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(*E*)-Vinylphosphonates: Nuclease-stable phosphate mimics for effective single-stranded RNA gene silencing

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Abstract

The therapeutic efficacy of double-stranded short interfering RNAs (ds-siRNAs) is limited by the formation of the lipid complex to deliver them to cells. Whereas if effective single-stranded siRNAs (ss-siRNAs) could be developed, they would offer an easier way of delivery and they can be produced at half price. Chemical modifications to ss-siRNAs are required to improve gene-silencing efficacy *in vivo* and to enhance their nuclease stability. This study describes a synthetic route that can access all the possible combinations of the modified (*E*)-vinylphosphonate ((*E*)-VP) linked-dinucleotides. Additionally, it demonstrates a method to synthesise a ss-siRNA contain a single 5′-terminus (*E*)-vinyphosphonate (5′-(*E*)-VP) using the standard phosphoramidite method that does not require additional deprotection steps. The study shows that the vinylphosphonate is well tolerated in the 5′-terminus between nucleotide 1 and nucleotide 2 of the ss-siRNA, and that shown efficient *in vitro* and *in vivo* knockdown of luciferase in MDA-MB fluc cells in comparison to a negative control.

This study shows that (*E*)-VP linked-dinucleotides can also be incorporated in the 3′-terminus of the oligonucleotide, this can extend the study to investigate the effect of the presence of multiple vinylphosphonates on the overall stability and activity of the oligonucleotide. Furthermore, the study describes a synthetic method to synthesise dinucleotide and trinucleotide that have two and three consecutive 5′-(*E*)-VP but can only be used in the 5′-termius of the oligonucleotide. Finally, this thesis will suggest a synthetic route to synthesise consecutive vinylphosphonates that possibly can be used at any position of the oligonucleotide.

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Abbreviations

A	Adenine
Ac	Acetyl
AGO	Argonaute
ASO	Antisense oligonucleotide
ATR	Attenuated total reflectance
Bz	Benzoyl
C	Cytosine
CE	2-Cyanoethyl
CM	Cross-metathesis
d	Day(s)
DMF	*N*,*N*'-Dimethylformamide
DMP	Dess-Martin periodinane
DMTr	4,4'-Dimethoxytrityl
DNA	Deoxyribonucleic acid
dppf	1,1'-Bis(diphenylphosphino)ferrocene
dsRNA	double-stranded ribonucleic acid
eq	Equivalent(s)
Et	Ethyl
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
G	Guanine
Grubbs II	Second-generation Grubbs catalyst
h	Hour(s)
Hoveyda Grubbs II	Second-generation Hoveyda-Grubbs catalyst
^{i}Bu	Isobutyryl
^{i}Pr	Isopropyl

kDa	Kilodalton
m.p	Melting point
m/z	Mass/charge (ratio)
m^6A	N-6-methyladenosine
Me	Methyl
min	Minute(s)
mmol	millimoles
MOE	methoxyethyl
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
NMI	*N*-Methylimidazole
NMR	Nuclear Magnetic Resonance
nt	Nucleotide
o	*ortho*
OMe	methoxy
Ph	Phenyl
PMO	phosphorodiamidate morpholino oligomer
PT	phosphorothioate
*R*f	Retention factor
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
RNase	Ribonuclease
rt	Room temperature
siRNA	Short interfering ribonucleic acid
SMN1	survival motor neuron 1
ss-RNA	Single-stranded ribonucleic acid
t	*tert*
T	Thymine

TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl
VP	vinylphosphonate

Contents

1 Introduction

1.1 RNA therapeutics

Recent developments in genome research have led to the use of short oligonucleotides (antisense oligonucleotides ASOs) as therapeutic agents to reduce disease-related protein function by targeting messenger ribonucleic acids (mRNA).[1] The therapeutic strategy of antisense technology is based on hybridizing target mRNAs with a complementary synthetic oligonucleotide *via* Watson-Crick base-pair interactions. Once the oligonucleotide base-pairs with the target mRNA, this results in degradation of the target RNA or sterically block the translation, which leads to protein synthesis being inhibited (**Figure 1**).[2]

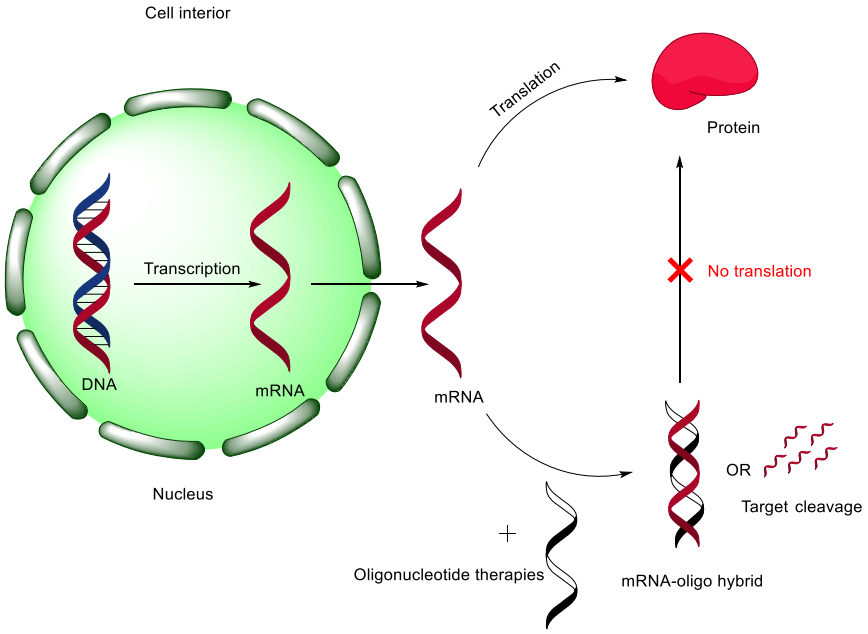


Figure 1. The general mechanism of action of the oligonucleotide therapies. The use of a sequence, complementary by Watson-Crick base pair hybridization, to a specific mRNA, can inhibit its expression and then induce a blockade in the transfer of genetic information from DNA to protein.[3]

The use of a synthetic short oligonucleotide to target a specific gene was first demonstrated by Zamecnik and Stephenson in 1978 were the 13mer d(A-A-T-G-G-T-A-A-A-A-T-G-G) was synthesized and used *in vitro* to inhibit the viral replication in the *Rous sarcoma* virus by blocking the RNA translation.[4,5] Two

decades after the Zamecnik and Stephenson research, the first antisense drug, Fomivirsen, was approved by the USA Food and Drug Administration (FDA) for marketing to treat cytomegalovirus (CMV) retinitis.

1.2 Antisense oligonucleotide's chemical modifications

Simple phosphodiester ASOs are rapidly degraded in cells by endonuclease and exonuclease enzymes, found in the serum and within the mammalian cells, leading to a shortened duration of activity.[6] The unmodified ASOs failed to reach the therapeutic end-point in clinical trials, mainly due to their poor *in vivo* stability,[1,3,4] rapid turnover and ineffective uptake of ASOs by the cell to suppress target genes.[1] Thus, several chemical modifications have now been incorporated in the ASOs, resulting in improved *in vitro* and *in vivo* pharmacological characteristics such as; nuclease stability, binding affinity for RNA targets and cellular uptake.[6–8]

1.2.1 First-generation antisense oligonucleotides

First-generation ASOs are the ones having a phosphorothioate (PT) modified backbone. The PT modification has been commonly used in ASOs therapies. Replacing one of the non-bridging phosphate oxygen **1**,**2** with a sulphur **3**,**4** creates a significant difference in the overall ASO characteristics (**Figure 2**).

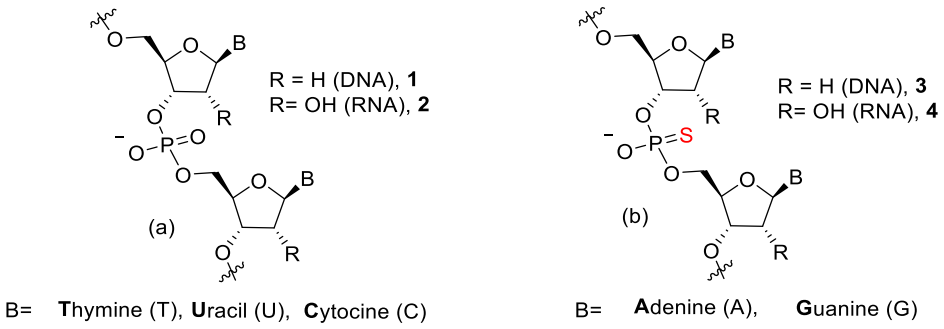


Figure 2. Phosphorothioate modification (PT). (a) Backbone structure of unmodified backbone 1, DNA (B= A (adenine), T (thymine), G (guanine), C (cytosine)) and, 2, RNA (B= A (adenine), U (uracil), C (cytosine), G (guanine)). (b) Backbone structure of PS modified backbone.

Phosphorothioate antisense oligonucleotides poses higher resistance against nuclease degradation, leading to higher bioavailability. Additionally, they are soluble in water and have good antisense activity.[9] Moreover, phosphorothioate oligonucleotides can be introduced to the cells by gymnotic "naked" uptake without using electroporation or transfection agents.[6,7]

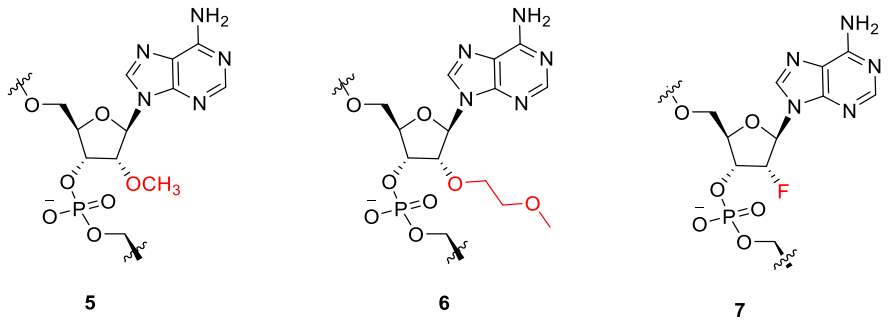
Although phosphorothioate modification promotes a modest reduction in binding affinity to the mRNA target,[6,10] in compensation, it improves resistance to nuclease degradation in the cells and blood and increases binding to albumin and other serum proteins. Thus increasing half-lives from minutes to days and retarding renal elimination.[4,6,7,10,11] Although, these features can enhance the pharmacokinetics and circulation time. There is significant toxicity resulting from PT oligonucleotides binding with serum proteins e.g. albumin and Apolipoproteina.[6,10]

The phosphorothioate modification can be synthesized using standard solid-phase oligonucleotide synthesis protocols.[6,12] PT modification appears in the FDA-approved drugs; Fomivirsen, Mipomersen, and Nusinersen. [7,13]

In order to overcome the issue with the decreased binding affinity of PT ASOs, more modifications have been developed to increase the binding affinity of ASOs to mRNA target.

1.2.2 Second-generation antisense oligonucleotides

The second-generation represents ASOs in which the structural modification is not limited to the backbone linkage but also includes structural modifications of the ribose; **2′-sugar modification** has been commonly used to provide one of the best ways to enhance antisense oligonucleotide drug properties. This single 2′-modification can improve nuclease resistance and increase the binding affinity to the mRNA target.[6] **2′-O-Methyl (2′-OMe), and 2′-O-methoxyethyl (2′-MOE)**, and **2′-Flouro (2′-F)** are the most common examples of 2′-sugar modifications (**Figure 3**) which have been widely used.



5 **6** **7**

Figure 3. 2′-OMe **5**, 2′-O-(2-MOE) **6**, and 2′-F modifications.

They increase the binding affinity to RNA in addition to enhancing nuclease resistance, and that is due to their ability to promote A-helix or RNA-like conformation.[6,7,10] 2′-modification can be found in the FDA-approved drugs in combination with PT modification such as; Mipomersen, Pegaptanib, and Nusinersen.[7,10,13]

1.2.3 Third-generation antisense oligonucleotides

Third-generation ASOs have been developed to further enhance nuclease resistance, increase binding affinity, and to improve pharmacokinetics and biostability. They are characterized by chemical modifications of the nucleotide, and more precisely to its furanose ring.[14] **phosphorodiamidate morpholino oligomer (PMO) modification** has been frequently used to provides neutral backbone and high resistance to nucleases. These are non-charged ASOs whose pentose sugar is substituted by a morpholino ring and the inter-nucleotide linkages are phosphorodiamidate bonds in place of phosphodiester bonds (**Figure 4**).[8] PMO modification can be found in Eteplirsen; recently FDA-approved oligonucleotides drug.[7,13]

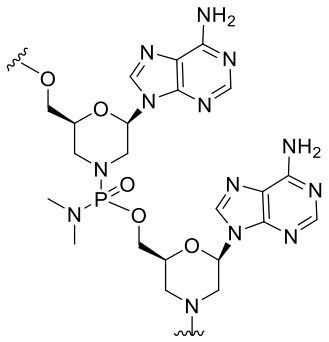


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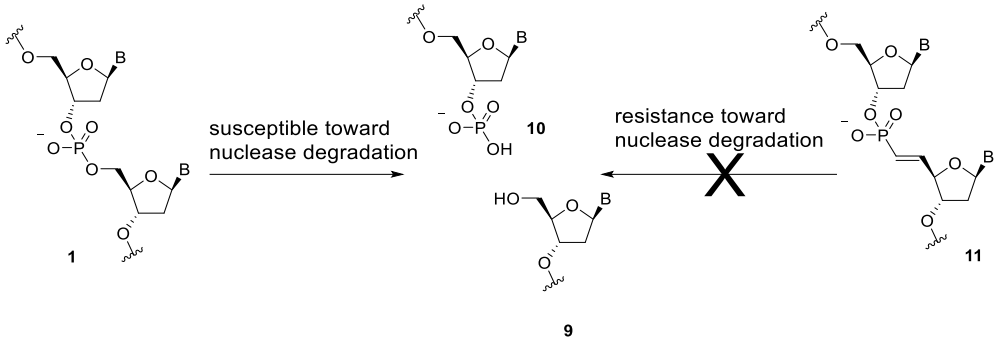
Figure 4. Phosphorodiamidate morpholino oligomer (PMO) modifications.

1.2.4 (*E*)-Vinylphosphonate modification

It was shown by Zhao and Caruthers, that vinylphosphonate-containing oligonucleotides **11** are more stable than the corresponding unmodified oligonucleotides towards nuclease degradation. By replacing the 5′-oxygen with a carbon in **1** results in a new 5′-phosphorus-carbon bond in **11** that is more stable (**Scheme 1**).[15]



Scheme 1. The difference in stability towards nuclease degradation of the natural **1** and modified backbone **11** of the nucleic acid.

They synthesized three oligonucleotides (**12**, **13**, and **14**); oligonucleotide **12** (dT_{15}) was unmodified, oligonucleotide **13** ($dT_6(T^*T)T_6$) has a single (*E*)-VP modification, and **14** ($dT(T^*T)_6T$) has six vinylphosphonate modification. The three oligonucleotides were tested *in vitro* with snake venom phosphodiesterase enzyme to evaluate their resistance towards hydrolysis. The results shown that; oligonucleotide **12** was completely degraded by the enzyme, **13** has shown significant stability against degradation. While, **14** remained undigested as only one band was observed by polyacrylamide gel electrophoresis analysis (**Figure 5**).[15]

12: 5′-TTT TTT TTT TTT TTT-3′

13: 5′-TTT TTT T*T TTT TTT-3′

14: 5′-TT*TT*TT*TT*TT*TT*T T-3′

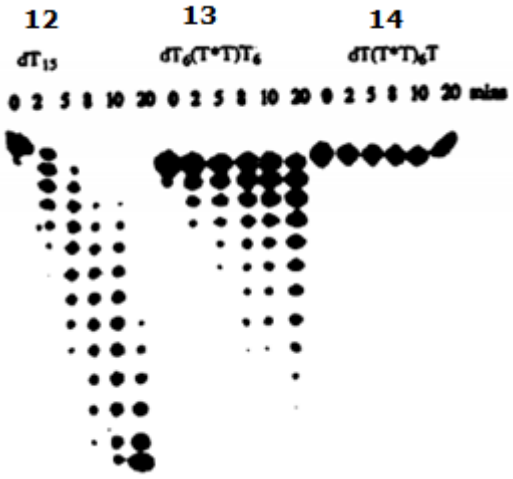


Figure 5: Denaturing polyacrylamide gel electrophoresis analysis of degraded deoxyoligonucleotides **12**, **13**, and **14** (* represent vinylphosphonate). 5′-32p end labelled oligonucleotides were treated with snake venom phosphodiesterase.[15]

The Hayes group in collaboration with the Soultanas group has been working on the synthesis and biological applications of vinylphosphonate containing oligonucleotides **11**. They have shown that a single vinylphosphonate modification has significantly inhibited the activity of the PcrA helicase enzyme on the translocating strand.[16] The more interesting result was the stability of these modified oligonucleotides against the nuclease enzymes. When three DNA substrates were synthesised, **15** had no modifications, **16** had four (*E*)-VP modifications in the centre of the sequence and **17** had one (*E*)-VP backbone.[17] All three substrates were exposed to endonuclease III digestion, and the products were run on a gel (**Figure 6**). No bands were observed for the unmodified substrate **15**, meaning the DNA was completely degraded. Whereas, undigested products were observed for substrates; **16** and **17**, and they corresponded to the position where the (*E*)-vinylphosphonate modification was placed. This result demonstrated that the modification alters cleavage specificity of exonuclease III.[17]

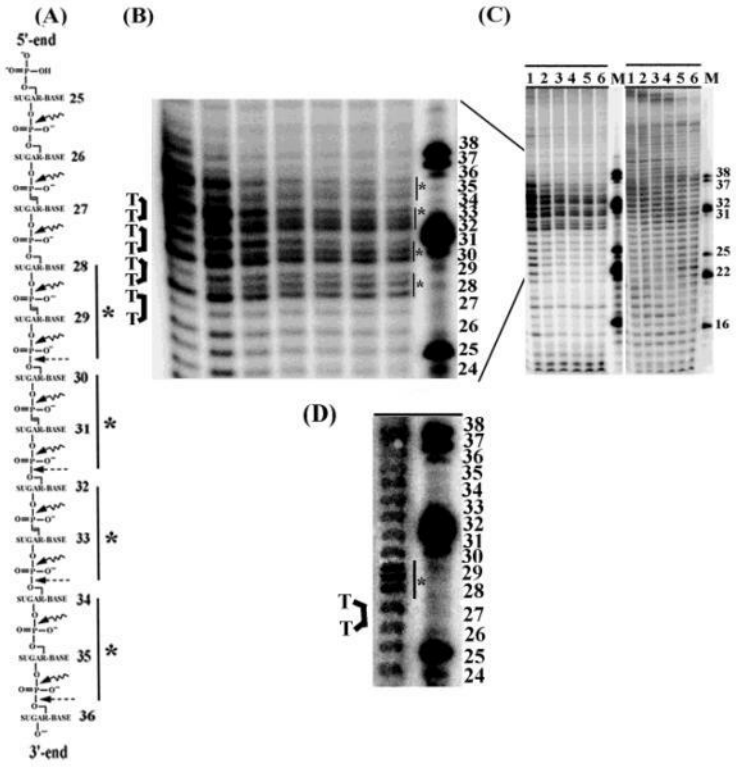


Figure 6. Time-course experiments of exonuclease III digestion of substrate **15**, **16** and **17**. The precise positions of the cleavages are shown by wiggly arrows, whereas the vinylphosphonate induced cleavages are shown by interrupted arrows in panel (**A**). The corresponding triplets are denoted with asterisks in both panels (**A**) and (**B**). The magnified region (nucleotides 24–38) containing the triplets is shown in panel (**B**). The complete gels showing the exonuclease III time course digestions using substrates **15** and **16** are shown in panel (**C**). Labels 1–6 indicate termination of the reactions 15, 30, 45, 60, 90, and 120 s after addition of the exonuclease III, respectively. Lanes marked M contained size markers. Panel (**D**) shows similar reactions with substrate **17**. All the samples from the time points were pooled and run in one lane alongside size markers. One triplet was apparent.[17]

In the last few years, (*E*)-VP modification has been incorporated in siRNA applications and shown to have a positive influence on the oligonucleotide activity and nuclease resistance. [18–26] (more details will be discussed later in section 1.5).

1.3 FDA-approved oligonucleotide drugs

After several years of slow-going progress, the pace of development for oligonucleotide therapeutics has dramatically-accelerated with many clinical trials reaching decisive stages during 2016-2019.

In the past three decades, oligonucleotides have been under clinical investigation beginning with ASOs and followed by siRNA in the last 15 years.[13] During that period most of the research in this field has been focused on designing strategies to produce more stable and effective oligonucleotides along with developing methods to study their delivery. It has led to investigating numerous oligonucleotide therapies in the clinic.[27–30] As a result, eight oligonucleotide drugs have been approved by the FDA.[13,7,31]

1.3.1 Fomivirsen (Vitravene)

Fomivirsen was the first antisense drug approved by FDA in 1998 under the trade name Vitravene, and it was the first commercially available antisense oligonucleotide drug. It was developed by the National Institutes of Health (NIH) and Isis Pharmaceuticals, and subsequently licensed to Novartis. Fomivirsen consists of 21 nucleotides and has phosphorothioate backbone to protect from endonuclease degradation. Fomivirsen has the following sequence; 5'-GCG TTT GCT CTT CTT CTT GCG-3' (**Figure 7**).

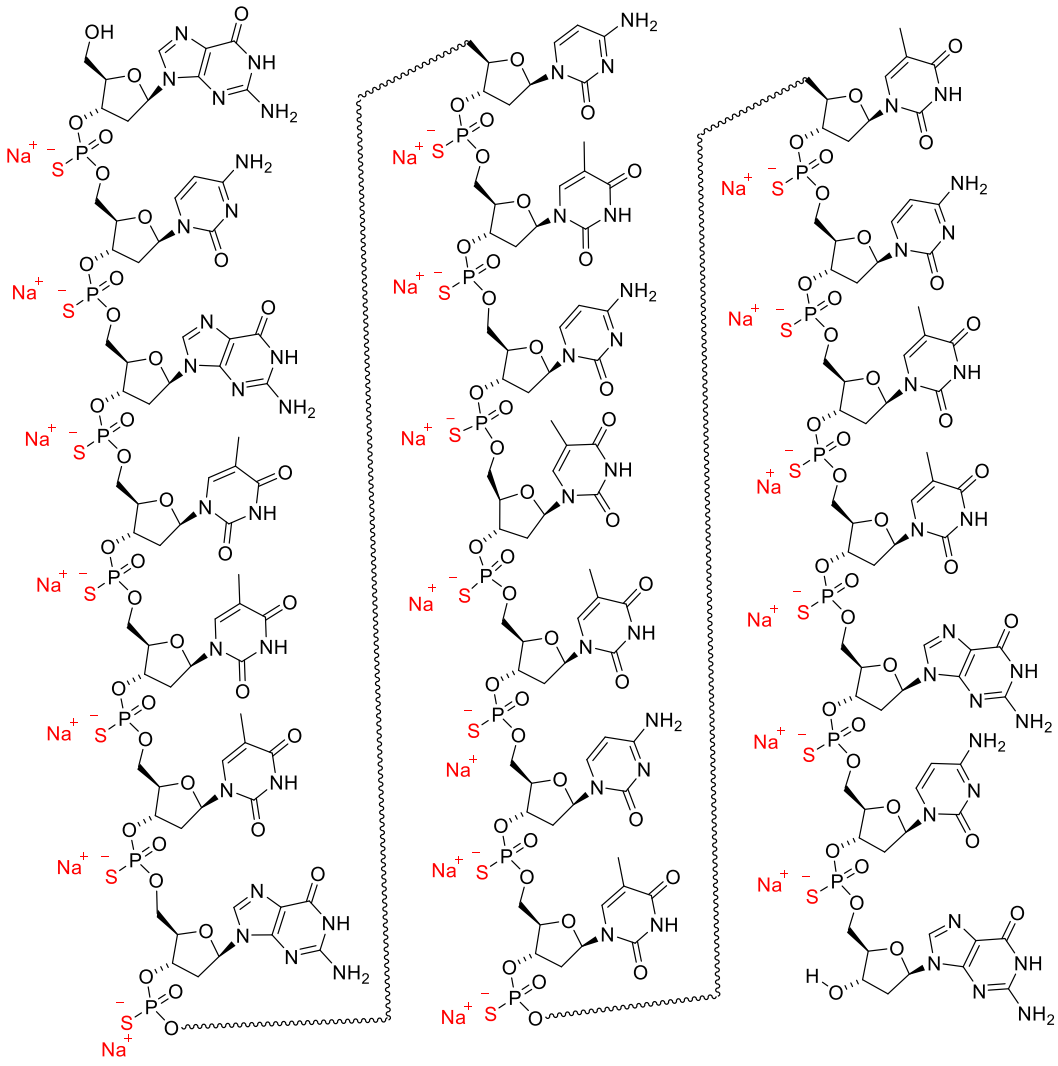


Figure 7. The chemical structure of 5′→3′ antisense oligonucleotide (Fomivirsen). Fully sulfurized backbone shown in red.

Fomivirsen is an antiviral drug used to treat cytomegalovirus (CMV) retinitis in immunocompromised patients, including those with AIDS.[32,33] It was designed to bind to the complementary sequence of human CMV immediate-early 2 (IE2) mRNA, which encodes several proteins responsible for the regulation of viral gene expression that is essential for viral replication. As a result, the translation of viral mRNA is blocked through steric blocking mechanism. In this mechanism, the antisense oligonucleotide binds to the complementary mRNA target without causing degradation.[34] The antisense oligonucleotides are usually chemically modified to enhance their affinity to the targeted mRNA. When the oligonucleotide is bound to the target mRNAs at a position near to the AUG start

codon and small ribosomal subunit (pink), it provides a steric hindrance, as a consequence, prevents the recruitment of the large ribosomal subunit (black) and thus blocks the translation of mRNA into protein. (**Figure 8**).[35,36]

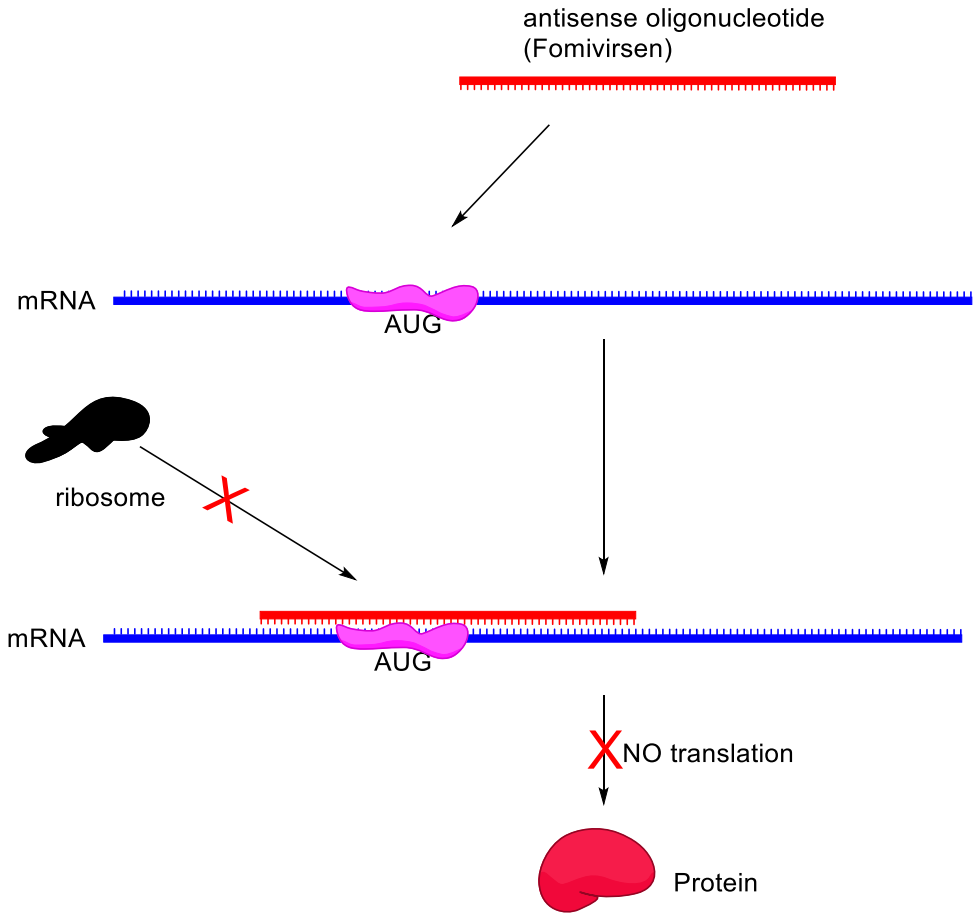


Figure 8. Steric blocking mechanism. Antisense oligonucleotide binds to the targeted mRNA close to the AUG start codon and small ribosomal subunit (pink). This binding prevents the recruitment of the large ribosomal subunit (black) and thus blocks the translation of mRNA into protein.[4,36]

The recommended dosage of Fomivirsen involves an induction dose on days 1 and 15, followed by a monthly intravitreal injection of 330 µg. This drug was discontinued from marketing by developers in 2006.[13] This is because the number of CMV cases has significantly decreased, and maybe because of the unfavourable delivery method of Fomivirsen which is ocular injection.

1.3.2 Pegaptanib (Macugen)

Pegaptanib was approved by the FDA in 2004 to treat age-related macular degeneration (AMD) of the retina; this disease causes blindness in people over 50 age. Pegaptanib is administrated in 0.3 mg dose intravitreous injection once every 6 weeks to the affected eye. [13,37] It was developed by NeXstar Pharmaceuticals and licensed in 2000 to EyeTech Pharmaceuticals in collaboration with Pfizer.

Pegaptanib is a pegylated anti-vascular endothelial growth factor (VEGF), has been designed to binds to the 165 isoform of VEGF, a protein that is involved in the growth of blood vessels and in making them more permeable. Pegaptanib injected into the eye blocks VEGF. This reduces the growth of blood vessels in the eye and controls the leakage and swelling. Pegaptanib is an ’aptamer’ (a short DNA, RNA oligonucleotide, or peptide that binds and blocks a specific molecular target protein) consists of 27 nucleotides, modified with 2’- fluoro and 2’-methoxy in combination. The chemical structure contains a 3’-3’ deoxythymidine cap to protect from nuclease enzyme and a 40 kDa polyethylene glycol substituent was linked to the 5’ terminus (**Figure 9**).

