_3)_2$) and –4.9 (2 x $Si(CH_3)_2$); δ_P (121 MHz, $CDCl_3$) minor 8.49 and major 7.82; *m/z* (ES+) 800.2743 (M+Na, $C_{39}H_{48}N_3O_{10}NaSiP^+$ requires 800.2744).

2'-*O*-methoxy-3'-*O*-(2-cyanoethyl)-*H*-phosphonate-5'-*O*-(4,4'-dimethoxytrityl) uridine 256

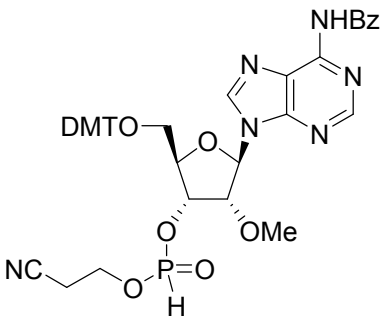


To a stirred solution of phosphoramidite **254** (973 mg, 1.28 mmol) in MeCN (10.7 mL) was added 5-methyl-1*H*-tetrazole (215 mg, 2.56 mmol). The solution was

stirred for 30 minutes then H_2O (1.78 mL) was added and stirring was maintained for a further 70 minutes. The reaction mixture was then diluted with CH_2Cl_2 (12 mL), washed with brine (12 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to obtain *H*-phosphonate **2** as a 1:1 mixture of diastereoisomers as a colourless foam (897 mg, 103%). The crude *H*-phosphonate **256** was used without further purification. Data is reported for the 1:1 mixture of diastereoisomers which were not separated. R_f 0.31 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{29}$ +19.9 (*c* 0.91, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3663 (N-H), 3390 (N-H), 3202 (O-H), 2959 (C-H), 2936 (C-H), 2908 (C-H), 2839 (C-H), 2411 (P-H), 2248 (C≡N), 1720 (C=O), 1694 (C=O), 1632 (C=C), 1609 (C=C) 1582 (C=C), 1507, 1492, 1460, 1389, 1369, 1303, 1272; δ_H (400 MHz, $CDCl_3$) 8.75 (2H, br s, 2 x N*H*), 7.84 (1H, d, *J* 8.2, 6-*H*), 7.78 (1H, d, *J* 8.2, 6-*H*), 7.40-7.22 (8H, m, Ar-*H*), 7.31-7.23 (10H, m, Ar-*H*), 7.11 (1H, d, *J* 748, P*H*), 6.92 (1H, d, *J* 728, P*H*), 6.88-6.86 (8H, m, Ar-*H*), 6.10 (1H, d, *J* 4.7, 1'-*H*), 6.05 (1H, d, *J* 3.9, 1'-*H*), 5.33 (1H, d, *J* 8.2, 5-*H*), 5.28 (1H, d, *J* 8.2, 5-*H*), 5.17 (1H, dd, *J* 10.3 and 4.9, 3'-*H*), 5.08 (1H, dd, *J* 7.7 and 5.1, 3'-*H*), 4.36-4.31 (4H, m, 2 x 4'-*H* and C*H*$_2$CH$_2$CN), 4.26-4.17 (2H, m, C*H*$_2$CH$_2$CN), 4.17 (1H, dd, *J* 4.9 and 4.7, 2'-*H*), 4.10 (1H, dd, *J* 5.1 and 3.9, 2'-*H*), 3.82 (6H, s, 2 x ArOC*H*$_3$), 3.81 (6H, s, 2 x ArOC*H*$_3$), 3.65 (2H, m, 2 x 5'-*H*), 3.61 (3H, s, 2'-OC*H*$_3$), 3.60 (3H, s, 2'-OC*H*$_3$), 3.50 (2H, m, 2 x 5'-*H*), 2.79 (2H, ddd, *J* 10.7, 6.5 and 6.3, CH$_2$C*H*$_2$CN), 2.69 (2H, ddd, *J* 10.7, 6.0 and 5.0, CH$_2$C*H*$_2$CN); δ_C (100 MHz, $CDCl_3$) 162.9 (4-*C*), 162.8 (4-*C*), 158.9 (2-*C*), 158.8 (2-*C*), 150.2 (2 X Ar-*C*), 144.0 (2

x Ar-*C*), 143.9 (2 x Ar-*C*), 139.7 (6-*C*H), 139.6 (6-*C*H), 134.8 (Ar-*C*), 134.7 (2 x Ar-*C*), 134.6 (Ar-*C*), 130.3 (3 x Ar-*C*H), 130.2 (5 x Ar-*C*H), 130.1 (3 x Ar-*C*H), 128.3 (3 x Ar-*C*H), 128.2 (6 x Ar-*C*H), 127.5 (Ar-*C*H), 127.4 (Ar-*C*H), 116.3 (*C*N), 116.2 (*C*N), 113.5 (4 x Ar-*C*H), 102.8 (5-*C*H), 102.7 (5-*C*H), 87.7 (*C*Ph(4-OMePh)$_2$), 87.6 (*C*Ph(4-OMePh)$_2$), 86.7 (1'-*C*H), 86.5 (1'-*C*H), 82.7 (2 x 4'-*C*H), 82.0 (2'-*C*H), 81.7 (2'-*C*H), 74.8 (3'-*C*H), 73.2 (3'-*C*H), 61.8 (5'-*C*H$_2$), 61.2 (5'-*C*H$_2$), 60.1 (*C*H$_2$CH$_2$CN), 60.0 (*C*H$_2$CH$_2$CN), 59.6 (CH$_2$*C*H$_2$CN), 59.5 (CH$_2$*C*H$_2$CN), 59.2 (2'-O*C*H$_3$), 58.9 (2'-O*C*H$_3$), 55.4 (4 x Ar-O*C*H$_3$); δ_P (121 MHz, $CDCl_3$) 8.10 (dd, *J* 749 and 6) and 7.19 (dd, *J* 734 and 8); *m/z* (ES+) 700.2041 (M+Na, $C_{34}H_{36}NaN_3O_{10}P^+$ requires 700.2036).

2'-*O*-methoxy-3'-*O*-(2-cyanoethyl)-*H*-phosphonate-5'-*O*-(4,4'-dimethoxytrityl) 6-*N*-benzoyladenosine 257



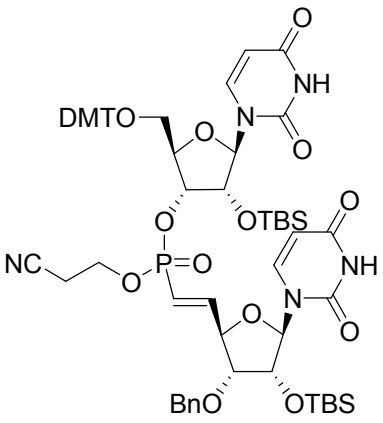
To a stirred solution of phosphoramidite **255** (500 mg, 563 mmol) in distilled MeCN (4.7 mL) at r.t. was added 5-methyl-1*H*-tetrazole (94.7 mg, 1.13 mmol). The resulting solution was stirred for 10 minutes then water (0.8 mL) added and stirring maintained for a further 50 minutes. The solution was then diluted with CH_2Cl_2 (10 mL) then washed with brine (5 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to obtain *H*-phosphonate **257** as a 1:1 mixture of diastereoisomers as a white foam (449 mg, 99%). Data is reported for the 1:1 mixture

of diastereoisomers (A and B) which were not separated. R_f 0.20 (98:2 CH_2Cl_2:MeOH); $[\alpha]_D^{20}$ -20.9 (*c* 0.43, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3662 (O-H), 3413 (N-H), 2992 (C-H), 2959 (C-H), 2936 (C-H), 2913 (C-H), 2839 (C-H), 2476 (P-H), 1708 (C=O), 1612 (C=O), 1587 (C=C), 1457, 1505, 1484, 1457, 1404, 1356, 1327; δ_H (500 MHz, $CDCl_3$) 9.11 (2H, app br s, 2 x N*H*, A and B), 8.73 (2H, s, 2 x 2-*H*, A and B), 8.18 (2H, d, *J* 9.5, 8-*H*, A and B), 8.04 (4H, app d, Ar-*H*, A and B), 7.63 (2H, app tt, *J* 8.0, 7.5 and 1.5, Ar-*H*, A and B), 7.55 (4H, app t, *J* 7.5, Ar-*H*, A and B), 7.45-7.39 (4H, m, Ar-*H*, A and B), 7.35-7.26 (12H, m, Ar-*H*, A and B), 7.25 (2H, ddd, *J* 7.5, 4.6 and 1.5, Ar-*H*, A and B), 7.02 (1H, d, *J* 745, P*H*, A), 7.02 (1H, d, *J* 735, P*H*), 6.83 (4H, m, Ar-*H*), 6.82 (4H, m, Ar-*H*), 6.18 (1H, d, *J* 6.5, 1'-*H*, A), 6.17 (1H, d, *J* 7.0, 1'-*H*, B), 5.32-5.29 (1H, m, 3'-*H*, B), 5.28-5.24 (1H, m, 3'-*H*, A), 5.01 (1H, dd, *J* 7.0 and 5.0, 2'-*H*, B), 4.97 (1H, dd, *J* 6.5 and 5.0, 2'-*H*, A), 4.47-4.44 (2H, m, 2 x 4'-*H*, A and B), 4.37 (2H, ddd, *J* 13.1, 6.5 and 6.0, C$H_2$$CH_2$CN, A), 4.34-4.21 (2H, m, C$H_2$$CH_2$CN, B), 3.79 (12H, s, 4 x ArOCH_3), 3.61-3.57 (2H, m, 5'-*Ha* and 5'-*Hb*), 3.51 and 3.50 (6H, s, 2 x 2'-OCH_3), 3.47 (2H, ddd, *J* 10.8, 3.5 and 3.0, 5'-*Ha*), 3.44 (2H, ddd, *J* 10.8, 3.5 and 2.5, 5'-*Hb*), 2.79 (2H, app dd, *J* ~6.5 and ~6.0, CH_2CH_2CN, A), 2.76-71 (2H, ddd, *J* 10.5, 6.5 and 4.4 CH_2CH_2CN, B); δ_C (100 MHz, $CDCl_3$) 164.7 (*C*OPh), 164.8 (*C*OPh), 159.3 (Ar-*C*OCH_3), 158.8 (2 x Ar-*C*OCH_3), 158.7 (Ar-*C*OCH_3), 152.9 (6-*C*), 152.4 (6-*C*), 149.6 (2 x 4-*C*), 144.3 (Ar-*C*), 143.4 (Ar-*C*), 144.2 (2 x 8-*C*H), 142.3 (2-*C*H), 142.1 (2-*C*H), 135.4 (2 x Ar-*C*), 135.3 (2 x Ar-*C*), 135.2 (2 x Ar-*C*), 133.3 (Ar-

*C*H), 133.1 (2 x Ar-*C*H), 131.7 (2 x Ar-*C*H), 130.2 (3 x Ar-*C*H), 130.1 (2 x Ar-*C*H), 129.9 (Ar-*C*H), 129.2 (Ar-*C*H), 129.1 (Ar-*C*H), 129.0 (2 x Ar-*C*H), 128.2 (3 x Ar-*C*H), 128.1 (3 x Ar-*C*H), 127.9 (4 x Ar-*C*H), 127.8 (Ar-*C*H), 127.3 (Ar-*C*H), 127.2 (Ar-*C*H), 123.6 (5-*C*), 123.5 (5-*C*), 113.4 (4 x Ar-*C*H), 113.2 (Ar-*C*H), 113.0 (Ar-*C*H), 87.2 (*C*Ph(4-OMe-Ph)$_2$), 87.1 (*C*Ph(4-OMe-Ph)$_2$), 86.2 (1'-*C*H), 86.0 (1'-*C*H), 83.2 4'-*C*H), 83.1 (4'-*C*H), 81.8 (2 x 2'-*C*H), 81.2 (2'-*C*H), 76.2 (3'-*C*H), 74.8 (3'-*C*H), 62.7 (5'-CH_2), 62.6 (5'-CH_2), 59.6 (CH_2*C*H_2CN), 59.4 (CH_2*C*H_2CN), 59.3 (*C*$H_2$$CH_2$CN), 59.2 (*C*$H_2$$CH_2$CN), 55.3 (4 x Ar-O*C*$H_3$), δ_P (121 MHz, $CDCl_3$) 8.16 and 7.15; *m/z* (ES+) 805.2765 (M+H, $C_{42}H_{42}N_6O_9P^+$ requires 805.2751), 827.2533 (M+Na, $C_{42}H_{41}N_6O_9PNa^+$ requires 827.2565).

4.5 Vinylphosphonate-linked Dinucleotides

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl)-uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-benzyl-2'-*O*-(*tert*-butyldimethylsilyl) uridine 258



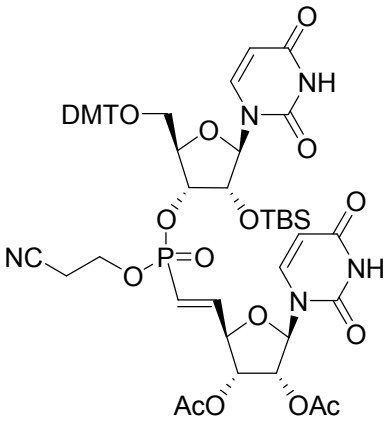
A solution of *H*-Phosphonate **253** (212 mg, 273 mmol) in dry THF (2.8 mL) was added to a stirred mixture of $Pd(OAc)_2$ (9.4 mg, 42 mmol), dppf (46 mg, 84 mmol), vinyl bromide **167** (110 mg, 210 mmol) and propylene oxide (0.29 mL, 4.22 mmol) in a screw cap reactivial. The reaction mixture was heated in the sealed vial at 70 °C for 6 hours. The

reaction mixture was cooled, further propylene oxide (0.29 mL) was added then stirring continued at r.t. for a further 16 hours. The solvent was removed *in vacuo* and the residue (416 mg) was dissolved in $CHCl_3$ (1 mL) and purification by gradient column chromatography was attempted (8:1 EtOAc-Petrol $\rightarrow$ neat EtOAc). All tlc spots co-eluted but later HPLC (9:1 MeOH-H_2O) showed product present in all fractions. Combined fractions were further purified by reverse phase HPLC (9:1 MeOH-H_2O), peak collected at 17 minutes. Combined fractions were evaporated and the U*U vinylphosphonate dimer **258** was obtained as a 2:1 mixture of diastereoisomers as white solid (63 mg, 25%). R_f 0.53 (EtOAc); $[\alpha]_D^{29}$ -74.1 (*c* 1.37, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3392 (N-H), 2954 (C-H), 2932 (C-H), 2903 (C-H), 2859 (C-H), 2839 (C-H), 1714 (C=O), 1697 (C=O), 1634 (C=O), 1609 (C=C), 1585 (C-H), 1508, 1461, 1456, 1389, 1363; **Major diastereoisomer:** δ_H (500 MHz, $CDCl_3$) 9.18-8.85 (2H, br s, N*H*), 7.90 (1H, d, *J* 8.2, U_1-6-*H*), 7.40-7.26 (15H, m, Ar-*H*), 7.06-6.95 (2H, m, U_2-5'-*H*), 6.90-6.86 (3H, m, 2 x Ar-*H* and U_2-5-*H*), 6.90-6.86 (3H, m, 2 x Ar-*H* and U_2-5-*H*), 6.15-6.03 (2H, m, U_2-6'-*H*), 6.01 (1H, d, *J* 4.7, U_1-1'-*H*), 5.79 (1H, d, *J* 8.1, U_2-5-*H*), 5.75 (1H, d, *J* 4.1, U_2-1'-*H*), 5.30 (1H, d, *J* 8.2, U_1-5-*H*), 4.95 (1H, m, U_1-3'-*H*), 4.74 (1H, d, *J* 11.6, U_2-3'-OC*H*$_2$Ph), 4.69-4.66 (1H, m, U_2-4'-H), 4.55-4.51 (3H, m, U_2-3'-OC*H*$_2$Ph, U_1-2'-*H*, U_2-2'-*H*), 4.34 (1H, m, U_1-4'-*H*), 4.30-4.16 (1H, m, C*H*$_2$CH$_2$CN), 4.10-3.89 (1H, m, C*H*$_2$CH$_2$CN), 3.83 (3H, s, Ar-OC*H*$_3$), 3.81 (3H, s, Ar-OC*H*$_3$), 3.83-3.78 (1H, obscured multiplet, U_2-3'-*H*), 3.59 (1H, dd, *J* 11.0 and 1.8, U_1-5'-*H*), 3.46 (1H, dd, *J*

11.0 and 1.8, U_1-5'-*H*), 2.79-2.68 (2H, m, CH_2CH_2CN), 0.93 (9H, s, $SiC(CH_3)_3$), 0.92 (9H, s, $SiC(CH_3)_3$), 0.18 (3H, s, $SiCH_3$), 0.17 (3H, s, $SiCH_3$), 0.14 (3H, s, $SiCH_3$), 0.11 (3H, s, $SiCH_3$); **Minor diastereoisomer:** δ_H (500 MHz, $CDCl_3$) 9.18-8.85 (2H, br s, N*H*), 7.93 (1H, d, *J* 8.2, U_1-6-*H*), 7.40-7.26 (15H, m, Ar-*H*), 7.06-6.95 (2H, m, U_2-5'-*H*), 6.90-6.86 (3H, m, 2 x Ar-*H* and U_2-6-*H*), 6.15-6.03 (2H, m, U_2-6'-*H*), 5.98 (1H, d, *J* 3.9, U_1-1'-*H*), 5.82 (1H, d, *J* 8.1, U_2-6-*H*), 5.81 (1H, obscured d, U_2-1'-*H*), 5.28 (1H, d, *J* 8.2, U_1-6-*H*), 5.02 (1H, m, U_1-3'-*H*), 4.79-4.76 (1H, obs m, U_2-4'-H), 4.76 (1H, d, *J* 11.6, U_2-3'-OCH_2Ph), 4.55-4.51 (3H, m, U_2-3'-OCH_2Ph, U_1-2'-*H*, U_2-2'-*H*), 4.40 (1H, m, U_1-4'-*H*), 4.30-4.16 (1H, m, CH_2CH_2CN), 4.10-3.89 (1H, m, CH_2CH_2CN), 3.84 (6H, app s, 2 x Ar-OCH_3), 3.83-3.78 (1H, obscured multiplet, U_2-3'-*H*), 3.72 (1H, dd, *J* 11.2 and 1.6, U_1-5'-*H*), 3.54 (1H, dd *J* 11.2 and 1.6, U_1-5'-*H*), 2.53 (1H, dt, *J* 17.1 and 5.8, CH_2CH_2CN), 2.44 (1H, dt, *J* 17.1 and 5.8, CH_2CH_2CN), 0.93 (9H, s, $SiC(CH_3)_3$), 0.89 (9H, s, $SiC(CH_3)_3$), 0.17 (3H, s, $SiCH_3$), 0.15 (3H, s, $SiCH_3$), 0.12 (6H, s, 2 x $SiCH_3$); **Major diastereoisomer:** δ_C (125 MHz, $CDCl_3$) 163.2 (4-*C*), 163.1 (4-*C*), 158.83 (Ar-*C*OCH_3), 158.81 (Ar-*C*OCH_3), 150.4 (U_2-5'-*C*H), 150.0 (2-*C*), 149.8 (2-*C*), 144.1 (Ar-*C*), 140.8 (6-*C*H), 139.9 (6-*C*H), 137.1 (2 x Ar-*C*), 128.6 (3 x Ar-*C*H), 128.3 (Ar-*C*H), 128.2 (2 x Ar-*C*H), 128.1 (Ar-*C*H), 128.09 (3 x Ar-*C*H) 127.7 (3 x Ar-*C*H), 127.3 (Ar-*C*H), 130.3 (Ar-*C*H), 130.27 (Ar-*C*H), 130.2 (Ar-*C*H), 130.2 (Ar-*C*H), 118.25 (U_2-6'-CH), 116.7 (*C*N), 116.5 (*C*N), 113.38 (4 x Ar-*C*H), 102.8 (5-*C*H), 102.5 (5-*C*H), 92.6 (U_2-1'-*C*H), 88.2 (U_1-1'-*C*H), 87.5 (*C*Ph(4-OMe-Ph)$_2$), 82.0 (U_1-4'-*C*H), 81.2 (U_2-4'-