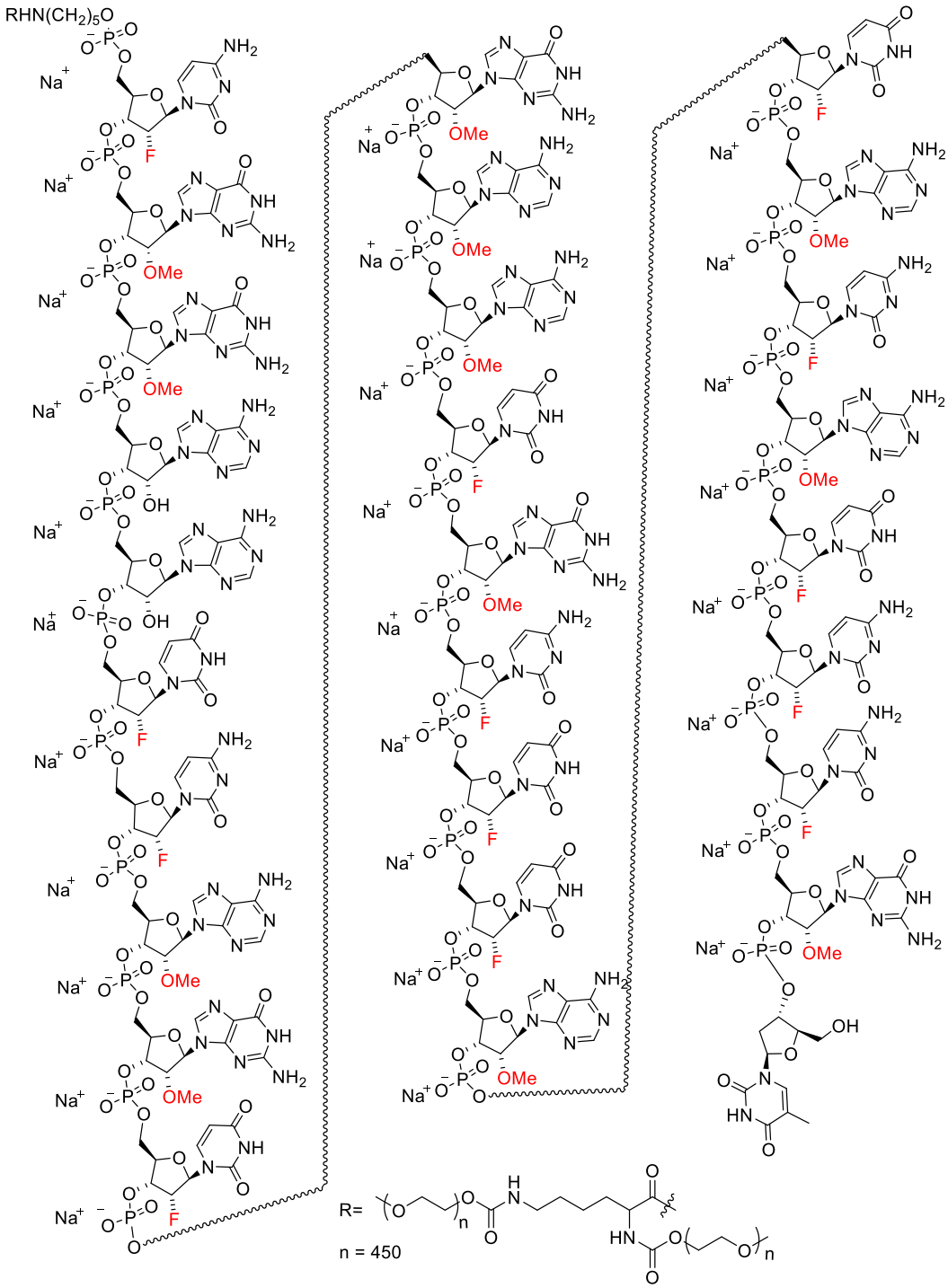


Figure 9. The chemical structure of 5′→5′ antisense oligonucleotide (Pegaptanib).

1.3.3 Mipomersen (Kynamro)

In 2013 the FDA approved Mipomersen, known under the trade name as Kynamro, designed by Isis Pharmaceuticals to reduce expression of apolipoprotein B (apoB), decrease cholesterol levels, and treat familial hypercholesterolemia.[38–40] It is administrated by 200 mg subcutaneous injection once weekly. Mipomersen is a 'gapmer' 20-mer antisense oligonucleotide, 2'-methoxyethoxy modification are incorporated in positions at 1-5 and 15-20 of the fully sulfurized oligonucleotide chain. Mipomersen has the following sequence: 5'-$\underline{G^{Me}C^{Me}C^{Me}U^{Me}C}$ $AGT^{Me}CTG^{Me}CTT^{Me}C\underline{G^{Me}CA^{Me}C^{Me}C}$-3', (Underlined letters are 2'-O-(2-methoxyethyl) ribonucleotides; non-underlined letters are 2'-deoxyribonucleotides) (**Figure 10**). ASOs that have only 2'-modification will not be able to cleave the target mRNA but can only block the translation. In order to make them able to cleave the target then the presence of ten central DNA nucleotides are required to allow the ASO work through the RNase H mechanism, which results in cleavage of the mRNA target.[13]

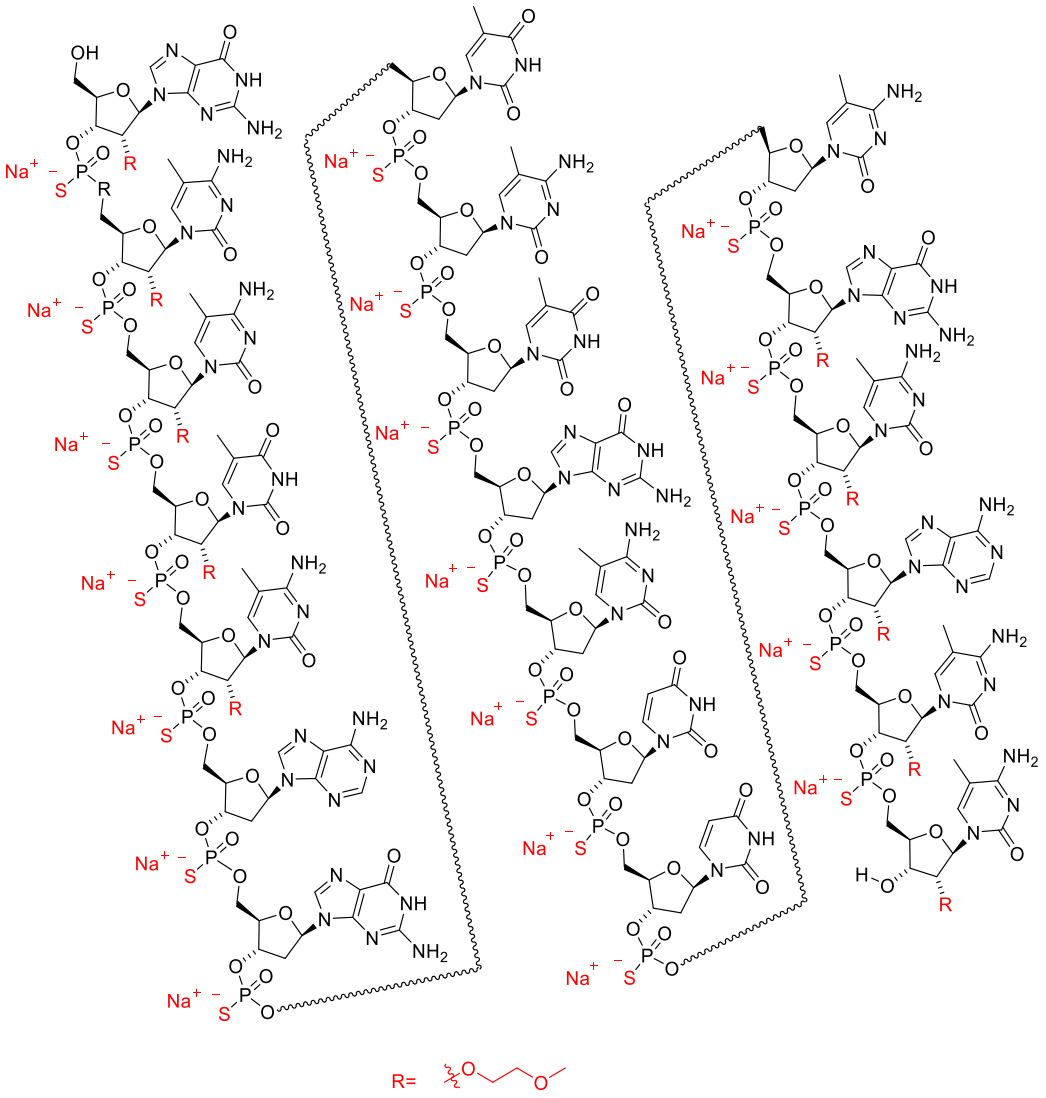


Figure 10. The chemical structure of 5′→3′ antisense oligonucleotide (Mipomersen). Fully sulfurized backbone shown in red, R represents methoxyethoxy modification.

The RNase H is an endonuclease enzyme that can hydrolyse RNA in RNA:DNA hybrids. Once bound to the mRNA target, the 2′-deoxyoligonucleotide can form an RNA:DNA duplex that is a substrate for an RNase H enzymes, which then stimulate mRNA cleavage. (**Figure 11**). Thus, inhibits protein synthesis.[41, 4,7]

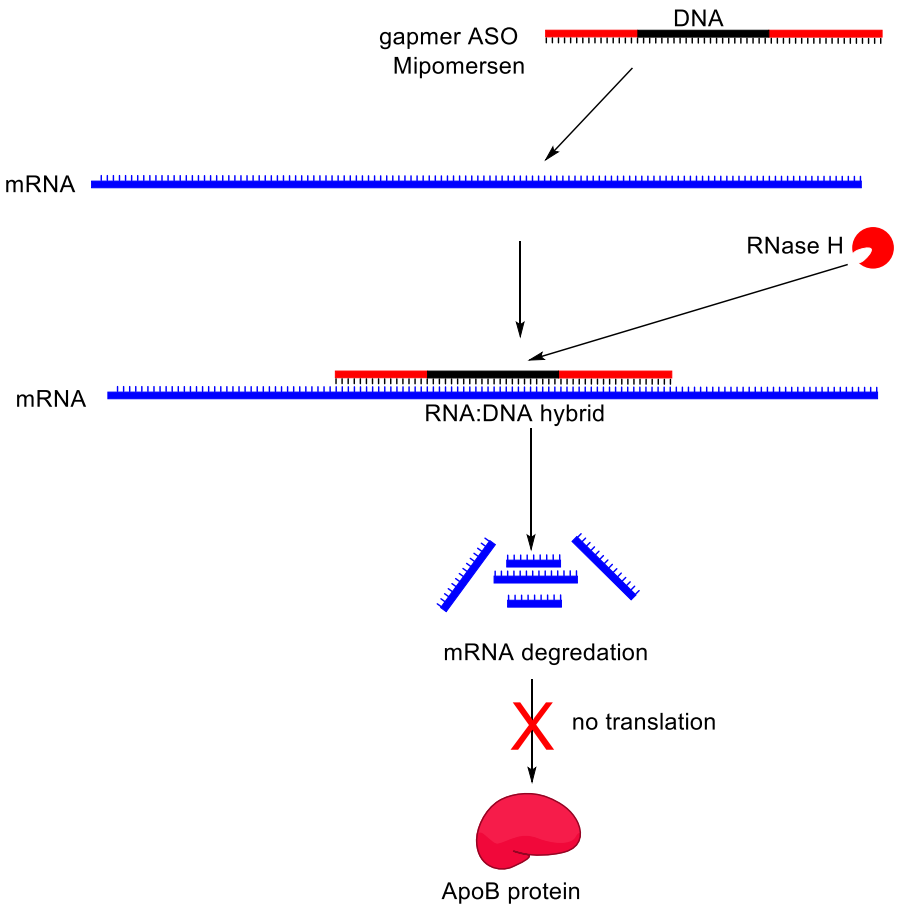


Figure 11. mRNA cleavage by the activating of the RNase.[4,7]

Antisense oligonucleotides that work through this approach should have at least five consecutive DNA nucleotides.[4] The presence of 2'deoxy residues is required to form the RNA:DNA hybrid which activates the RNase H to cleave the target. The most efficient design for ASOs that activate RNase H is the one that has sulfurized backbone to increase the nuclease stability with 2'-modification in both oligonucleotide terminus to increase the affinity and central DNA chain "gapmer".[7]

1.3.4 Eteplirsen (Exondys 51)

Eteplirsen was developed by Sarepta Therapeutics and approved by the FDA in 2016 to treat Duchenne muscular dystrophy (DMD) in young male patients.[7,13,] Eteplirsen is a 30-nucleotide phosphorodiamidate morpholino oligomer (PMO), with the sequence 5'-CTCCAACATCAAGGAAGATGGCATTTCT-3'. In contrast to

regular RNA or DNA, subunits are connected *via* phosphorodiamidate linkages that are neutrally charged at physiological pH and the PMO bases are attached to a morpholine moiety[42] (**Figure 12**).

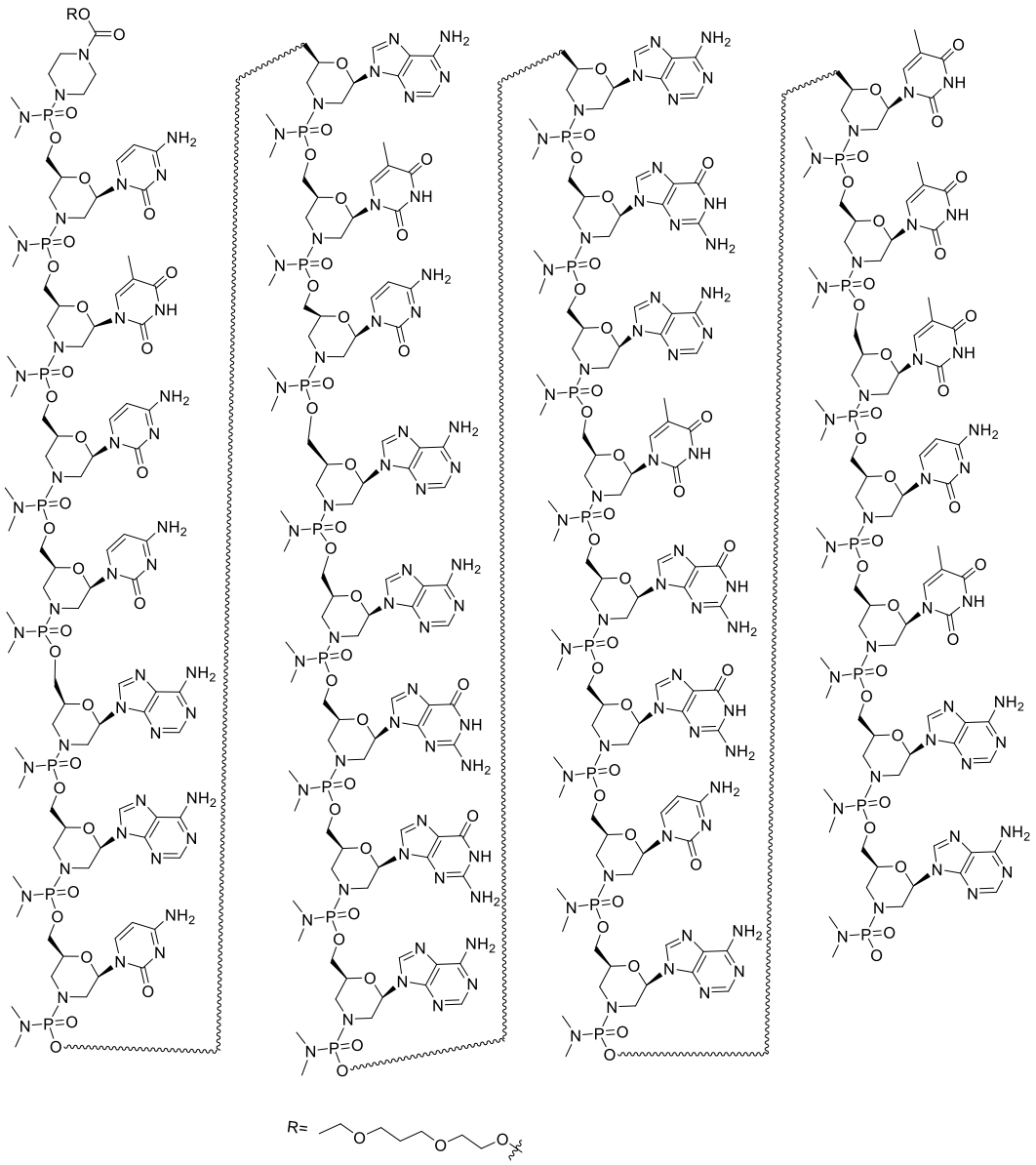


Figure 12. The structure of 5′→3′ ASO (Eteplirsen); A PMO oligonucleotide.

DMD is a genetic disorder characterized by progressive muscle degeneration and weakness caused by an absence of dystrophin; this protein is required to keep muscle cells intact. Thus, the lack of this protein will cause muscle disproportionation, and patients with DMD disease lose mobility within 10-12 years from birth and they die afterwards suffering from heart and respiratory

failure.[36] Eteplirsen can treat DMD through a splice-switching mechanism; DNA is transcribed into pre-mRNA, which contains both Exon and Intron units. The information for protein synthesis is stored in the exons, while introns must be removed and degraded. The pre-mRNA is processed by proteins, and small nuclear RNAs in the spliceosome attaches to an exon-intron boundary, removes intron and joint exon to form the mature mRNA.[43] Sometimes, an intron is retained in the mature mRNA, or an exon is skipped or extended, this alteration can cause disease. In DMP patients, the inclusion of mutant exon 51 leads to the creation of a non-productive protein if not corrected (left, **Figure 13**). Eteplirsen is designed to target mutated-exon 51 and that creates a steric block to the binding of the splicing factor to its cognate binding site.[44] This block causes the spliceosome to skip exon 51 and read the transcript in frame to produce a shorter but functional dystrophin-like protein (right, **Figure 13**).[4,7]

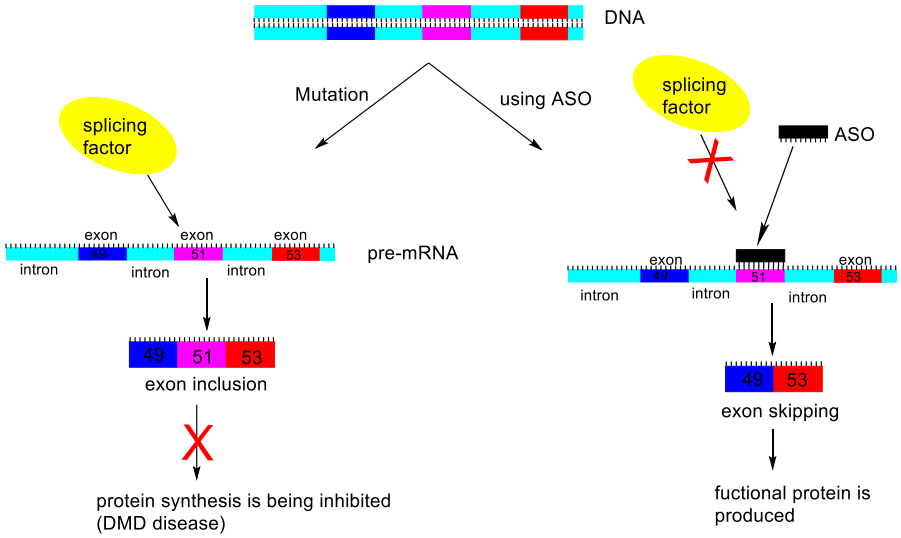


Figure 13. Splice switching mechanism. ASOs can control alternative splicing by binding to pre-mRNA and disrupting recognition by splicing factors.[7,3]

1.3.5 Defibrotide (Defitelio)

Defibrotide by Jazz Pharmaceuticals was approved by the FDA in 2016 to treat severe hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS). Defibrotide is a polydisperse mixture of 90% single-stranded and 10% double-stranded phosphodiester oligonucleotide, approximate length 9-80-mer; average50-mer; average molecular mass 16.5±2.5 kDa. This drug is a natural product, cannot be produced *via* DNA solid-phase synthesis, and extracted from intestinal mucosal DNA of pigs. It works through very complicated non-specific mechanisms of action.

Defibrotide seems to suppress the formation of blood clots and to dissolve blood clots *via* increasing levels of prostaglandin I2 and E2, altering platelet activity, increasing tissue plasminogen activator function, and decreasing activity of tissue plasminogen activator inhibitor. Prostaglandin I2 relaxes the smooth muscle of blood vessels and helps to prevent platelets from sticking to each other. While Prostaglandin E2 inhibits platelet aggregation together. Furthermore, Defibrotide has been shown to have anti-inflammatory, anti-ischemic, and antithrombotic properties.[13,45]

1.3.6 Nusinersen (Spinraza)

Nusinersen was approved by the FDA in December-2016 to treat infants with spinal muscular atrophy (SMA).[46] About 400 infants every year are born with this disease in the United States; these infants suffer from muscle weakness, difficulty swallowing, and difficulty breathing. [13] Nusinersen is an antisense 18-mer oligonucleotide, and it has the following sequence: 5'-TCACTTTCATAATGCTGG-3', cytidines are 5-methylated, all sugars are 2'-O-methoxyethyl (MOE) modified, and the backbone is fully sulfurized (**Figure 14**).

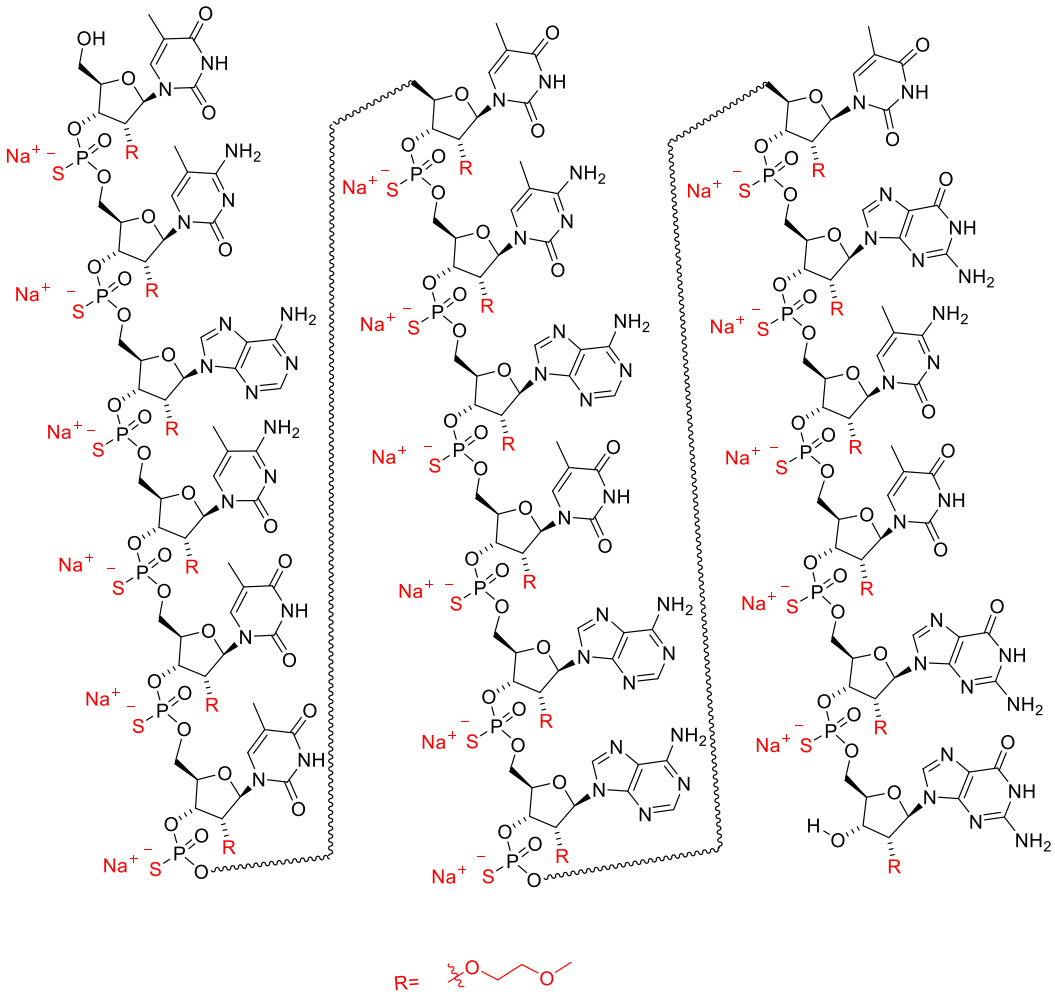


Figure 14. The chemical structure of 5′→3′ oligonucleotide (Nusinersen).

In individuals with SMA, a deleted survival motor neuron 1 (SMN1) gene does not produce SMN protein. This protein is essential for muscle function, and its absence can lead to progressive muscle weakness and atrophy. SMN2, closely related to the SMN1 gene, it does not produce enough full-length SMN protein, the exclusion of exon 7 leading to produce unfunctional SMN protein that is rapidly degraded. Nusinersen works through splice-switching mechanism by binding to a specific sequence in the intron downstream of exon 7 (between exons 7 and 8) of the SMN2 transcript, which forces the inclusion of exon 7 into the mRNA and thereby increases the amount of full-length SMN2 produced by SMN2, rescuing the cell from SMN deficiency (**Figure 15**).[47] Nusinersen was discovered and developed by Biogen and Ionis Pharmaceuticals, and it should

be administrated by 12 mg intrathecally per dose, the initial dose involves 4 loading doses; the first 3 doses at 14-day intervals and the fourth dose 30 days after the third dose. The Maintenance dose is once every 4 months.

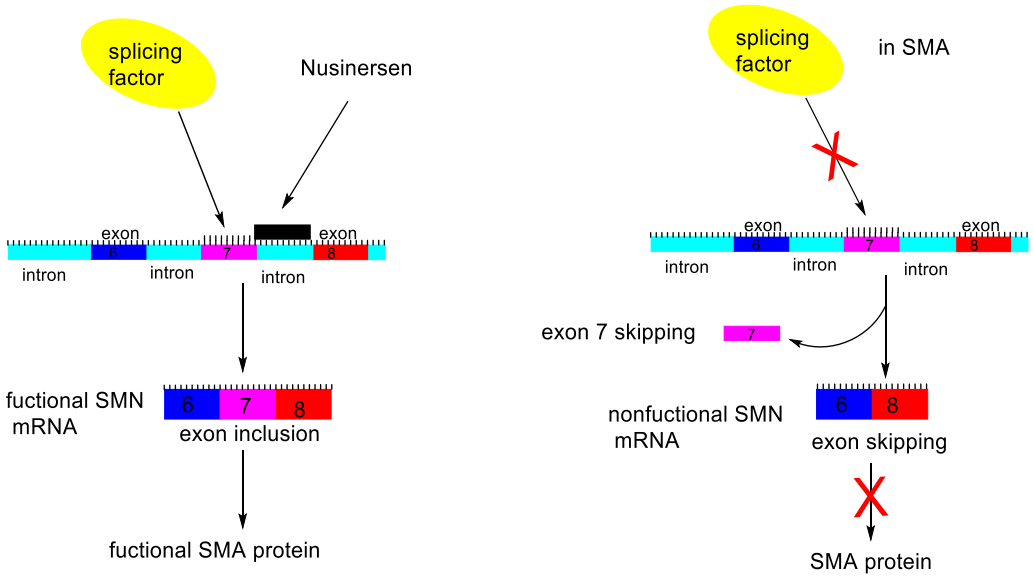


Figure 15. Skipping exon 7 cause SMA protein deficiency and can lead to SMA (on the right), using Nusinersen ASO can induce the inclusion of exon 7 and produce the full length of protein (on the left).

1.3.7 Tegsedi (Inotersen)

Tegsedi was approved by the FDA in 2018,[48] to treat polyneuropathy of hereditary transthyretin-mediated amyloidosis (hATTR) in adults.[49] A rare fatal genetic disease that is estimated to affect 50,000 people worldwide, by reducing the expression level of the disease-causing protein transthyretin. It was developed by Ionis Pharmaceuticals, together with its subsidiary Akcea Therapeutics under trade name Inotersen.[50] Tegsedi is a 20-mer antisense oligonucleotide, 2'-methoxyethoxy modification are incorporated in positions at 1-5 and 16-20 of the fully-sulfurized oligonucleotide backbone, with a central deoxynucleotide region, and all cytosine bases are 5-methylated. The sequence of the oligonucleotide is: 5'-^{Me}U^{Me}C^{Me}U^{Me}UGGTTA^{Me}CATGAAA^{Me}U^{Me}C^{Me}C^{Me}C-3', Underlined letters are 2'-O-(2-methoxyethyl) ribonucleotides; non-underlined letters are 2'-deoxyribonucleotides; all pyrimidines are 5-methylated (**Figure**

16). This antisense oligonucleotide binds to TTR mRNA to inhibit the production of TTR protein for cleavage by RNase H activity. The recommended dose of Tegsedi is 284 mg injected subcutaneously once weekly.

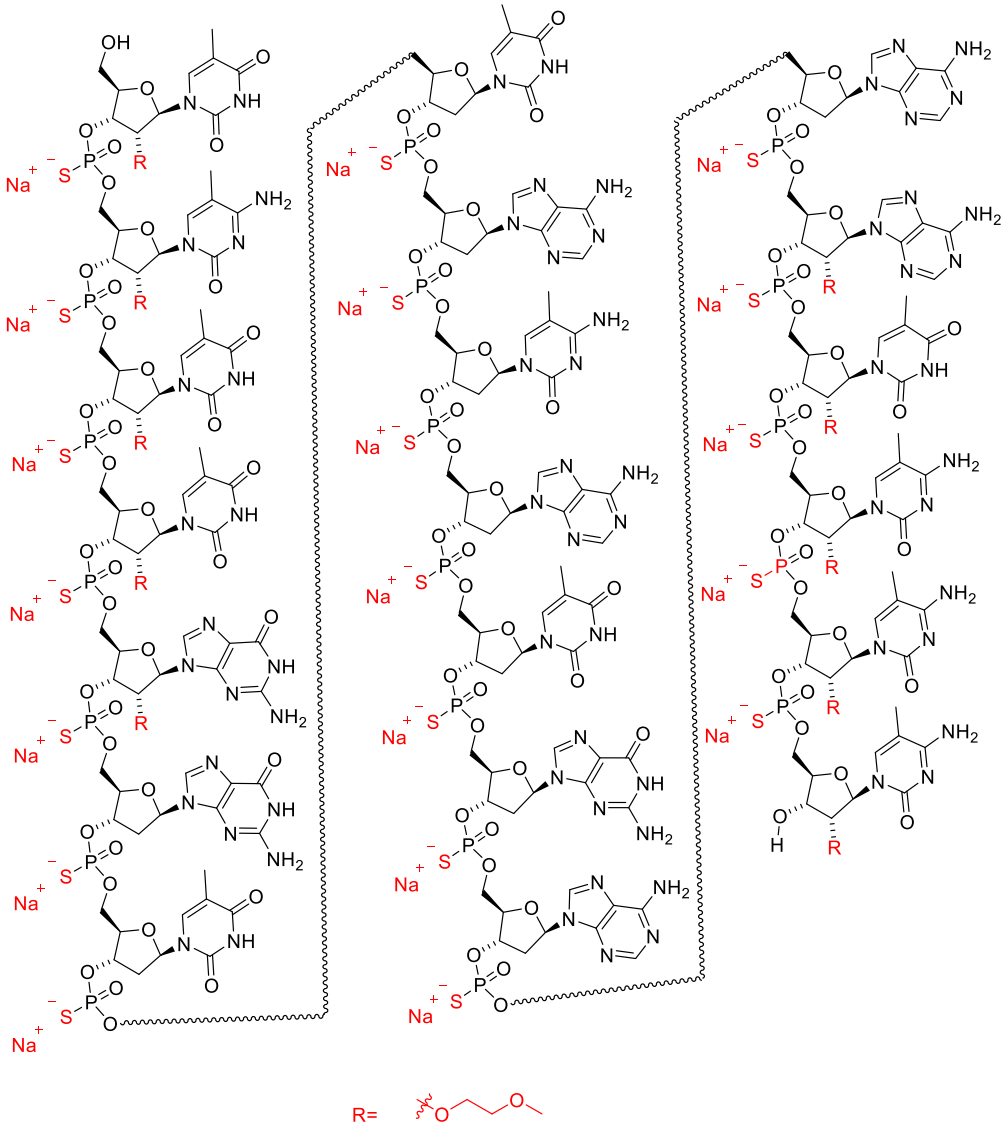


Figure 16. The chemical structure of 5′→3′ antisense oligonucleotide (Tegsedi). Fully sulfurized backbone shown in red, R represents methoxyethoxy modification.

1.3.8 Patisiran (Onpattro)

While all the previously-mentioned oligonucleotide drugs are single-stranded antisense oligonucleotides, Patisiran, approved by the FDA in 2018,[31,51] is the first double-stranded (**Figure 17**) short or small interfering RNA (siRNA) drug, encapsulated in a lipid nanoparticle for delivery to hepatocytes.[31,52]

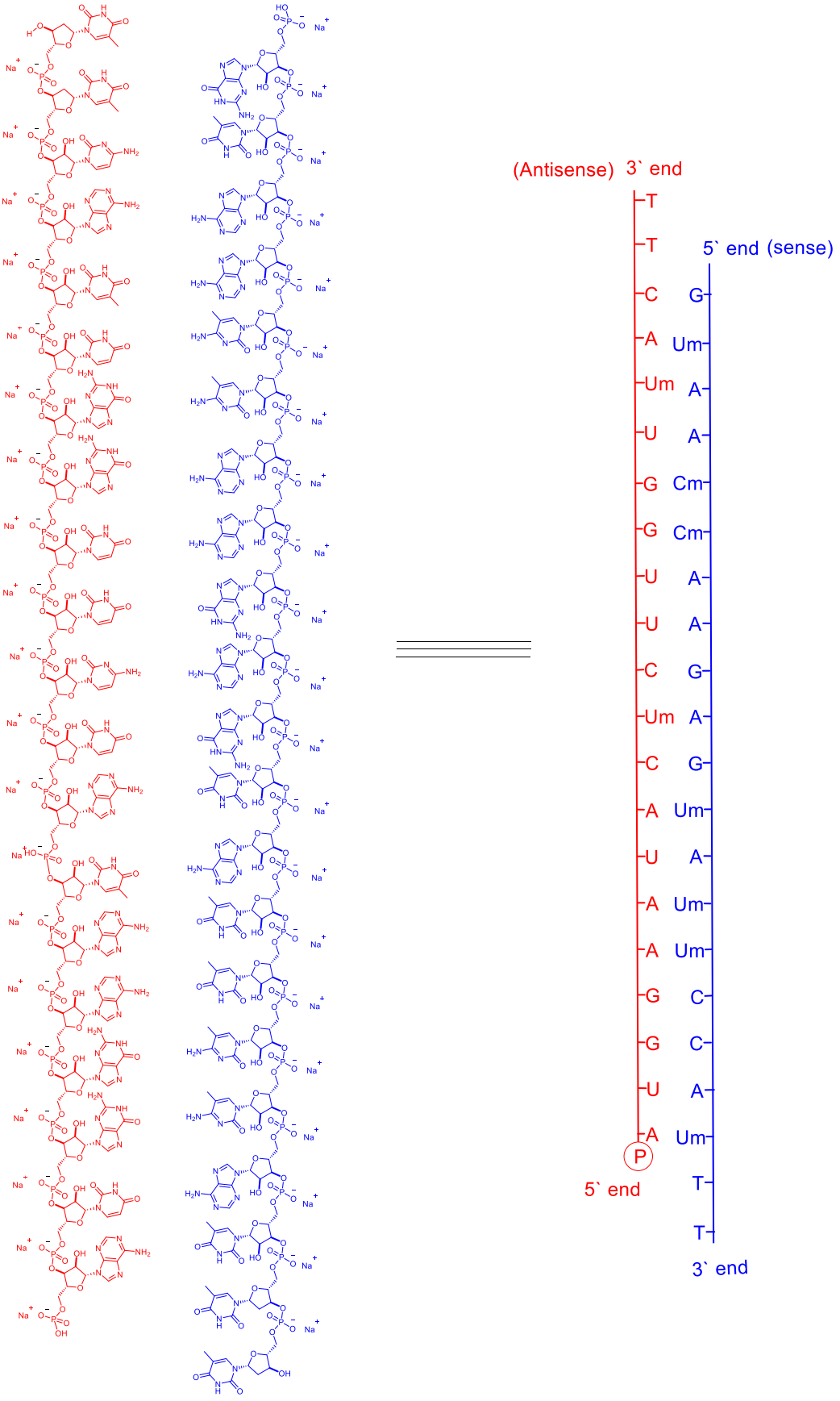


Figure 17. The chemical structure of Patisiran. Antisense strand (red); oxidised backbone. Sense strand (blue) oxidized backbone, (m) represents methylation.

Patisiran is developed by Alnylam to treat polyneuropathy in people with Hereditary ATTR Amyloidosis, by reducing the level of the disease-causing protein transthyretin.[52] Patisiran specifically binds to a genetically conserved sequence in the 3'-untranslated region (3'UTR) of mutant and wild type transthyretin (TTR) messenger RNA (mRNA). Dose depend on body weight, <100 kg: 0.3 mg/kg IV every 3 weeks and for ≥100 kg: 30 mg IV every 3 weeks. Whereas all last-addressed drugs work through antisense mechanisms, patisiran was developed to work through the RNA interference mechanism (RNAi).

1.4 RNAi mechanism of action

The RNAi is a process by which a double-stranded RNAs (dsRNAs) is used can cleave the target mRNA. RNAi is an important pathway that is used in many different organisms to manipulate gene expression. In 1998 Fire *et al.* were first discovered RNAi process in the nematode worms *Caenorhabditis Elegans.*[53] they noticed that, when dsRNAs injected into the worm to target the mRNAs, gene carrying the same sequenced were silenced and specific protein level was decreased.[53,54] In 2006 they were awarded Nobel Prize for their research on the discovery of the RNAi. siRNA was first used in cultured mammalian cells by Elbashir *et al*. in 2001.[55] After one year, the first successful use of short interfering RNA (siRNA) *in vivo* in mice was reported.[56] After 2006, most of the oligonucleotide therapies research was focused on using siRNA to treat human diseases.[57] This research has led a number of RNAi- based therapies advancing into clinical studies and finally approval first RNAi therapy, Patisiran (Onpattro) and at least six more siRNA drugs are already in phase 3 trials.[31]

The RNAi pathway is initiated by Dicer, an endoribonuclease protein, which binds to and cuts siRNAs into short segments, approximately 21 nucleotides long. The short double-stranded of RNA are then binds to Argonaute (AGO)

protein, one strand of the RNA is selected and remain bound to AGO protein and this is called "the guide strand" or "antisense", while the other strand which is called the "passenger strand" or "sense" is degraded by nuclease enzyme. The combination of the guide strand and AGO along with other proteins is called RNA-induced silencing complex (RISC). Single-stranded siRNAs direct RISC to bind to specific mRNAs, the targeting is precisely because it has determined by base pairing between the ss-siRNA and the target mRNA (siRNAs often have perfect complimentarily to their target sites), once bound, AGO protein catalyses the cleavage of mRNA which will then be degraded. As a result, specific protein levels are decreased (**Figure 18**).[3]

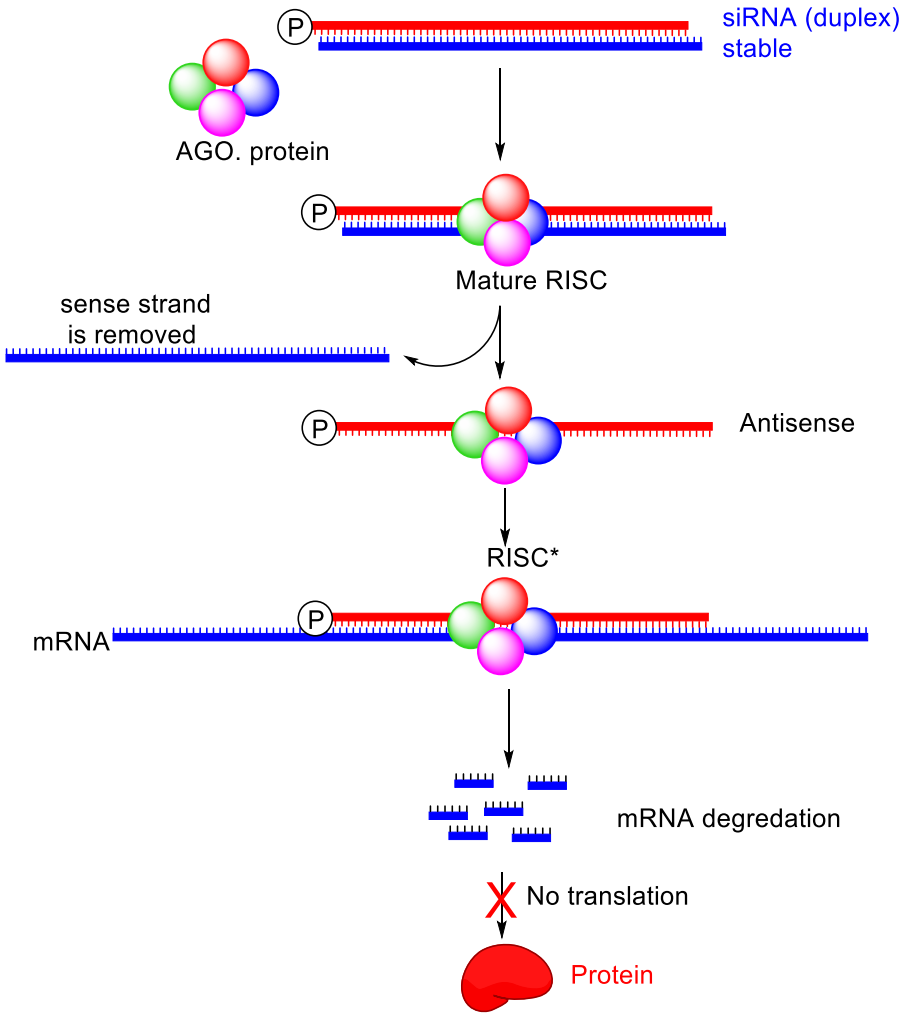


Figure 18. RNA interference to regulate gene expression within the cell. The activated RISC includes the antisense strand and AGO with other proteins interacts with a specific mRNA and cause its degradation.[54,57]

siRNAs that are processed by Dicer should have a unique structure (**Figure 19**) including the presence of a phosphate group at 5′-end, a hydroxyl group at 3′-end and two nucleotides 3′-overhang.[6,58,59] This unique structure is required so that AGO can recognize the siRNAs and all these features must be considered when synthetic dsRNAs are designed.

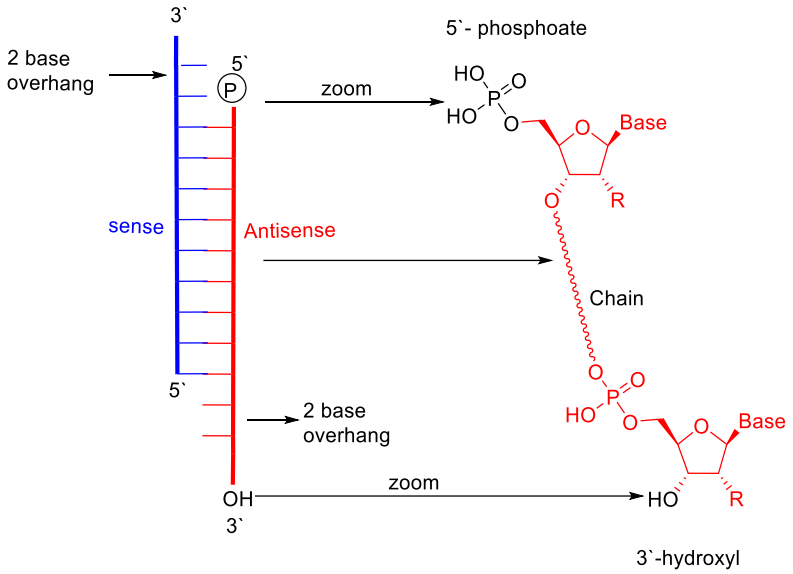


Figure 19. Classical siRNA structure. The antisense strand is complementary to target mRNA. On the left side, the cartoon structure shows two overhang nucleotides are present in 3′ ends. On the right side the chemical structure of the antisense strand where a phosphate group at present in 5′ end and hydroxyl group present in 3′.[6]

The crystal structure of human Argonaute was published in 2012 and was first discovered in plants.[60] AGO is a bilobed protein consisted of four domains: amino terminus (N), PAZ, Mid, and PIWI connected through two linker domains (L2 and L2).[60,61] A PIWI domain is located at the amino terminus, a Mid domain, and a PAZ domain are both located at the carboxyl terminus (**Figure 20**).[62,63]

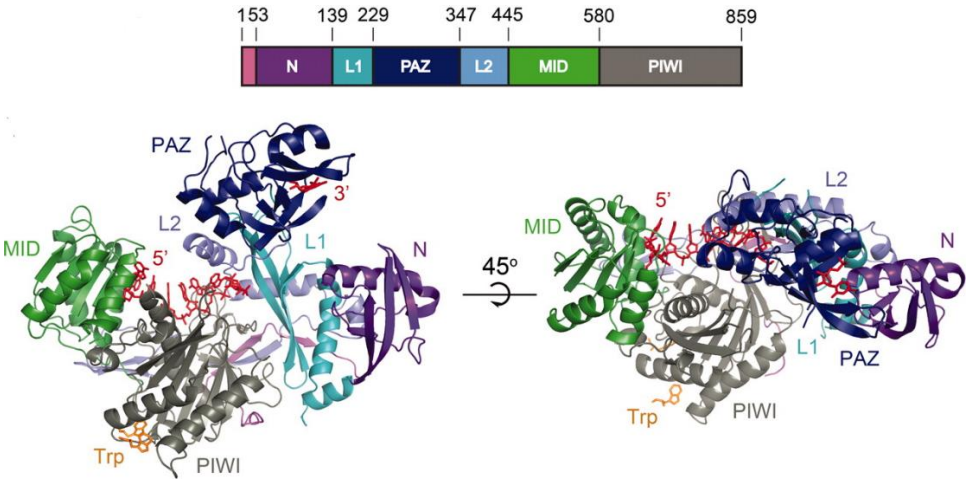


Figure 20. Crystal Structure of human Argonaute-2 protein. Front and top views of AGO2. The picture was taken from N. T. Schirle publication.[60]

Mid and PIWI domains bind specifically to the 5′ terminal phosphate group (**Figure 21**) and the backbone at the 5′ pole of the guide strand.[64,21] The two-terminal nucleotides on the 3′ end were found to bind to the PAZ domain.[65]

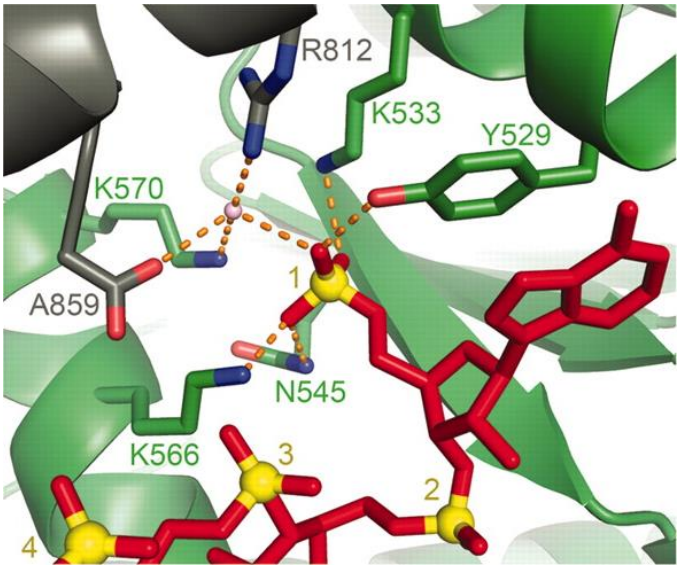


Figure 21. Structure of human Argonaute-2 protein. The MID and PIWI domains recognize the 5′ nucleotides of the siRNA. Pictures were taken from N. T. Schirle publication.[60]

There are four types of Argonaute protein in mammals, only the Argonaute 2 (AGO2) has catalytic character and functions as an endonuclease protein.[66] RNAi has remarkably proved to be effective *in vitro*, and that results in increasing the hopes of developing the most promising therapies to treat viral

or genetic disease.[3] However, the clinical utility of siRNA therapeutics has been limited by *in vivo* stability, unwanted off-target, immunogenicity effects and effective delivery to tissues.[25, 67] Thus, more research should be done to overcome these challenges.

While Patisiran uses a lipid complex to deliver the medication into the cells, next-generation of RNA interference (RNAi) therapies are aiming for an improved delivery approach with fewer side effects as we are looking for in this project.

1.5 Developing Single-stranded siRNAs

siRNA duplexes have been shown to potently decrease the target mRNA level in tissues by activating the RNAi mechanism. Recently, the approval of first RNAi drugs and the positive results from the phase 3 trial of siRNA therapies has paved the way for siRNA compounds to become a reality for patients.[20] Nevertheless, in their current state, the siRNA-based therapies are required for complex lipid formulations to deliver the siRNA to cells.[22,68] These lipid complexes are expensive to make, complicated, and have been shown to be toxic.[69,22] In contrast, single-stranded antisense siRNAs do not need the requirement for transfection reagents to improve the delivery, are cheaper to manufacture, and the off-target effects caused by sense strand will be eliminated.[7,70,21] It was suggested that ss-siRNA might be able to activate the RNAi pathway since Argonaute-2 binds only to the short single-stranded RNAs (antisense strand) (**Figure 22**).[22]

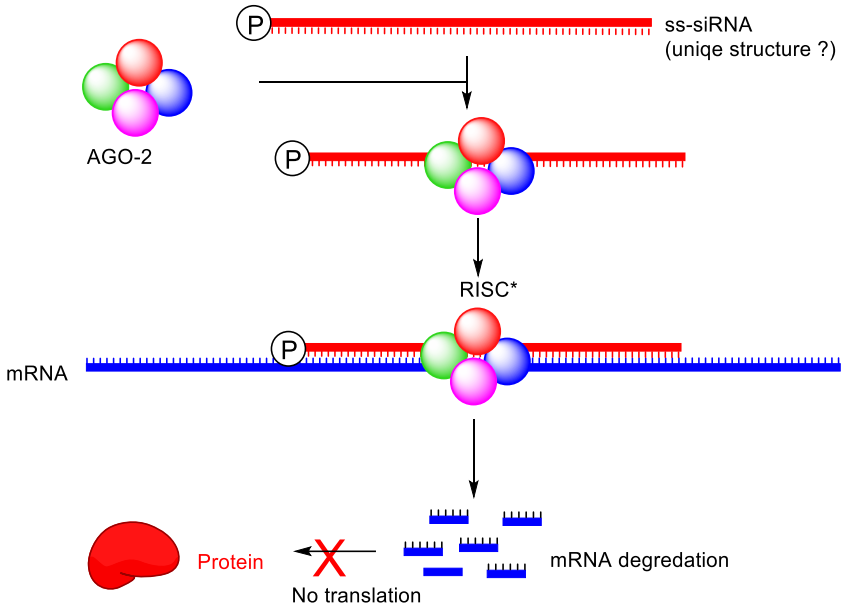


Figure 22. Suggested ss-siRNA mechanism of action.

Synthetic ss-siRNAs have been used to target mRNA *in vitro* in Hela Cells. However, their efficacy was poor when compared to ds-siRNAs. [71,72] The lower activity of the ss-siRNAs is mainly related to their metabolic instability, incorporation of chemical modifications are predicted to increase their nuclease stability.[73–76] However, the problem is how to design a ss-siRNA that is not only stable to nuclease hydrolysis but can also induce RNAi response. Not much research has been investigated what type of modifications could increase ssRNA activity.

In the early years of ss-siRNA and ds-siRNA development, in 2003, Holen *et al*. demonstrated that by using ssRNA with 2′-OMe modification in Hela cells, the IC_{50} value is approximately 5 fold higher compared to the corresponding ds-siRNA and most probably results from its lower intracellular stability.[77] Further research has been aimed at designing a chemical modification that has a dual function, which can increase the ss-siRNA stability against nuclease hydrolysis and activating RNAi.[77–81] The results have shown that incorporating 2′-F and 2′-OMe at multiple positions can increase the stability, affinity, and reduce the immunogenicity. Furthermore, it was demonstrated that a full modified ds-

siRNA with 2′-F and 2′-OMe modifications possess a higher efficacy than the unmodified one. Whereas, when these modifications have been incorporated in ss-siRNAs, they did not work as well as the corresponding ds-siRNA.[77–81]

In 2005 a study by Rivas *et al.* showed, that although the presence of phosphate at 5′ terminus of ds-siRNA is not essential, its presence significantly enhances ds-siRNA's activity in comparison to the 5′-hydroxyl (5′-OH).[82] In addition, it was demonstrated that the presence of phosphate at the 5′ end stabilizes the Argonaute-siRNA complex and antisense strand-siRNA must bear a 5′-phosphate so as to bind to the AGO.[82–87] Furthermore, another study performed experiments in Hela cells and found that siRNAs with terminal 5′-hydroxyl groups are phosphorylated by the cellular kinase enzyme and thus activate the RNAi pathway. On the other hand, the siRNA with 5′-methoxy group (5′-OMe), did not activate the RNAi (cannot be phosphorylated).[88] The same experiments were carried out for ss-siRNAs with identical results.[88] These results showed that the 5′-phosphate is important to activate RNAi in animals and should be present in synthetic ss-siRNA.

In 2012, Haringsma *et al.* demonstrated that by using 5′-end phosphorylation in combination with 2′-F modifications in ss-siRNA, significant activity is observed *in vitro* and *in vivo*.[89] Nevertheless, the modified ss-siRNAs were still less effective than ds-siRNAs, which suggests that more research need to be done to support the metabolic stability of si-RNAs. Overall, these results suggest that two factors should be considered before developing effective ss-siRNAs therapeutics: first, identifying chemical modifications to increase the ss-siRNA resistance to endonuclease and exonuclease, and second stabilizing the RISC complex and activating RNAi pathway.[22]

In general, 2′-sugar modifications have shown their ability to protect the internal stability of the ss-siRNA as mentioned previously. However, the critical

question of this study is how to protect the tails with a modification that is not only stable to nuclease hydrolysis but also can activate the RNAi pathway?

In fact, the phosphate group at the 5′ terminus of the ss-siRNA is not stable, when dephosphorylation occurs by nuclease, it can lead to ss-siRNA loss of function.[26] In 2012 Lima *et al.* demonstrated that incorporation of seven consecutive phosphorothioate backbones from the 3′ terminus, the ss-siRNA had increased stability while still being unable of efficient cleavage of the mRNAs.[22] To additionally protect the 3′-end, two purine nucleotides (2′-MOE modified) were incorporated, as the presence of a two purine overhang was previously reported to enhance siRNAs *in vivo* activity.[90] However, none of the above alterations to the chemical structure of ss-siRNA have contributed to the enhancement in metabolic half-lives (~30 minutes).[22] More interestingly, Lima confirmed that incorporation of a 5′-phosphate enhances ss-siRNA activity *in vitro*.[22] Experiments performed in mice found that 5′-phosphate groups are required for ss-siRNA *in vivo* activity since ss-siRNAs with phosphorylated 5′-ends were inactive *in vivo* due to their degradation by nucleases.[22] These results prompted studies into 5′-phosphate analogues that can resist to nuclease degradation. As a result, it was decided to test 5′-methylenephosphonate (5′-MP) and 5′-(*E*)-VP modification, where the phosphorus is directly attached to the carbon (P-C) instead of phosphorus-oxygen (P-O) bound in the corresponding natural phosphate (**Figure 23**), the new P-C bond cannot be cleaved since there are no known enzymes capable of doing so.[22,19]

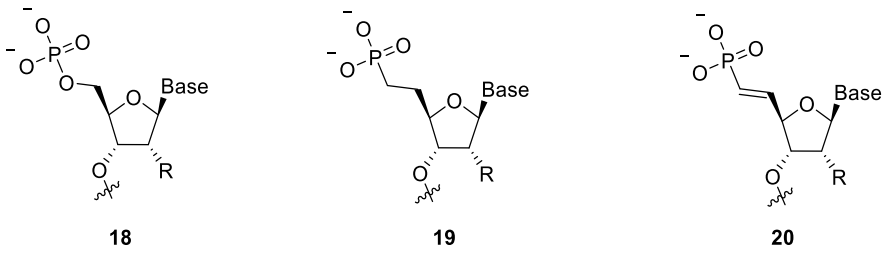


Figure 23. The chemical structures of; natural phosphate **18**, methylene phosphonate **19,** and vinlyphosphonate analogues **20**.

Importantly, the results of this study had shown that 5′-(*E*)-VP was the most effective in comparison to natural phosphate and MP modification when used *in vivo* and *in vitro*.[22] The activity difference between VP and MP is mainly due to their conformational and stereoelectronic differences.

Structural analysis of the Argonaute-2 loaded with the antisense strand carrying the 5′-(*E*)-VP modification was published by two different studies in 2016 and 2017 (**Figure 24**).[24,21] It has shown the conformational and stereoelectronic properties in a *trans* configuration of 5′-(*E*)-VP are closer to the natural phosphate which makes it the best metabolically stable mimic and this structural similarity supports successful RISC loading of the ss-siRNAs carrying 5′-(*E*)-VP moiety. The β–torsion angle of the 5′-terminal nucleotide (nt 1) is 167.9° (**A**, **Figure 24**), which is closely mimicked by the (*E*)-VP that has a β–torsion angle constrained to 180° (**B**, **Figure 24**).[24,22]

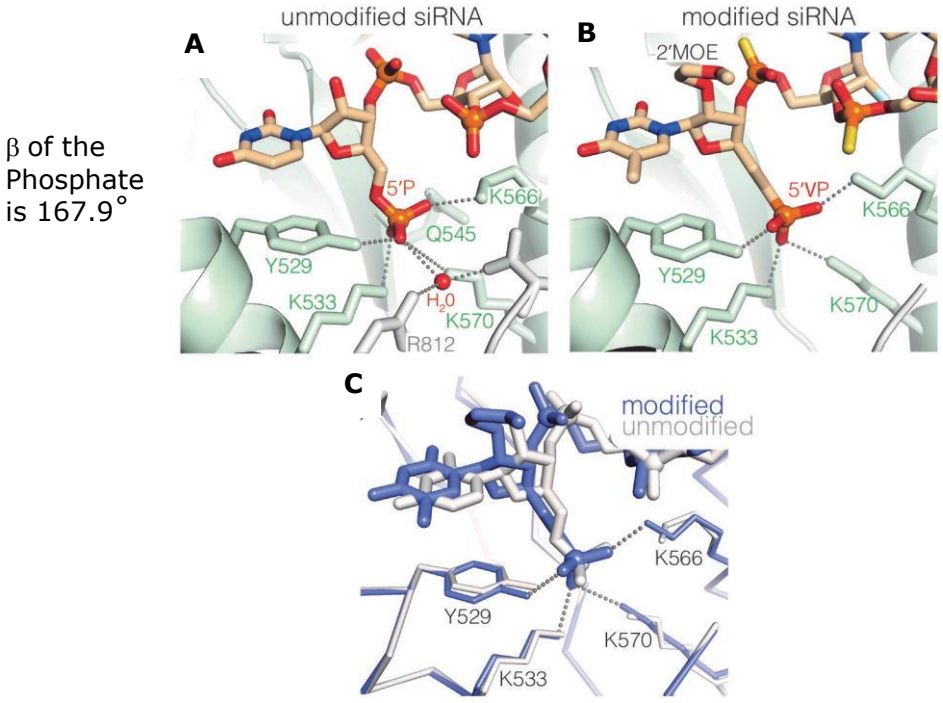
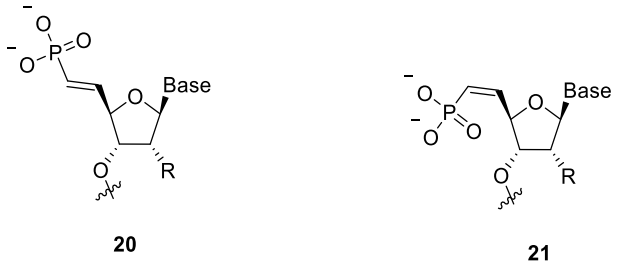


Figure 24. Crystal-structure comparison for 5′- end modified and unmodified ss-siRNA complex with Argonaute-2. (**A**) natural phosphate at 5′-end of unmodified siRNA. (**B**) vinylphosphonate modification is incorporated in the 5′- end of the ss-siRNA, obviously seems VP is well accommodating in the AGO. (**C**) Superposition of the 5′-phosphate and 5′-VP ss-siRNAs with surrounding protein atoms displayed. Pictures were taken from N. T. Schirle publication.[24]

In 2018, another study confirmed the importance of the similarity conformational in stereoelectronic properties of 5′-(*E*)-VP to the natural phosphate.[20] This study compared two ss-siRNAs one of them has 5′-(*E*)-VP modification while 5′-(*Z*)-VP in the other one (**Figure 25**). The results of this study have shown that despite their similar tissue accumulation, both ss-siRNAs were metabolically stable. However, 5′-(*E*)-VP ss-siRNA has shown higher gene silencing, *in vivo* and *in vitro*, in comparison to the corresponding *Z*- isomer. Furthermore, it was suggested that the higher activity of 5′-(*E*)-VP was due to increased "RISC-loading" of modified ss-siRNA, whereas, the poor *in vivo* activity of the corresponding 5′-(*Z*)-VP ss-siRNA was related to its poor "RISC-loading".[20]



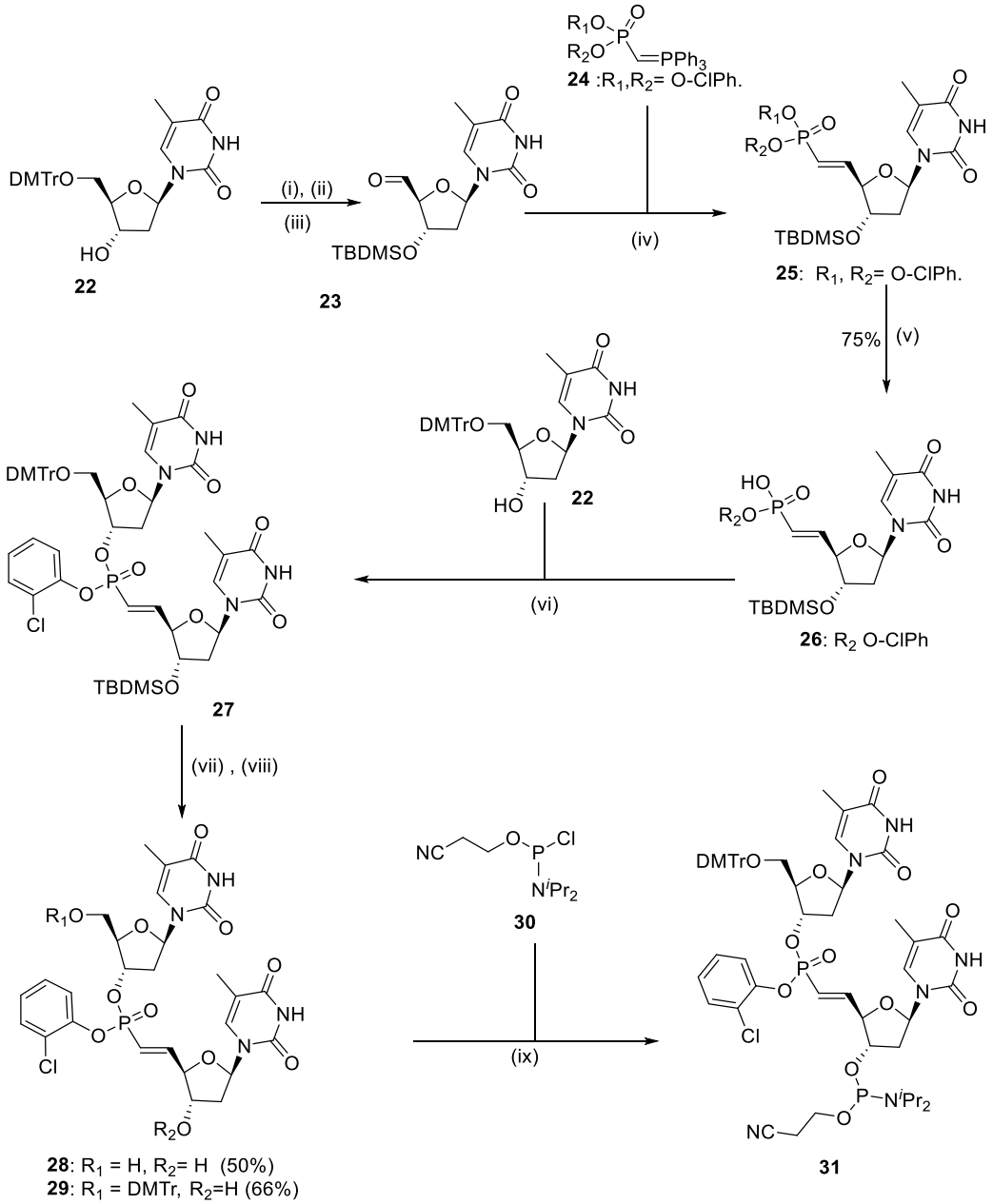
20 21

Figure 25. The chemical structures of; (*E*)-vinylphosphonate **20** and (*Z*)-vinylphosphonate **21** analogues.