*C*H), 80.3 (U_2-3'-*C*H), 74.4 (U_1-2'-*C*H), 73.5 (U_2-2'-*C*H), 73.1 (*C*H_2Ph), 72.8 (U_1-3'-CH), 61.9 (U_1-5'-CH_2), 60.8 (d, J_{CP} 4.7, *C*$H_2$$CH_2$CN), 55.3 (2 x Ar-O*C*$H_3$), 25.6 (2 x SiC(*C*$H_3$)$_3$), 19.9 (d, $^2J_{CP}$ 6.5, CH_2*C*H_2CN), 18.1 (Si*C*$(CH_3)_3$), 18.05 (Si*C*$(CH_3)_3$), -4.75 (Si*C*H_3), -4.8 (Si*C*H_3), -4.9 (2 x Si*C*H_3); **Minor diastereoisomer:** δ_C (125 MHz, $CDCl_3$) 163.1 (2 x 4-*C*), 158.89 (Ar-*C*OCH_3), 158.86 (Ar-*C*OCH_3), 150.3 (U_2-5'-*C*H), 150.0 (2-*C*), 149.7 (2-*C*), 144.2 (Ar-*C*), 140.1 (6-*C*H), 139.8 (6-*C*H), 137.1 (2 x Ar-*C*), 134.9 (Ar-*C*), 134.8 (Ar-*C*), 128.6 (2 x Ar-*C*H), 128.2 (2 x Ar-*C*H), 128.1 (Ar-*C*H), 128.09 (2 x Ar-*C*H), 127.6 (2 x Ar-*C*H), 127.4 (Ar-*C*H), 30.3 (Ar-*C*H), 130.27 (Ar-*C*H), 130.2 (Ar-*C*H), 130.2 (Ar-*C*H), 118.3 (U_2-6'-*C*H), 116.7 (*C*N), 116.4 (*C*N), 113.40 (4 x Ar-*C*H), 102.8 (5-*C*H), 102.4 (5-*C*H), 92.4 (U_2-1'-*C*H), 88.7 (U_1-1'-*C*H), 87.5 *C*Ph(4-OMe-Ph)$_2$), 81.6 (U_1-4'-*C*H), 81.5 (U_2-4'-*C*H), 80.2 (U_2-3'-*C*H), 74.8 (U_1-2'-*C*H), 74.5 (U_2-2'-*C*H), 73.4 (*C*H_2Ph), 72.77 (U_1-3'-*C*H), 61.7 (U_1-5'-CH_2), 60.7 (d, J_{CP} 4.1, *C*$H_2$$CH_2$CN), 55.3 (2 x Ar-O*C*$H_3$), 25.7 (SiC(*C*$H_3$)$_3$), 25.6 (SiC(*C*$H_3$)$_3$), 19.7 (d, $^2J_{CP}$ 6.8, CH_2*C*H_2CN), 18.0 (2 x Si*C*$(CH_3)_3$), -4.7 (Si*C*H_3), -4.82 (Si*C*H_3), 4.85 (Si*C*H_3), -5.0 (Si*C*H_3); δ_P (121 MHz, $CDCl_3$) minor 19.7 and major 19.4; *m/z* (ES+) 1242.4624 (M+Na, $C_{62}H_{78}N_5NaO_{15}SiP_2^+$ requires 1242.4668).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl) uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-acetyl-2'-*O*-acetyl uridine 259

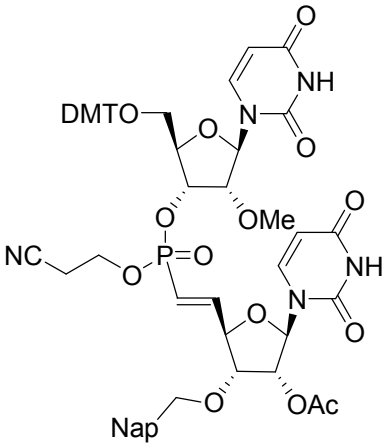


Uridine *bis*-acetate **211** (81 mg, 201 μmol), dppf (45 mg, μmol), $Pd(OAc)_2$ (9 mg, μmol) and propylene oxide (280 μL, 4.02 mmol) were charged to a 5 mL reactivial flushed with argon. To this mixture was added a solution of *H*-phosphonate **253** (203 mg, 261 μmol) in dry THF (2.7 mL). The reactivial was sealed and the reaction mixture was heated at 70 °C for 6 hours, then cooled to r.t. and concentrated *in vacuo* to an orange foam (303 mg). Purification by column chromatography (4:1 $CHCl_3$:EtOAc) produced a pale orange foam U*U dimer **53** as a 1:1 mixture of diastereoisomers (95 mg, 43%). R_f 0.23 (4:1 $CHCl_3$:EtOAc); $[\alpha]_D^{28}$ -82.4 (*c* 0.40, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3696 (N-H), 3604 (N-H), 3390 (O-H), 2954 (C-H), 2932 (C-H), 2858 (C-H), 1748 (C=O), 1698 (C=O), 1634 (C=C), 1608 (C=C), 1508, 1456, 1377; δ_H (400 MHz, $CDCl_3$) 9.38 (1H, s, N*H*), 9.36 (1H, s, N*H*), 9.24 (1H, s, N*H*), 9.18 (1H, s, N*H*), 7.90 (1H, d, *J* 7.8, U_1-C*H*), 7.88 (1H, d, *J* 7.7, U_1-C*H*), 7.38-7.23 (20H, m, 2 x U_2-C*H* and 18 x Ar-*H*), 7.00 (1H, ddd, J 11.6, 6.2 and 4.3, U_2-5'-*H*), 6.90 (1H, ddd, *J* 10.7, 6.3 and 4.1, U_2-5'-*H*), 6.88-6.83 (8H, m, Ar-*H*), 6.10 (1H, app td, *J* 17.4 and 1.7, U_2-6'-*H*), 6.05 (1H, app td, 17.1 and 1.7, U_2-6'-*H*), 5.94 (1H, d, *J* 4.4, U_1-1'-*H*), 5.92 (1H, d, *J* 3.8, U_1-1'-*H*), 5.86 (1H, d, *J* 4.4, U_2-2'-*H*), 5.81

(1H, dd, *J* 7.8 and 1.1, U_2-C*H*), 5.80 (1H, d, *J* 4.4, U_2-1'-*H*), 5.78 (1H, dd, *J* 7.7 and 1.3, U_2-C*H*), 5.43-5.38 (2H, app dd, *J* 10.6 and 5.9, 2 x U_2-2'-*H*), 5.29-5.25 (1H, obs m, U_1-C*H*), 5.27 (2H, app dd, *J* 6.2 and 5.5, 2 x U_2-3'-*H*), 5.23 (1H, dd, *J* 8.2 and 1.6, U_1-C*H*), 5.01 (1H, ddd, *J* 9.5, 9.3 and 4.8, U_1-3'-*H*), 4.92 (1H, ddd, *J* 8.8, 8.6 and 4.0, U_1-3'-*H*), 4.65-4.61 (1H, m, U_2-4'-*H*), 4.54-4.50 (1H, m, U_2-4'-*H*), 4.48 (1H, dd, *J* 4.8 and 4.4, U_1-2'-*H*), 4.46 (1H, dd, *J* 4.3 and 4.1, U_1-2'-*H*), 4.37 (1H, ddd, *J* 5.4, 4.8 and 2.7, U_1-4'-*H*), 4.33 (1H, ddd, *J* 4.6, 4.4 and 2.3, U_1-4'-*H*), 4.30-4.25 (1H, m, C*H*$_2$CH$_2$CN), 4.21-4.15 (1H, m, C*H*$_2$CH$_2$CN), 3.98-3.88 (2H, m, C*H*$_2$CH$_2$CN), 3.81 (6H, s, 2 x ArOC*H*$_3$), 3.80 (6H, s, 2 x ArOC*H*$_3$), 3.69 (1H, dd, *J* 11.2 and 2.0, U_1-5'-*H*), 3.61-3.57 (1H, m, U_1-5'-*H*), 3.55-3.49 (1H, m, U_1-5'-*H*), 3.45 (1H, dd, *J* 11.2 and 2.0, U_1-5'-*H*), 2.73 (1H, app t, *J* 5.9, CH$_2$C*H*$_2$CN), 2.59 (1H, app ddd, *J* 24.3, 12.4 and 6.2, CH$_2$C*H*$_2$CN), 2.56 (1H, app ddd, *J* 23.4, 12.0 and 6.0, CH$_2$C*H*$_2$CN), 2.45 (1H, app ddd, *J* 17.1, 7.4 and 5.4, CH$_2$C*H*$_2$CN), 2.13 (3H, s, 2 x U_2-2'-COC*H*$_3$), 2.12 (3H, s, 2 x U_2-2'-COC*H*$_3$), 2.11 (6H, s, 2 x U_2-2'-COC*H*$_3$), 0.89 (9H, s, SiC(C*H*$_3$)$_3$), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.15 (3H, s, SiC*H*$_3$), 0.14 (6H, s, 2 x SiC*H*$_3$) and 0.11 (3H, s, SiC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 169.66 (*C*OCH$_3$), 169.65 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.5 (*C*OCH$_3$), 163.1 (2 x U-4-*C*), 162.85 (2 x U-4-*C*), 158.9 (2 x Ar-*C*OCH$_3$), 158.8 (Ar-*C*OCH$_3$), 158.7 (Ar-*C*OCH$_3$) 150.45 (U-2-*C*), 150.01 (U-2-*C*), 148.2 (U-2-*C*), 148.0 (U-2-*C*), 144.2 (4 x Ar-*C*), 141.0 (U-6-*C*H), 140.7 (U-6-*C*H), 139.9 (U-6-*C*H), 139.8 (U-6-*C*H), 135.04 (2 x Ar-*C*), 130.4 (3 x Ar-*C*H), 130.3 (3 x Ar-*C*H), 130.2 (2 x Ar-*C*H), 129.2 (Ar-*C*H), 128.6

(Ar-*C*H), 128.3 (2 x Ar-*C*H), 128.2 (2 x Ar-*C*H), 128.1 (2 x Ar-*C*H), 127.9 (Ar-*C*H), 127.5 (Ar-*C*H), 118.9 (d, *J* 56.9, U_2-5'-*C*H), 117.4 (d, *J* 52.8, U_2-5'-*C*H), 116.9 (*C*N), 116.6 (*C*N), 113.5 (3 x Ar-CH), 113.4 (3 x Ar-*C*H), 113.2 (2 x Ar-*C*H), 103.8 (U-5-*C*H), 103.6 (U-5-*C*H), 102.5 (U-5-*C*H), 102.4 (U-5-*C*H), 90.2 (2 x U_2-1'-*C*H), 88.8 (U_1-1'-*C*H), 88.5 (U_1-1'-*C*H), 87.6 (2 x *C*Ph(4-OMe-Ph)$_2$), 81.8 (U_1-4'-*C*H), 81.5 (U_1-4'-*C*H), 81.0 (U_2-4'-*C*H), 80.8 (U_2-4'-*C*H), 80.9 (d, *J* 23.9, U_2-6'-*C*H), 80.5 (d, *J* 23.6, U_2-6'-*C*H), 74.9 (2 x U_1-2'-*C*H), 74.3 (U_1-3'-*C*H), 73.6 (U_1-3'-*C*H), 72.7 (2 x U_2-2'-*C*H), 72.5 (U_2-3'-*C*H), 72.4 (U_2-3'-*C*H), 61.8 (U_1-5'-*C*H_2), 61.7 (U_1-5'-*C*H_2), 60.9 (d, *J* 3.8, *C*H_2CH_2CN), 60.80 (d, *J* 5.0, *C*H_2CH_2CN), 55.4 (2 x Ar-O*C*H_3), 55.3 (2 x Ar-O*C*H_3), 25.7 (SiC(*C*H_3)$_3$), 25.6 (SiC(*C*H_3)$_3$), 20.5 (4 x CO*C*H_3), 20.0 (d, *J* 7.5, CH_2*C*H_2CN), 19.8 (d, *J* 6.3, CH_2*C*H_2CN), -4.76 (Si*C*H_3), -4.79 (Si*C*H_3), -4.86 (Si*C*H_3), -4.91 (Si*C*H_3); δ_P (121 MHz, $CDCl_3$) 19.1 and 18.9; m/z (ES+) 1122.3551 (M+Na, $C_{53}H_{62}N_5NaO_{17}PSi^+$ requires 1122.3545).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl uridinyl 2-cyanoethyl-phosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-methyl(2-naphthyl)-2'-*O*-acetyl uridine 260



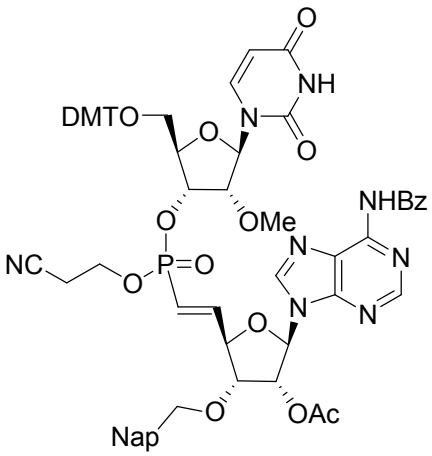
Vinyl bromide **218** (102 mg, 203 μmol), $Pd(OAc)_2$ (9.1 mg, 40.7 μmol) and dppf (45.1 mg, 81.4 μmol) were combined in a Radleys® Carousel tube flushed with argon. A solution of *H*-phosphonate **254** (179 mg, 264 μmol) in freshly distilled THF (2.71 mL) was added followed by propylene oxide (285 μL, 4.07 mmol). The tube was then sealed, placed in the carousel and heated at 70 °C. After 6 hours the tube was removed from the heat, then diluted with CH_2Cl_2 (2 x 1.5 mL) to transfer to a flask and concentrated *in vacuo* then purified by column chromatography (96:4 CH_2Cl_2:MeOH) to obtain U*U dimer **260** as a pale yellow foam (120 mg, 54%). R_f 0.04 (96:4 CH_2Cl_2:MeOH); $[\alpha]_D^{24}$ +588 (*c* 0.76, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3673 (N-H), 3602 (N-H), 3415 (O-H), 3390 (O-H), 3177 (C-H), 3060 (C-H), 3011 (C-H), 2960 (C-H), 2937 (C-H), 2912 (C-H), 2878 (C-H), 2839 (C-H), 1752 (C=O), 1701 (C=O), 1635 (C=C), 1609 (C=C), 1583 (C=C), 1510, 1459, 1416, 1379, 1335; δ_H (500 MHz, $CDCl_3$) 9.93 (1H, s, N*H*), 9.85 (1H, s, N*H*), 9.76 (1H, s, N*H*), 9.69 (1H, s, N*H*), 7.90-7.83 (8H, m, Ar-*H*), 7.79 (2H, app d, *J* 7.5, Ar-*H*), 7.48-7.45 (4H, m, Ar-*H*), 7.42 (1H, d, *J* 8.2, U_1-*CH*), 7.42 (1H, d, *J* 8.2, U_1-C*H*), 7.37-7.26

(10H, m, Ar-*H*), 7.25-7.22 (8H, m, Ar-*H*), 7.21 (1H, d, *J* 8.1, U_2-C*H*), 7.16 (1H, d, *J* 8.1, U_2-C*H*), 7.00 (1H, ddd, *J* 17.2, 4.5 and 3.8, U_2-5'-*H*), 6.95 (1H, ddd, *J* 17.2, 4.6 and 3.8, U_2-5'-*H*), 6.87 (4H, d, *J* 8.9, Ar-*C*H), 6.83 (4H, *J* 8.9, Ar-*C*H), 6.14 (1H, ddd, *J* 17.2, 16.4 and 1.6, U_2-6'-*H*), 6.04 (1H, d, *J* 3.8, U_1-1'-*H*), 6.02 (1H, d, *J* 3.5, U_1-1'-*H*), 6.02 (1H, ddd, *J* 37.6, 17.2 and 1.6, U_2-6'-*H*), 5.77-5.75 (3H, m, 2 x U_2-1'-*H* and U_2-5-C*H*), 5.73 (1H, dd, *J* 8.1 and 1.3, U_2-5-*C*H), 5.51 (1H, dd, *J* 5.9 and 2.8, U_2-2'-*H*), 5.47 (1H, dd, *J* 5.8 and 3.1, U_2-2'-*H*), 5.28 (2H, *J* 8.2 and 1.6, 2 x U_1-5-*C*H), 5.13 (1H, ddd, *J* 10.7, 5.3 and 1.6, U_1-3'-*H*), 5.06 (1H, ddd, *J* 11.6, 6.2 and 5.4, U_1-3'-*H*), 4.75 (1H, d, *J* 11.5, U_2-3'-OC*H*H), 4.70 (1H, d, *J* 11.6, U_2-3'-OC*H*H), 4.66 (1H, d, *J* 11.5, U_2-3'-OCH*H*), 4.59 (1H, d, *J* 11.6, U_2-3'-OCH*H*), 4.61-4.58 (1H, m, U_2-3'-*H*), 4.44 (1H, m, U_2-3'-*H*), 4.30 (1H, ddd, *J* 5.1, 3.8 and 1.6, U_1-4'-*H*), 4.28 (1H, ddd, *J* 7.6 and 5.9, U_2-4'-*H*), 4.24 (1H, ddd, *J* 4.6, 3.8 and 1.6, U_1-4'-*H*), 4.20 (1H, dd, *J* 7.6 and 5.9, U_2-4'-*H*), 4.09 (1H, dd, *J* 4.6 and 3.8, U_1-2'-*H*), 4.08-3.95 (3H, obs m, CH_2CH_2CN), 3.90-3.84 (1H, m, CH_2CH_2CN), 4.02 (1H, app dd, *J* 4.3 and 4.2, U_1-2'-*H*), 3.80 (3H, s, Ar-OCH_3), 3.79 (3H, s, Ar-OCH_3), 3.75 (3H, s, Ar-OCH_3), 3.74 (3H, s, Ar-OCH_3), 3.64 (1H, dd, *J* 11.0 and 2.2, U_1-5'-*H*), 3.59 (3H, s, U_1-2'-OCH_3), 3.54 (1H, dd, *J* 11.0 and 2.2, U_1-5'-*H*), 3.48 (1H, dd, *J* 11.1 and 2.2, U_1-5'-*H*), 3.40 (1H, dd, *J* 11.1 and 2.2, U_1-5'-*H*), 3.40 (3H, s, U_1-2'-OCH_3), 2.53 (1H, ddd, *J* 10.4, 7.2 and 5.4, OCH_2CH_2CN), 2.48 (1H, ddd, *J* 11.0, 6.1 and 5.5, OCH_2CH_2CN), 2.45 (1H, ddd, *J* 11.3, 7.2 and 5.4, OCH_2CH_2CN), 2.40 (1H, ddd, *J* 17.0, 6.1 and 5.5, OCH_2CH_2CN),

2.12 (3H, s, U_2-2'-COC*H*$_3$), 2.11 (3H, s, U_2-2'-COC*H*$_3$); δ_C (121 MHz, $CDCl_3$) 170.04 (U_2-2'-*C*OCH$_3$), 170.02 (U_2-2'-*C*OCH$_3$), 163.31 (U-4-*C*), 163.29 (2 x U-4-*C*), 163.17 (U-4-*C*), 158.89 (Ar-*C*OCH$_3$), 158.86 (Ar-*C*OCH$_3$), 158.83 (Ar-*C*OCH$_3$), 158.78 (Ar-*C*OCH$_3$), 150.6 (U-2-*C*), 150.4 (U-2-*C*), 150.0 (U-2-*C*), 149.9 (U-2-*C*), 148.9 (d, *J* 6.8, U_2-5'-*C*H), 148.2 (d, *J* 6.0, U_2-5'-*C*H), 144.2 (Ar-*C*), 144.0 (Ar-*C*), 141.31 (U-6-*C*H), 141.28 (U-6-*C*H), 139.6 (U-6-*C*H), 139.5 (U-6-*C*H), 134.97 (Ar-*C*), 134.95 (Ar-*C*), 134.86 (Ar-*C*), 134.77 (Ar-*C*), 134.36 (Ar-*C*), 134.30 (Ar-*C*), 133.18 (Ar-*C*), 133.16 (2 x Ar-*C*), 133.14 (Ar-*C*), 130.4 (2 x Ar-*C*H), 130.3 (2 x Ar-*C*H), 130.2 (2 x Ar-*C*H), 130.1 (2 x Ar-*C*H), 128.51 (Ar-*C*H), 128.48 (Ar-*C*H), 128.4 (2 x Ar-*C*H), 128.1 (6 x Ar-*C*H), 128.05 (Ar-*C*H), 128.01 (Ar-*C*H), 127.8 (Ar-*C*H), 127.7 (Ar-*C*H), 127.4 (Ar-*C*H), 127.33 (Ar-*C*H), 127.26 (Ar-*C*H), 127.1 (Ar-*C*H), 126.37 (Ar-*C*H), 126.35 (Ar-*C*H), 126.30 (Ar-*C*H), 126.29 (Ar-*C*H), 125.96 (Ar-*C*H), 125.84 (Ar-*C*H), 118.0 (d, *J* 140, U_2-6'-*C*H), 116.8 (CH_2CH_2*C*N), 116.5 (d, *J* 139, U_2-6'-*C*H), 116.5 (CH_2CH_2*C*N), 113.4 (8 x Ar-*C*H), 103.3 (U-5-*C*H), 103.2 (U-5-*C*H), 102.7 (U-5-*C*H), 102.6 (U-5-*C*H), 91.9 (U_2-1'-*C*H), 91.4 (U_2-1'-*C*H), 87.5 (*C*Ph(4-OMePh)$_2$), 87.3 (*C*Ph(4-OMePh)$_2$), 86.8 (U_1-1'-*C*H), 86.6 (U_1-1'-*C*H), 82.7 (U_1-2'-*C*H), 82.3 (U_1-2'-*C*H), 81.7 (U_1-4'-*C*H), 81.6 (U_1-4'-*C*H), 81.4 (U_2-3'-*C*H), 81.2 (U_2-3'-*C*H), 80.8 (U_2'-3'-*C*H), 80.6 (U_2'-3'-*C*H), 79.2 (U_2-4'-*C*H), 78.9 (U_2-4'-*C*H), 73.9 (*C*H_2Nap), 73.7 (*C*H_2Nap), 73.6 (U_2-2'-*C*H), 73.5 (U_2-2'-*C*H), 72.8 (d, *J* 5.4, U_1-3'-*C*H), 72.0 (d, *J* 5.3, U_1-3'-*C*H), 61.4 (U_1-5'-CH_2), 61.2 (U_1-5'-CH_2), 60.4 (d, *J* 5.2, *C*H_2CH_2CN), 60.1 (d, *J* 5.5, *C*H_2CH_2CN), 58.8 (U_1-2'-

O*C*H$_3$), 58.6 (U$_1$-2'-O*C*H$_3$), 55.4 (2 x Ar-O*C*H$_3$), 55.3 (2 x Ar-O*C*H$_3$), 20.8 (U$_2$-2'-CO*C*H$_3$), 20.7 (U$_2$-2'-CO*C*H$_3$), 19.7 (d, *J* 8.0, CH$_2$*C*H$_2$CN), 19.6 (d, *J* 7.0, CH$_2$*C*H$_2$CN); δ_P (121 MHz, CDCl$_3$) 19.5 and 19.3; *m/z* (ES+) 1120.3375 (M+Na, $C_{57}H_{56}N_5NaO_{16}P^+$ requires 1120.3357).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl uridinyl 2-cyanoethyl-phosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-methyl-(2-naphthyl)-2'-*O*-acetyl (*N*-6-benzoyl)adenosine 261



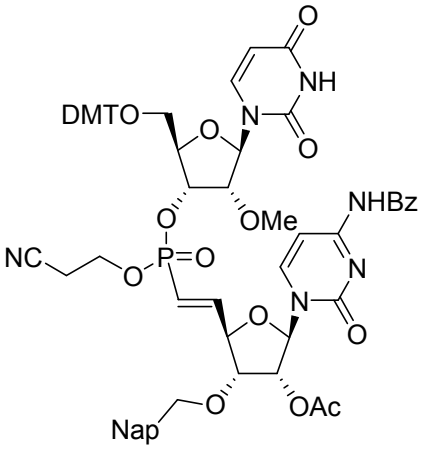
Vinyl bromide **220** (81.0 mg, 129 μmol), dppf (28.6 mg, 51.6 μmol) and Pd(OAc)$_2$ (5.8 mg, 25.8 μmol) were combined in an oven-dried carousel tube purged with nitrogen. A solution of *H*-phosphonate **254** (130 mg, 167 μmol) in dry THF (1.75 mL) was added followed by propylene oxide (180 μL, 2.58 mmol). The tube was sealed and placed in a Radleys® Carousel at 70 °C for 6 hours. The reaction mixture was then removed from heat, transferred to a flask, diluting with CH_2Cl_2 (2 mL) and concentrated *in vacuo* to a orange foam. Purification by column chromatography (95:5 CH_2Cl_2:MeOH) obtained U*A dimer **261** as an orange foam (155 mg, 92%). R_f 0.11 (95:5 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +26.5 (*c* 0.95, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3698 (N-H), 3606 (N-H), 3394 (O-H), 2954 (C-H), 2935 (C-H), 2875 (C-H), 2839 (C-

H), 1747 (C=O), 1711 (C=O), 1694 (C=O), 1636 (C=C), 1610 (C=C), 1587 (C=C), 1487, 1456, 1372, 1303; δ_H (500 MHz, $CDCl_3$) 9.47 (1H, br s, N*H*), 9.44 (1H, br s, N*H*), 8.78 (2H, app d, *J* 6.9, 2 x A-N*H*), 8.17 (2H, app d, *J* 13.5, 2 x Ar-*H*), 8.12 (1H, s, A-8-*H*), 8.10 (1H, s, A-8-*H*), 7.95-7.84 (5H, m, 2 x A-2-*H*, 3 x Ar-*H*), 7.68-7.62 (1H, m, Ar-*H*), 7.59-7.51 (6H, m, 2 x U-6-*C*H and Ar-*H*), 7.49-7.42 (1H, m, Ar-*H*), 7.40-7.35 (4H, m, Ar-*H*), 7.34-7.20 (16H, m, Ar-*H*), 7.11 (2H, m, 2 x A-5'-*H*), 6.93-6.84 (8H, m, Ar-*H*), 6.23 (1H, dd, *J* 25.5 and 3.2, A-1'-*H*), 6.09 (1H, dd, *J* 20.9 and 3.7, A-6'-*H*), 6.03 (2H, app dd, *J* 3.9 and 3.7, A-1'-*H* and A-2'-*H*), 6.00 (1H, dd, *J* 4.6 and 3.2, A-2'-*H*), 6.00-5.95 (1H, m, A-6'-*H*), 5.33-5.29 (2H, m, 2 x U-5-*C*H), 5.17 (1H, dd, *J* 11.7 and 5.8, U-1'-*H*), 5.07 (1H, dd, *J* 11.7 and 6.6, U-1'-*H*), 4.89 (1H, d, *J* 11.6, A-3'-OC*H*H), 4.84-4.78 (2H, m, A-3'-OCH_2 and A-3'-*H*), 4.77-4.73 (1H, m, A-3'-*H*), 4.63 (1H, m, A-4'-*H*), 4.33 (1H, dd, *J* 5.7 and 2.6, U-4'-*H*), 4.25-4.23 (1H, m, U-3'-*H*), 4.11 (1H, dd, *J* 4.2 and 3.3, A-4'-*H*), 4.09-3.90 (2H, obs m, CH_2CH_2CN), 4.03 (2H, dd, *J* 15.7 and 4.1, 2 x U-2'-*H*), 3.97-3.91 (1H, m, U-3'-*H*), 3.86-3.78 (2H, obs m, CH_2CH_2CN), 3.86 (6H, s, (6H, s, 2 x Ar-OCH_3), 3.85 (6H, s, 2 x Ar-OCH_3), 3.83 (1H obs m, U-3'-H), 3.80 (3H, s, U-2'-OCH_3), 3.79 (3H, s, U-2'-OCH_3), 3.72-3.69 (1H, m, U-5'-*H*a), 3.61-3.58 (1H, m, U-5'-*H*a), 3.53-3.50 (1H, m, U-5'-*H*b), 3.44-3.40 (1H, m, U-5'-*H*b), 2.63 (1H, ddd, *J* 12.6, 6.2 and 6.0, CH_2CH_2CN), 2.56 (1H, ddd, *J* 17.4, 6.0 and 5.8, CH_2CH_2CN), 2.48 (1H, ddd, *J* 11.9, 6.2 and 6.0, CH_2CH_2CN), 2.41 (6H, s, 2 x A-2'-COCH_3), 2.40 (1H, obs m, CH_2CH_2CN); δ_C (125 MHz, $CDCl_3$) 169.9 (A-2'-*C*OCH_3),

169.8 (A-2'- *C*OCH$_3$), 165.0 (U-4-*C*), 164.9 (U-4-*C*), 163.04 (A-N*C*OPh), 163.01 (A-N*C*OPh), 158.86 (A-6-*C*), 158.80 (A-6-*C*), 152.82 (A-2-*C*H), 152.79 (A-2-*C*H), 152.74 (U-2-*C*), 152.73 (U-2-*C*), 151.50 (Ar-*C*), 150.20 (Ar-*C*), 150.14 (2 x A-4-*C*), 149.1 (d, *J* 6.9, A-5'-*C*H), 148.1 (d, *J* 6.5, A-5'-*C*H), 144.2 (2 x Ar-*C*), 144.0 (2 x A-*C*), 142.3 (A-8-*C*H), 140.0 (A-8-*C*H), 139.7 (2 x U-N*C*H), 137.9 (4 x Ar-*C*), 135.03 (Ar-*C*), 135.00 (Ar-*C*), 134.91 (Ar-*C*), 134.81 (Ar-*C*), 134.28 (Ar-*C*), 134.24 (Ar-*C*), 133.25 (2 x Ar-*C*), 133.20 (Ar-*C*), 133.19 (Ar-*C*), 132.93 (Ar-*C*H), 132.88 (Ar-*C*H), 132.5 (Ar-*C*H), 131.89 (Ar-*C*H), 130.37 (2 x Ar-*C*H), 130.34 (3 x Ar-*C*H), 130.26 (Ar-*C*H), 130.21 (2 x Ar-*C*H), 130.19 (Ar-*C*H), 130.16 (Ar-*C*H), 130.08 (2 x Ar-*C*H), 129.10 (2 x Ar-*C*H), 128.86 (Ar-*C*H), 128.83 (Ar-*C*H), 128.60 (Ar-*C*H), 128.38 (Ar-*C*H), 128.29 (2 x Ar-*C*H), 128.27 (Ar-*C*H), 128.2 (Ar-*C*H), 128.16 (4 x Ar-*C*H), 128.13 (Ar-*C*H), 128.06 (Ar-*C*H), 127.84 (Ar-*C*H), 127.83 (Ar-*C*H), 127.67 (Ar-*C*H), 127.48 (Ar-*C*H), 127.34 (Ar-*C*H), 127.12 (Ar-*C*H), 126.51 (Ar-*C*H), 126.43 (Ar-*C*H), 126.03 (Ar-*C*H), 125.9 (A-5-*C*), 125.36 (Ar-*C*H), 124.0 (A-5-*C*), 116.5 (*C*N), 116.39 (*C*N), 113.4 (8 x Ar-*C*H), 113.27 d, *J* 16.5, A-6'-*C*H), 113.16 (d, *J* 16.3, A-6'-*C*H), 102.6 (U-5-*C*H), 102.5 (U-5-*C*H), 88.1 (A-1'-*C*H), 88.0 (A-1'-*C*H), 87.5 (*C*Ph(4-OMePh)$_2$), 87.3 (*C*Ph(4-OMePh)$_2$), 87.0 (2 x U-1'-*C*H), 84.1 (U-4'-*C*H), 83.3 (U-4'-*C*H), 82.8 (A-4'-*C*H), 82.30 (A-4'-*C*H), 81.7 (U-2'-*C*H), 81.3 (U-2'-*C*H), 79.2 (A-3'-*C*H), 78.8 (A-3'-*C*H), 73.8 (O*C*H$_2$Nap), 73.6 (O*C*H$_2$Nap), 73.50 (A-2'-*C*H), 73.47 (A-2'-*C*H), 72.6 (d, *J* 6.0, U-3'-*C*H), 71.91 (d, *J* 5.6, U-3'-*C*H), 61.3 (U-5'-CH$_2$), 61.0 (U-5'-CH$_2$), 60.3 (d, *J* 5.2, *C*H$_2$CH$_2$CN), 60.0 (d, *J* 5.4,

CH_2CH_2CN), 58.7 (U-2'-OCH_3), 58.6 (U-2'-OCH_3), 55.4 (2 x Ar-OCH_3), 55.3 (2 x Ar-OCH_3), 21.5 (2 x A-2'-COCH_3), 19.7 (d, *J* 7.0, OCH_2CH_2CN), 19.6 (d, *J* 6.3, OCH_2CH_2CN); δ_P (121 MHz, $CDCl_3$) 19.7 and 19.3; *m/z* (ES+) 1225.4026 (M+H, $C_{65}H_{62}N_8O_{15}P^+$ 1225.4072), (M+Na, $C_{65}H_{61}N_8NaO_{15}P^+$ requires 1247.3892).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-methyl(2-naphthyl)-2'-*O*-acetyl (*N*-6-benzoyl)cytidine 262



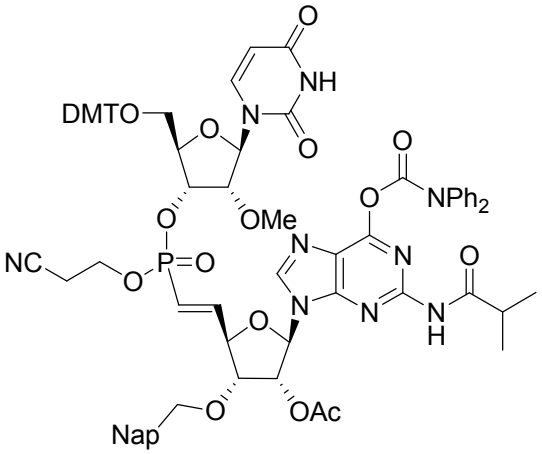
To an oven dried Carousel tube purged with argon was added vinyl bromide **226** (150 mg, 248 μmol), dppf (55 mg, 99.3 μmol), $Pd(OAc)_2$ (11 mg, 49.6 μmol). A solution of *H*-phosphonate **254** (251 mg, 323 μmol) in dry THF (3.30 mL) was added, followed by propylene oxide (350 μL, 4.96 mmol). The tube was sealed and placed in the Radleys® Carousel at 70 °C for 6 hours. The tube was then removed from the heat, cooled to r.t., and transferred to a flask, diluting with CH_2Cl_2 (2 x 1 mL) and concentrated *in vacuo* to a pale brown foam. Purification by column chromatography (95:5 CH_2Cl_2:MeOH) followed by trituration (Et_2O) produced the U*C dimer **262** (286 mg, 96%) as a 1:1 mixture of diastereoisomers as a pale yellow solid. R_f 0.17 (95:5 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +64.5 (*c* 2.05, $CHCl_3$); ν_{max}/cm^{-1}