1.6 Synthesis of (*E*)-vinylphosphonate nucleotides

Caruthers and Zhao first synthesized (*E*)-vinylphosphonate dinucleotide **31** in 1996. The aldehyde **23** was prepared from commercially available 5′-DMTr-protected thymidine **22**. A wittig reaction between the aldehyde **23** and ylide **24** to form vinylphosphonate **25** was the key step in their synthesis of dinucleotide **31**. After the selective removal of one *o*-chlorophenyl group, the resulting acid **26** was esterified with alcohol **22** to give dinucleotide **27** as a mixture of (1:1) diastereoisomers at phosphorus centre. The selective removal of 3′-TBDMS group in **26** was not possible without affecting the *o*-chlorophenyl protection on phosphorus. Instead, both 3′ and 5′ protections were removed simultaneously with 0.5 M HCl in methanol to give diol **28** (50%). Diol **28** was

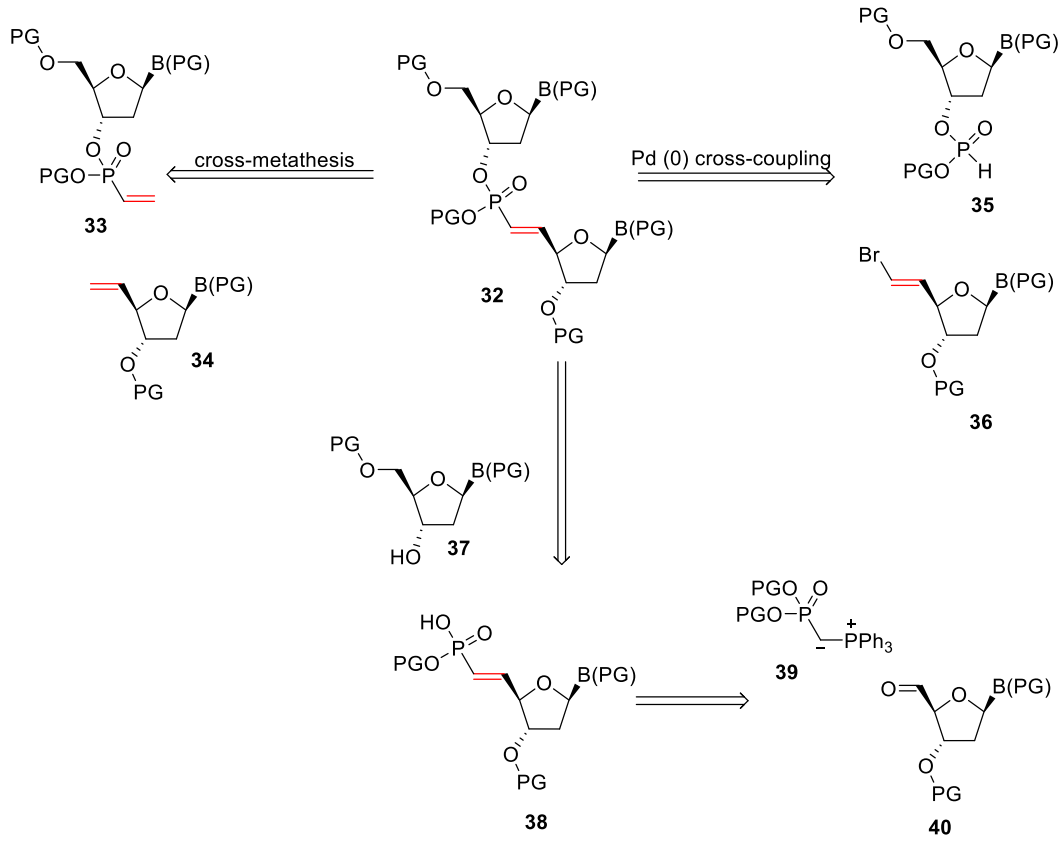
then reacted with dimethoxytritylchloride and tetrabutylammonium perchlorate in dry pyridine to give the 3′-DMTr protected alcohol **29** (66%). Finally, phosphoramidite **31** was isolated in 56% yield as a 1:1:1:1: a mixture of four diastereomers when dinucleotide **29** reacted with 2-cyanoethyl-*N*,*N* diisopropylchlorophosphoramidite **30** (**Scheme 2**).



Scheme 2. Caruthers synthesis of vinylphosphonate-linked dimer **31**.[15] Reagents: (i) *tert*-butyldimethylsilyltrifluoromethanesulfonate, pyridine (95%); (ii) 80% acetic acid (82%) (iii) Pfitzner-Moffat oxidation conditions (iv) CH_2Cl_2, RT (64%); (v) 2-nitrobenzaldoxime, tetramethylguandinine, triethylamine, dioxane (75%); (vi) 2,4,6-triisopropylbenzenesulfonyl chloride, *N*-methylimidazole, pyridine (61%); (vii) 0.5 M HCl in methanol (50%); (viii) DMTrCl, tetrabutylammonium perchlorate (66%); (ix) 2-cyanoethyl-N,N diisopropylchlorophosphoramidite **30** (56%).

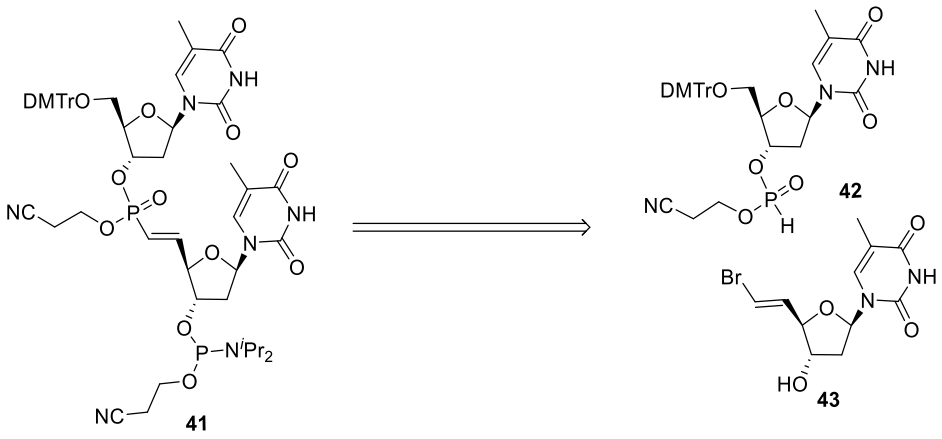
The Caruthers synthetic route is suitable to make T*T dinucleotide **31**. However, it cannot provide access to all the possible combination of the dinucleotides, since the use of HCl causes depurination.

The Hayes group suggested three approaches to access to the (*E*)-vinylphosphonate linked-dinucleotides **32**. Firstly, a cross-coupling, secondly cross-metathesis, and thirdly a Wittig reaction and esterification approaches. (**Scheme 3**).



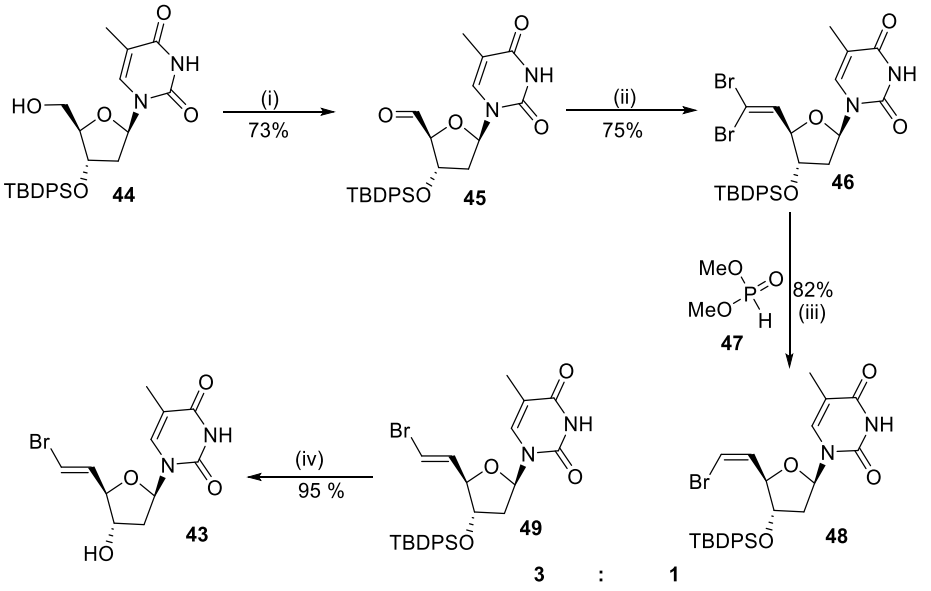
Scheme 3. Retrosynthesis approaches for the synthesis of (*E*)-vinylpohosphonate modification **32**.

The Hayes group,[91] started with a cross-coupling approach, which involves a palladium (0) catalyst cross-coupling reaction between *H*-phosphonate **42** and (*E*)-vinyl bromide **43** to afford the phosphoramidite **41** (**Scheme 4**).



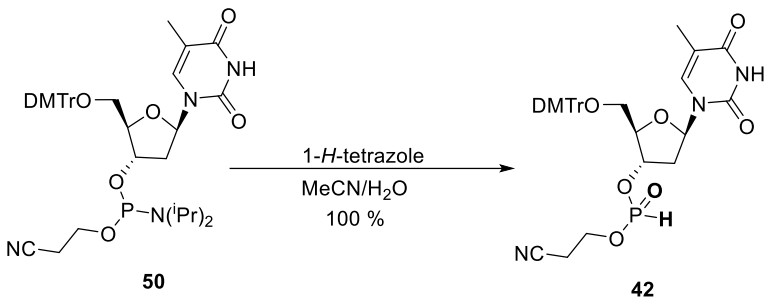
Scheme 4. The retrosynthesis of dinucleotide **41** *via* cross-coupling of *H*-phosphonate **42** and (*E*)-vinyl bromide **43**.

(*E*)-vinyl bromide **43** can be obtained as illustrated in **Scheme 5**. Alcohol **44** was oxidized to aldehyde **45** using Dess-Martin periodinane[92], and dibromo vinylphosphonate **46** was generated from the Ramirez's[93] Wittig olefination of aldehyde **45.** Then, by using the method of Hirao *et al.*[94], the dibromo olefin **46** was reduced to a 1:3 mixture of separable *E:Z* isomers **48** and **49**, respectively. The two isomers were separated by column chromatography to give the desired (*E*)-vinylphosphonate **42**. The corresponding alcohol **43** was then obtained by deprotection of the 3'-TBDPS group using TBAF in 95%.



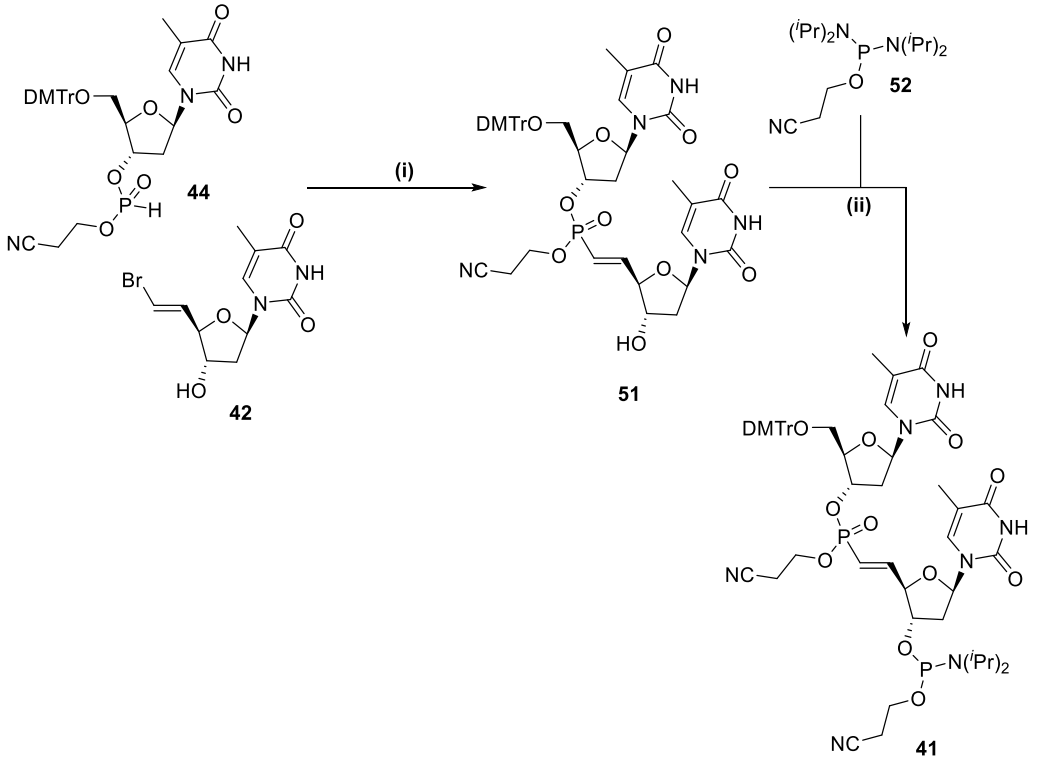
Scheme 5. Synthesis of thymine-bearing (*E*)-vinylphosphonate **43**. Reagents: (i) DMP, CH_2Cl_2 (73%); (ii) PPh_3, CBr_4, CH_2Cl_2 (88%); (iii) Dimethyl phosphite **47** (82%), Et_3N; (iv) TBAF, THF (95%).[91]

The other fragment, *H*-phosphonate nucleoside **42**, was obtained from hydrolysis of the commercially available phosphoramidite **50** using 1-*H*-tetrazole in aqueous acetonitrile (**Scheme 6**).



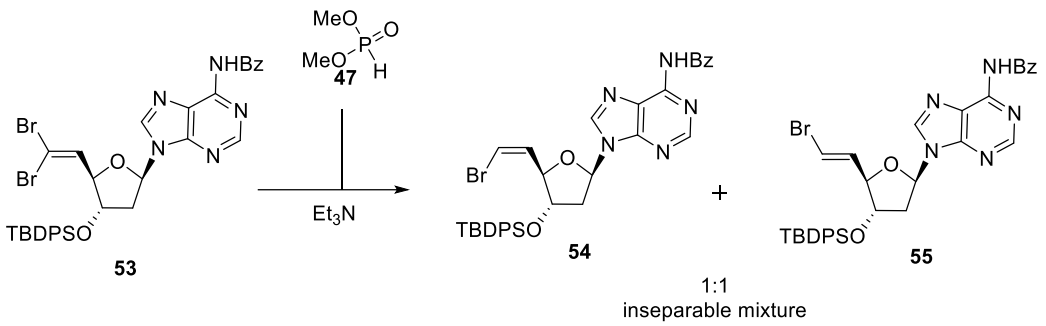
Scheme 6. The hydrolysis of phosphoramidite **50** to *H*-phosphonate **42**.[91]

Finally, Vinylphosphonate **51** was obtained *via* palladium-catalyzed cross-coupling between *H*-phosphonate **42** and vinyl bromide **43** in 73% yield. The produced dinucleotide **51** was reacted with phosphoramidite **52** to give the final product **41** (**Scheme 7**).



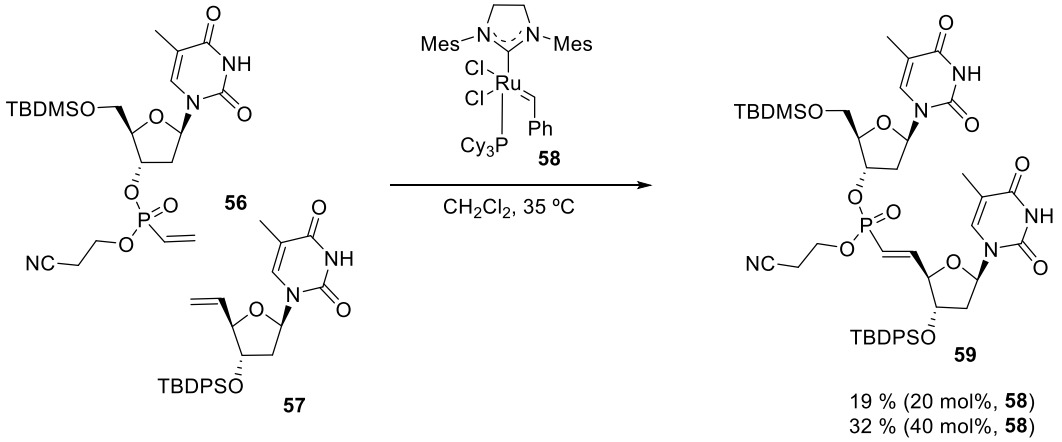
Scheme 7. Synthesis of phosphoramidite **41**. Reagents**:** (i) 0.2 eq. $Pd(OAc)_2$, 0.4 eq. dppf, 10 eq. propylene oxide, THF, 70 °C (59%); (ii) **52**, 1-*H*-tetrazole, CH_2Cl_2 (70%).[91]

Disappointingly, the reduction of dibromo vinylbromide **53** was less selective than **43**, and the separation of the (*Z*) **54** and (*E*)-vinlybromide **55** (1:1) mixture was not possible in this case (**Scheme 8**). Hence, another route required to be investigated to overcome this challenge.



Scheme 8. The Hayes group attempt to reduce the dibromo olefin **53**.

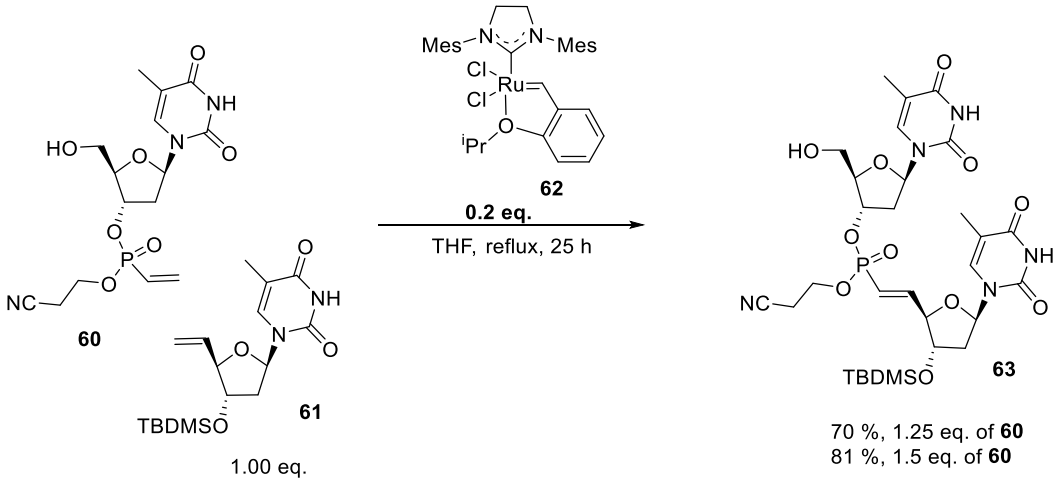
Our group then investigated the cross-metathesis approach, to get access to all possible vinylphosphonate dinucleotides combinations. A cross metathesis reaction of second-generation Grubbs catalyst II **58** with olefins **56** and **57**, resulted in formation of dinucleotide **59** in 19% and 32% when 20 and 40 mol% Of Grubbs II catalyst **58** used, respectively (**Scheme 9**).[95]



Scheme 9. Using the second generation of Grubbs catalyst II to synthesize the vinylphosphonate **59**.

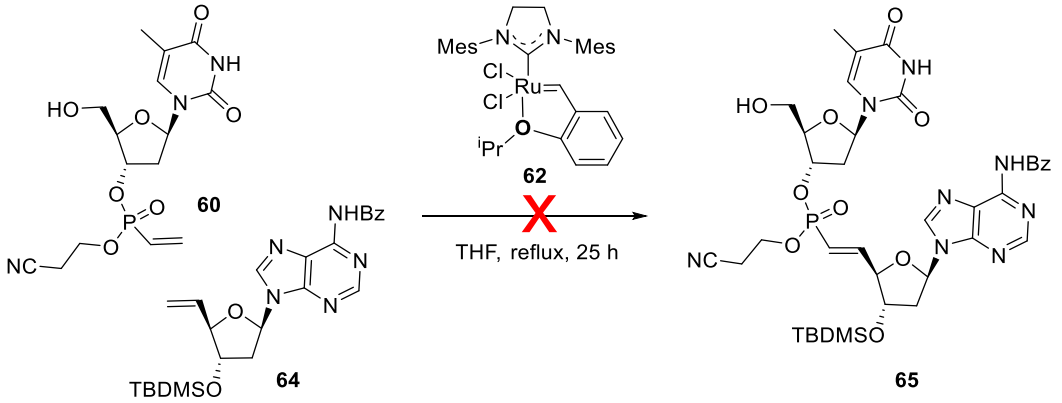
However, further optimisation studies showed replacing starting materials **56** and **57** with **60** and **61** and using Hoveyda-Grubbs catalysts **62** in refluxing THF afforded dinucleotide **63** in a pleasingly 70% yield (**Scheme 10**). The

reaction was repeated using increased excess of **60** (1.5 eq. instead of 1.25 eq.), resulted in an increased 81% yield (**Scheme 10**).



Scheme 10. The optimised condition for the synthesis of dinucleotide **63**.

The same optimised conditions were used with adenosine nucleoside in the aim of make dinucleotide **65**. However, the reaction was not successful, and dinucleotide **65** could not be isolated at all, significant amounts of free adenine were separated during the purification (**Scheme 11**).

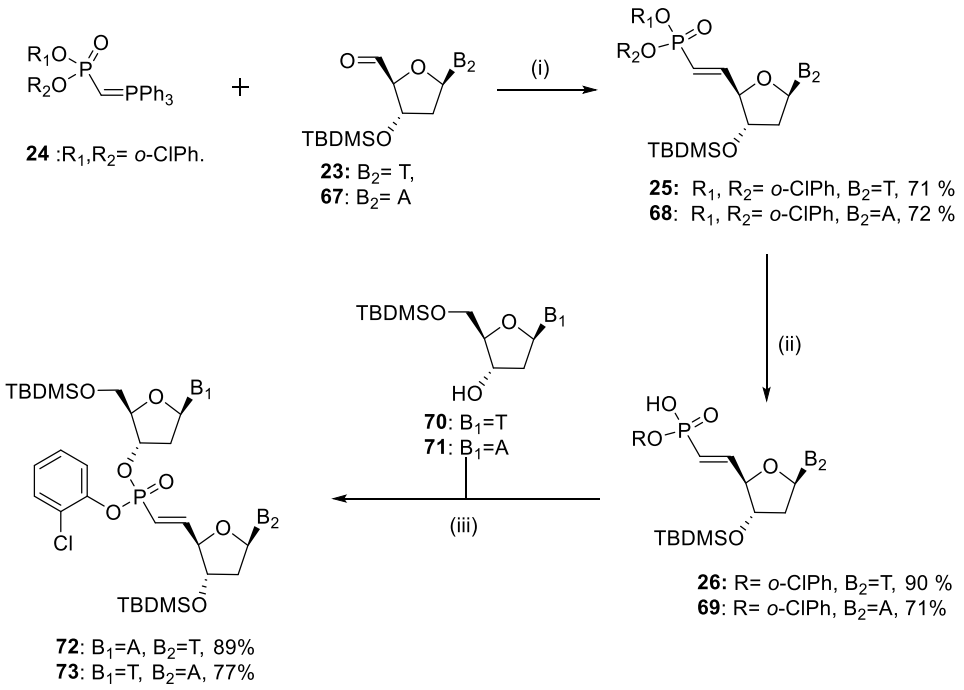


Scheme 11. Our groups attempt to make T*A dinucleotide **65**.

It seems that the high temperature induces depurination of adenosine as already reported in the literature.[96] In an attempt to avoid depurination, the reaction of **60** with **64** was carried out in THF at 25 °C, and it was stirred for 6

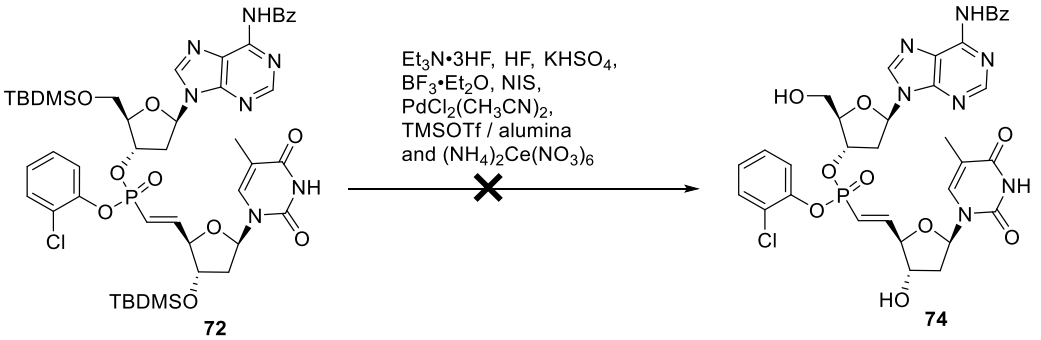
days. Although the formation of free adenosine could not be detected in this case, the desired dinucleotide **65** was obtained in a poor yield of 11%.

As such, it was not possible to make all the different combinations of the dinucleotides *via* cross-metathesis and cross-coupling approaches. Therefore, our group investigated the Wittig reaction and esterification method following the Caruthers's route with replacing the 3'-DMTr protected alcohol **22** to the TBDMS versions **70** and **71**, thus our group has successfully synthesized dinucleotides; (A*T) **72** and (T*A) **73**. (**Scheme 12**).



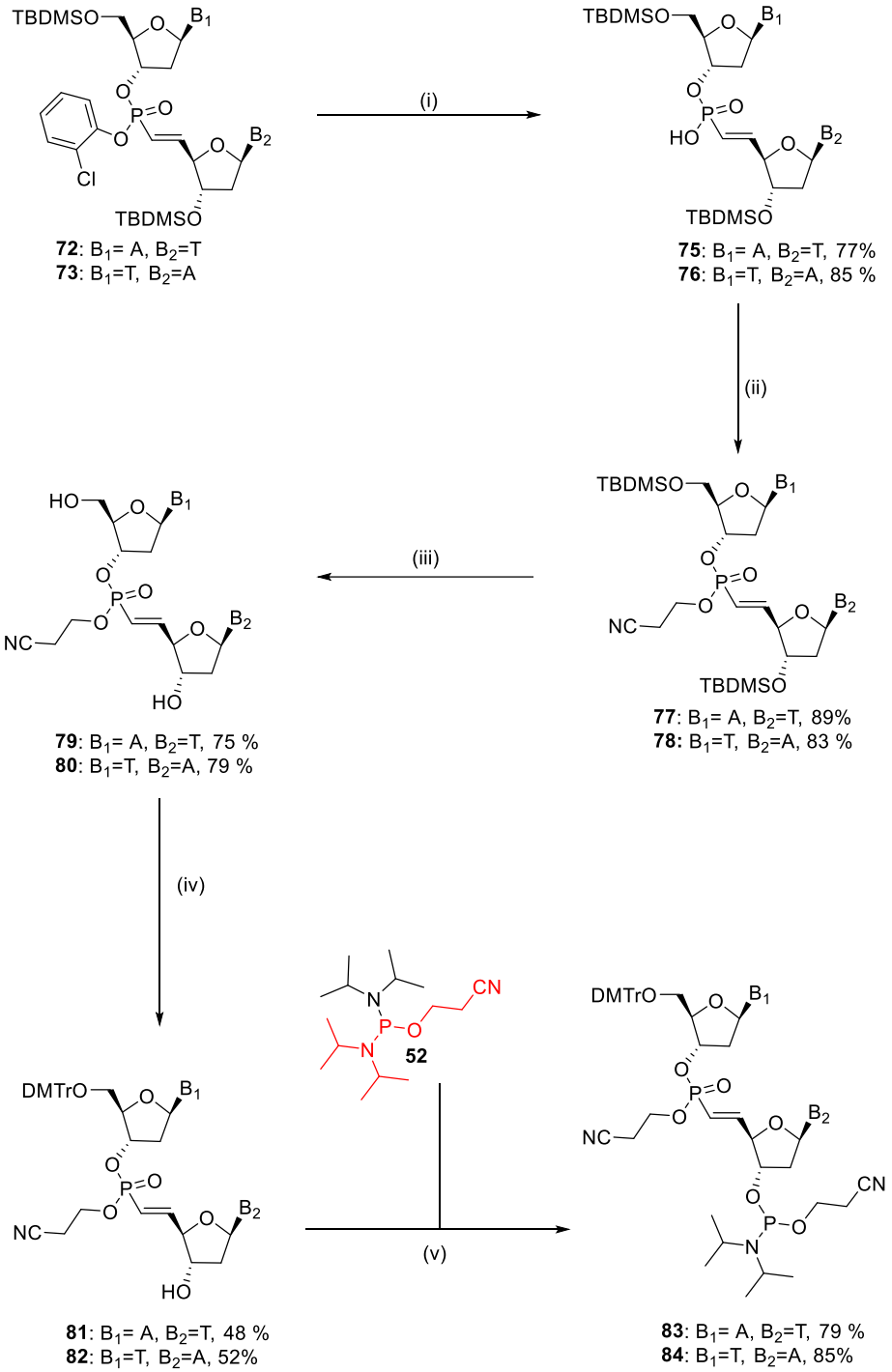
Scheme 12. Our group investigation to Wittig route to make dinucleotides **72** and **75**. Reagents: (i) CH_2Cl_2, 20 h, RT; (ii) 2-nitrobenzaldoxime (1.2 eq), tetramethylguanidine (1.2 eq), triethylamine (6.0 eq), dioxane, 3 d, RT; (iii) 2,4,6-triisopropylbenzenesulfonyl chloride (2.0 eq), NMI (6.0 eq), pyridine, 2.5 h, RT.[97]

The removal of 3' and 5' TBDMS groups of **72** and **73** was not possible without affecting the remaining *o*-chlorophenyl group as already reported in Caruthers's publication. Our group screened various methods for selective removal of the TBDMS groups, resulting in either no reaction or decomposition of the dimeric material (**Scheme 13**).[97]



Scheme 13. Hayes group attempt selectively removes the TBDMS groups.[97]

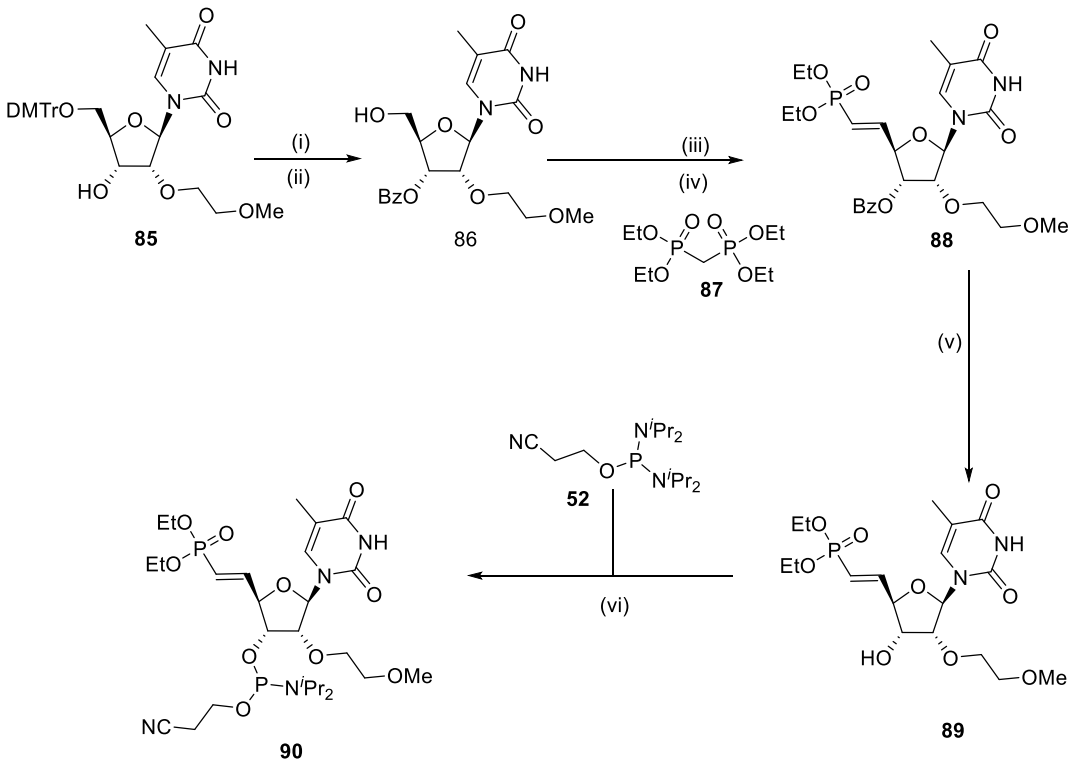
Since using 0.5 M HCl in methanol can lead to depurination. It was then decided to replace the *o*-chlorophenyl PG with cyanoethyl group, and this can be removed after the oligonucleotide is synthesized *via* post-synthesis treatments. Thus, the *o*-chlorophenyl group of dinucleotide **72** was removed to form the acid **75.** After protecting the phosphate with cyanoethyl to form **77**, the two TBDMS groups were removed from cyanoethyl dinucleotide **77** using $Et_3N.3HF$ to afford diol **79**. The 5′-DMTr-protected dinucleotide **81** was prepared (48%) from reacting diol **79** with DMTrCl. The desired phosphoramidite **83** was obtained (85%) when dinucleotide **81** reacted with **52** (**Scheme 14**). (T*A) dinucleotide **84** was also obtained by using the same strategy used in synthesizing dinucleotide **83** (A*T).



Scheme 14. Our group's developed route to synthesis the phosphoraimidate **83** and **84**. Reagents: (i) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguandinine, 1.0 eq. triethylamine, dioxane, 20 h, RT; (ii) 5.5 eq. 3-hydroxypropionitrile, 5.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 17 eq. *N*-methylimidazole, pyridine, 3 h, RT; (iii) 10 eq. $Et_3N{\cdot}3HF$, THF, 4 h, RT; (iv) 1.0 eq. DMTrCl, pyridine, 2 d, RT; (v) 1.0 eq. **52**, 1.0 eq. 5-methyl-1*H*-tetrazole, CH_2Cl_2, 20 h, RT.[97]

Lima *et al*. (Ionis pharmaceuticals) synthesise 5′-(*E*)-vinylphosphonate that is shown in **Scheme 15**; substrate **85** is not commercially available and it was made by 10 steps starting from 1-O-methyl ribose and following the synthetic route described in the published patent.[98]

The 3′-OH of substrate **85** was protected with Bz group followed by DMTr deprotection to give alcohol **86**. The alcohol **86** was then was oxidised, and the resulting aldehyde was subsequently reacted with phosphonate **87** to give (*E*)-vinylphosphonate **88.** Benzoyl group was then removed and the resulting alcohol **89** was reacted with phosphoramidite **52** to afford the phosphoramidite **90 (Scheme 15)**.



Scheme 15. Synthesis of phosphoramidite with the terminal 5′-(*E*)-vinylphosphonate **90** proposed by Ionis pharmaceuticals. Reagents: (i) benzoylchlroride, Pyridine; (ii) TFA, CH_2Cl_2, $(C_2H_5)_3SiH$, 87% (over two steps); (iii) DCC, DMSO, Pyridine-TFA; (iv) phosphonate **87**, potassium-*tert*-butoxide, THF, 47%; (v) NH_3, MeOH, 95%; (vi) (2-cyanoethoxy)-tetraisopropylphosphorrdiamidite **52**, tetrazole, *N*-methylimidazole, DMF, 90%.[99]

1.7 Solid-phase oligonucleotide synthesis

Solid-phase synthesis is carried out on a solid support (also called resins) held between filters, in columns that enable all reagents and solvents to pass through freely. Solid supports are the insoluble particles, typically 50-200 μm in diameter, to which the oligonucleotide is bound during synthesis. Many types of solid support have been used, but controlled pore glass (CPG) and polystyrene have proved to be the most useful. Controlled-pore glass is rigid and non-swelling with deep pores in which oligonucleotide synthesis takes place. The standard phosphoramidite solid-phase synthesis (**Figure 26**) involves four main steps, first DMTr deprotection for the first nucleotide at the 3' position of the sequence which is on the solid support. When the DMTr group is cleaved from the oligo, the resulting DMTr cation has a bright orange colour, which can be measured by the UV-Vis spectroscopy to quantify the coupling yield. The second step of the synthesis is activating the phosphoramidite of the first delivered base (second nucleotide in the sequence) with a suitable BTT activator and then coupling with the hydroxyl of first nucleotide to make the dinucleotide. Then capping the unreacted and failed sequences by protecting the hydroxyls with an acyl group. Finally, either oxidation or sulfurization of the phosphorus atom depends on the desired oligo characteristics using a suitable sulfurizing reagent or oxidiser. Once the first cycle is complete, the cycle will be repeated many times depending on the oligonucleotide length. When the synthesis is complete, some post synthesis steps are required to get the free oligonucleotide ready for analysis and biological study. These steps include cleaving the oligo from the solid support, deprotecting the protective groups on nitrogen bases and phosphates, and purification if required. After desalting the oligonucleotide using a NAP-5^{TM} column to get the exact mass spec data for the synthesized oligonucleotide.

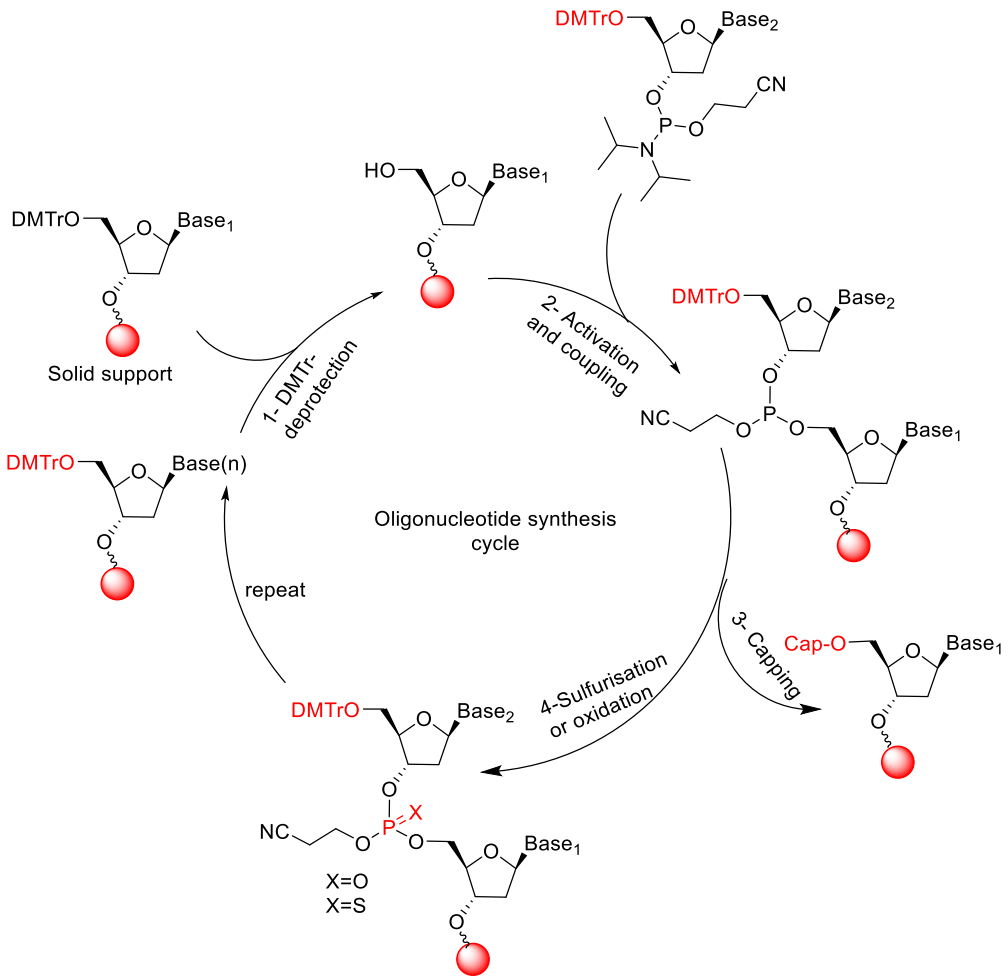


Figure 26. Phosphoramidite method for oligonucleotide synthesis.

1.8 Delivery of oligonucleotides

The cell membranes of living organisms are composed of phospholipids and proteins. Phospholipids are amphipathic molecules that consist of two fatty acid chains, which are linked to the phosphate hydrophilic head group. Because their fatty acid tails are poorly soluble in water, phospholipids spontaneously form bilayers in aqueous solutions with the polar head groups exposed on both sides in contact with water and the hydrophobic tails buried in the interior of the membrane. This forms a stable barrier between two aqueous compartments and represents the basic structure of all biological membranes. The structure of the cell membrane is responsible for its primary function, control of the movement of substances in and out of the cells. in order to influence gene

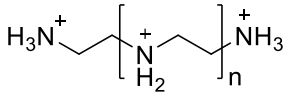
silencing, oligonucleotides have to pass through the cell membrane. Oligonucleotides have high molecular weight and are negatively charged, hence are very unlikely to cross the membrane *via* passive diffusion. Many strategies have been developed to increase the rate and selectivity of cellular internalisation of oligonucleotides. It has been proposed that delivery methods should be classified into two groups. The first group focuses on the cellular uptake process and improving the binding properties of oligonucleotides to the membrane. This group includes a conjugation of various compounds (e.g. cholesterol), complexation with cationic compounds (e.g. cationic polymers and cationic lipids) and encapsulation. The second group focuses on entry into the cytoplasm (and/or nucleus) and includes cytoplasmic transfer techniques (e.g. microinjection, electroporation). In this research we will focus on the cationic polymers, encapsulation and injection, as they been used or mentioned in this research.

1.8.1 Injection

Injection is a preferred method of delivering naked oligonucleotides *in vivo*. Delivery can be performed by intravenous, subcutaneous, intravenous, intraocular, intrathecal or intramuscular injection. To produce a therapeutic effect, injection requires several doses. Some FDA approved oligonucleotide drugs, Vitravene (intraocular) and Kynamro (subcutaneous), Exondys 51 (intravenous), Spinraza (intrathecal) and Inotersen (subcutaneous) are delivered by injection.

1.8.2 Cationic polymers

Cationic polymers are referred to positively charged macromolecules that can be inherently present in the polymer backbone or in the side chains. Most cationic polymers have primary, secondary or tertiary amine functional groups that can be protonated. They are also very different in the polymer structure (linear, branched, hyperbranched and dendrimer-like) and can be different by placing positive charges (backbone or side chains). As both cell membrane and oligonucleotide are negatively charged, cationic polymers have attracted the most attention as delivery reagents and are probably the most widely used. Cationic polymers form nanocomplexes with oligonucleotides and as result compensate their negative charge. Polyethyleneimine (PEI) (**Figure 27**) is a cationic polymer that is commonly used in gene delivery. Due to their easy synthesis and low-cost modification, PEI can be effectively assembled with siRNA through electrostatic interaction and this positively charged condensed complex can improve cellular uptake and siRNA-mediated gene-silencing. PEI is prepared as an effective carrier for a broad range of gene medicine, including DNA plasmids, siRNAs, mRNAs.[100] In addition, continuous improvement of the physical properties and biological function of polyelectrolyte complex achieved by nanoparticles made of PEI and nucleic acids. PEI is a polymer that contains amines isolated by ethylene groups and is hydrophobic because of its rich ethylene-backbone. The PEI chain is charged positively in acidic environments, which is due to secondary amine protonation along the backbone. Thus, they can readily form nanocomplexes with oligonucleotides.



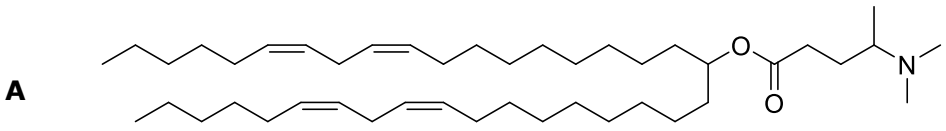
Polyethyleneimine (PEI)

Figure 27. polyethyleneimine structure.

1.8.3 Encapsulation (lipid nanoparticles)

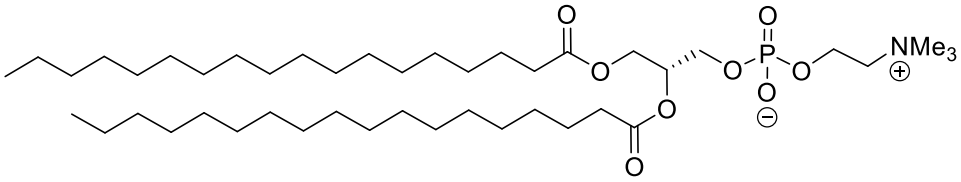
Lipid nanoparticles (LNPs), also known as lipid carriers, are used to transport inefficient hydrophobic drugs as they are composed of solid lipids, glycerides, waxes, fatty acids, and fixed by surface active agents. LNPs are able to encapsulate a variety of materials such as low molecular compounds, gold nanoparticles, peptides, DNA, and RNA.

Patisiran (first class of si-RNA drug) is formulated as lipid nanoparticles (patisiran LNP) to target delivery to hepatocytes in the liver, which is the site of expression, synthesis, and secretion of TTR. The nanoparticles are composed of four lipid excipients: DLin-MC3-DMA; PEG-DMG; DSPC; and cholesterol. (**Figure 28**).



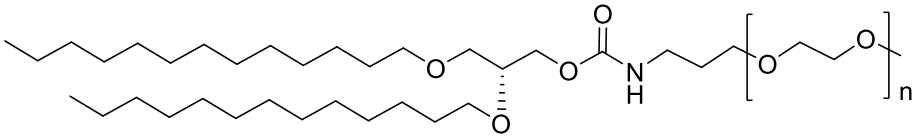
A

(6Z, 9Z, 28Z, 31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate
(DSPC)



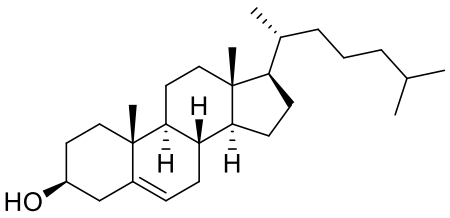
B

1,2-Distearoyl-sn-Glycero-3-Phosphocholine
(DLin-MC3-DMA)



C

α-(3'-{[1,2-di(myristyloxy)proponoxyy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene
(PEG-DMG)



D

Cholesterol

E

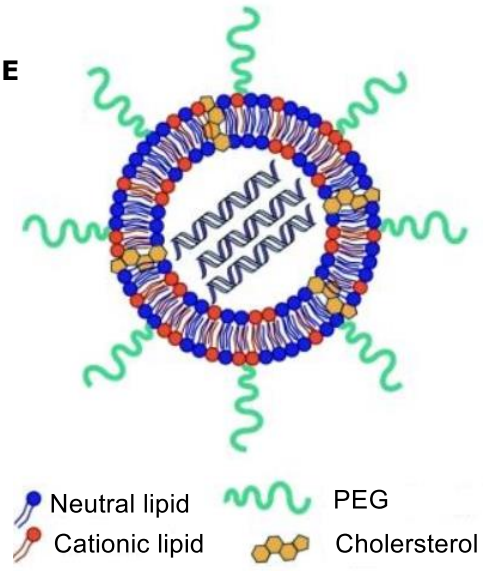
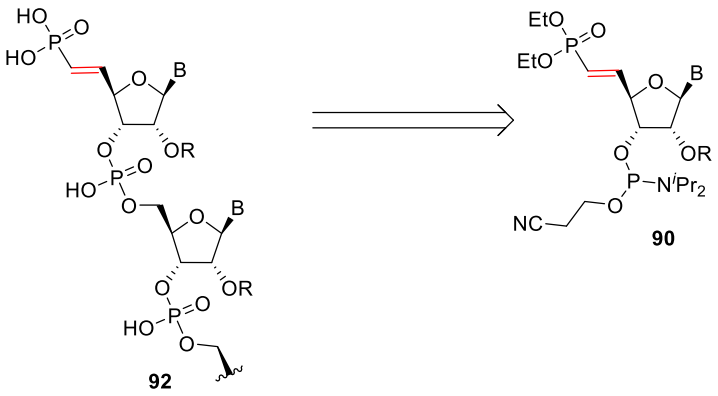


Figure 28. (**A**, **B**, **C**, and **D);** chemical structures of lipid excipients in Patisiran finished product. (**E**) Stable nucleic acid lipid particles (SNALPs) encapsulate siRNA as cargo. They are formulated with cationic and neutral lipids, cholesterol, and poly(ethylene glycol) (PEG). picture (**E**) were reproduced from Kanasty publication.[101]

2 Aims and objectives

Lima *et a*l. recently showed that single-stranded siRNAs containing a single (*E*)-vinylphosphonate at the 5'terminus (position nt 1) (**92**, **Scheme 16)** efficiently activate the RNAi response. The current chemistry, however, involves 16 steps and is protected by patent rights.[99] In addition, it is limited to modification of only the nt 1 position at the 5'-terminus of the oligonucleotide **92**, and the published synthetic route using phosphoramidite **90** (**Scheme 15**) cannot be used to incorporate modifications at other desirable internucleotide positions. Furthermore, the ethyl ester-protecting group used for the terminal vinylphosphonate is not compatible with standard deprotection protocols, which makes the synthesis and purification of the oligonucleotide **92** more difficult compared to when standard phosphoramidite reagents are used (**Scheme 16)**.



Scheme 16. The retrosynthesis of oligonucleotide **92**. The VP modification can be only at position 1 at 5'-end.

X-ray crystal structures of Ago2 bound to both unmodified and 5′-(*E*)-VP-containing ssRNA have recently been reported (**Figure 24,** page 32). The Ago2/unmodified ssRNA X-ray structure (PDB 5JS1, **Figure 29**) shows that the β–torsion angle of the 5′-terminal nucleotide (nt 1) is 167.9°, which is closely mimicked by the (*E*)-VP that has a β–torsion angle constrained to 180°.

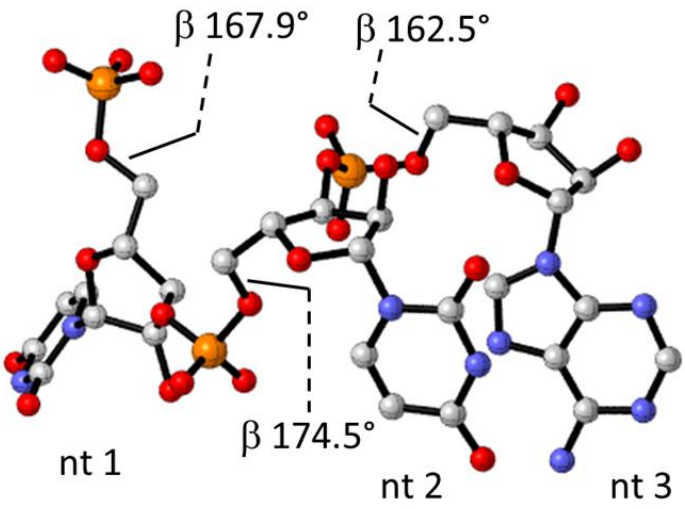
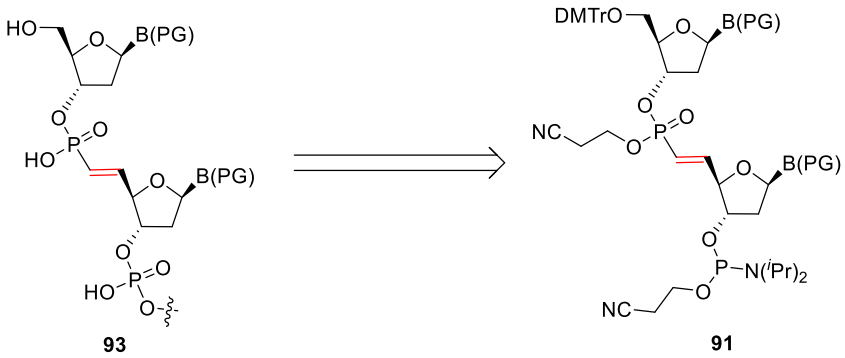


Figure 29 β-Torsion angles for the first three nucleotides (nt 1, nt 2, nt 3) from the 5′-terminus taken from the X-ray crystal structure of unmodified RNA bound to human AGO2 (PDB 5JS1). Hydrogen atoms, and AGO2 have been omitted for clarity.

Our own further analysis of the X-ray structure shows that the 180° β–torsion angle of the (*E*)-vinylphosphonate modification is an even better match for the 174.5° β–torsion angle of the second nucleotide (nt 2, **Figure 29**) in the AGO2 complex. Therefore, we aimed to study the inclusion of an (*E*)-vinylphosphonate modification at the nt 2 position. Furthermore, in this project we, aimed to examine the incorporation of both single and multiple vinylphosphonates (and combination thereof) into a wider variety of positions along the ssRNAs backbone, to see if further nuclease protection can be conferred.

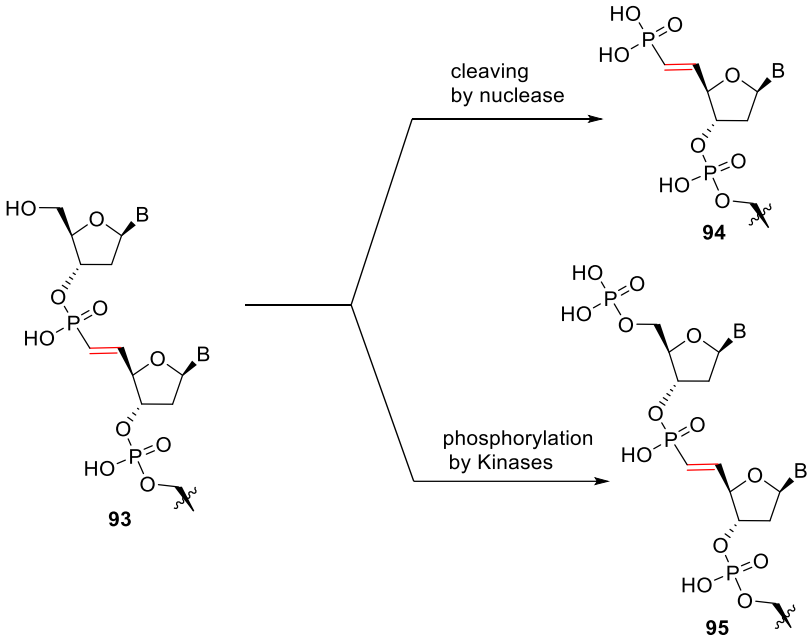
In order to test our hypothesis that an (*E*)-vinylphosphonate would be tolerated at the nt 2 position, we first needed to develop a synthesis of a suitable phosphoramidite reagent (**91**, **Scheme 17)** that could be used to construct the ssRNA antisense strand **93**. The presence of at 5'-DMTr group makes it entirely suitable for the solid phase synthesis and compatible with standard synthesis and deprotection protocols of DNA/RNA oligonucleotide synthesis and purification.



Scheme 17. The retrosynthesis of oligonucleotide **93** form our proposed phosphoramidite **91**.

We proposed to use the DNA derived phosphoramidite **91** instead of an RNA derived variant since the crystal structure shows that the presence of modification at 2' is not essential to stabilize the arganoute/ss-siRNA complex as there is no interaction between these modifications with AGO protein residues (**Figure 24**). Furthermore, incorporating the DNA phosphoramidite dimer **91** in the ss-siRNA would significantly simplify synthesis and handling as standard coupling, deprotection, and purification protocols could be used.

Although (*E*)-VP is not located at the terminal nucleotide, it is possible that once the ss-siRNA **93** is inside the cell, nuclease cleavage of the 5′-terminal nucleoside, could give an oligomer that bears a terminal 5′-(*E*)-VP **94.** The resultant oligomer **94** will still be able to activate the RNAi response although it is one nucleotide shorter (**Scheme 18**).

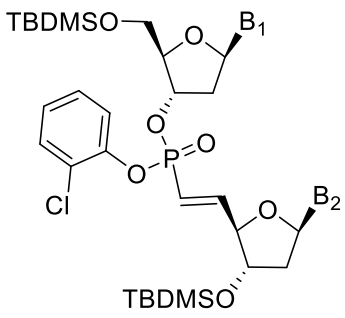


Scheme 18. The two possible pathways of how vinylphosphonate-linked DNA dinucleotide can be processed in the cell. We hypothesised that once the oligonucleotide is inside the cell the (*E*)-VP dinucleotide **93** will be either partially digested by nucleases which results in loss of the terminal nucleotide and production of oligonucleotide that is shorter **94**, but still has the 5′-(*E*)-vinylphosphonate modification **94**. Or the terminal nucleotide in dimer can be phosphorylated on its 5′-end **95**.

It has been shown that for the siRNAs, the first three and last five nucleotides do not bind to the target mRNA, whereas the central nucleotides are more important.[102] Therefore, even if one nucleotide is cleaved, it should not affect the activity of the oligonucleotide. An alternative mode of RNAi activation could be the terminal nucleotide with 5′-hydroxyl **93** can be phosphorylated in the cell by the cellular kinases to afford the phosphorylated form of ss-siRNA **95** (**Scheme 18**).