($CHCl_3$) 3395 (N-H), 2995 (C-H), 2954 (C-H), 2933 (C-H), 2913 (C-H), 2882 (C-H), 2841 (C-H), 1744 (C=O), 1692 (C=O), 1631 (C=C), 1610 (C=C), 1580 (C=C), 1554, 1482, 1462, 1369, 1323, 1303; δ_H (500 MHz, $CDCl_3$) 9.18-8.72 (2H, obs br s, 2 x N*H*Bz), 9.15 (1H, br s, U-*N*H), 8.89 (1H, br s, U-N*H*), 7.96-7.91 (4H, m, 4 x Ar-*H*), 7.86-7.80 (6H, m, U-6-*H* and 5 x Ar-*H*), 7.76 (1H, d, *J* 8.1, U-6-*H*), 7.74 (1H, s, Ar-*H*), 7.70 (1H, s, Ar-C*H*), 7.68 (1, d, *J* 7.0, C-6-*H*), 7.64-7.59 (3H, m, Ar-*H*), 7.62 (1H, d, *J* 8.4, C-6-*H*), 7.53-7.42 (10H, m, Ar-*H*), 7.40-7.32 (8H, m, Ar-*H*), 7.31-7.23 (14H, m, Ar-*H*), 7.02 (1H, ddd, *J* 1.73, 5.9 and 4.0, C-5'-*H*), 6.98 (1H, ddd, *J* 17.0, 5.9 and 3.7, C-5'-*H*), 6.88-6.81 (8H, m, Ar-*H*), 6.16-5.95 (2, m, 2 x C-6'-*H*), 6.08 (1H, d, *J* 4.3, U-1'-*H*), 6.01 (1H, d, *J* 2.7, U-1'-*H*), 5.90 (1H, app s, C-5-*H*), 5.84 (1H, app s, C-5-*H*), 5.69 (1H, d, *J* 4.8, C-1'-*H*), 5.64 (1H, d, *J* 4.6, C-1'-*H*), 5.28 (1H, d, *J* 8.1, U-5-*C*H), 5.27 (1H, d, *J* 8.3, U-5-*C*H), 5.17 (1H, dd, *J* 11.8 and 5.0, U-3'-*H*), 5.05 (1H, dd, *J* 11.5 and 6.3, U-3'-*H*), 4.76-4.69 (1H, obs m, C-3'-*H*), 4.75 (1H, d, *J* 11.4, OCH_2Nap), 4.74 (1H, d, *J* 11.4, OCH_2Nap), 4.64-4.48 (1H, obs m, C-3'-*H*), 4.57 (1H, d, *J* 11.4, OCH_2Nap), 4.52 (1H, d, *J* 11.4, OCH_2Nap), 4.32 (1H, app d, *J* 2.2, U-4'-*H*), 4.25-4.20 (2H, m, U-4'-*H* and C-2'-*H*), 4.17-4.12 (1H, obs m, U-2'-*H*), 4.15 (1H, dd, *J* 8.5 and 5.4, OC$H_2$$CH_2$CN), 4.10-4.03 (1H, obs m, U-2'-*H*), 4.09 (1H, app dd, *J* 3.8 and 3.7, C-4'-*H*), 4.04 (1H, dd, *J* 4.5 and 4.0, C-2'-*H*), 4.07-3.97 (3H, obs m, C-4'-*H* and 2 x OCH_2CH_2CN), 3.96-3.90 (1H, m, OC$H_2$$CH_2$CN), 3.81 (3H, s, Ar-OC$H_3$), 3.80 (3H, s, Ar-OC$H_3$), 3.75 (3H, s, Ar-OC$H_3$), 3.74 (3H, s, Ar-OC$H_3$), 3.64-3.46 (1H, obs m, U-

5'-*H*), 3.59 (3H, s, U-2'-OC*H*$_3$), 3.26 (3H, s, U-2'-OC*H*$_3$), 3.39 (1H, dd, *J* 13.9 and 4.6, U-5'-*H*), 2.57 (1H, ddd, *J* 16.7, 7.2 and 6.2, OCH$_2$C*H*$_2$CN), 2.57-2.51 (2H, obs m, OCH$_2$C*H*$_2$CN), 2.47 (1H, d, *J* 16.8, 6.1 and 5.6, OCH$_2$C*H*$_2$CN), 2.17 (3H, s, C-2'-COC*H*$_3$), 2.16 (3H, s, C-2'-COC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 169.9 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 162.9 (2 x C-4-*C*), 162.8 (2 x U-4-*C*), 158.94 (Ar-*C*OCH$_3$), 158.92 (Ar-*C*OCH$_3$), 158.86 (Ar-*C*OCH$_3$), 158.81 (Ar-*C*OCH$_3$), 150.7 (Ar-*C*), 150.6 (Ar-*C*), 150.2 (2 x C-2-*C*), 149.0 (d, *J* 6.7, C-5'-*C*H), 147.8 (d, *J* 6.0, C-5'-*C*H), 144.9 (C-6-*C*H), 144.2 (Ar-*C*), 144.1 (Ar-*C*), 139.67 (2 x U-6-*C*H), 139.42 (C-6-*C*H), 134.98 (2 x Ar-*C*), 134.84 (Ar-*C*), 134.35 (Ar-*C*), 134.28 (Ar-*C*), 133.4 (Ar-*C*H), 133.3 (Ar-*C*H), 133.2 (3 x Ar-*C*), 130.4 (2 x Ar-*C*H), 130.3 (2 x Ar-*C*H), 130.2 (2 x Ar-*C*H), 130.1 (2 x Ar-*C*H), 129.1 (2 x Ar-*C*H), 129.0 (2 x Ar-*C*H), 128.5 (2 x Ar-*C*H), 128.4 (2 x Ar-*C*H), 128.19 (6 x Ar-*C*H), 128.15 (2 x Ar-*C*H), 128.13 (Ar-*C*H), 128.07 (Ar-*C*H), 127.96 (Ar-*C*H), 127.78 (2 x Ar-*C*H), 127.72 (Ar-*C*H), 127.5 (Ar-*C*H), 127.38 (Ar-*C*H), 127.36 (Ar-*C*H), 127.2 (Ar-*C*H), 126.36 (Ar-*C*H), 126.34 (Ar-*C*H), 126.31 (2 x Ar-*C*H), 126.1 (Ar-*C*H), 125.9 (Ar-*C*H), 118.1 (d, *J* 171, C-6'-*C*H), 116.6 (d, *J* 171, C-6'-*C*H), 116.7 (OCH$_2$CH$_2$*C*N), 116.5 (OCH$_2$CH$_2$*C*N), 113.5 (4 x Ar-*C*H), 113.4 (4 x Ar-*C*H), 103.0 (U-5-*C*H), 102.5 (U-5-*C*H), 93.1 (C-5-*C*H), 92.9 (C-5-*C*H), 87.5 (*C*Ph(4-OMePh)$_2$, 87.4 (*C*Ph(4-OMePh)$_2$, 87.03 (U-1'-*C*H), 86.28 (U-1'-*C*H), 82.8 (U-2'-*C*H), 82.4 (U-2'-*C*H), 82.0 (d, *J* 6.8, C-4'-*C*H), 81.6 (d, *J* 8.4, C-4'-*C*H), 81.3 (U-4'-*C*H), 81.1 (U-4'-*C*H), 80.9 (C-1'-*C*H), 80.7 (C-1'-*C*H), 78.9 (2 x C-2'-*C*H), 73.8 (C-3'-*C*H), 73.7 (2 x OCH_2Nap), 73.4 (C-3'-*C*H),

72.0 (U-3′-*C*H), 71.9 (U-3′-*C*H), 61.7 (U-5′-*C*H_2), 61.1 (U-5′-*C*H_2), 60.4 (d, *J* 5.3, O*C*$H_2$$CH_2$CN), 60.1 (d, *J* 5.3, O*C*$H_2$$CH_2$CN), 58.8 (U-2′-O*C*$H_3$), 58.6 (U-2′-O*C*$H_3$), 55.4 (2 x Ar-O*C*$H_3$), 55.3 (2 x Ar-O*C*$H_3$), 20.9 (C-2′-CO*C*$H_3$), 20.8 (C-2′-CO*C*$H_3$), 19.8 (d, *J* 7.1, OCH_2*C*H_2CN), 19.7 (d, *J* 6.6, OCH_2*C*H_2CN), 14.3 (C-2′-CO*C*H_3), 14.1 (C-2′-CO*C*H_3); δ_P (121 MHz, $CDCl_3$) 19.5 and 19.1; *m/z* (ES+) 1223.3742 (M+Na, $C_{64}H_{61}N_6NaO_{16}P^+$ requires 1223.3779).

5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methyl uridinyl 2-cyanoethylphosphono-[3′(O)→5′(C)]-5′-deoxy-5′-methylidene-3′-*O*-methyl(2-naphthyl)-2′-*O*-acetyl (*O*-6-(*N,N*-diphenylcarbamoyl *N*-2-*iso*-butyryl)guanosine 263



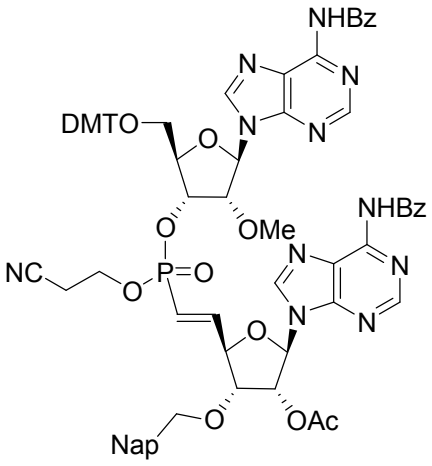
Vinyl bromide **230** (104 mg, 129 μmol), $Pd(OAc)_2$ (5.8 mg, 25.8 μmol) and dppf (28.6 mg, 51.6 μmol) were combined in a Radleys® Carousel tube flushed with argon. A solution of *H*-phosphonate **254** (114 mg, 168 μmol) in freshly distilled THF (1.7 mL) was added followed by propylene oxide (181 μL, 2.58 mmol). The tube was then sealed, placed in the carousel and heated at 70 °C. After 6 hours the tube was removed from the heat, diluted with CH_2Cl_2 (2 x 1 mL) to transfer to a flask then concentrated *in vacuo* and purified by column chromatography (96:4 CH_2Cl_2:MeOH) to a pale yellow foam of U*G dimer **263** (140 mg, 83%). R_f 0.34

(97:3 CH_2Cl_2:MeOH); $[\alpha]_D^{28}$ +271 (*c* 0.37, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3411 (N-H), 3392 (N-H), 2948 (C-H), 2936 (C-H), 2903 (C-H), 2864 (C-H), 1746 (C=O), 1721 (C=O), 1707 (C=O), 1694 (C=O), 1621 (C=C), 1609 (C=C), 1589 (C=C), 1496, 1491, 1455, 1386, 1373, 1342, 1323, 1304; δ_H (500 MHz, $CDCl_3$) 9.50-9.15 (2H, 2 x br s, 2 x N*H*CO^{i}Pr), 8.76 (1H, br s, U-N*H*), 8.59 (1H, br s, U-N*H*), 7.98 (1H, app d, *J* 2.8, G-8-*H*), 7.97 (1H, app d, *J* 2.7, G-8-*H*), 7.86-7.81 (8H, m, 2 x U-6-*H* and 6 x Ar-*H*), 7.80 (1H, s, Ar-*H*), 7.77 (1H, s, Ar-*H*), 7.61 (1H, s, Ar-*H*), 7.60 (1H, s, Ar-*H*), 7.59 (1H, s, Ar-*H*), 7.58 (1H, s, Ar-*H*), 7.51-7.40 (16H, m, 16 x Ar-*H*), 7.39-7.29 (13H, m, 13 x Ar-*H*), 7.27-7.20 (11H, m, 11 x Ar-*H*), 7.21-7.07 (2H, m, G-5'-*H*), 6.88-6.80 (8H, m, 8 x Ar-*H*), 6.07-5.90 (6H, m, 2 x G-1'-*H*, *2* x G-6'-*H*, 2 x U-1'-*H*), 5.90-5.85 (2H, m, 2 x G-2'-*H*), 5.28-2.22 (1H, obs dd, G-3'-*H*), 5.26 (1H, d, *J* 8.5, U-5-*H*), 5.24 (1H, d, *J* 8.7, U-5-*H*), 5.18 (1H, dd, *J* 11.6 and 5.4, G-3'-*H*), 5.12 (1H, dd, *J* 11.6 and 5.7, U-3'-*H*), 5.05 (1H, dd, *J* 11.6 and 5.7, U-3'-*H*), 4.70 (1H, dd, *J* 7.4 and 7.0, G-4'-*H*), 4.59-4.55 (1H, m, G-4'-*H*), 4.29 (1H, app d, *J* 5.7, U-2'-*H*), 4.21 (1H, app d, *J* 5.7, U-2'-*H*) 4.16-4.07 (3H, m, U-4'-*H* and OC$H_2$$CH_2$CN), 4.03-3.92 (3H, m, U-4'-*H* and OC$H_2$$CH_2$CN), 3.80 (3H, d, *J* 4.8, Ar-OCH_3), 3.79 (3H, d, 5.5, Ar-OCH_3), 3.73 (3H, s, Ar-OCH_3), 3.72 (3H, s, Ar-OCH_3), 3.58 (3H, s, U-2'-OCH_3), 3.54-3.44 (4H, m, 2 x U-5'-C*H*H), 3.37 (3H, s, U-2'-OCH_3), 2.62 (1H, dd, *J* 6.8 and 5.5, OCH$_2$*C*H$_2$CN), 2.60 (1H, dd, *J* 5.8 and 5.2, OCH$_2$*C*H$_2$CN), 2.44 (1H, dd, *J* 6.7 and 6.5, OCH$_2$*C*H$_2$CN), 2.43 (1H, dd, *J* 6.7 and 6.5, OCH$_2$*C*H$_2$CN), 2.10 (3H, s, G-2'-OCOCH_3), 2.09 (3H, s, G-2'-OCOCH_3), 1.30 (3H, d, *J*

6.4, COC*H*$(CH_3)_2$), 1.24 (3H, d, *J* 6.8, COC*H*$(CH_3)_2$), 1.22 (3H, d, *J* 6.8, COC*H*$(CH_3)_2$), 1.21 (3H, d, *J* 6.4, COC*H*$(CH_3)_2$); $\square_C$ (125 MHz, $CDCl_3$) 175.4 (NH*C*O^{i}Pr), 175.2 (NH*C*O^{i}Pr), 169.93 (G-2'-*C*OCH_3), 169.88 (G-2'-*C*OCH_3), 163.2 (U-4-*C*), 163.1 (U-4-*C*), 158.91 (Ar-*C*), 158.90 (Ar-*C*), 158.86 (Ar-*C*), 158.81 (Ar-*C*), 156.46 (G-2-*C*), 156.44 (G-2-*C*), 154.0 (2 x G-N*C*), 152.34 (U-2-*C*), 152.32 (U-2-*C*), 150.44 (G-4-*C*), 150.42 (G-4-*C*), 150.3 (2 x G-6-*C*), 149.8 (d, *J* 6.8, G-5'-*C*H), 149.1 (d, *J* 5.3, G-5'-*C*H), 144.15 (Ar-*C*), 143.53 (G-8-*C*H), 143.45 (G-8-*C*H), 141.81 (Ar-*C*), 139.75 (2 x U-6-*C*H), 137.92 (Ar-*C*), 135.39 (Ar-*C*), 135.17 (Ar-*C*), 135.11 (Ar-*C*), 135.07 (Ar-*C*), 135.02 (Ar-*C*), 135.00 (Ar-*C*), 134.97 (Ar-*C*), 134.84 (Ar-*C*), 134.07 (Ar-*C*), 133.28 (Ar-*C*), 133.26 (Ar-*C*), 133.22 (Ar-*C*), 133.14 (Ar-*C*), 131.93 (Ar-*C*H), 131.8 (2 x Ar-*C*H), 131.6 (2 x Ar-*C*H), 131.4 (2 x Ar-*C*H), 131.3 (2 x Ar-*C*H), 130.36 (2 x Ar-*C*H), 130.33 (2 x Ar-*C*H), 130.28 (2 x Ar-*C*H), 130.21 (2 x Ar-*C*H), 130.09 (2 x Ar-*C*H), 129.3 (2 x Ar-*C*H), 129.2 (2 x Ar-*C*H), 129.10 (2 x Ar-*C*H), 128.44 (2 x Ar-*C*H), 128.37 (2 x Ar-*C*H), 128.36 (2 x Ar-*C*H), 128.29 (2 x Ar-*C*H), 128.25 (2 x Ar-*C*H), 128.19 (2 x Ar-*C*H), 128.15 (2 x Ar-*C*H), 128.09 (2 x Ar-*C*H), 127.98 (2 x Ar-*C*H), 127.79 (2 x Ar-*C*H), 127.4 (d, *J* 11.0,Ar-*C*H), 127.0 (d, *J* 3.2, Ar-*C*H), 126.3 (d, *J* 2.9, Ar-*C*H), 126.2 (d, *J* 4.0, Ar-*C*H), 126.0 (d, *J* 3.3, Ar-*C*H), 125.4 (2 x Ar-*C*H), 121.96 (G-5-*C*), 121.92 (G-5-*C*), 118.0 (d, *J* 179, G-6'-*C*H), 117.5 (d, *J* 178, G-6'-*C*H), 116.6 (*C*N), 116.5 (*C*N), 113.43 (8 x Ar-*C*H), 102.6 (U-5-*C*H), 102.5 (U-5-*C*H), 88.6 (d, *J* 12.6, U-1'-*C*H), 88.5 (d, *J* 11.3, U-1'-*C*H), 87.45 (*C*Ph(4-OMePh)$_2$, 87.36 (*C*Ph(4-OMePh)$_2$, 87.0 (G-1'-

*C*H), 86.9 (G-1'-*C*H), 82.9 (U-4'-*C*H), 82.5 (d, *J* 15.0, G-4'-*C*H), 82.3 (d, *J* 9.9, G-4'-*C*H), 82.3 (U-4'-*C*H), 81.75 (d, *J* 3.1, U-2'-*C*H), 81.67 (d, *J* 1.7, U-2'-*C*H), 79.6 (G-3'-*C*H), 79.4 (G-3'-*C*H), 74.2 (G-2'-*C*H), 74.1 (G-2'-*C*H), 73.9 (d, *J* 4.7,U-2'-*C*H), 73.74 (*C*H_2Nap), 73.66 (*C*H_2Nap), 73.56 (d, *J* 6.3, U-2'-*C*H), 72.6 (d, *J* 5.6, U-3'-*C*H), 72.0 (d, *J* 5.2, U-3'-*C*H), 61.4 (U-5'-CH_2), 61.1 (U-5'-CH_2), 60.5 (d, *J* 5.1, O*C*$H_2$$CH_2$CN), 60.1 (d, *J* 4.7, O*C*$H_2$$CH_2$CN), 58.8 (U-2'-O*C*$H_3$), 58.6 (U-2'-O*C*$H_3$), 55.4 (2 x Ar-O*C*$H_3$), 55.3 (2 x Ar-O*C*$H_3$), 36.0 (CO*C*H($CH_3$)$_2$), 35.9 (CO*C*H($CH_3$)$_2$), 21.5 (d, *J* 3.8, G-2'-CO*C*H_3), 20.7 (d, *J* 3.6, G-2'-CO*C*H_3), 19.64 (d, *J* 6.1, OCH$_2$*C*H_2CN), 19.59 (d, *J* 5.8, OCH$_2$*C*H_2CN), 19.5 (COCH(*C*H_3)$_2$), 19.41 (COCH(*C*H_3)$_2$), 19.36 (COCH(*C*H_3)$_2$), 19.30 (COCH(*C*H_3)$_2$); δ_P (121 MHz, $CDCl_3$) 19.86 and 19.58; *m/z* (ES+) 1402.4801 (M+H, $C_{75}H_{73}N_9O_{17}P^+$ requires 1402.4857), 1424.4460 (M+Na, $C_{75}H_{73}N_9NaO_{17}P^+$ requires 1424.4676).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl (*N*-6-benzoyl)-adenosine 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-methyl(2-naphthyl)-2'-*O*-acetyl (*N*-6-benzoyl)adenosine 264