In order to make the phosphoramidite reagent **91**, we need first to develop a reliable and scalable synthetic route from readily available DNA nucleosides. Therefore, we will start this project aiming to make all the possible combinations of dinucleotides (**96** general structure, **Table 1**).

Table 1. our aim of making all the possible combination of (*E*)-vinylphosphonate linked-dinucleotide. general structure of dinucleotides **96** on the left. All the possible combination of dinucleotide on the right, star (*) represent VP modification.

 **96**	B_1*B_2 **96**	B_1*B_2 **96**	B_1*B_2 **96**	B_1*B_2 **96**
	T*T **105**	T*A **84**	T*C **128**	T*G **137**
	A*T **72**	A*A **119**	A*C **127**	A*G **136**
	C*T **106**	C*A **120**	C*C **129**	C*G **138**
	G*T **107**	G*A **121**	G*C **130**	G*G **139**

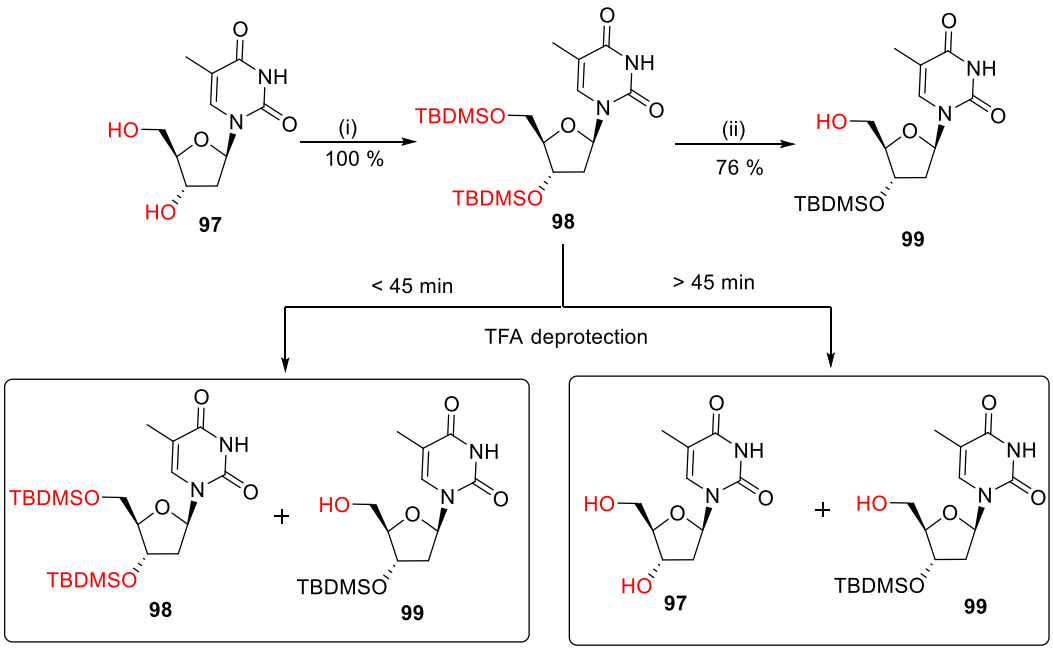
We will then investigate methods for vinylphosphonate dimer incorporation into ssRNA sequences, and we will access their gene silencing ability *in vitro* and *in vivo*.

3 Results and discussion

3.1 Synthesis of **72**, **105**, **106** and **107** dinucleotides

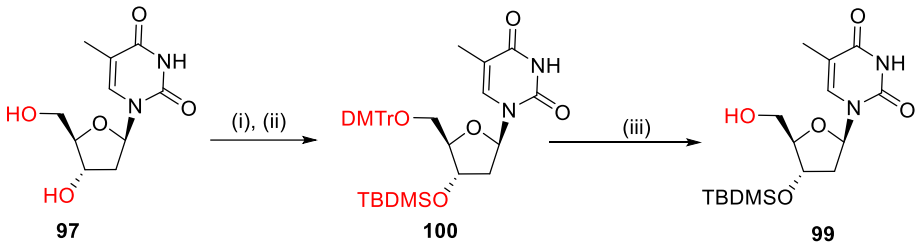
It was not possible for both cross-metathesis and cross-coupling methods to provide the access to all the possible combinations of the (*E*)-VP linked-dinucleotides. Based on our group's encouraging results with the Wittig reaction and esterification method, we therefore decided to follow this approach in the aim of making all the sixteen possible combinations of dinucleotides.

Thymidine **97** was TBDMS protected in the presence of imidazole, forming 3',5'-bis(OTBDMS) thymidine **98** in a quantitative yield. This was deprotected to primary alcohol **99** using two different conditions; firstly, TFA/H_2O (10/1 v/v) to provide alcohol **99** in 76% and 15% of recovered starting material, secondly using $CuSO_4.H_2O$ at 50 °C in methanol to give alcohol **99** in 55%. The TFA/H_2O condition need to be controlled to 45 min, otherwise deprotection of both TBDMS groups occurred at time more than 1 hr. While, incomplete deprotection was observed at time less than 30 min, which led to substrate **98** is being recovered (**Scheme 19**).



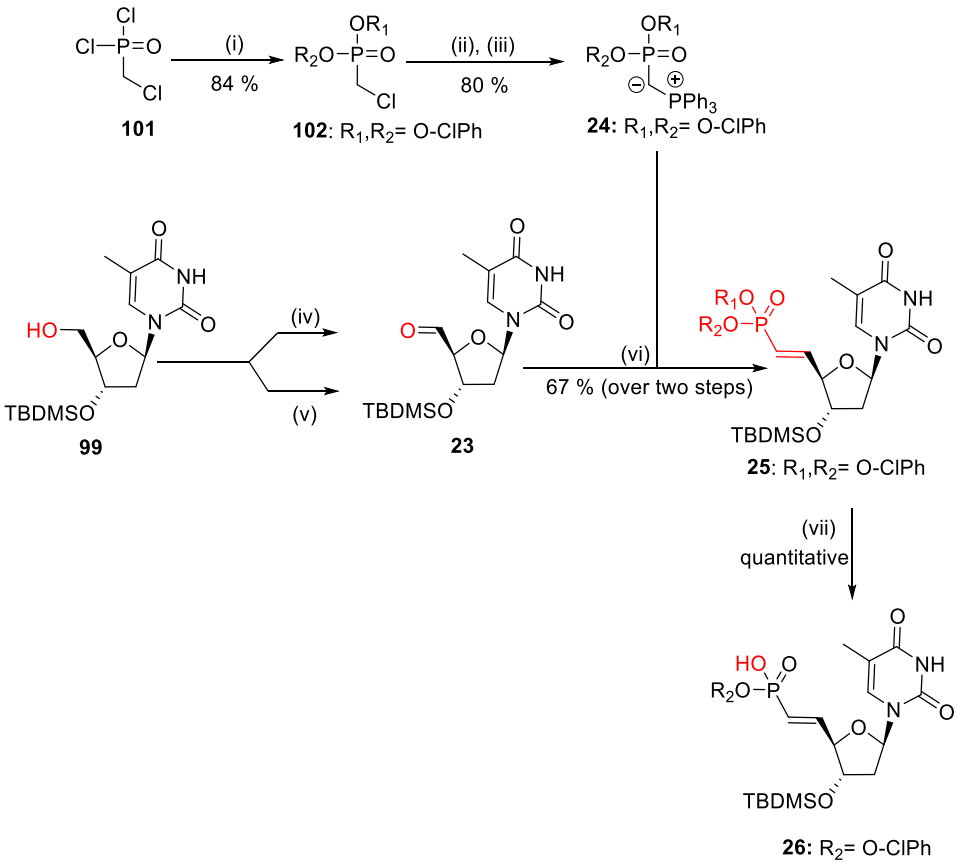
Scheme 19. Synthesises of alcohol **99.** Reagents: (i) 2.5 eq. TBDMSCl, 5.0 eq. imidazole, DMF, RT, 18h; (ii) TFA/H_2O (10/1, v/v), CH_2Cl_2, RT, 45 min.

A more consistent method was used to make alcohol **99** which involved 5'-DMTr protection of thymidine **97** to give **22,** then TBDMS protection of the 3'- OH followed by DMTr deprotection using dichloroacetic acid/CH_2Cl_2 (3%, v/v). Alcohol **99** was obtained in 88% as a white foam, and 11% recovery of starting material **100** (**Scheme 20**).



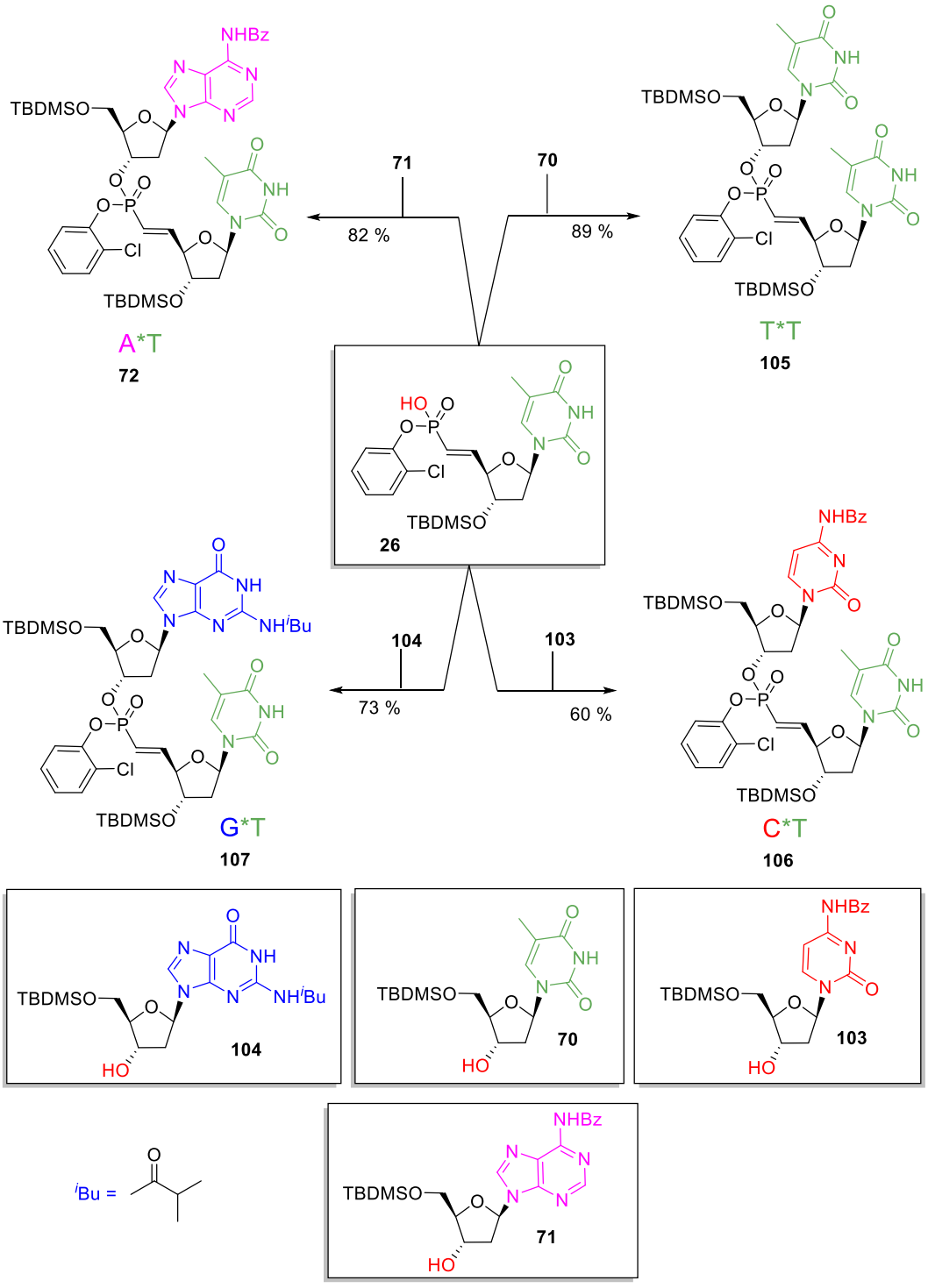
Scheme 20. Synthesis of alcohol **99** from DMTr deprotection. Reagents: (i) DMTrCl, pyridine, rt, 22h; (ii) 1.5 eq. TBDMSCl, 3.5 eq. imidazole, DMF, rt, 3 d; (iii) dichloroacetic acid/CH_2Cl_2 (3%, v/v), RT, 15 min.

Alcohol **99** was then oxidised to aldehyde **23** in quantitative yield using Dess-martin periodinane (DMP). Using stabilized IBX reagent gave a more straightforward workup in which the unreacted IBX and its derivatives were insoluble in cold acetonitrile and thus were easily filtered. The freshly prepared aldehyde **23** was used directly for the next step without any further purification. The key step in the vinylphosphonate-linked dinucleotide synthesis is the reaction between the aldehyde **23** and the previously made ylide **24**, which leads to generation the trans carbon-carbon double bond **25** in (67% over two steps) (**Scheme 21**). Phosphonic acid **26** was generated by the selective deprotection of one of the two *O*-chlorophenyl groups in (*E*)-vinylphoaphonate **25** using 2-nitrobenzadoxime, tetramethylguanidine, and triethylamine in dioxane.



Scheme 21. Synthesis of (*E*)-vinylphosphonate *via* a Wittig reaction and esterification approach. Reagents: (i) 2.0 eq. 2-chlorophoenol, 2.0 eq. triethylamine, RT, 20; (ii) 1.0 eq. PPh_3, 160 °C, 18h; (iii) 2M NaOH, CH_2Cl_2, RT, 30 min; (iv) 1.5 eq. DMP, CH_2Cl_2, RT, 3h; (v) 3.0 eq. S-IBX, MeCN, 80 °C, 3h; (vi) 1.5 eq. Ylide, CH_2Cl_2, RT, 18 h; (vii) 1.2 eq. 2-nitrobenzaldoxime, 1.2 eq. tetramethylguanidine, 6.0 eq. triethylamine, dioxane, RT, 3d.

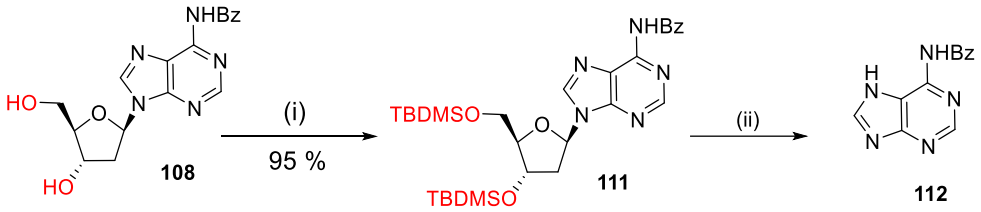
The phosphonic acid **26** was then used in the esterification reaction to generate the phosphodiester bond. Alcohols **71**, **70**, **103** and **104** were synthesized from mono TBDMS protection of their corresponding commercially available diols **108**, **97**, **109** and **110** respectively (**Scheme 22**). The coupling between these alcohols and phosphonic acid **26** was carried out in dry pyridine, in the presence of molecular sieves. Furthermore, water was removed from alcohols, phosphonic acid and methylimidazole using azeotropic distillation with pyridine 3 times. Dinucleotide **72**, **105**, **106** and **107** were obtained in good yield and were isolated as a mixture of two diastereoisomers due to the chiral phosphorus centre as determined by ^{31}P and ^{1}H NMR (**Scheme 22**).



Scheme 22. Esterification reactions to make dinucleotides **72**, **105**, **106**, and **107**. Reagents; 1.0-5.0 eq. triisopropylbenzenesulphonyl chloride, 6.0-10 eq. *N*-methylimidazole, pyridine, RT, overnight (18-22 h).

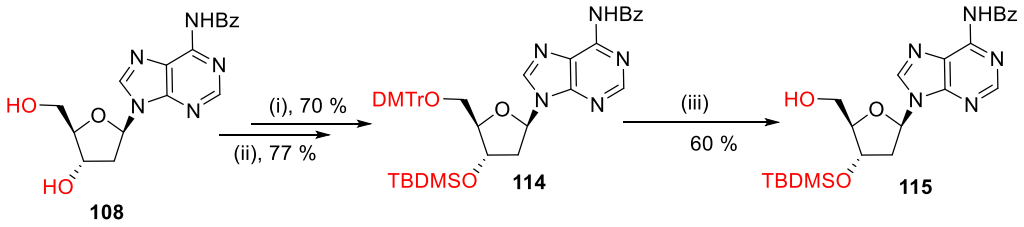
3.1.1 Synthesis of **119**, **73**, **120**, and **121** dinucleotides

Starting from commercially available *N*-benzoyldeoxyadenosine **108** and following method developed for thymidine, the starting material was protected with TBDMS group at both 5′ and 3′-positions **111** It was not possible to make alcohol **115** from *via* using the same condition that used before in 3′,5′-Bis(OTBDMS) thymidine **98**, as depurination occurred and this has led to the isolation of free adenine **112** instead of alcohol **115** (**Scheme 23**).



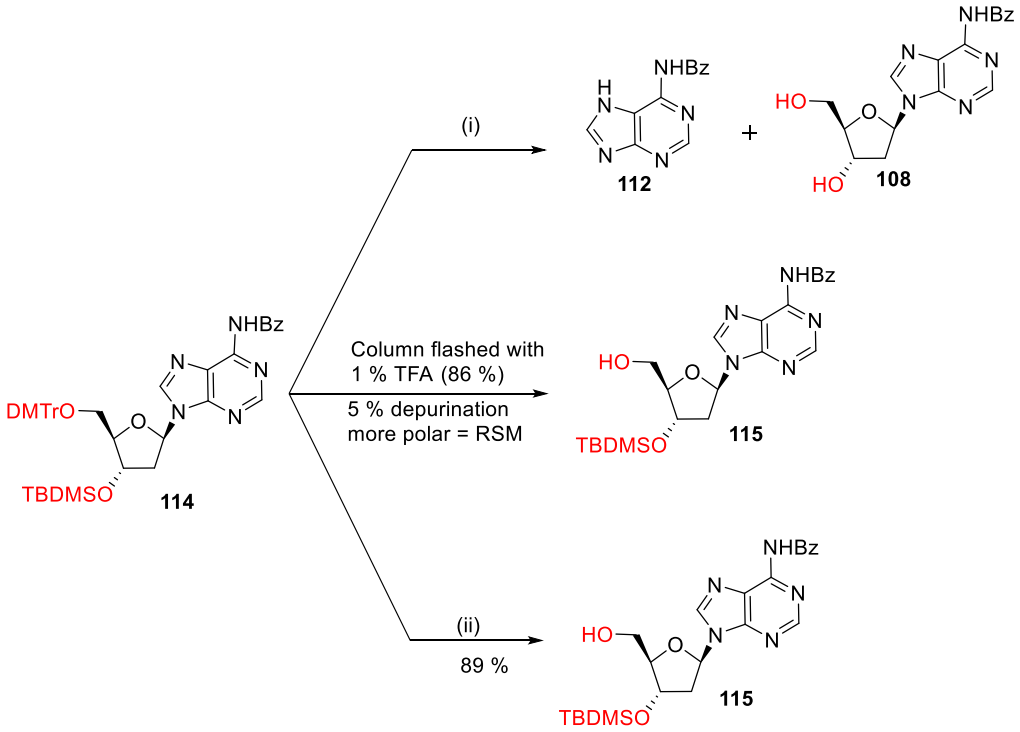
Scheme 23. First attempt of making alcohol **115** using TFA/H_2O (10:1, v/v). Reagents: (i) 2.5 eq. TBDMSCl, 5.0 eq. imidazole, DMF, RT, 22h; (ii) TFA/H_2O (10:1, v/v), CH_2Cl_2, RT, 30 min.

As it was already reported in the literature[103] that depurination can occur in concentrated acidic medium, we decided to use milder acidic conditions. The starting material **108** was protected with DMTr at 5′-position to form 5′-DMTr alcohol **113** followed by TBDMS protection at 3′-position to give **114**, selective deprotection of DMTr group were performed using TFA/H_2O, 1:1 in THF, at 0°C for 1 hr, where the alcohol **115** was obtained in 60% yield (**Scheme 24**).



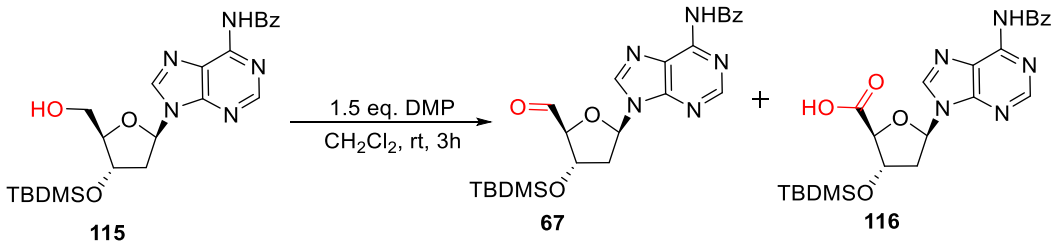
Scheme 24. Selective deprotection condition for DMTr group in the presence of TBDMS group **114**. Reagents: (i) 1.0 eq. DMTrCl, pyridine, RT, 17 h; (ii) 1.5 eq. TBDMSCl, 3.0 eq. imidazole, DMF, RT, 20 h; (iii) TFA/H_2O (1/1, v/v), THF, 0°C, 1h.

According to literature[104], another method has been also tested in the aim of selective removal of DMTr in the presence of TBDMS **114** using $NaIO_4$ in 70% aqueous acetone, but that led to depurination and fully TBDMS deprotection. Another attempt was also made when compound **114** was dry-loaded on silica gel and the column was flushed with a solvent system containing 1% TFA, alcohol **115** was obtained in 86% but the column requires a long time to complete and a large volume of solvent amount required to achieve efficient deprotection. When the polarity of the solvent system was increased it led to recovering starting material **114** while when the percentage of TFA was increased to 5%, depurination was observed. Finally, efficient selective DMTr deprotection was achieved using 3% dichloroacetic acid/dichloromethane, the reaction mixture was stirred at room temperature for 10-20 min. However, depurination **112** occurred at a time more than 20 min. Therefore, the optimum time found was 10 min. (**Scheme 25**).



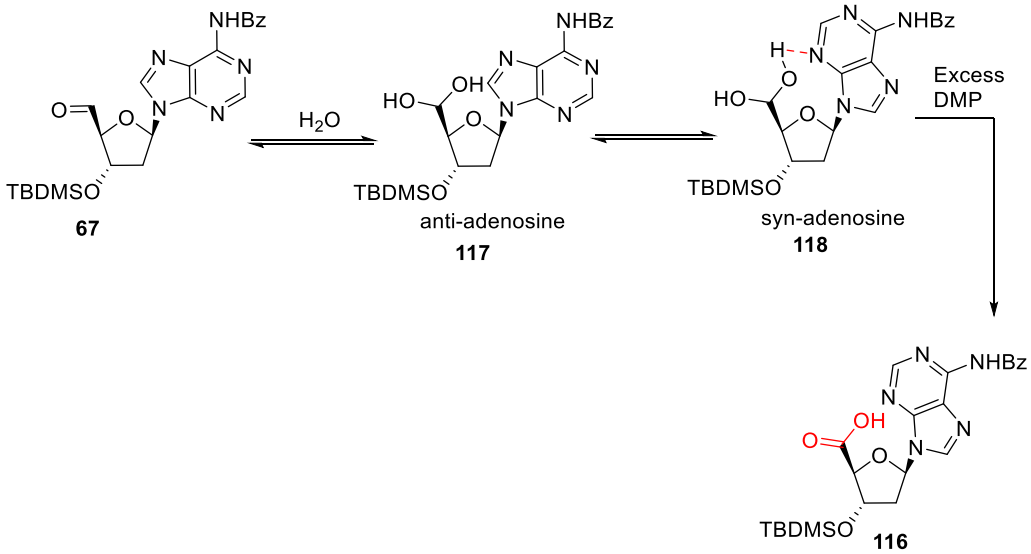
Scheme 25. Screening different methods for selective deprotection of DMTr group in **114**. Reagent: (i) 10 eq. $NaIO_4$, 70% aq. acetone, RT, 20h; (ii) dichloroacetic acid/ CH_2Cl_2 (3%, v/v), RT, 10 min.

Unexpectedly, using an excess of DMP reagent (1.5 eq.), to oxidize alcohol **115** into aldehyde **67**, led to the formation of the carboxylic acid **116** in 40% and aldehyde **67** in 60% (**Scheme 26**).



Scheme 26. Aldehyde **67** overoxidation using an excess of DMP.

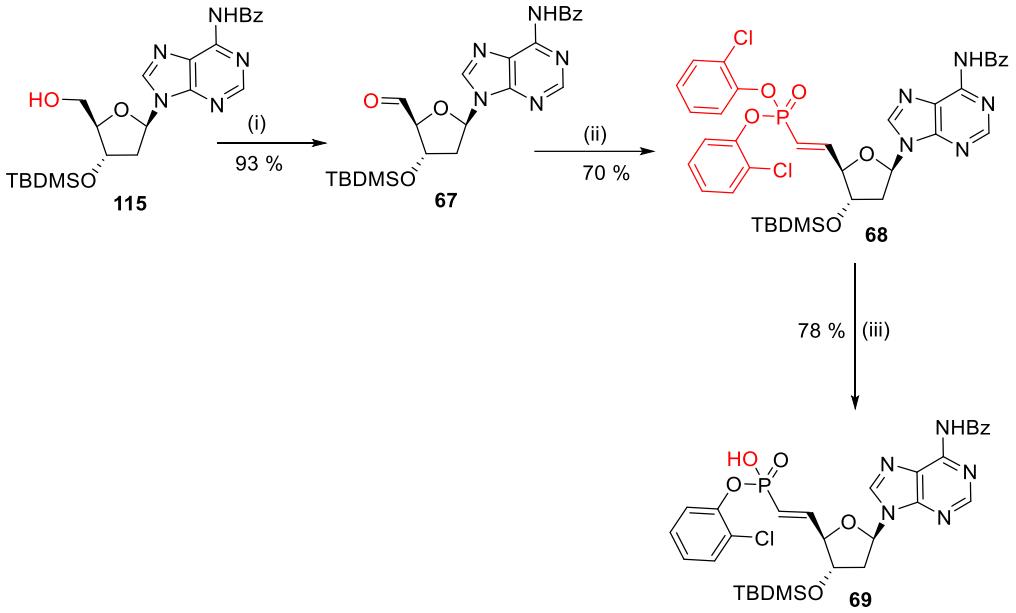
It was known that DMP offers selective and very mild oxidation of alcohols to aldehydes or ketones. However, the overoxidation of aldehyde **67** can be explained due to the stability of the hydrate **117** form of aldehyde **67**. The rotation of adenine heterocycle from anti **117** to syn-form **118** promotes hydrogen bonding between the nitrogen in the adenine and the hydrate hydroxyl in **118**, and that makes the hydrate **118** more stable. Thus, hydrate **118** can be oxidized by the access of the DMP reagent into a carboxylic acid **116** (**Scheme 27**).



Scheme 27. Hypothesis of overoxidation by DMP.

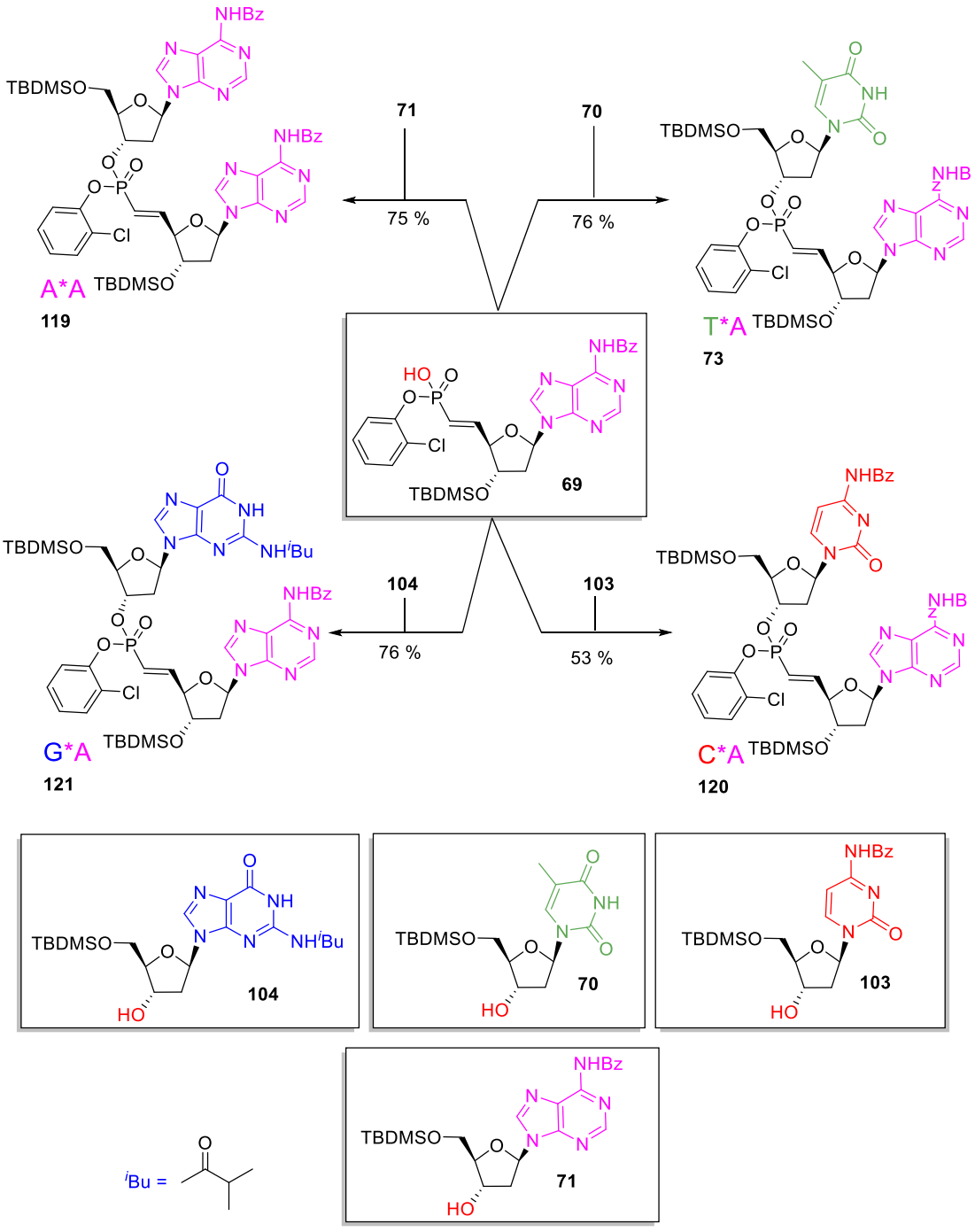
The formation of carboxylic acid **116** was confirmed mass spectrometry, ^{13}C and HMBC spectra, the carboxylic acid carbonyl at nearly 177 ppm on ^{13}C are coupled to the CH-4' proton.

The reaction was repeated using only one equivalent of DMP and clean oxidation was observed with no carboxylic acid **116** was observed. Aldehyde **67** was used in the next step without any further purification and was reacted with ylide **24** to form (*E*)-vinylphosphonate **68**. Deprotection using the same previous conditions then generated the phosphonic acid **69** (**Scheme 28**).



Scheme 28. Synthesis of phosphonic acid **69**. Reagents: (i) 1.0 eq. DMP, CH_2Cl_2, RT, 3h; (ii) 1.5 eq. Ylide **24**, CH_2Cl_2, RT, 21 h; (iii) 1.2 eq. 2-nitrobenzaldoxime, 1.2 eq. tetramethylguanidine, 6.0 eq. triethylamine, dioxane, RT, 3d.

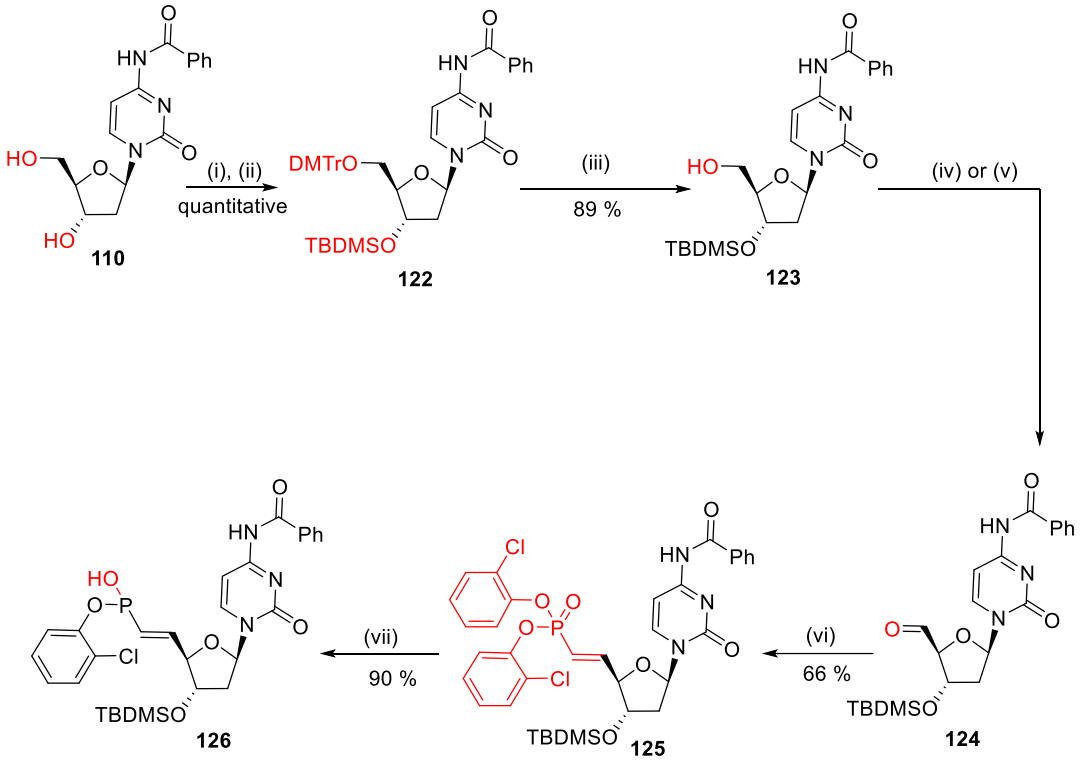
Phosphonic acid **69** was then coupled with alcohols **71**, **70**, **103,** and **104** using the same conditions used before for **26**. Thus, dinucleotides **119**, **73**, **120** and **121** were obtained respectively in good yield, and were isolated as mixtures of two diastereoisomers (**Scheme 29**).



Scheme 29. Synthesis of dinucleotides; **119**, **77**, **120,** and **121**. Reagents; 1.0-5.0 eq. triisopropylbenzenesulphonyl chloride, 6.0-10.0 eq. *N*-methylimidazole, pyridine, RT, overnight (19-25 h).

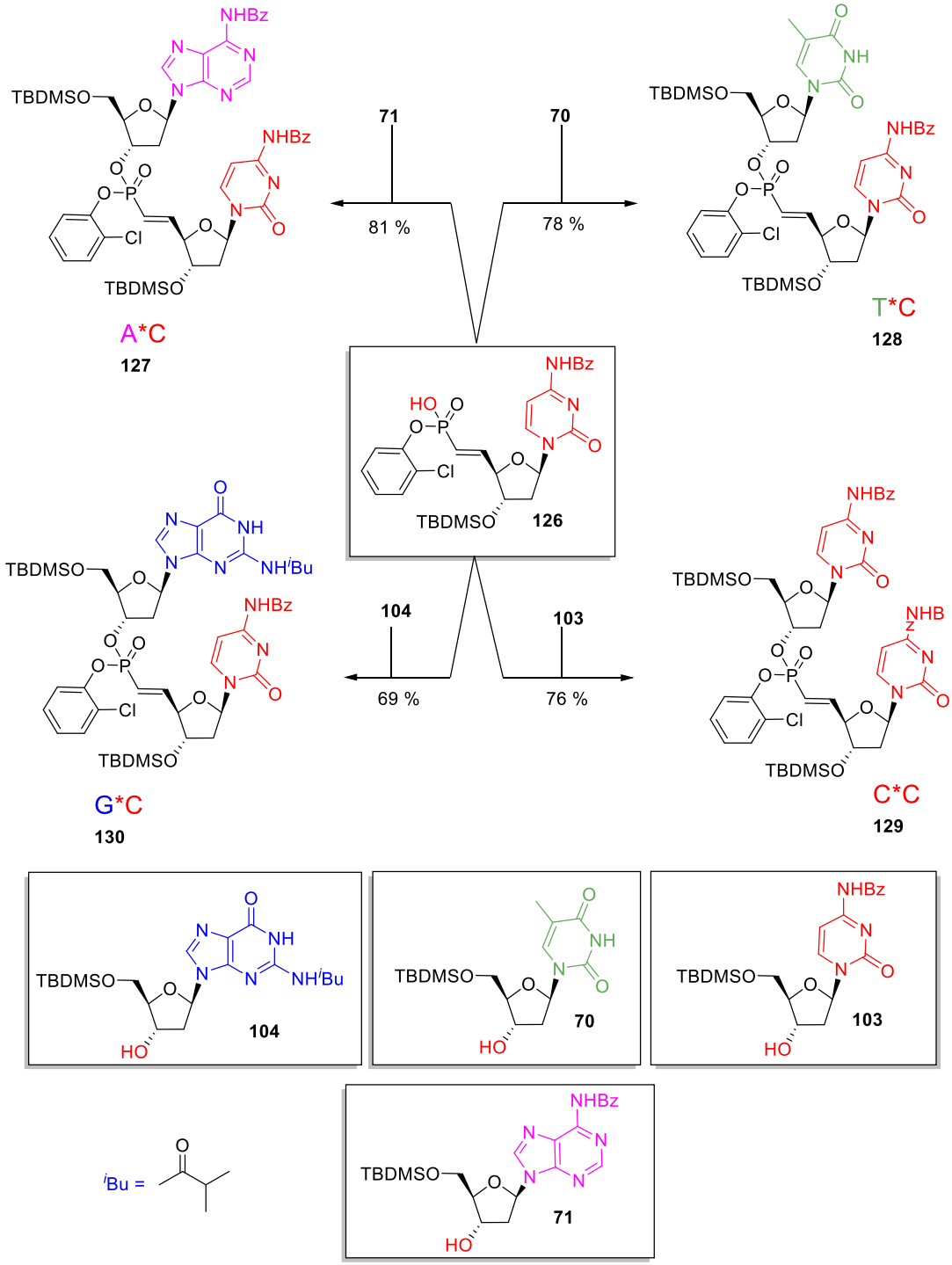
3.1.2 Synthesis of 127, 128, 129 and 130 dinucleotides

Both cytidine and thymidine are pyrimidine bases, and they have similar chemistry in term of stability. Starting from commercially available *N*-benzoyldeoxycytidine **110** and following the same procedures that have been used in thymidine chemistry, phosphonic acid **126** was prepared as illustrated in (**Scheme 30**).



Scheme 30. Synthesis of phosphonic acid **126**. **Reagents**: (i) 1.0 eq. DMTrCl, pyridine, RT, 21 h; (ii) 1.5 eq. TBDMSCl, 3.0 eq. imidazole, DMF, RT, 23 h; (iii) dichloroacetic acid/ CH_2Cl_2 (3%, v/v), RT, 20 min; (iv) 1.5 eq. DMP, CH_2Cl_2, RT, 3h; (v) 3.0 eq. S-IBX, MeCN, 80 °C, 3h; (vi) 1.5 eq. Ylide **24**, CH_2Cl_2, RT, 19 h; (vii) 1.2 eq. 2-nitrobenzaldoxime, 1.2 eq. tetramethylguanidine, 6.0 eq. triethylamine, dioxane, RT, 3d.

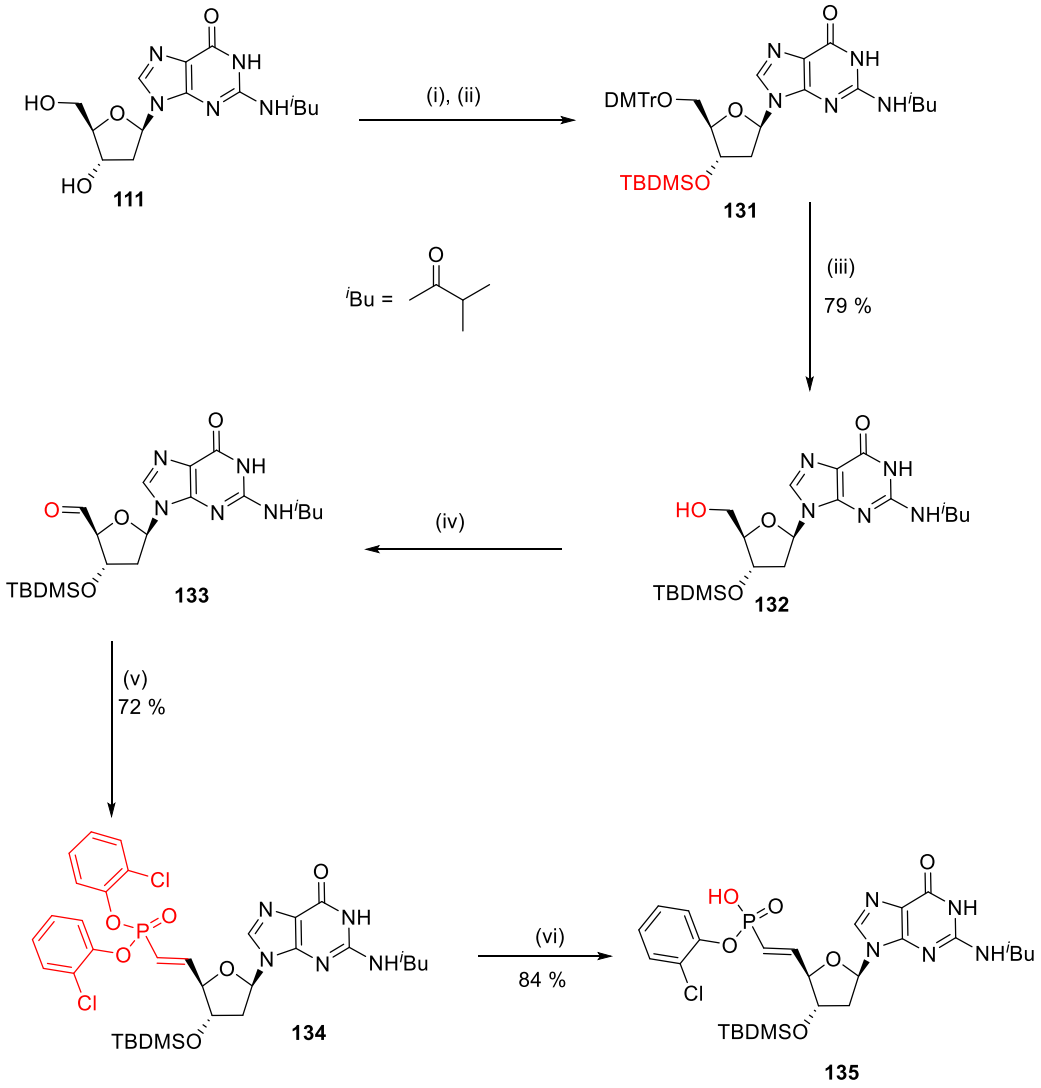
Phosphonic acid **126** was then coupled with alcohols **71**, **70**, **103** and **104** using the conditions used previously to give the dinucleotides **127**, **128**, **129** and **130** as mixtures of two diastereoisomers (**Scheme 31**).



Scheme 31. Synthesis of dinucleotides; **127**, **128**, **129,** and **130**. Reagents; 1.0-5.0 eq. triisopropylbenzenesulphonyl chloride, 6.0-10.0 eq. *N*-methylimidazole, pyridine, RT, overnight (19-26 h).

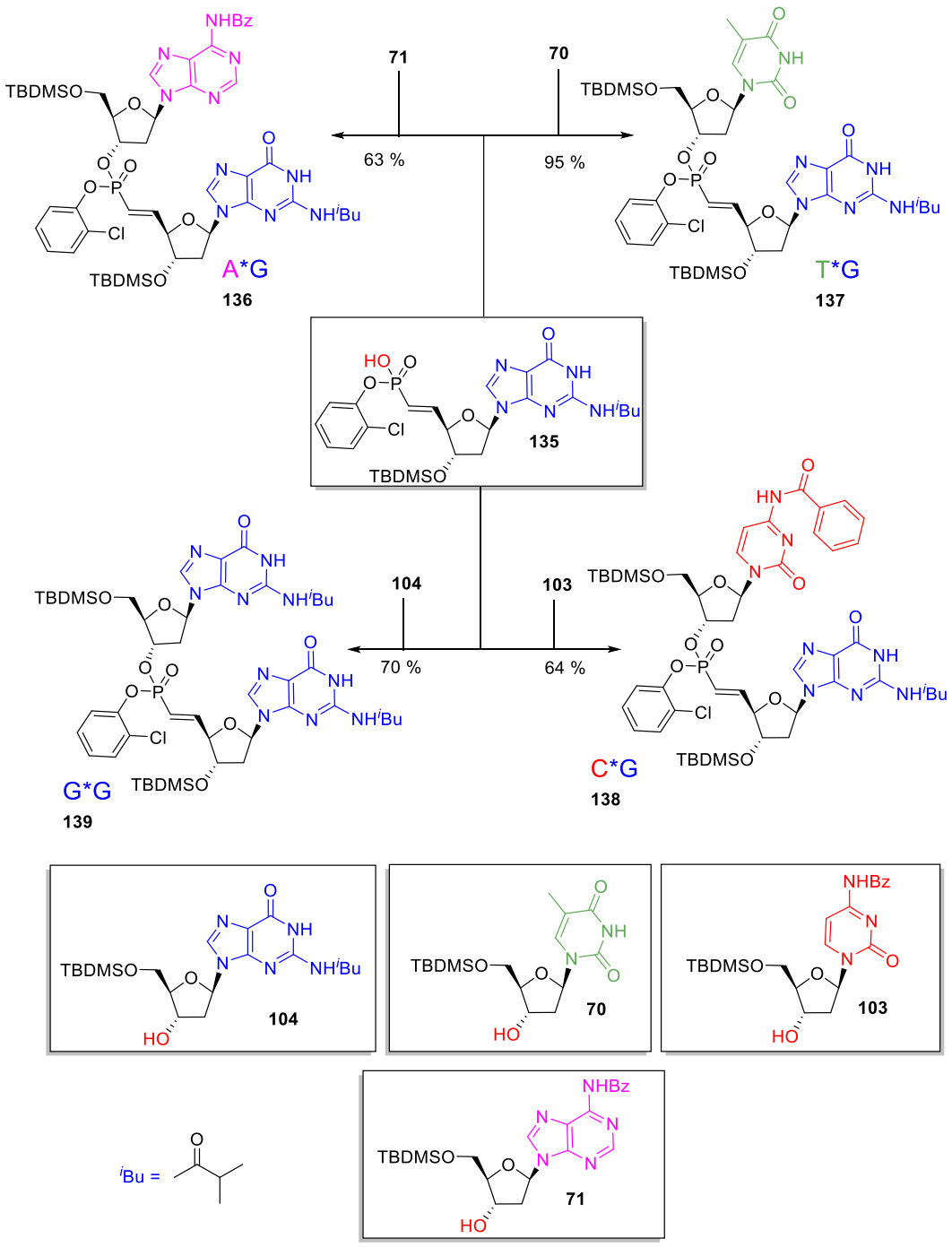
3.1.3 Synthesis of 136, 137, 138, and 139 dinucleotides

2-*N*-isobutyryl deoxyguanosine **111** was used as a commercially available starting material and following the same conditions and procedures that have been used in the adenosine chemistry, the phosphonic acid **135** was synthesised as shown in **Scheme 32**.



Scheme 32. Synthesis of phosphonic acid **135**. **Reagents**: (i) 1.0 eq. DMTrCl, pyridine, RT, 24 h; (ii) 1.5 eq. TBDMSCl, 3.0 eq. imidazole, DMF, RT, 26 h; (iii) dichloroacetic acid/ CH_2Cl_2 (3%, v/v), RT, 10 min; (iv) 1.0 eq. DMP, CH_2Cl_2, RT, 3h; (v) 1.5 eq. Ylide, CH_2Cl_2, RT, 20 h; (vi) 1.2 eq. 2-nitrobenzaldoxime, 1.2 eq. tetramethylguanidine, 6.0 eq. triethylamine, dioxane, RT, 26 h.

Phosphonic acid **135** was then reacted with alcohols **71**, **70**, **103,** and **104** using the same conditions used previously (**Figure 33**).

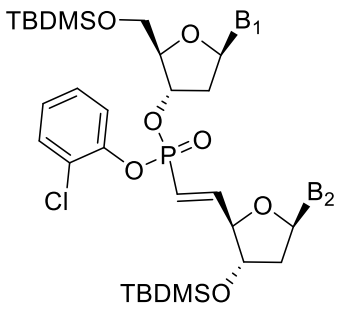


Scheme 33. Synthesis of dinucleotides; **136**, **137**, **138**, and **139**. Reagents; 1.0-5.0 eq. triisopropylbenzenesulphonyl chloride, 6.0-10.0 eq. *N*-methylimidazole, pyridine, RT, overnight (18-26 h).

3.1.4 Synthesis of phosphoramidites T*T, T*A, A*T and A*A

The main aim of this research has been now achieved, which is making all the 16 possible combinations of nucleotides *via* a Wittig reaction and esterification approach. All dimers were obtained in acceptable yields (**Table 2**).

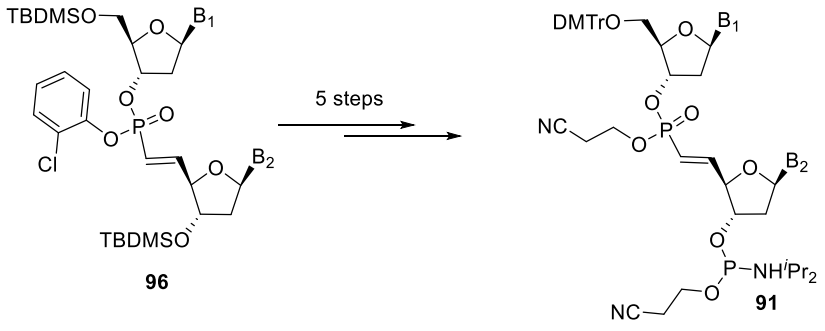
Table 2. Yield percentage for the 16 different combinations of dinucleotides from the coupling step.



96

B_1 / B_2	A	G	C	T
A	**119**, 75%	**121**, 76%	**120**, 53%	**73**, 76%
G	**136**, 63%	**139**, 70%	**138**, 64%	**137**, 95%
C	**127**, 81%	**130**, 69%	**129**, 76%	**128**, 78%
T	**72** 82%	**107**, 73%	**106**, 60%	**105**, 89%

The dimers then needed to be further functionalized in order to be compatible with DNA/RNA synthesizer protocols. They should have a phosphoramidite at 3'-position, DMTr group at 5'-position and cyanoethyl protection at the phosphate **91**. Therefore, five more chemical steps are required before the required phosphoramidite is generated (**Scheme 34**).



Scheme 34. Functionalization of nucleotide **96** for DNA/RNA synthesis.

Four dimers were selected to be functionalized into their corresponding phosphoramidites. The selected dimers have varying purine-pyrimidine combinations. T*T **41**, which has a pyrimidine at the top and the bottom of the dimer, A*T **83** has purine at the top and pyrimidine at the bottom, T*A **84** has pyrimidine at the top and purine at the bottom while A*A **140** has purine at the top and the bottom of the molecule A*A **140** (**Figure 30**).

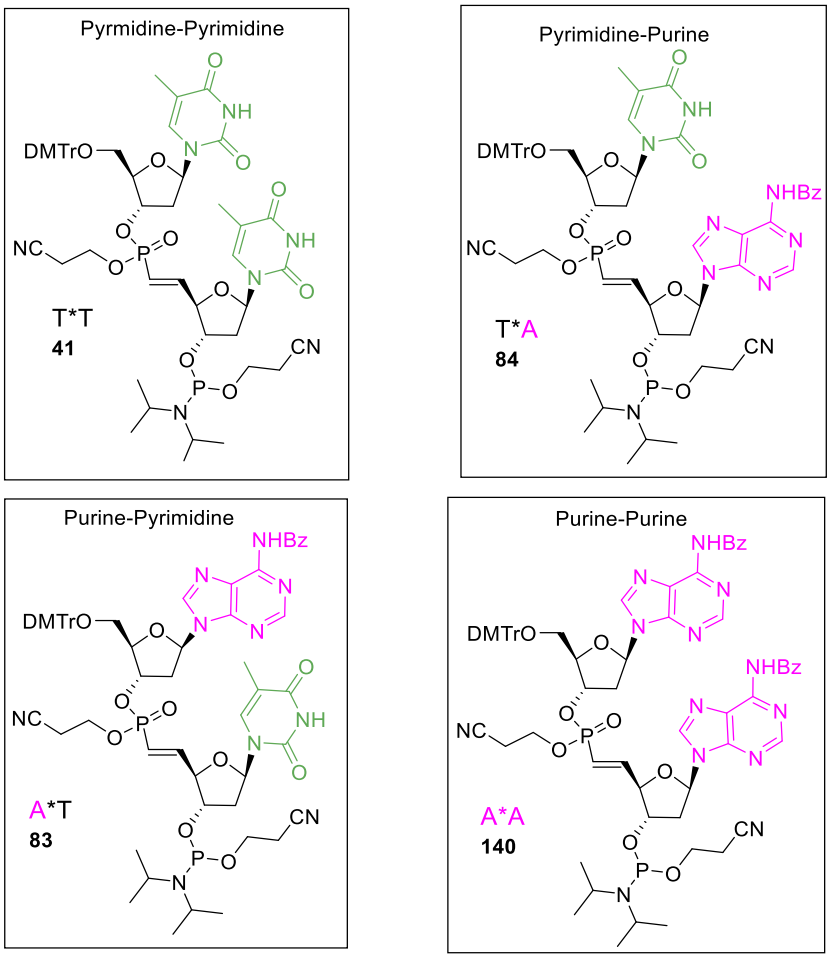
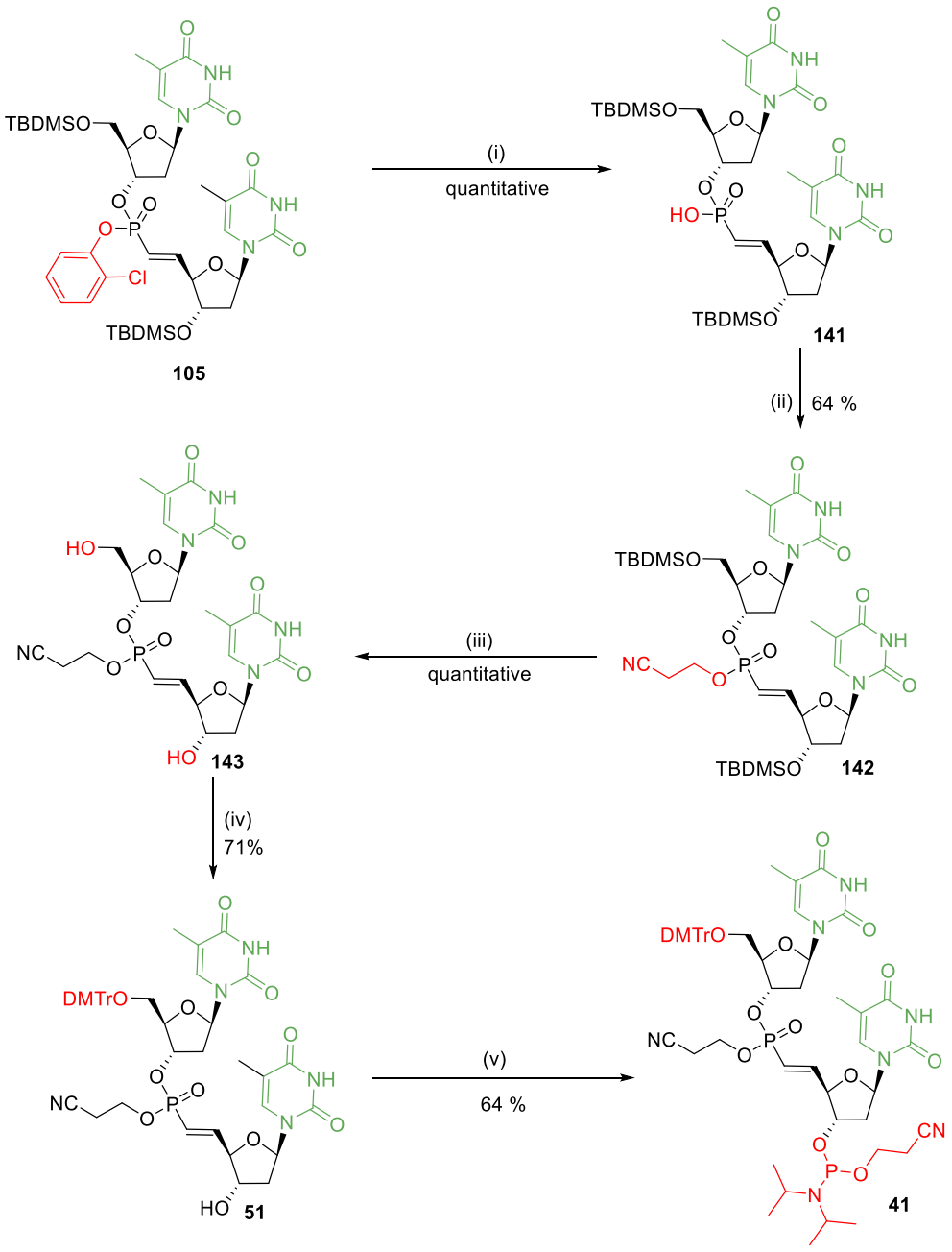


Figure 30. (*E*)-VP-modified Phosphoramidite reagents.

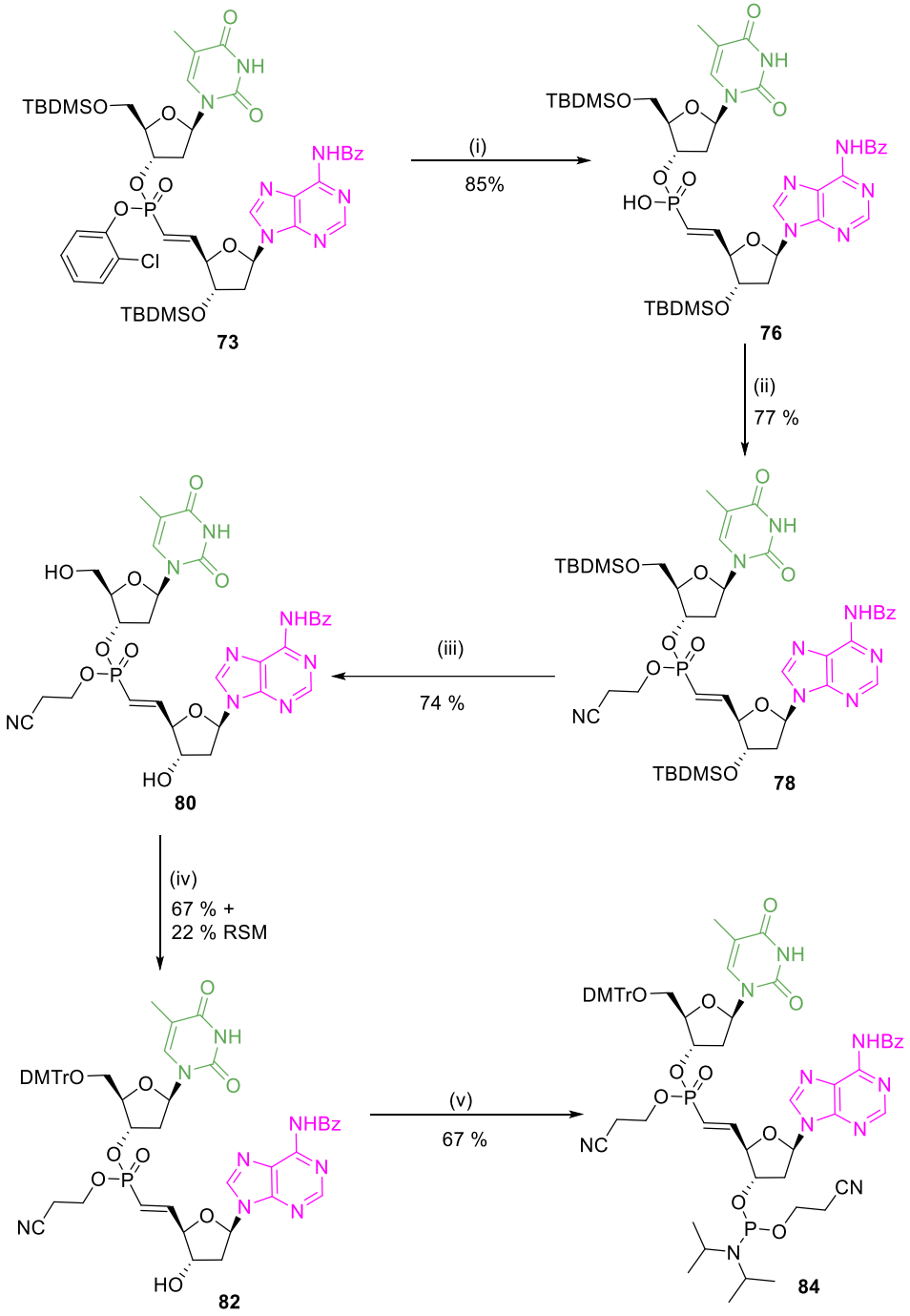
The last chlorophenyl group of dinucleotide **105** was removed using the same condition that previously used to remove the first one from **25** (**Scheme 35**). Thus, phosphonic acid **141** was obtained as one isomer and was protected with cyanoethyl alcohol to generate dinucleotide **142** in a mixture of two diastereoisomers. The reason of choosing this group is related to its stability in the acidic medium during TBDMS deprotection and it is compatible with

DNA/RNA synthesis protocols and can be removed easily from the oligonucleotide backbone by using ammonia solution. Both TBDMS group were selectively removed using $Et_3N.3HF$ to form diol **141**. The diol was then protected with a DMTr group at 5'-position to form **51**. The DMTr group is susceptible to acidic conditions and can be even deprotected by silica gel. Therefore, silica gel was flashed with ammonia dissolved in dichloromethane and dichloromethane afterwards to remove ammonia to avoid cyanoethyl deprotection. The purification was performed as quickly as possible to avoid loss of the DMTr group. Finally, phosphoramidite **41** was generated at 3'-position and the final product was obtained as a mixture of four diastereoisomers (**Scheme 35**).