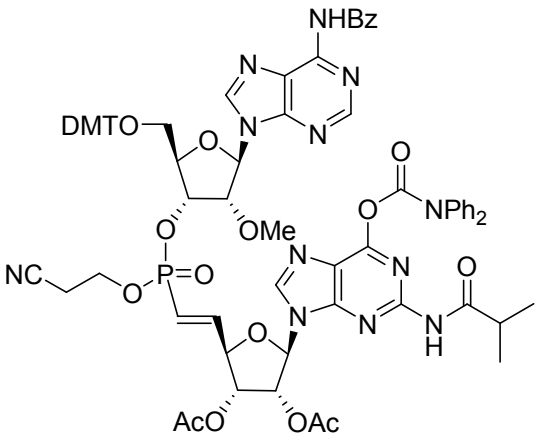
Vinyl bromide **238** (120 mg, 191 μmol), $Pd(OAc)_2$ (8.6 mg, 38.2 μmol), dppf (42.3 mg, 76.4 μmol) and *H*-phosphonate **255** (184 mg, 229 μmol) were all combined in a Radleys® Carousel tube purged with

argon. Freshly distilled THF (2.55 mL) was added followed by propylene oxide (267 μL, 3.82 mmol), the tube was then sealed and heated at 70 °C for 6 hours. After this time, the tube was removed from the heat, cooled to r.t. and transferred to a flask, diluting with CH_2Cl_2 (2 mL). The solution was evaporated to dryness and the residue purified by column chromatography (96:4 CH_2Cl_2:MeOH) to obtain the pale yellow solid A*A dimer **264** as a 1.66:1.00 mixture of diastereoisomers (260 mg, 99%). R_f 0.11 (96:4 CH_2Cl_2:MeOH); $[\alpha]_D^{25}$ +3.81 (*c* 1.20, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3668 (N-H), 3626 (N-H), 3403 (N-H), 2990 (C-H), 2936 (C-H), 2838 (C-H), 1748 (C=O), 1709 (C=O), 1612 (C=C), 1587 (C=C), 1504, 1485, 1456, 1401, 1356, 1328, 1301; δ_H (500 MHz, $CDCl_3$) 9.21 (1H, br s, N*H*), 9.12 (3H, br s, 3 x N*H*), 8.75 (1H, s, A-*H*), 8.73 (1H, s, A-2-*H*), 8.70 (1H, s, A-2-*H*), 8.66 (1H, s, A-2-*H*), 8.33 (1H, s, A-2-*H*), 8.18 (1H, s, A-8-*H*), 8.17 (1H, s, A-8-*H*), 8.15 (1H, s, A-8-*H*), 8.11-8.04 (8H, m, Ar-8-*H*), 7.96-7.85 (8H, m, Ar-*H*), 7.68-7.62 (4H, m, Ar-*H*), 7.60-7.52 (14H, m, Ar-*H*), 7.50-7.42 (6H, m, Ar-*H*), 7.39-7.22 (12H, m, Ar-*H*), 7.06-6.96 (2H, m, A_2-5'-*H*), 6.88-6.82 (8H, m, Ar-*H*), 6.30 (1H, d, *J* 3.5, A_1-1'-*H*), 6.23 (1H, d, *J* 3.1, A_1-1'-*H*), 6.16 (1H, d, *J* 2.7, A_2-1'-*H*), 6.15 (1H, d, *J* 2.5, A_2-1'-*H*), 6.16-6.04 (2H, obs m, 2 x A_2-6'-*H*), 6.00-5.97 (2H, m, 2 x A_1-2'-*H*), 5.33-5.29 (1H, m, A_2-3'-*H*), 5.25-5.22 (1H, m, A_2-3'-*H*), 4.94 (2H, app dd, *J* 11.5 and 6.5, 2 x A_2-2'-*H*), 4.91-4.75 (6H, m, 2 x A_1-3'-*H*, 1 x A_1-4'-*H*, 2 x A_2-3'-OC*H*H), 4.68-4.64 (1H, m, A_1-4'-*H*), 4.48-4.47 (1H, m, A_2-4'-*H*), 4.39-4.35 (1H, m, A_2-4'-*H*), 4.18-3.91 (4H, m, 2 x OC*H*$_2$CH$_2$CN), 3.83 (6H, s, 2 x Ar-OC*H*$_3$),

3.79 (6H, s, 2 x Ar-OC*H*$_3$), 3.65 (1H, dd, *J* 11.1 and 4.6, A$_1$-5'-*H*), 3.57 (1H, dd, *J* 13.6 and 8.9, A$_1$-5'-*H*), 3.54-3.48 (1H, m, A$_1$-5'-*H*), 3.52 (3H, s, A$_1$-2'-OC*H*$_3$), 3.38 (1H, dd, *J* 10.6 and 3.7, A$_1$-5'-*H*), 3.30 (3H, s, A$_1$-2'-OC*H*$_3$), 2.68-2.48 (4H, m, 2 x OCH$_2$C*H*$_2$CN), 2.22 (3H, s, A$_2$-2'-OCOC*H*$_3$), 2.20 (3H, s, A$_2$-2'-OCOC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 169.88 (A$_2$-2'-*C*OCH$_3$), 169.85 (A$_2$-2'-*C*OCH$_3$), 164.6 (2 x NH*C*OPh), 158.74 (Ar-*C*OCH$_3$), 158.71 (Ar-*C*OCH$_3$), 158.69 (Ar-*C*OCH$_3$), 158.64 (Ar-*C*OCH$_3$), 152.8 (2 x A-2-*C*H), 152.7 (A-2-*C*H), 152.6 (A-2-*C*H), 151.8 (2 x A-6-*C*), 151.5 (A-6-*C*), 151.4 (A-6-*C*), 150.0 (A-4-*C*), 149.7 (A-4-*C*), 148.7 (d, *J* 6.9, A$_2$-5'-*C*H), 147.8 (d, *J* 6.7, A$_2$-5'-*C*H), 144.33 (A-4-*C*), 144.29 (A-4-*C*), 142.8 (A-8-*C*H), 142.2 (A-8-*C*H), 142.1 (2 x A-8-*C*H), 135.5 (Ar-*C*), 135.4 (Ar-*C*), 135.35 (2 x Ar-*C*), 134.22 (Ar-*C*), 134.18 (Ar-*C*), 133.6 (Ar-*C*), 133.4 (Ar-*C*), 133.3 (2 x Ar-*C*), 133.2 (2 x Ar-*C*), 132.99 (Ar-*C*H), 132.93 (Ar-*C*H), 132.88 (Ar-*C*H), 132.85 (Ar-*C*H), 132.5 (2 x Ar-*C*), 131.9 (2 x Ar-*C*), 130.20 (Ar-*C*H), 130.18 (Ar-*C*H), 130.13 (4 x Ar-*C*H), 130.08 (2 x Ar-*C*H), 128.96 (2 x Ar-*C*H), 128.93 (3 x Ar-*C*H), 128.91 (4 x Ar-*C*H), 128.63 (2 x Ar-*C*H), 128.28 (2 x Ar-*C*H), 128.21 (3 x Ar-*C*H), 128.09 (2 x Ar-*C*H), 128.07 (2 x Ar-*C*H), 127.99 (7 x Ar-*C*H), 127.95 (5 x Ar-*C*H), 127.83 (Ar-*C*H), 127.81 (Ar-*C*H), 127.5 (Ar-*C*H), 127.4 (Ar-*C*H), 127.21 (Ar-*C*H), 127.16 (Ar-*C*H), 126.55 (Ar-*C*H), 126.53 (Ar-*C*H), 126.47 (Ar-*C*H), 126.45 (Ar-*C*H), 126.1 (Ar-*C*H), 125.9 (Ar-*C*H), 123.94 (A-5-*C*), 123.88 (A-5-*C*), 123.81 (A-5-*C*), 123.71 (A-5-*C*), 118.5 (d, *J* 116, A$_2$-6'-*C*H), 117.0 (d, *J* 113, A$_2$-6'-*C*H), 116.5 (OCH$_2$CH$_2$*C*N), 116.4 (OCH$_2$CH$_2$*C*N), 113.29 (4 x Ar-*C*H), 113.27 (4 x Ar-*C*H), 88.0 (A$_1$-

1'-*C*H), 87.9 (A_1-1'-*C*H), 87.1 (*C*Ph(4-OMePh)$_2$, 87.0 (*C*Ph(4-OMePh)$_2$, 86.3 (A_2-1'-*C*H), 86.3 (A_2-1'-*C*H), 83.5 (d, *J* 5.2, A_2-4'-*H*), 83.3 (d, *J* 5.0, A_2-4'-*C*H), 81.8 (d, *J* 23.8, A_1-4'-*C*H), 81.2 (d, *J* 22.9, A_1-4'-*C*H), 81.0 (d, *J* 1.5, A_2-2'-*C*H), 80.7 (d, *J* 1.9, A_2-2'-*C*H), 79.0 (A_1-3'-*C*H), 78.8 (A_1-3'-*C*H), 74.2 (A_2-3'-*C*H), 74.1 (A_2-3'-*C*H), 73.63 (d, *J* 25.3, A_1-2'-*C*H), 73.55 (d, *J* 18.1, A_1-2'-*C*H), 62.8 (A_1-5'-*C*H_2), 62.5 (A_1-5'-*C*H_2), 60.2 (d, *J* 5.3, O*C*H_2CH_2CN), 60.0 (d, *J* 5.0, O*C*H_2CH_2CN), 58.9 (A_1-2'-O*C*H_3), 58.8 (A_1-2'-O*C*H_3), 55.34 (2 x Ar-O*C*H_3), 55.28 (2 x Ar-O*C*H_3), 22.7 (A_2-2'-CO*C*H_3), 20.8 (A_2-2'-CO*C*H_3), 19.8 (d, *J* 2.0, OCH_2*C*H_2CN), 19.7 (d, *J* 1.8, OCH_2*C*H_2CN); δ_P (121 MHz, $CDCl_3$) 19.6 (minor) and 19.3 (major); *m/z (ES+)* 1353.4558 (M+H, $C_{73}H_{67}N_{11}O_{14}P^+$ requires 1353.4607), 1374.4418 (M+Na, $C_{73}H_{66}N_{11}NaO_{14}P^+$ requires 1374.4421).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl (*N*-6-benzoyl)-adenosine 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-3'-*O*-acetyl-2'-*O*-acetyl (*O*-6-(*N,N*-diphenylcarb-amoyl *N*-2-*iso*-butyryl)guanosine 265



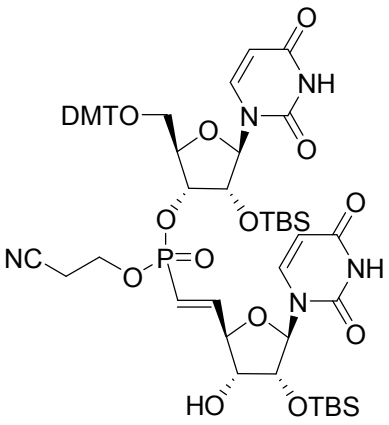
Vinyl bromide **249** (75 mg, 106 μmol), *H*-phosphonate **255** (103 mg, 127 μmol), $Pd(OAc)_2$ (4.8 mg, 21.2 μmol) and dppf (23.5 mg, 42.4 μmol) were combined in an oven dried Carousel tube purged with Argon. Freshly distilled THF (1.4 mL) was added

followed by propylene oxide (148 μL, 212 μmol) and the tube was then sealed and heated at 70 °C for 6 hours. The reaction mixture was then cooled to r.t., transferred to a flask, diluting with CH_2Cl_2 (2 mL) and concentrated *in vacuo* to an orange foam. Purification by column chromatography (95:5 CH_2Cl_2:MeOH) obtained A*G dimer **265** (138 mg, 91%) as a pale yellow foam. R_f 0.32 and 0.32 (95:5 CH_2Cl_2:MeOH); $[\alpha]_D^{22}$ +0.34 (*c* 1.27, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3697 (N-H), 3605 (N-H), 3414 (O-H), 2961 (C-H), 2935 (C-H), 2910 (C-H), 2874 (C-H), 2838 (C-H), 1749 (C=O), 1713 (C=O), 1611 (C=C), 1588 (C=C), 1490, 1456, 1388, 1373, 1336, 1324, 1303; δ_H (500 MHz, $CDCl_3$) 9.22 (1H, br s, N*H*), 9.20 (1H, br s, N*H*), 8.87-8.61 (4H, m, 2 x N*H* and 2 x A-2-*H*), 8.28-8.14 (2H, m, 2 x 8-*H*), 8.04-7.98 (8H, m, 2 x 8-*H*, 6 x Ar-*H*), 7.90-7.80 (3H, m, 2 x Ar-*H*), 7.60-7.57 (3H, m, 3 x Ar-*H*), 7.51-7.15 (36H, m, 36 x Ar-*H*), 7.08-6.91 (2H, m, 2 x G-5'-*H*), 6.82-6.77 (8H, m, Ar-*H*), 6.26-6.01 (6H, m, 2 x A-1'-*H*, 2 x G-1'-*H*, 2 x G-6'-*H*), 6.00-5.95 (1H, dd, *J* 5.7 and 5.4, G-2'-*H*), 5.95-5.92 (1H, dd, *J* 6.1 and 5.4, G-2'-*H*), 5.88-5.78 (1H, m, G-3'-*H*), 5.67-5.63 (1H, m, G-3'-*H*), 5.32-5.28 (1H, m, A-3'-*H*), 5.25-5.20 (1H, m, A-3'-*H*), 4.99-4.95 (2H, m, 2 x G-4'-*H*), 4.78-4.65 (1H, m, G-4'-*H*), 4.63-4.59 (1H, m, G-4'-*H*), 4.48-4.44 (1H, m, A-4'-*H*), 4.41-4.38 (1H, m, A-4'-*H*), 4.30-4.25 (2H, m, OC*H*$_2$CH$_2$CN), 4.24-4.08 (2H, m, OC*H*$_2$CH$_2$CN), 3.78 (3H, s, Ar-OC*H*$_3$), 3.77 (3H, s, Ar-OC*H*$_3$), 3.76 (3H, s, Ar-OC*H*$_3$), 3.75 (3H, s, Ar-OC*H*$_3$), 3.52-3.32 (4H, m, 2 x A-5'-C*H*H), 3.49 (3H, s, A-2'-OC*H*$_3$), 3.33 (3H, s, A-2'-OC*H*$_3$), 3.06-2.94 (2H, m, COC*H*(CH$_3$)$_2$), 2.73 (1H, dd, *J* 6.3 and 6.2, OCH$_2$C*H*$_2$CN), 2.68-

2.55 (2H, m, OCH_2CH_2CN), 2.52-2.47 (1H, m, OCH_2CH_2CN), 2.18 (3H, s, G-3'-$COCH_3$), 2.16 (3H, s, G-3'-$COCH_3$), 2.06 (3H, s, G-2'-$COCH_3$), 2.04 (3H, s, G-2'-$COCH_3$), 1.27-1.14 (12H, m, 2 x G-$CH(CH_3)_2$); δ_C (125 MHz, $CDCl_3$) 176.0 ($NHCO^iPr$), 174.6 ($NHCO^iPr$), 169.7 (G-3'-$COCH_3$), 169.5 (G-3'-$COCH_3$), 169.4 (G-2'-$COCH_3$), 169.3 (G-2'-$COCH_3$), 164.8 (2 x A-NHCOPh), 158.74 (2 x Ar-C), 158.71 (2 x Ar-C), 156.54 (G-4-C), 156.51 (G-4-C), 154.5 (G-6-C), 154.4 (G-6-C), 152.7 (G-2-C), 152.63 (G-2-C), 152.62 (A-2-CH), 152.5 (A-2-CH), 151.9 (A-6-C), 151.8 (A-6-C), 150.44 ($CONPh_2$), 150.41 ($CONPh_2$), 149.8 (2 x A-4-C), 148.1 (d, *J* 7.2, G-5'-CH), 147.5 (d, *J* 6.9, G-5'-CH), 144.5 (Ar-C), 144.32 (2 X Ar-C), 144.30 (Ar-C), 143.02 (8-CH), 142.94 (8-CH), 142.87 (8-CH), 142.3 (8-CH), 141.8 (2 x Ar-C), 135.5 (Ar-C), 135.44 (Ar-C), 135.40 (2 x Ar-C), 133.7 (Ar-C), 133.6 (Ar-C), 132.84 (2 x Ar-CH), 130.18 (4 x Ar-CH), 138.15 (5 x Ar-CH), 130.10 (4 x Ar-CH), 129.24 (3 x Ar-CH), 129.21 (2 x Ar-CH), 128.86 (6 x Ar-CH), 128.4 (Ar-CH), 128.3 (3 x Ar-CH), 128.2 (3 x Ar-CH), 128.05 (4 x Ar-CH), 128.01 (3 x Ar-CH), 127.99 (5 x Ar-CH), 127.78 (Ar-CH), 127.19 (Ar-CH), 127.15 (Ar-CH), 123.81 (5-C), 123.78 (5-C), 121.9 (2 x 5-C), 119.6 (d, *J* 78.5, G-6'-CH), 118.0 (d, *J* 76.7, G-6'-CH), 116.6 (CN), 116.4 (CN), 113.2 (8 x Ar-CH), 86.8 (1'-CH), 86.4 (2 x 1'-CH), 86.3 (1'-CH), 83.42 (d, *J* 6.5, A-4'-CH), 83.36 (d, *J* A-4'-CH), 82.4 (d, *J* 2.45, G-4'-CH), 81.8 (d, *J* 23.9, G-4'-CH), 80.9 (d, *J* 2.1, A-2'-CH), 80.6 (d, *J* 1.7, A-2'-H), 74.5 (d, *J* 5.8, A-3'-CH), 74.0 (d, *J* 6.2, A-3'-CH), 73.5 (G-3'-CH), 73.1 (G-3'-CH), 71.9 (G-2'-CH), 71.8 (G-2'-CH), 62.7 (A-5'-CH_2), 62.6 (A-5'-CH_2),

60.7 (d, *J* 5.3, O*C*$H_2$$CH_2$CN), 60.4 (d, *J* 5.1, O*C*$H_2$$CH_2$CN), 59.0 (A-2'-O*C*$H_3$), 58.9 (A-2'-O*C*$H_3$), 55.33 (2 x Ar-O*C*$H_3$), 55.29 (2 x Ar-O*C*$H_3$), 35.7 (CO*C*H($CH_3$)$_2$), 35.6 (CO*C*H($CH_3$)$_2$), 20.7 (G-3'-CO*C*$H_3$), 20.6 (G-3'-CO*C*$H_3$), 20.4 (2 x G-2'-CO*C*$H_3$), 19.80 (d, *J* 6.9, OCH$_2$*C*H_2CN), 19.78 (d, *J* 5.8, OCH$_2$*C*H_2CN), 19.44 (COCH(*C*H_3)$_2$), 19.39 (COCH(*C*H_3)$_2$), 19.34 (COCH(*C*H_3)$_2$), 19.32 (COCH(*C*H_3)$_2$); δ_P (121 MHz, $CDCl_3$) 19.5 and 19.0; *m/z* (ES+) 1431.4834 (M+H, $C_{74}H_{72}N_{12}O_{17}P^+$ requires 1431.4876), 1453.4639 (M+Na, $C_{74}H_{71}N_{12}NaO_{17}P^+$ requires 1453.4695)

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl) uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-2'-*O*-(*tert*-butyldimethylsilyl) uridine 266



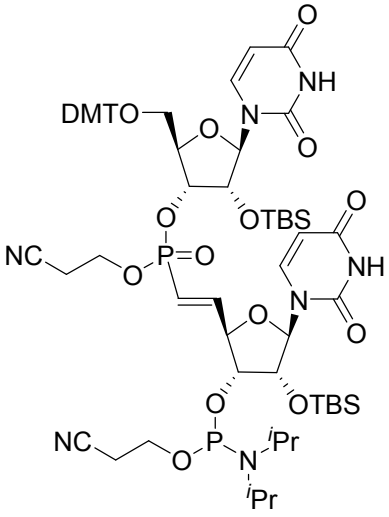
Vinyl bromide **235** (58 mg, 134 μmol), dppf (29.7 mg, 53.5 μmol) and $Pd(OAc)_2$ (6.0 mg, 26.8 μmol) were combined in a Carousel tube. A solution of *H*-phosphonate **251** (135 mg, 174 μmol) in freshly distilled THF (1.78 mL) was added followed by propylene oxide (187 mL, 2.68 μmol). The carousel tube was sealed and heated at 70 °C for 6 hours, then cooled to r.t. and evaporated to an orange foam. Purification by column chromatography (96:4 CH_2Cl_2:MeOH) obtained a 7:3 mixture of diastereoisomers of U*U dimer **266** (117 mg, 77%) as a pale yellow solid. R_f 0.29 and 0.21 (97:3 CH_2Cl_2:MeOH); $[\alpha]_D^{29}$ -38.7 (*c*

0.17, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3392 (N-H), 3215 (O-H), 3062 (C-H), 2976 (C-H), 2959 (C-H), 2932 (C-H), 2859 (C-H), 2714 (C-H), 1697 (C=O), 1636 (C=C), 1608 (C=C), 1485, 1459, 1438, 1389, 1364, 1310; δ_H (400 MHz, $CDCl_3$) 9.56 (1H, br s, N*H*), 9.51 (1H, br s, N*H*), 9.49 (1H, br s, N*H*), 9.45 (1H, br s, N*H*), 7.88 (2H, app d, *J* 8.2, U_1-6-*H* major and U_1-6-*H* minor), 7.86-7.42 (2H, m, Ar-*H* major and Ar-*H* minor), 7.37-7.33 (4H, m, 2 x Ar-*H* major and 2 x Ar-*H* minor), 7.32-7.22 (14H, m, U_2-6-*H* major, U_2-6-*H* minor, 6 x Ar-*H* major and 6 x Ar-*H* minor), 7.05 (1H, ddd, *J* 23.6, 17.3 and 4.0, U_2-5'-*H* minor), 6.99 (1H, ddd, *J* 23.4, 17.1 and 4.0, U_2-5'-*H* major), 6.88-6.83 (8H, m, 4 x Ar-*H* major and 4 x Ar-*H* minor), 6.10 (1H, ddd, *J* 20.8, 17.3 and 1.7, U_2-6'-*H* minor), 6.03 (1H, ddd, *J* 20.6, 17.4 and 1.8, U_2-6'-*H* major), 5.97 (1H, d, *J* 4.6, U_1-1'-*H* major), 5.93 (1H, d, *J* 3.9, U_1-1'-*H* minor), 5.78 (1H, app dd, *J* 8.2 and 2.0, U_2-5-*H* minor), 5.77 (1H, app dd, *J* 8.2 and 2.0, U_2-5-*H* major), 5.76 (1H, d, *J* 2.6, U_2-1'-*H* minor), 5.72 (1H, d, *J* 3.4, U_2-1'-*H* major), 5.27 (1H, app dd, *J* 8.2 and 2.0, U_1-5-*H* major), 5.02 (1H, app ddd, *J* 9.3, 5.0 and 4.5, U_1-3'-*H* minor), 5.01 (1H, app dd, *J* 8.2 and 2.0, U_1-5-*H* minor), 4.92 1H, app ddd, *J* 8.8, 4.5 and 4.3, U_1-3'-*H* major), 4.51-4.46 (1H, obs m, U_1-2'-*H* minor), 4.48 (1H, app dd, 4.5 and 4.6, U_1-2'-*H* major), 4.38-4.36 (2H, m, U_1-4'-*H* major and U_2-4'-*H* minor), 4.35-4.30 (4H, m, U_1-4'-*H* minor, U_2-4'-*H* major, C*H*$_2$CH$_2$CN major and C*H*$_2$CH$_2$CN minor), 4.34-4.31 (2H, m, U_2-3'-*H* major and U_2-3'-*H* minor), 4.22-4.10 (2H, m, C*H*$_2$CH$_2$CN major and C*H*$_2$CH$_2$CN minor), 4.01-3.91 (2H, U_2-2'-*H* major and U_2-2'-*H* minor), 3.80 (6H, app s, 2 x Ar-OC*H*$_3$

minor), 3.79 (3H, Ar-OC*H*$_3$, major), 3.78 3H, Ar-OC*H*$_3$, major), 3.69 (1H, dd, *J* 11.1 and 2.0, U$_1$-5'-*H* minor), 3.60 (1H, dd, *J* 11.1 and 2.1, U$_1$-5'-*H* major), 3.53 (1H, dd, *J* 11.1 and 2.0, U$_1$-5'-*H* minor), 3.43 (1H, dd, *J* 11.1 and 1.9, U$_1$-5'-*H* major), 2.82 (1H, app dddd, *J* 17.1, 7.7, 7.3 and 1.8, CH$_2$C*H*$_2$CN major), 2.73 (1H, app ddd, *J* 6.5, 5.7 and 4.4, CH$_2$C*H*$_2$CN major), 2.52 (1H, app dddd, *J* 37.9, 12.9, 7.5 and 5.5, CH$_2$C*H*$_2$CN minor), 2.48 1H, app dddd, *J* 38.6, 12.2, 6.2 and 5.7, CH$_2$C*H*$_2$CN minor), 0.93 (9H, s, SiC(C*H*$_3$)$_3$ minor), 0.91 (9H, s, SiC(C*H*$_3$)$_3$ major), 0.90 (9H, s, SiC(C*H*$_3$)$_3$ major), 0.88 (9H, s, SiC(C*H*$_3$)$_3$ minor), 0.15 (6H, s, SiC*H*$_3$ major and SiC*H*$_3$ minor), 0.14 6H, s, SiC*H*$_3$ major and SiC*H*$_3$ minor), 0.13 (6H, 2 x s, 2 x SiC*H*$_3$ minor), 0.12 (3H, s, SiC*H*$_3$ major), (3H, s, SiC*H*$_3$ major); δ_C (125 MHz, $CDCl_3$) 163.51 (U$_1$-4-*C*, minor), 163.47 (U$_1$-4-*C*, major), 163.43 (U$_2$-4-*C*, minor), 163.40 (U$_2$-4-*C*, major), 158.90 (Ar-*C*OCH$_3$, minor), 158.88 (Ar-*C*OCH$_3$, minor), 158.84 (Ar-*C*OCH$_3$, major), 158.81 (Ar-*C*OCH$_3$, major), 150.7 (U$_2$-2-*C*, major), 150.6 (U$_2$-2-*C*, minor), 150.14 (U$_1$-2-*C*, major), 150.10 (U$_1$-2-*C*, minor), 149.8 (d, *J* 6.8, U$_2$-5'-*C*H, major), 149.7 (d, *J* 6.7, U$_2$-5'-*C*H, minor), 144.2 (Ar-*C* major and Ar-*C* minor), 140.6 (U$_2$-6-*C*H, major), 140.2 (U$_2$-6-*C*H, minor), 139.91 (U$_1$-6-*C*H, major), 139.89 (U$_1$-6-*C*H, minor), 134.98 (Ar-*C*, minor), 134.96 (Ar-*C*, minor), 134.92 (Ar-*C*, major), 134.90 (Ar-*C*, major), 130.34 (2 x Ar-*C*H, major), 130.31 (4 x Ar-*C*H, minor), 130.28 (2 x Ar-*C*H, major), 128.31 (2 x Ar-*C*H, minor), 128.23 (2 x Ar-*C*H, major), 128.2 (2 x Ar-*C*H, minor), 128.1 (2 x Ar-*C*H, major), 127.45 (Ar-*C*H, minor), 127.37 (Ar-*C*H, major), 117.12 (d, *J* 190,

U_2-6'-*C*H, major), 117.07 (d, *J* 191, U_2-6'-*C*H, minor), 116.9 (CH_2CH_2*C*N, major), 116.7 (CH_2CH_2*C*N, minor), 113.37 (4 x Ar-*C*H major and 4 x Ar-*C*H minor), 103.21 (U_2-5-*C*H, minor), 103.17 (U_2-5-*C*H, major), 102.6 (U_1-5-*C*H, major), 102.4 (U_1-5-*C*H, minor), 91.76 (U_2-1'-*C*H, minor), 91.74 (U_2-1'-*C*H, major), 88.8 (U_1-1'-*C*H, minor), 88.4 (U_1-1'-*C*H, major), 87.5 (*C*Ph(4-OMePh)$_2$, major and minor), 83.0 (d, *J* 22.4, U_2-4'-*C*H, minor), 82.8 (d, *J* 22.0, U_2-4'-*C*H, major), 81.9 (d, *J* 4.4, U_1-4'-*C*H, major), 81.5 (d, *J* 5.3, U_1-4'-*C*H, minor), 74.91 (U_1-2'-*C*H, major), 74.88 (U_1-2'-*C*H, minor), 74.5 (U_2-3'-*C*H, major and minor), 74.3 (d, *J* 5.1, U_1-3'-*C*H, major), 73.5 (d, *J* 6.3, U_1-3'-*C*H, minor), 73.3 (U_2-2'-*C*H, major and minor), 61.9 (major, U_1-5'-*C*H_2), 61.6 (minor, U_1-5'-*C*H_2), 60.9 (major, d, *J* 4.4, *C*H_2CH_2CN), 60.8 (minor, d, *J* 4.5, *C*H_2CH_2CN), 55.4 (Ar-O*C*H_3, 2 x major and 2 x minor), 25.74 (SiC(*C*H_3)$_3$, minor), 25.73 (SiC(*C*H_3)$_3$, major and minor), 25.64 (SiC(*C*H_3)$_3$, major), 20.0 (d, *J* 6.4, CH_2*C*H_2CN, major), 19.8 (d, *J* 6.8, CH_2*C*H_2CN, minor), 18.12 (Si*C*, major and minor), 18.08 (Si*C*(CH$_3$)$_3$, major and minor), -4.61 (Si*C*H$_3$, minor), -4.66 (Si*C*H$_3$, major), -4.76 (Si*C*H$_3$, major), -4.78 (Si*C*H$_3$, minor), -4.86 (Si*C*H$_3$, minor), -4.90 (Si*C*H$_3$, major), -4.91 (Si*C*H$_3$, minor), -5.04 (Si*C*H$_3$, major); δ_P (121 MHz, $CDCl_3$) 19.7 and 19.5; *m/z* (ES+) 1152.4255 (M+Na, $C_{55}H_{73}N_3NaO_{15}PSi_2^+$ requires 1152.4199).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl) uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-2'-*O*-(*tert*-butyldimethylsilyl)-3'-*O*-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite uridine 268



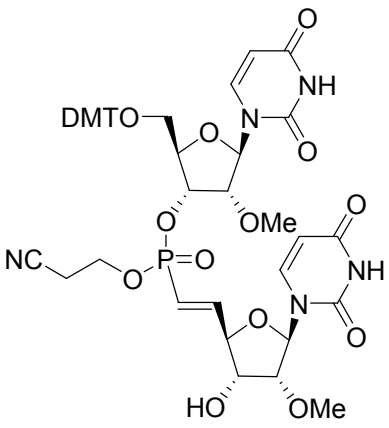
A solution of U*U dimer **266** (68 mg, 60.5 μmol) in dry CH_2Cl_2 (680 μL) was added to a flame-dried flask containing freshly activated 3 Å mol. sieves under Argon. Neat DIPEA (42 μL, 242 μmol), DMAP (1.5 mg, 12.1 μmol), and 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite (14.8 μL, 66.5 μmol) were added. Stirring under an argon atmosphere was maintained for 3 hours then the reaction mixture was filtered, diluted with CH_2Cl_2 (2 mL), washed with brine (1 mL), dried (Na_2SO_4), filtered and evaporated to a pale yellow foam. Purification by column chromatography (97:3 CH_2Cl_2:MeOH) obtained U*U phosphoramidite **268** (31 mg, 39%) as an off-white solid. R_f 0.30 (97:3 CH_2Cl_2:MeOH); δ_H (500 MHz, $CDCl_3$) 9.75-8.98 (8H, app br s, 8 x N-*H*), 7.90 (1H, d, *J* 8.1, U_1-6-*H*), 7.87 (2H, app d, *J* 8.1, U_1-6-*H*), 7.86 (1H, d, *J* 8.1, U_1-6-*H*), 7.37-7.34 (9H, m, Ar-*H*), 7.33-7.28 (9H, m, Ar-*H*), 7.28-7.24 (20H, m, 2 x U_2-6-*H* and Ar-*H*), 7.22 (2H, app d, *J* 8.0, 2 x U_2-6-*H*), 7.07 (1H, dd, *J* 17.6 and 4.2, U_2-5'-*H*), 7.02 (1H, dd, *J* 17.6 and 4.2, U_2-5'-*H*), 7.01 (1H, dd, *J* 17.3 and 4.2, U_2-5'-*H*), 6.96 (1H, dd, *J* 17.3 and 4.2, U_2-5'-*H*),

6.89-6.82 (16H, m, Ar-*H*), 6.13-6.01 (2H, m, 2 x U_2-6'-*H*), 6.03 (2H, ddd, *J* 20.7, 17.2 and 2.0, 2 x U_2-6'-*H*), 5.97-5.95 (2H, d, *J* 4.7, 2 x U_1-1'-*H*), 5.95 (1H, d, *J* 4.8, U_1-1'-*H*), 5.94 (1H, d, *J* 3.9, U_1-1'-*H*), 5.81 (1H, d, *J* 8.0, U-5-*H*), 5.77 (2H, d, *J* 8.1, U-5-*H*), 5.76-5.71 (2H, obs m, U-5-*H*), 5.72 (1H, d, *J* 8.3, U-5-*H*), 5.68 (2H, d, *J* 3.6, U_2-1'-*H*), 5.57 (2H, d, *J* 3.4, U_2-1'-*H*), 5.27 (3H, app d, *J* 8.1, U-5-*H*), 5.24 (1H, d, *J* 8.1, U-5-*H*), 5.02-4.97 (2H, m, U_1-3'-*H*), 4.93-4.87 (2H, m, U_1-3'-*H*), 4.50-4.46 (8H, 4 x U_1-2'-*H*, 2 x U_1-4'-*H*, 2 x U_2-4'-*H*), 4.38-4.31 (12H, 2 x U_1-4'-*H*, 2 x U_2-4'-*H*, 8 x C*H*$_2$CH$_2$CN), 4.31-4.26 (4H, m, 4 x U_2-3'-*H*), 4.25-4.11 (4H, 2 x C*H*$_2$CH$_2$CN), 4.10-4.02 (2H, C*H*$_2$CH$_2$CN), 3.97-3.93 (4H, 4 x U_2-2'-*H*), 3.90-3.81 (2H, m, C*H*$_2$CH$_2$CN), 3.81 (6H, s, Ar-OC*H*$_3$), 3.80 (6H, s, Ar-OC*H*$_3$), 3.80 (6H, s, Ar-OC*H*$_3$), 3.79 (6H, s, Ar-OC*H*$_3$), 3.69-3.62 (2H, m, 2 x U_1-5'-*H*), 3.60 (2H, dd, *J* 11.1 and 2.3, 2 x U_1-5'-*H*), 3.58-3.55 (1H, obs m, U_1-5'-*H*), 3.55 (2H, app d, *J* 6.9, 2 x PNC*H*), 3.55-3.49 (1H, obs m, U_1-5'-*H*), 3.52 (2H, app dd, *J* 6.9 and 2.1, 2 x PNC*H*), 3.50 (2H, app d, *J* 6.8, 2 x PNC*H*), 3.50-3.45 (2H, obs m, 2 x PNC*H*), 3.44 (2H, app ddd, *J* 11.2, 2.0 and 1.5, 2 x U_1-5'-*H*), 2.92-2.82 (4H, m, CH$_2$C*H*$_2$CN), 2.82-2.77 (4H, m, CH$_2$C*H*$_2$CN), 2.78-2.71 (8H, m, CH$_2$C*H*$_2$CN), 1.40-1.30 (6H, m, 2 x NCH(C*H*$_3$)$_2$), 1.28 (18H, d, *J* 6.9, 6 x NCH(C*H*$_3$)$_2$), 1.27 (18H, d, *J* 6.9, 6 x NCH(C*H*$_3$)$_2$), 1.21 (3H, d, *J* 6.7, NCH(C*H*$_3$)$_2$), 1.18 (3H, d, *J* 6.2, NCH(C*H*$_3$)$_2$), 0.93 (6H, s, SiC(C*H*$_3$)$_3$), 0.92 (6H, s, SiC(C*H*$_3$)$_3$), 0.91 (18H, s, SiC(C*H*$_3$)$_3$), 0.90 (9H, s, SiC(C*H*$_3$)$_3$), 0.89 (24H, s, SiC(C*H*$_3$)$_3$), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.143 (12H, s, 4 x SiC*H*$_3$), 0.137 (6H, s, 2 x SiC*H*$_3$), 0.131 (12H, s, 4 x SiC*H*$_3$), 0.128 (3H, s,

SiC*H*$_3$), 0.118 (3H, s, SiC*H*$_3$), 0.114 (6H, s, 2 x SiC*H*$_3$), 0.112 (6H, s, 2 x SiC*H*$_3$); δ_C (126 MHz, $CDCl_3$) 163.3 (U-4-*C*), 163.2 (U-4-*C*), 163.13 (3 x U-4-*C*), 163.11 (U-4-*C*), 163.04 (2 x U-4-*C*), 158.93 (Ar-*C*OCH$_3$), 158.91 (Ar-*C*OCH$_3$), 158.88 (2 x Ar-*C*OCH$_3$), 158.87 (Ar-*C*OCH$_3$), 158.85 (2 x Ar-*C*OCH$_3$), 158.81 (Ar-*C*OCH$_3$), 150.57 (U-2-*C*), 150.54 (3 x U-2-*C*), 150.49 (U-2-*C*), 150.0 (3 x U-2-*C*), 149.8 (2 x U$_2$-5'-*C*H), 149.7 (2 x U$_2$-5'-*C*H), 144.22 (2 x Ar-*C*), 144.19 (2 x Ar-*C*), 140.80 (3 x U-6-*C*H), 140.48 (U-6-*C*H), 139.91 (3 x U-6-*C*H), 139.83 (U-6-*C*H), 135.00 (Ar-*C*), 134.97 (5 x Ar-*C*), 134.94 (2 x Ar-*C*), 130.35 (5 x Ar-*C*H), 130.31 (4 x Ar-*C*H), 130.28 (5 x Ar-*C*H), 130.25 (2 x Ar-*C*H), 128.32 (2 x Ar-*C*H), 128.26 (6 x Ar-*C*H), 128.16 (Ar-*C*H), 128.13 (7 x Ar-*C*H), 127.46 (Ar-*C*H), 127.39 (3 x Ar-*C*H), 117.1 (d, *J* 190, 4 x U$_2$-6'-*C*H), 116.96 (2 x *C*N), 116.82 (4 x *C*N), 116.77 (*C*N), 116.63 (*C*N), 113.45 (16 x Ar-*C*H), 103.20 (2 x U-5-*C*H), 103.16 (U-5-*C*H), 102.94 (U-2-*C*H), 102.56 (2 x U-5-*C*H), 102.52 (U-5-*C*H), 102.49 (U-5-*C*H), 92.35 (U$_2$-1'-*C*H), 92.23 (3 x U$_2$-1'-*C*H), 88.64 (U$_1$-1'-*C*H), 88.37 (2 x U$_1$-1'-*C*H), 88.33 (U$_1$-1'-*C*H), 87.58 (4 x *C*Ph(4-OMePh)$_2$), 83.2 (d, *J* 23.1, U$_2$-4'-*C*H), 83.1 (d, *J* 22.9, U$_2$-4'-*C*H), 82.9 (d, *J* 22.5, 2 x U$_2$-4'-*C*H), 82.0 (d, *J* 4.6, 3 x U$_1$-4'-*C*H), 81.7 (d, *J* 4.6, U$_1$-4'-*C*H), 74.9 (app d, *J* 3.6, 2 x U$_1$-2'-*C*H and 3 x U$_2$-3'-*C*H), 74.8 (app d, *J* 3.7, 2 x U$_1$-2'-*C*H and U$_2$-3'-*C*H), 74.44 (U$_1$-3'-*C*H), 74.39 (3 x U$_1$-3'-*C*H), 73.7 (U$_2$-2'-*C*H), 73.6 (3 x U$_2$-2'-*C*H), 62.0 (3 x U$_1$-5'-*C*H$_2$), 61.8 (U$_1$-5'-*C*H$_2$), 60.9 (d, *J* 4.5, 3 x P(O)*C*H$_2$CH$_2$CN), 60.7 (d, *J* 4.5, P(O)*C*H$_2$CH$_2$CN), 58.9 (d, *J* 5.6, P(N^{i}Pr)*C*H$_2$CH$_2$CN), 58.3 (d, *J* 5.3, 3 x P(N^{i}Pr)*C*H$_2$CH$_2$CN), 55.37 (8

x Ar-O*C*H$_3$), 47.54 (2 x PN*C*H), 47.01 (PN*C*H), 45.45 (3 x PN*C*H), 45.40 (2 x PN*C*H), 25.7 (14 x SiC(*C*H$_3$)$_3$), 25.6 (10 x SiC(*C*H$_3$)$_3$), 23.05 (4 x PNCH*C*H$_3$), 23.04 (4 x PNCH*C*H$_3$), 22.97 (4 x PNCH*C*H$_3$), 22.95 (4 x PNCH*C*H$_3$), 20.2 (d, *J* 6.8, 2 x CH$_2$*C*H$_2$CN), 20.0 (d, *J* 6.3, 5 x CH$_2$*C*H$_2$CN), 19.8 (d, *J* 6.3, 1 x CH$_2$*C*H$_2$CN), 8.11 (4 x Si*C*), 18.09 (3 x Si*C*), 18.06 (Si*C*), -4.48 (Si*C*H$_3$), -4.63 (Si*C*H$_3$), -4.67 (2 x Si*C*H$_3$), -4.75 (2 x Si*C*H$_3$), -4.79 (2 x Si*C*H$_3$), -4.82 (Si*C*H$_3$), -4.83 (Si*C*H$_3$), -4.87 (4 x Si*C*H$_3$), -5.02 (2 x Si*C*H$_3$); δ_P (121 MHz, CDCl$_3$) 151.7 (CEPN^{i}Pr$_2$), 151.4 (CEPN^{i}Pr$_2$), 151.1 (CEPN^{i}Pr$_2$), 150.4 (CEPN^{i}Pr$_2$), 19.83 (P=O), 19.77 (P=O), 19.6 (P=O), 19.5 (P=O); *m/z* (ES+) 1352.5301 (M+Na, $C_{64}H_{89}N_7NaO_{16}P_2Si^+$ requires 1352.5272).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl uridinyl 2-cyano-ethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-2'-*O*-methyl uridine 267

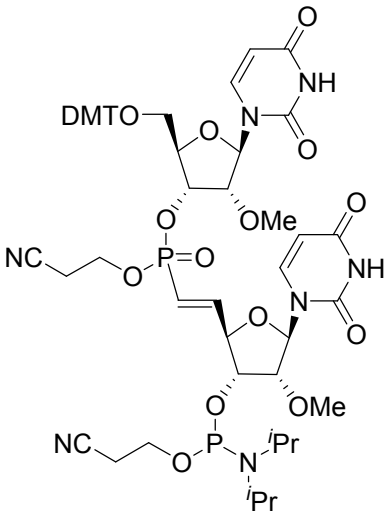


Vinyl bromide **236** (155 mg, 465 μmol), Pd(OAc)$_2$ (21 mg, 93 μmol) and dppf (103 mg, 186 μmol) were charged to a carousel tube purged with Argon. A solution of *H*-phosphonate **254** (410 mg, 605 μmol) in freshly distilled THF (6.2 mL) was added followed by propylene oxide (650 μL, 9.30 mmol). The tube was sealed and heated at 70 °C for 6 hours then cooled and evaporated to an orange foam. Purification by column chromatography (96:4 →

90:10 CH_2Cl_2:MeOH) obtained the 2'-OMe *U dimer **267** as yellow foam (420 mg, 97%). R_f 0.04 (96:4 CH_2Cl_2:MeOH); $[\alpha]_D^{29}$ +11.6 (*c* 0.35, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3392 (N-H), 2959 (C-H), 2936 (C-H), 2910 (C-H), 2839 (C-H), 1712 (C=O), 1694 (C=O), 1632 (C=C), 1608 (C=C), 1508 (C=C), 1489, 1460, 1456, 1390, 1377, 1364, 1326, 1301; δ_H (500 MHz, $CDCl_3$) 9.75-9.40 (2H, 2 x br s, N*H*), 9.28 (2H, app br s, 2 x N*H*), 7.84 (1H, d, *J* 7.9, U_1-6-*H*), 7.82 (1H, d, *J* 7.9, U_1-6-*H*), 7.53-7.45 (1H, m, Ar-*H*), 7.44-7.38 (1H, m, Ar-*H*), 7.38-7.30 (8H, m, Ar-*H*), 7.30-7.20 (10H, m, 2 x U_2-6-*H* and 8 x Ar-*H*), 7.11-6.96 (2H, m, 2 x U_2-5'-*H*), 6.91-6.81 (8H, m, Ar-*H*), 6.14 (1H, dd, *J* 19.6 and 1.11, U_2-5'-*H*), 6.05 (1H, obs dd, *J* U_2-5'-*H*), 6.04 (2H, d, *J* 3.6, 2 x U_1-1'-*H*), 5.87-5.80 (3H, m, 2 x U_2-1'-*H* and U_2-5-*H*), 5.29 (1H, d, *J* 7.9, U_1-5-*H*), 5.28 (1H, d, *J* 7.9, U_1-5-*H*), 5.17 (1H, dd, *J* 10.1 and 6.3, U_1-3'-*H*), 5.04 (1H, dd, *J* 10.6 and 5.7, U_1-3'-*H*), 4.47-4.42 (1H, m, U_2-4'-*H*), 4.36-4.31 (2H, m, U_1-4'-*H* and OC*H*$_2$$CH_2CN$), 4.34-4.24 (1H, obs m, U_1-4'-*H*), 4.30-4.25 (2H, m, U_2-4'-*H* and OC*H*$_2$$CH_2CN$), 4.15-4.09 (2H, m, U_1-2'-*H* and OC*H*$_2$$CH_2CN$), 4.08-4.02 (2H, m, U_1-2'-*H* and U_2-3'-*H*), 4.01-3.94 (2H, m, U_2-3'-*H* and OC*H*$_2$$CH_2CN$), 3.89 (1H, dd, *J* 4.1 and 3.0, U_2-2'-*H*), 3.85 (1H, dd, *J* 4.8 and 3.5, U_2-2'-*H*), 3.67-3.64 (1H, m, U_1-5'-*H*), 3.61-3.58 (1H, m, U_1-5'-*H*), 3.61 (3H, s, U_2-2'-OC*H*$_3$), 3.60 (3H, s, U_2-2'-OC*H*$_3$), 3.57 (3H, s, U_1-2'-OC*H*$_3$), 3.53 (3H, s, U_1-2'-OC*H*$_3$), 3.42-3.38 (2H, m, 2 x U_1-5'-*H*), 2.80-2.78 (2H, m, CH_2C*H*$_2$CN), 2.66-2.53 (2H, m, CH_2C*H*$_2$CN); δ_C (125 MHz, $CDCl_3$) 163.3 (U_2-4-*C*), 163.2 (U_1-4-*C*), 163.08 (U_1-4-*C*), 162.9 (U_2-4-*C*), 158.94 (Ar-*C*OCH_3), 158.91 (Ar-*C*OCH_3), 158.88 (Ar-

*C*OCH$_3$), 158.82 (Ar-*C*OCH$_3$), 150.6 (U-2-*C*), 150.4 (U-2-*C*), 150.0 (U-2-*C*), 149.9 (U-2-*C*), 149.5 (d, *J* 5.9, U$_2$-5'-*C*H), 148.7 (d, *J* 6.0, U$_2$-5'-*C*H), 144.1 (Ar-*C*), 144.0 (Ar-*C*), 140.0 (U-5-*H*), 139.9 (U-5-*H*), 139.7 (U-5-*H*), 139.6 (U-5-*H*), 135.1 (Ar-*C*), 134.94 (Ar-*C*), 134.86 (Ar-*C*), 134.80 (Ar-*C*), 130.4 (2 x Ar-*C*H), 130.3 (4 x Ar-*C*H), 130.2 (2 x Ar-*C*H), 128.4 (2 x Ar-*C*H), 128.21 (2 x Ar-*C*H), 128.17 (4 x Ar-*C*H), 127.5 (Ar-*C*H), 127.4 (Ar-*C*H), 118.1 (d, *J* 95.1, U$_2$-6'-*C*H), 116.8 (CH$_2$CH$_2$*C*N), 116.59 (CH$_2$CH$_2$*C*N), 116.56 (d, *J* 93.7, U$_2$-6'-*C*H), 113.5 (8 x Ar-*C*H), 103.2 (U$_2$-5-*C*H), 103.1 (U$_2$-5-*C*H), 102.8 (U$_1$-5-*C*H), 102.6 (U$_1$-5-*C*H), 89.8 (U$_2$-1'-*C*H), 89.5 (U$_2$-1'-*C*H), 87.6 (*C*Ph(4-OMePh)$_2$), 87.4 (*C*Ph(4-OMePh)$_2$), 86.9 (U$_1$-1'-*C*H), 86.5 (U$_1$-1'-*C*H), 82.9 (d, *J* 18.1, U$_2$-4'-*C*H), 82.73 (d, *J* 19.0, U$_2$-4'-*C*H), 82.76 (d, *J* 18.0, U$_2$-2'-*C*H), 82.70 (d, *J* 17.1, U$_2$-2'-*C*H), 82.52 (d, *J* 12.2, U$_1$-2'-*C*H), 82.45 (d, *J* 10.2, U$_1$-2'-*C*H), 81.9 (d, *J* 7.3, U$_1$-4'-*C*H), 81.8 (d, *J* 7.38, U$_1$-4'-*C*H), 73.0 (U$_1$-3'-*C*H), 72.9 (U$_1$-3'-*C*H), 72.4 (U$_2$-3'-*C*H), 72.3 (U$_2$-3'-*C*H), 61.5 (U$_1$-5'-*C*H$_2$), 61.4 (U$_1$-5'-*C*H$_2$), 60.6 (d, *J* 5.6, O*C*H$_2$CH$_2$CN), 60.2 (d, *J* 4.9, O*C*H$_2$CH$_2$CN), 59.01 (U$_2$-2'-O*C*H$_3$), 58.98 (U$_2$-2'-O*C*H$_3$), 58.83 (U$_1$-2'-O*C*H$_3$), 58.81 (U$_1$-2'-O*C*H$_3$), 55.4 (4 x Ar-O*C*H$_3$), 20.0 (d, *J* 6.4, OCH$_2$*C*H$_2$CN), 19.8 (d, *J* 7.0, OCH$_2$*C*H$_2$CN); δ_P (121 MHz, CDCl$_3$) 19.5 and 19.4; *m/z* (ES+) 952.2793 (M+Na, $C_{45}H_{48}N_5NaO_{15}P^+$ requires 952.2782).

5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl uridinyl 2-cyanoethylphosphono-[3'(O)→5'(C)]-5'-deoxy-5'-methylidene-2'-*O*-methyl-3'-*O*-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite uridine 269



To a flame dried flask containing freshly activated 3Å mol. sieves was added a solution of U*U dimer **267** (160 mg, 172 μmol) in dry CH_2Cl_2 (1.60 mL). Freshly distilled DIPEA (120 μL, 689 μmol), DMAP (4.2 μg, 34.4 μmol) then 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite (42 μL, 189 μmol) were added and stirring was maintained for 2 ½ hours. The reaction mixture was then filtered, eluting with CH_2Cl_2 (2 x 2 mL) and the combined organics were washed with brine (2 mL), dried (Na_2SO_4), filtered and concentrated to a pale brown solid. Purification by column chromatography (95:5 CH_2Cl_2:MeOH) obtained the U*U CEP **269** as an off-white foam (81 mg, 42%). R_f 0.06 95:5 CH_2Cl_2:MeOH); δ_H (400 MHz, $CDCl_3$) 9.95-8.75 (br s, 8 x N*H*), 7.86 (1H, d, *J* 8.2, U_1-6-*H*), 7.85 (1H, d, *J* 8.1, U_1-6-*H*), 7.83 (1H, d, 8.2, U_1-6-*H*), 7.81 (1H, d, *J* 8.1, U_1-6-*H*), 7.38-7.30 (16H, m, Ar-*H*), 7.30-7.21 (24H, m, 4 x U_2-6-*H* and 20 x Ar-*H*), 7.22-6.93 (4H, m, 4 x U_2-5'-*H*), 6.89-6.82 (16H, m, Ar-*H*), 6.17-6.04 (4H, m, 4 x U_2-6'-*H*), 6.05 (1H, d, *J* 4.2, U_1-1'-*H*), 6.04 (2H, app d, *J* 3.9, 2 x U_1-1'-*H*), 6.03 (1H, d, *J* 3.5, U_1-1'-*H*), 5.85 (1H, d, *J* 3.7, U_2-1'-*H*), 5.84 (1H, d, *J* 4.0, U_2-1'-*H*), 5.83 (1H, d, *J* 3.7, U_2-1'-*H*),

5.82 (1H, d, *J* 3.9, U_2-1'-*H*), 5.78 (2H, app d, *J* 7.5, 2 x U_2-5-*H*), 5.75 (1H, d, *J* 8.1, U_2-5-*H*), 5.74 (1H, d, *J* 8.0, U_2-5-*H*), 5.29 (3H, app d, *J* 8.2, 3 x U_1-5-*H*), 5.25 (1H, app d, *J* 8.0, U_1-5-*H*), 5.18-5.12 (2H, m, 2 x U_1-3'-*H*), 5.10-5.05 (2H, m, 2 x U_1-3'-*H*), 4.74-4.69 (1H, m, U_2-4'-*H*), 4.68-4.63 (1H, m, U_2-4'-*H*), 4.65-4.60 (1H, m, U_2-4'-*H*), 4.60-4.55 (1H, m, U_2-4'-*H*), 4.40-4.05 (8H, obs m, 4 x OC*H*$_2$CH$_2$CN), 4.35-4.31 (2H, obs m, 2 x U_1-4'-*H*), 4.32-4.25 (4H, obs m, 2 x U_1-4'-*H* and 2 x U_2-3'-*H*), 4.25-4.15 (2H, obs m, 2 x U_2-3'-*H*), 4.15-4.09 (2H, obs m, 2 x U_1-2'-*H*), 4.09-4.04 (2H, obs m, 2 x U_1-2'-*H*), 4.05-3.92 (4H, obs m, 4 x U_2-2'-*H*), 4.05-3.84 (6H, obs m, 3 x OC*H*$_2$CH$_2$CN), 3.81 (12H, s, 4 x Ar-OC*H*$_3$), 3.80 (6H, s, 2 x Ar-OC*H*$_3$), 3.79 (6H, s, 2x Ar-OC*H*$_3$), 3.71-3.60 (12H, obs m, 2 x U_1-5'-*H*, 8 x PNC*H*, OCH$_2$C*H*$_2$CN), 3.61 (3H, s, 2'-OC*H*$_3$), 3.60, (3H, s, 2'-OC*H*$_3$), 3.58-3.42 (6H, m, 6 x U_1-5'-*H*), 3.55 (3H, s, 2'-OC*H*$_3$), 3.54 (3H, s, 2'-OC*H*$_3$), 3.53 (6H, 2 x s, 2 x 2'-OC*H*$_3$), 3.52 (3H, s, 2'-OC*H*$_3$), 3.51 (3H, s, 2'-OC*H*$_3$), 2.81-2.77 (4H, app dd, *J* 12.2 and 6.0, 2 x OCH$_2$C*H*$_2$CN), 2.78-2.70 (4H, m, 4 x OCH$_2$C*H*$_2$CN), 2.67-2.53 (8H, m, 2 x OCH$_2$C*H*$_2$CN), 1.29 (3H, d, *J* 6.8, NCH(C*H*$_3$)$_2$), 1.27 (3H, d, *J* 7.2, NCH(C*H*$_3$)$_2$), 1.26 (3H, d, *J* 8.1, NCH(C*H*$_3$)$_2$), 1.22 (12H, app d, *J* 6.7, 4 x NCH(C*H*$_3$)$_2$), 1.19 (27H, app d, *J* 6.7, 9 x NCH(C*H*$_3$)$_2$); δ_C (125 MHz, $CDCl_3$) 163.12 (U-4-*C*), 163.09 (U-4-*C*), 163.01 (2 x U-4-*C*), 162.93 (2 x U-4-*C*), 162.86 (2 x U-4-*C*), 158.95 (2 x Ar-*C*), 158.92 (2 x Ar-*C*), 158.91 (2 x Ar-*C*), 158.89 (Ar-*C*), 158.87 (Ar-*C*), 150.43 (U-2-*C*), 150.41 (U-2-*C*), 150.39 (U-2-*C*), 150.36 (U-2-*C*), 150.00 (2 x U-2-*C*), 149.96 (2 x U-2-*C*), 149.6 (d, *J* 6.7, U_2-5'-*H*), 149.1 (d, *J* 6.7, U_2-5'-*H*), 148.9 (d, *J* 6.5,

U_2-5'-*H*), 148.5 (d, *J* 6.3, U_2-5'-*H*), 144.2 (Ar-*C*), 144.1 (Ar-*C*), 144.04 (Ar-*C*), 143.99 (Ar-*C*), 140.8 (U_2-6-*C*H), 140.53 (U_2-6-*C*H), 140.50 (2 x U_2-6-*C*H), 139.8 (U_1-6-*C*H), 139.68 (U_1-6-*C*H), 139.66 (U_1-6-*C*H), 139.57 (U_1-6-*C*H), 134.97 (Ar-*C*), 134.94 (Ar-*C*), 134.90 (Ar-*C*), 134.87 (2 x Ar-*C*), 134.82 (2 x Ar-*C*), 134.80 (Ar-*C*), 130.41 (4 x Ar-*C*H), 130.37 (4 x Ar-*C*H), 130.27 (4 x Ar-*C*H), 130.17 (4 x Ar-*C*H), 128.39 (4 x Ar-*C*H), 128.19 (6 x Ar-*C*H), 128.16 (6 x Ar-*C*H), 127.48 (Ar-*C*H), 127.45 (Ar-*C*H), 127.41 (Ar-*C*H), 127.36 (Ar-*C*H), 118.6 (d, *J* 94.6, U_2-6'-*C*H), 118.3 (2 x OCH_2CH_2*C*N), 117.9 (2 x OCH_2CH_2*C*N), 117.6 (d, *J* 122.8, U_2-6'-*C*H), 117.1 (d, *J* 94.4, U_2-6'-*C*H), 116.9 (OCH_2CH_2*C*N), 116.8 (OCH_2CH_2*C*N), 116.6 (OCH_2CH_2*C*N), 116.4 (OCH_2CH_2*C*N), 116.1 (d, *J* 122.3, U_2-6'-*C*H), 113.4 (16 x Ar-*C*H), 103.15 (U_1-5-*C*H), 103.14 (U_1-5-*C*H), 103.05 (2 x U_1-5-*C*H), 102.69 (2 x U_2-5-*C*H), 102.63 (U_2-5-*C*H), 102.58 (U_2-5-*C*H), 90.76 (U_2-1'-*C*H), 90.65 (U_2-1'-*C*H), 90.3 (U_2-1'-*C*H), 90.0 (U_2-1'-*C*H), 87.5 (3 x *C*Ph(4-OMePh)$_2$), 87.4 (*C*Ph(4-OMePh)$_2$), 86.92 (U_1-1'-*C*H), 86.87 (U_1-1'-*C*H), 86.83 (U_1-1'-*C*H), 86.6 (U_1-1'-*C*H), 82.7 (d, *J* 5.1, 2 x U_1-2'-*C*H), 82.40 (d, *J* 3.1, 2 x U_2-4'-*C*H), 82.37 (d, *J* 8.9, U_1-2'-*C*H), 82.24 (d, *J* 3.8, U_1-2'-*C*H), 82.23 (d, *J* 3.9, U_1-2'-*C*H), 82.04 (U_1-4'-*C*H), 82.00 (U_1-4'-*C*H), 81.97 (d, *J* 4.9, U_2-2'-*C*H), 81.92 (d, *J* 5.0, U_2-2'-*C*H), 81.8 (app s, 2 x U_2-4'-*C*H), 81.76 (U_1-4'-*C*H), 81.70 (U_1-4'-*C*H), 81.5 (d, *J* 4.4, U_2-2'-*C*H), 81.4 (d, *J* 4.5, U_2-2'-*C*H), 74.20 (d, *J* 14.8, U_2-3'-*C*H), 74.16 (d, *J* 15.4, U_2-3'-*C*H), 73.8 (d, *J* 15.9, U_2-3'-*C*H), 73.7 (d, *J* 14.9, U_2-3'-*C*H), 72.6 (d, *J* 5.4, U_1-3'-*C*H), 72.5 (d, *J* 5.6, U_1-3'-*C*H), 72.40 (d, *J* 4.2, U_1-3'-*C*H), 72.36 (d, *J* 4.3, U_1-3'-*C*H), 61.5

(U_1-5'-*C*H_2), 61.35 (U_1-5'-*C*H_2), 61.26 (2 x U_1-5'-*C*H_2), 60.7 (app d, *J* 5.4, 2 x P(O)O*C*H_2CH$_2$CN), 60.2 (d, *J* 5.4, (PO)O*C*H_2CH$_2$CN), 60.1 (d, *J* 5.1, (PO)O*C*H_2CH$_2$CN), 58.8 (2 x 2'-O*C*H_3), 58.82 (2 x 2'-O*C*H_3), 58.77 (2'-O*C*H_3), 58.75 (2'-O*C*H_3), 58.74 (2'-O*C*H_3), 58.70 (2'-O*C*H_3), 58.54 (d, *J* 19.0, P(N^{i}Pr$_2$)O*C*H_2CH$_2$CN), 58.53 (d, *J* 18.9, P(N^{i}Pr$_2$)O*C*H_2CH$_2$CN), 57.9 (d, *J* 20.2, P(N^{i}Pr$_2$)O*C*H_2CH$_2$CN), 57.8 (d, *J* 20.3, P(N^{i}Pr$_2$)O*C*H_2CH$_2$CN), 55.4 (4 x Ar-O*C*H_3), 55.3 (4 x Ar-O*C*H_3), 43.6 (2 x PN*C*H), 43.5 (2 x PN*C*H), 43.5 (2 x PN*C*H), 43.4 (2 x PN*C*H), 24.78 (2 x PNCH(*C*H_3)), 24.73 (6 x PNCH(*C*H_3)), 24.69 (6 x PNCH(*C*H_3)), 24.63 (2 x PNCH(*C*H_3)), 20.50 (app d, *J* 6.1, 2 x OCH$_2$*C*H$_2$CN), 20.49 (app d, *J* 6.3, 2 x OCH$_2$*C*H$_2$CN), 19.9 (app d, *J* 6.5, 2 x OCH$_2$*C*H$_2$CN), 19.8 (app d, *J* 6.5, 2 x OCH$_2$*C*H$_2$CN); δ_P (121 MHz, $CDCl_3$) 151.8 (CEPN^{i}Pr$_2$), 151.7 (2 x CEPN^{i}Pr$_2$), 150.9 (CEPN^{i}Pr$_2$), 19.7 (P=O), 19.5 (P=O), 19.3 (P=O), 18.9 (P=O); *m/z (ES+)* 1152.3895 (M+Na, $C_{54}H_{65}N_7NaO_{16}P_2^+$ requires 1152.3861).

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- APPENDIX -

6. Appendix

X-ray Crystallographic Data

6-*Z*-bromo-(5-deoxy-5-methylidene)-1,2-*O*-isopropylidene-α-D-ribofuranose

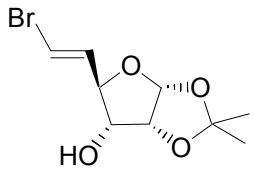

Table 1. Crystal data and structure refinement for BRETGL at 150(2)K.

Empirical formula	$C_9 H_{13} Br O_4$
Formula weight	265.10
Crystal description	colourless plate
Crystal size	0.74 x 0.36 x 0.06 mm
Crystal system	Orthorhombic
Space group	P 21 21 21
Unit cell dimensions	a = 5.5264(10) Å α = 90 deg.
	b = 8.611(2) Å β = 90 deg.
	c = 22.806(4) Å γ = 90 deg.
Volume	1085.3(6) $Å^3$
Reflections for cell refinement	4240
Range in theta	2.5 to 27.0°.
Z	4
Density (calculated)	1.622 Mg/m^3
Absorption coefficient	3.775 mm^{-1}
F(000)	536
Diffractometer type	Bruker SMART APEX CCD area detector
Wavelength	0.71073 Å

Scan type	omega
Reflections collected	9613
Theta range for data collection	2.53 to 26.33°
Index ranges	-7<=h<=7, -11<=k<=11, -28<=l<=29
Independent reflections	2208 [R(int) = 0.055]
Observed reflections	1903 [I>2σ(I)]
Absorption correction	Semi-empirical from equivalents (T_{min} = 0.503, T_{max} = 1.000)
Decay correction	none
Structure solution by	direct and difference Fourier methods
Hydrogen atom location	OH from delta-F; others geometrically placed
Hydrogen atom treatment	rigid rotor; riding model
Data / restraints / parameters	2208/0/129 (least-squares on F^2)
Final R indices [I>2σ(I)]	R1 = 0.0446, wR2 = 0.108
Final R indices (all data)	R1 = 0.0518, wR2 = 0.111
Goodness-of-fit on F^2	1.04
Absolute structure parameter	0.03(2)
Final maximum delta/sigma	0.001
Weighting scheme	Calc w=1/[\s^2^(Fo^2^)+(0.067P)^2^] where P=(Fo^2^+2Fc^2^)/3
Largest diff. peak and hole	1.14 and -0.53 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for BRETGL. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	*y*	*z*	*U(eq)*
O	328(5)	3548(4)	7133(1)	26(1)
C1	522(7)	4001(5)	6570(2)	24(1)
C2	2797(7)	4960(4)	6661(2)	22(1)
O1'	1293(5)	2703(4)	6247(2)	29(1)
C1'	3700(7)	2986(5)	6031(2)	25(1)
O2'	4718(4)	4030(4)	6449(1)	24(1)
C2'	5094(8)	1512(6)	6041(2)	32(1)
C3'	3568(9)	3747(6)	5438(2)	36(1)
C3	3045(7)	5060(5)	7326(2)	24(1)
O3	5453(5)	5162(4)	7529(1)	28(1)
C4	1714(7)	3630(5)	7531(2)	22(1)
C5	820(8)	3698(5)	8144(2)	26(1)
C6	1717(8)	2799(6)	8547(2)	29(1)
Br	803(1)	2897(1)	9346(1)	47(1)

Table 3. Bond lengths [Å], angles and torsions [°] for BRETGL.

O-C1	1.421(5)
O-C4	1.450(5)
C1-O1'	1.405(5)
C1-C2	1.519(6)
C1-H1	1.0000
C2-O2'	1.415(5)
C2-C3	1.525(6)
C2-H2	1.0000
O1'-C1'	1.439(5)
C1'-O2'	1.427(5)
C1'-C2'	1.485(6)

C1'-C3'	1.505(6)
C2'-H2'A	0.9800
C2'-H2'B	0.9800
C2'-H2'C	0.9800
C3'-H3'A	0.9800
C3'-H3'B	0.9800
C3'-H3'C	0.9800
C3-O3	1.412(5)
C3-C4	1.508(6)
C3-H3	1.0000
O3-H3O	0.8400
C4-C5	1.484(6)
C4-H4	1.0000
C5-C6	1.300(6)
C5-H5	0.9500
C6-Br	1.892(4)
C6-H6	0.9500
C1-O-C4	107.1(3)
O1'-C1-O	110.8(3)
O1'-C1-C2	104.7(3)
O-C1-C2	107.4(3)
O1'-C1-H1	111.2
O-C1-H1	111.2
C2-C1-H1	111.2
O2'-C2-C1	105.4(3)
O2'-C2-C3	107.7(3)
C1-C2-C3	103.9(3)
O2'-C2-H2	113.0
C1-C2-H2	113.0
C3-C2-H2	113.0
C1-O1'-C1'	109.0(3)
O2'-C1'-O1'	104.0(3)
O2'-C1'-C2'	108.9(3)
O1'-C1'-C2'	109.3(4)
O2'-C1'-C3'	110.3(4)
O1'-C1'-C3'	109.7(4)
C2'-C1'-C3'	114.3(4)
C2-O2'-C1'	106.8(3)

C1'-C2'-H2'A	109.5
C1'-C2'-H2'B	109.5
H2'A-C2'-H2'B	109.5
C1'-C2'-H2'C	109.5
H2'A-C2'-H2'C	109.5
H2'B-C2'-H2'C	109.5
C1'-C3'-H3'A	109.5
C1'-C3'-H3'B	109.5
H3'A-C3'-H3'B	109.5
C1'-C3'-H3'C	109.5
H3'A-C3'-H3'C	109.5
H3'B-C3'-H3'C	109.5
O3-C3-C4	114.2(4)
O3-C3-C2	114.4(3)
C4-C3-C2	102.6(3)
O3-C3-H3	108.4
C4-C3-H3	108.4
C2-C3-H3	108.4
C3-O3-H3O	109.5
O-C4-C5	109.4(3)
O-C4-C3	103.0(3)
C5-C4-C3	114.9(4)
O-C4-H4	109.7
C5-C4-H4	109.7
C3-C4-H4	109.7
C6-C5-C4	121.0(4)
C6-C5-H5	119.5
C4-C5-H5	119.5
C5-C6-Br	123.5(4)
C5-C6-H6	118.2
Br-C6-H6	118.2
C4-O-C1-O1'	91.7(4)
C4-O-C1-C2	-22.1(4)
O1'-C1-C2-O2'	-7.4(4)
O-C1-C2-O2'	110.4(4)
O1'-C1-C2-C3	-120.6(3)
O-C1-C2-C3	-2.7(4)
O-C1-O1'-C1'	-128.1(3)

C2-C1-O1'-C1'	-12.6(4)
C1-O1'-C1'-O2'	27.8(4)
C1-O1'-C1'-C2'	144.0(4)
C1-O1'-C1'-C3'	-90.1(4)
C1-C2-O2'-C1'	24.8(4)
C3-C2-O2'-C1'	135.3(3)
O1'-C1'-O2'-C2	-32.4(4)
C2'-C1'-O2'-C2	-148.8(3)
C3'-C1'-O2'-C2	85.2(4)
O2'-C2-C3-O3	37.7(5)
C1-C2-C3-O3	149.3(3)
O2'-C2-C3-C4	-86.5(3)
C1-C2-C3-C4	25.1(4)
C1-O-C4-C5	161.0(3)
C1-O-C4-C3	38.2(4)
O3-C3-C4-O	-162.9(3)
C2-C3-C4-O	-38.5(4)
O3-C3-C4-C5	78.2(5)
C2-C3-C4-C5	-157.4(3)
O-C4-C5-C6	131.8(4)
C3-C4-C5-C6	-112.9(5)
C4-C5-C6-Br	176.3(3)

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for BRETGL. The anisotropic displacement factor exponent takes the form:

$$-2\pi^2 [h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$$

	U11	*U22*	*U33*	*U23*	*U13*	*U12*
O	9(1)	36(2)	33(2)	2(1)	-2(1)	-2(1)
C1	16(2)	26(2)	29(2)	3(2)	-1(2)	1(2)
C2	13(2)	13(2)	41(3)	-1(2)	3(2)	1(2)
O1'	12(1)	32(2)	43(2)	6(1)	-2(1)	-4(1)
C1'	15(2)	34(2)	25(2)	-1(2)	2(2)	-1(2)
O2'	11(1)	29(2)	33(2)	-1(1)	2(1)	1(1)
C2'	26(3)	36(2)	35(3)	0(2)	5(2)	6(2)
C3'	37(3)	37(3)	34(3)	3(2)	-5(2)	9(2)
C3	11(2)	23(2)	37(3)	-5(2)	-2(2)	1(2)

O3	14(1)	30(2)	39(2)	-9(1)	-2(1)	-2(1)
C4	13(2)	21(2)	32(2)	-2(2)	-4(2)	1(2)
C5	22(2)	21(2)	36(2)	-4(2)	5(2)	-1(2)
C6	29(2)	32(2)	28(2)	0(2)	-2(2)	-5(2)
Br	62(1)	49(1)	30(1)	-2(1)	1(1)	-16(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for BRETGL.

	x	*y*	*z*	*U(eq)*
H1	-737	4603	6352	28
H2	2717	6002	6469	27
H2'A	5101	1090	6440	48
H2'B	6761	1712	5915	48
H2'C	4340	762	5774	48
H3'A	2637	4712	5467	54
H3'B	2773	3043	5160	54
H3'C	5207	3980	5299	54
H3	2142	5996	7466	29
H3O	6246	4396	7406	41
H4	2764	2694	7479	26
H5	-426	4411	8244	32
H6	2893	2052	8435	35