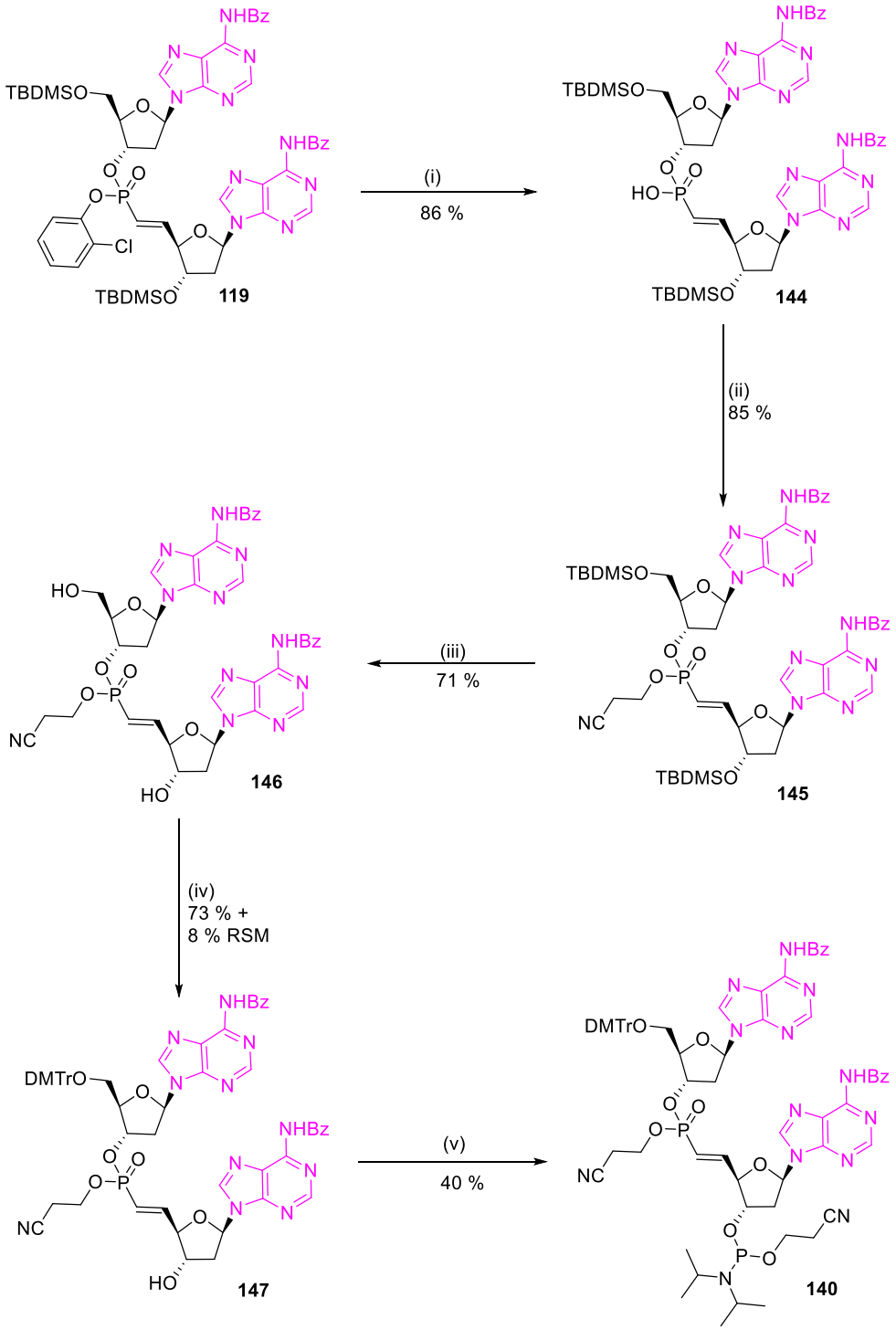


Scheme 35. Synthesis of (T*T) phosphoramidite **41**. Reagents: (i) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguandinine, 10 eq. triethylamine, dioxane, 20 h, RT; (ii) 5.5 eq. 3-hydroxypropionitrile, 5.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 17 eq. *N*-methylimidazole, pyridine, 3 h, RT; (iii) 10 eq. $Et_3N{\cdot}3HF$, THF, 4 h, RT; (iv) 1.0 eq. DMTrCl, pyridine, 2 d, RT; (v) 1.0 eq. **52**, 1.0 eq. 5-methyl-1*H*-tetrazole, CH_2Cl_2, 20 h, RT.

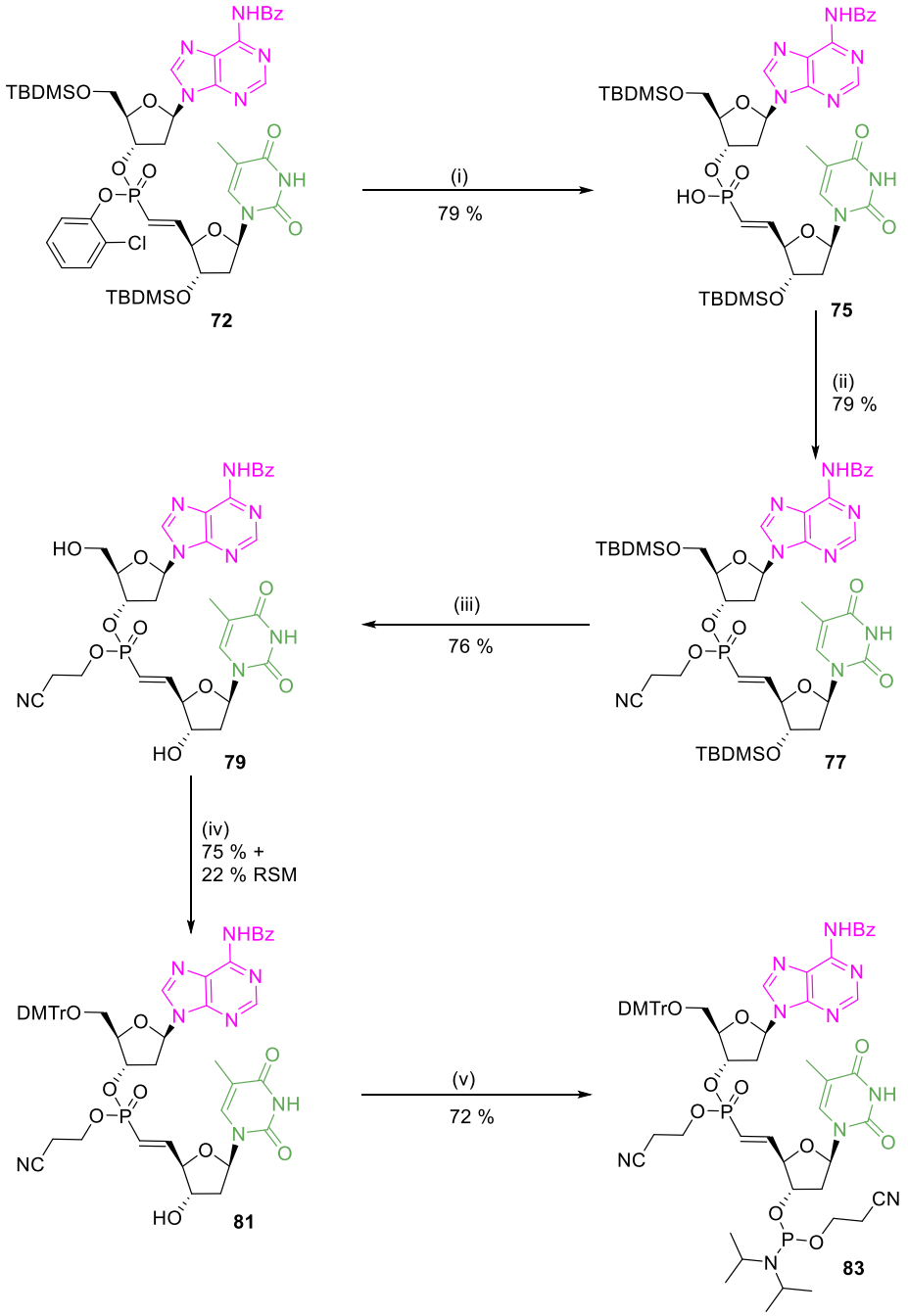
Phosphoramidites **83**, **84** and **140** were obtained as four mixture of diastereoisomers following the same procedures of making of phosphoramidite **41** as demonstrated in **schemes 36-38**.



Scheme 36. Synthesis of (T*A) phosphoraimidate **84**. Reagents: (i) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguandinine, 10 eq. triethylamine, dioxane, 20 h, rt; (ii) 5.0 eq. 3-hydroxypropionitrile, 5.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, 19 h, rt; (iii) 10 eq. $Et_3N{\cdot}3HF$, THF, 4 h, rt; (iv) 1.2 eq. DMTrCl, pyridine, 23 h, rt; (v) 1.2 eq. **52**, 1.2 eq. 5-methyl-1*H*-tetrazole, CH_2Cl_2, 20 h, rt.



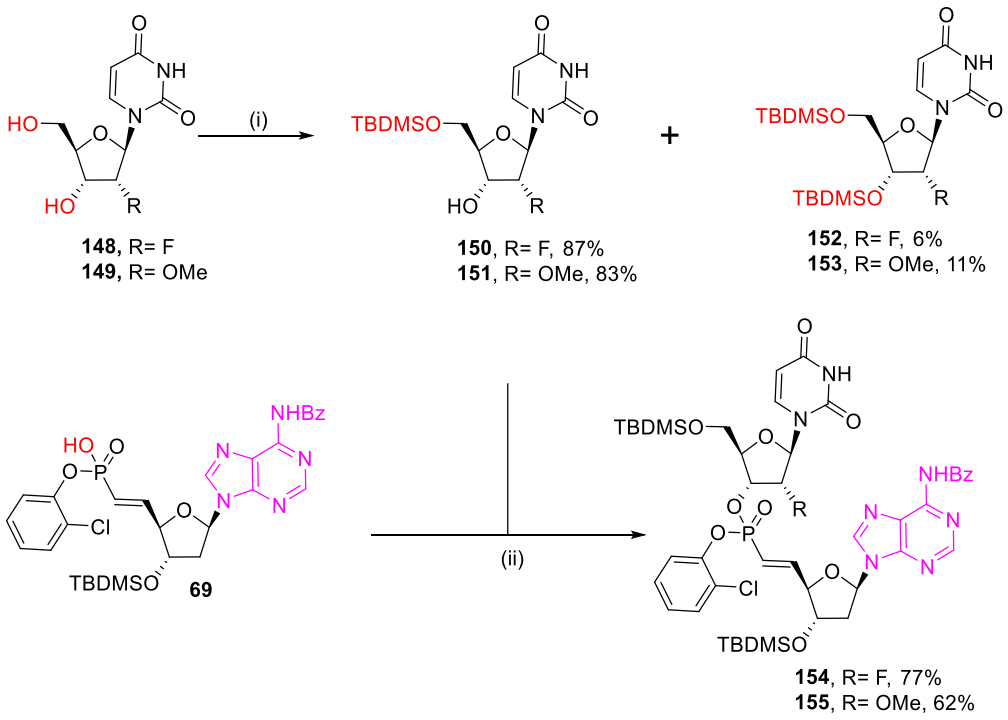
Scheme 37. Synthesis of (A*A) phosphoraimidate **140**. Reagents: (i) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguandinine, 10 eq. triethylamine, dioxane, 20 h, rt; (ii) 5.0 eq. 3-hydroxypropionitrile, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, 2 d, rt; (iii) 10 eq. $Et_3N{\cdot}3HF$, THF, 5 h, rt; (iv) 1.0 eq. DMTrCl, pyridine, 24 h, rt; (v) 1.2 eq. **52**, 1.2 eq. 5-methyl-1*H*-tetrazole, CH_2Cl_2, 19 h, rt.



Scheme 38. Synthesis of (A*T) phosphoraimidate **83**. Reagents: (i) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguandinine, 10 eq. triethylamine, dioxane, 19 h, rt; (ii) 5.0 eq. 3-hydroxypropionitrile, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, 19 h, rt; (iii) 10 eq. $Et_3N{\cdot}3HF$, THF, 4 h, rt; (iv) 1.1 eq. DMTrCl, pyridine, 4 d, rt; (v) 0.9 eq. **52**, 0.9 eq. 5-methyl-1*H*-tetrazole, CH_2Cl_2, 22 h, rt.

3.2 Synthesis of 2’-substituted dinucleotide

Having shown that the Wittig reaction and esterification approach could give access to all possible combinations of DNA nucleotides, the question now is what about RNA nucleotides? where steric and electronic effects might make the esterification more difficult than in DNA nucleotide. In order to answer this question, we have chosen two commercially available modified RNA-nucleosides; the first one has a fluorine atom at 2’-position, 2’-F-U **148** and 2’-methoxy for the other one, 2’-OMe-U **149**. These diols **148** and **149** were protected with TBDM*S*, and the primary products were isolated as mono protected alcohols **150** and **151** respectively, and minor by-products were also separated as bis(OTBDMS) nucleotides **152** and **153** respectively. The secondary alcohols **150** and **151** were coupled with phosphonic acid **69** using our previously developed conditions giving two diastereoisomers of dinucleotides **154** and **155** respectively as white foams (**Scheme 39**). The two diastereoisomers were confirmed by ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR data.



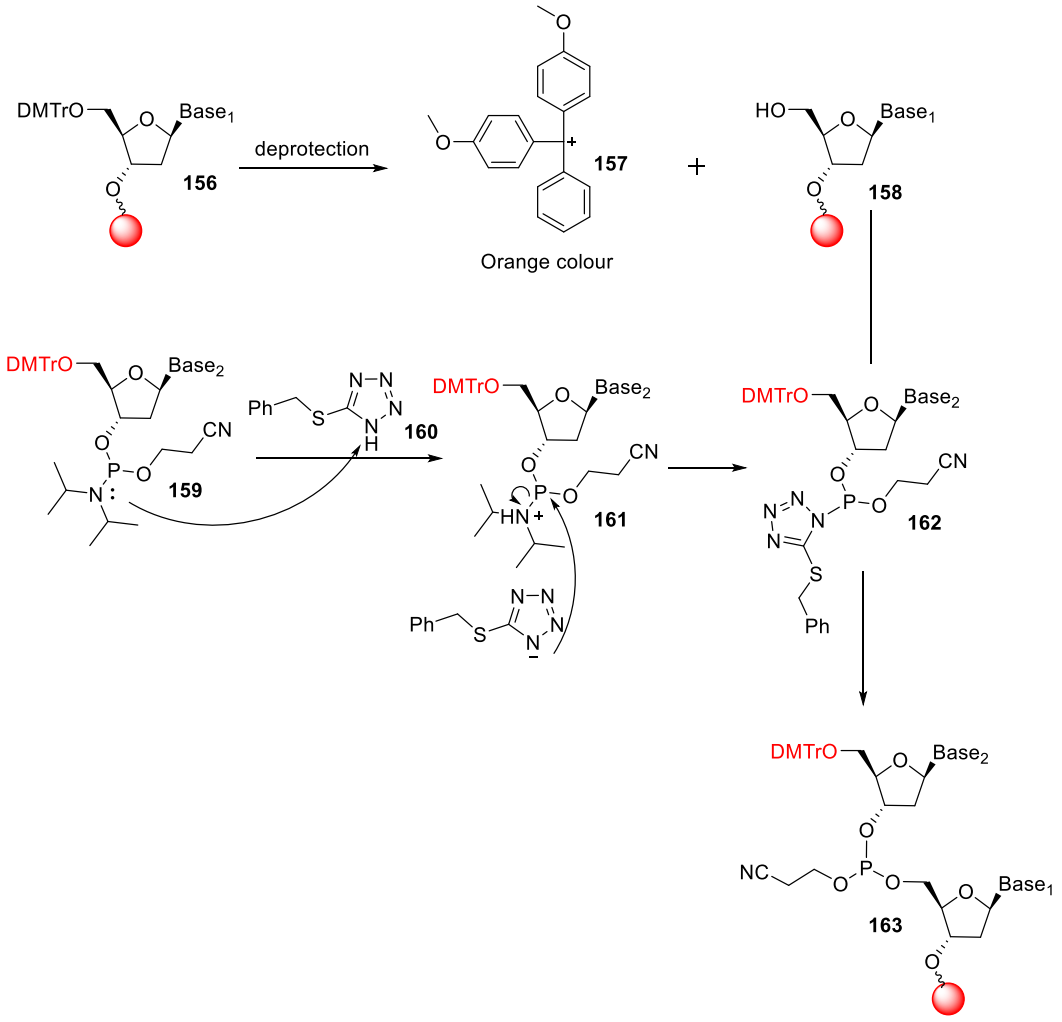
Scheme 39. Synthesis of RNA-DNA dinucleotides **142** and **143**. Reagents; (i) 1.1 eq. TBDMSCl, 2.2 eq. imidazole, DMF, RT, 17-24; (ii) 1.2 eq. triisopropylbenzenesulphonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, RT, overnight (21-22 h).

3.3 Solid-phase synthesis

Oligonucleotide synthesis was performed in our laboratory using an ABI394 DNA/RNA synthesizer (0.2 µM scale), by the phosphoramidite coupling method on a CPG Solid Supports (**Figure 26**, page 45). The following solutions were used:

1- 3% trichloroacetic acid in dichloromethane (Deblock Mix): This reagent was used for the 5′-DMTr deprotection in the beginning of each cycle.

2- Anhydrous 0.3M 5-Benzylthio-1-H-tetrazole in MeCN (BTT activator): This reagent is very important to activate the phosphoramidite before the coupling reaction is taking place as illustrated in **scheme 40**.



Scheme 40. Deprotection, activation and coupling steps of solid-phase synthesis of oligonucleotides.

3- THF/pyridine/acetic anhydride (8:1:1) (Cap Mix A): Cap mix A was used for the acylation of all unreacted oligomers that still have 5'-OH.

4- 10% Methylimidazole in THF (Cap Mix B): Cap mix B was used as a catalyst for the acylation in capping step.

5- 3H-1,2-Benzodithiol-3-one-1,1-dioxide (sulphurasing reagent): This reagent is required for the synthesis of phosphorathioate oligonucleotides.

6- 0.1M Iodine in THF/pyridine/water (78:20:2) (oxidiser): Iodine was used for the synthesis of phosphodiester oligonucleotides.

7- 0.1 M solutions of phosphoramidite in MeCN.

3.3.1 Synthesis and purification of DNA oligonucleotides with using the solid support

Unmodified parts of DNA oligonucleotides were synthesized using the standard 0.2 µM scale protocol provided by the manufacturer. The vinylphosphonate dinucleotides were coupled with a largely increased coupling time of 900 s. After the desired sequence was synthesised the column was dried under vacuum for 20 minutes. The content of the synthesis column was transferred to a vial, and the oligonucleotide was cleaved from the solid support and the base-labile protecting groups were deprotected (35 % NH_4OH, 1 mL) at 55 °C overnight. The synthesised products were purified using the oligonucleotide purification cartridges OPC® and by following the manufacturer's protocol to remove the failed sequences and other impurities oligonucleotide purification. MeCN HPLC Grade, TEAA (2 M), NH_4OH (1.5 M), 3% TFA, Milli-Q water and 20% MeCN were used as stock solutions in the purification protocol. Oligo samples were diluted with Milli-Q water (1 mL) prior to OPC® and loaded twice on the cartridge. Detritylation occurred using 3% TFA, DMTr-off samples are eluted upon addition of 20% MeCN and freeze-dried prior to further purification/analysis. The

resultant oligonucleotide was desalted using a Nap-5 column and supplied protocol.

3.3.2 Synthesis and purification of 2'-OMe modified oligonucleotides

2'-OMe modified oligonucleotides were synthesized using the standard, manufacturer's 0.2 μM DNA synthesis cycle with coupling time increased to 360 s. The post-synthesis procedure that was used is described in (3.3.1).

3.3.3 Synthesis and purification of ssRNAs with DNA nucleotides or the vinylphosphonate dinucleotide

For the sequences **165** and **166** that contain both phosphodiester and phosphorothioate backbones the synthesis was divided into two cycles. First, the 0.2 μM DNA phosphorathioate synthesis cycle was selected with the coupling step extended to 360 seconds, all other steps in the protocol were not modified. For the phosphodiester backbones the standard 0.2 μM DNA phosphodiester synthesis cycle was selected with the coupling step extended to 360 seconds, all other steps remained unmodified. For the chemically modified ssRNAs that contain a vinylphosphonate dimer **165** in a last step of synthesis (3 × 900 sec.) couplings were performed to ensure sufficient coupling of the dimer to the rest of the oligo. The post-synthesis procedure that was used is described in (3.3.1).

For the sequences that only contain phosphodiester backbone **172**, **173** and **174**, the procedure described in 3.3.2 was used. With coupling time increased to 900 sec. for vinylphosphonate dinucleotides to ensure sufficient coupling of the dimer to the rest of the oligo. The first DMTr deprotection was repeated three times, when universal support is used. The post-synthesis procedure that was used is described in (3.3.1).

3.4 HPLC analysis

Sample analysis to check oligonucleotides purity was performed by reverse phase (RP) HPLC on the Phenomenex Calrity Oligo-RP column (50 × 4.6 mm), 3 µm particle sized beads, C18 hydrocarbon chain length, at rt. using the following condition:

buffer A: 0.1M TEAA, buffer B: CH_3CN. The following gradient of CH_3CN (B) was used: 0 min – 5%, 5 min – 5%, 20 min – 30%, 30 min – 30% with a run rate of 1.0 mL/min. UV absorption at 260 nm and 290 nm.

3.5 *In vitro* and *In vivo* performance of (*E*)-VP linked-dinucleotide

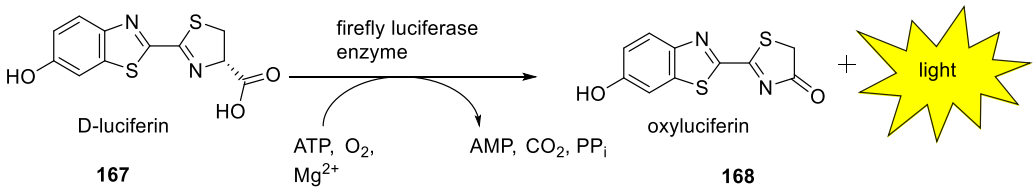
3.5.1 Previous work – *in vitro* silencing

Our group has previously worked on targeting the luciferase gene using the principle of siRNA gene silencing. The effective target region was selected, and the antisense sequence was identified (**164**, **Table 3**) However, our group showed that whilst effective as dsRNA the antisense unmodified ssRNA sequence **164** did not shown a significant activity when it was used as a single strand. Therefore, our group developed the most effective design of the ssRNA backbone (**165**, **Table 3**) to target the luciferase gene. Similar to the approach adopted by Lima,[22] replacing the unmodified RNA nucleotides (black) to 2′-F (green) and 2′-OMe (blue) modifications has supported the nuclease stability and target affinity. Further nuclease stability was provided by the incorporation of 7 consecutive phosphorothioate (s) modifications to the backbone at the 3′-terminus. Additionally, the (*E*)-VP dinucleotide (T*A, red, DNA) was incorporated in the 5′-end to increase the nuclease resistance and the gene silencing activity. A negative control **166** was also synthesised, which was constructed to contain a similar degree of modification to the modified sense strands described above.

Table 3. The selected antisense sequence for luciferase targeting. Black stands for unmodified RNA nucleotides, green for 2'-F and blue - 2'-OMe modified nucleotides, underlined – AA overhangs, "s" phosphorothioate linkage, DNA nucleotides are marked in red, DNA dinucleotide linked by the (*E*)-vinylphosphonate is labelled with * (T*A).

ID	**Structure 5'→3'**	Length
antisense **164**	UAA UGU UUU UGG CAU CUU CCA AA	23
antisense **165**	T*AA UGU UUU UGG CAU CsUsUs CsCsAs AsA	23
-ve control **166**	AAU UCU CCG AAC GUG UsCsAs CsGsUs AsA	23

In order to test our hypothesis, phosphoramidite **84** was incorporated in the solid phase synthesis to make the ssRNA-containing (*E*)-VP at position 2 nucleotide **165**. Both ssRNA **165** and the negative control **166** were synthesised by following the phosphoramidite method (**Figure 26**, page 45). The purity was checked by HPLC, and the oligomers were analysed by mass spectrometry. The synthesised oligonucleotide **165** was designed to the target luciferase gene in human breast using adenocarcinoma cell line (MDA-MB 231 fluc), which stably express luciferase. Luciferase is an enzyme that catalyses the oxidation of D-luciferin **167** into oxyluciferin **168** and light is released from the enzymatic reaction; the light intensity represents the activity of enzyme and gene silencing efficiency (less light represents good gene silencing) (**Scheme 41**).



Scheme 41. Luciferase enzyme activity.

The *in vitro* study was carried out by Paulina Powalowska; our group member.[105] The results of the *in vitro* study indicate that the (*E*)-VP contained ss-siRNA showed 95% knockdown of luciferase after 3-day treatment.

Moreover, the effect was long-lasting as 82% inhibition in luciferase production was achieved after a 6-day exposure to a single dose of ss-siRNA (**Figure 31**).

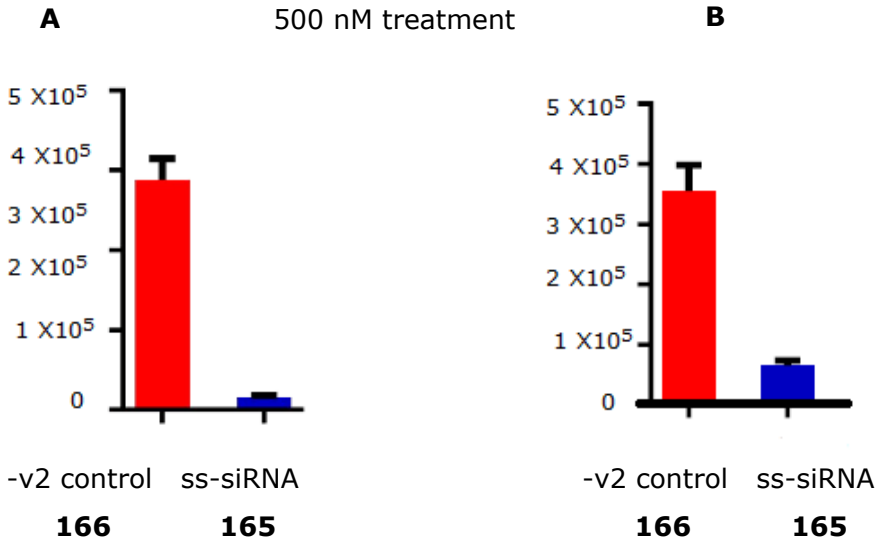


Figure 31. ssRNAs knockdown activity *in vitro*. a (**A**) 3-day post treatment of MDA-MB-231-fluc cells with ss-siRNAs; (**B**) 6-day treatment of MDA-MB-231-fluc cells with ss-siRNAs. The Y axis represents firefly luciferase activity normalised against 1 µg of total protein. Data is shown as mean with SD. Treatments were run in biological triplicates. Figure reproduced from Paulina thesis.[105]

3.6 Current work- *in vivo*

The *in vitro* knowckdown of leuceferase was very encouraging and suggests that significant silencing of the target can be achieved at low concentration using a modified ss-siRNA, which may make it favourable for use *in vivo* as the possibility of off-target effects caused by the sense strand is eliminated. Therefore, in this study, we have repeated the synthesis of the oligonucleotides **165** and **166** in larger scale (approximatley 7 mg), we have followed the same condition used by our group previously. The oligonucleotides were then used by our collaborators to evaluate their *in vivo* activity.

The study was carried out in accordance with the NCRI guidelines for welfare and use of animals in cancer research, LASA good practice and FELASA working group on pain and distress guidelines, under UK Home Office project licence, PPL P435A9CF8. MDA-MB-231-fluc tumours were established by subcutaneous grafting of 2 × 10^6 cells/mL in 100 µL of growth factor-reduced matrigel into

the left flank of female CD1-nude mice (Charles River, UK). Tumour size was monitored by calliper measurements and mice were imaged under anaesthesia using the IVIS®Spectrum imaging system (Caliper Life Sciences) 15 minutes after administration of luciferase substrate, D-Luciferin (intraperitoneal, 150 µL of 30 mg/mL in sterile PBS per mouse, Xenogen, New Jersey, USA). Areas of luminescence were identified as Regions of Interest (ROIs) and quantified as photons emitted (Total flux) using Living Image/Igor Pro Software (Caliper Life Sciences).

Mice were divided into 4 groups of 10 mice, randomly distributed based on pre-treatment luminescent signal. 24 hrs after pre-treatment imaging, 50 µg of luciferase-targeting or control oligonucleotide, with or without 50 µg of PEI delivery agent in a total volume of 100 µL per mouse was administered by injection into the tail vein, then imaging was carried out at 24 and 72 h. At 144 hrs after the first injection, the mice were imaged again to afford a 24 hr pre-treatment reference a second intra-tumoral (IT) administration of the ss-siRNA formulations (two 50 µL injections into different locations in the tumour), again followed by 24 and 72 hrs post-injection imaging; bioluminescence post-treatment was expressed as a percentage of Total flux prior to ss-siRNA administration (**Figures 32** and **33**).

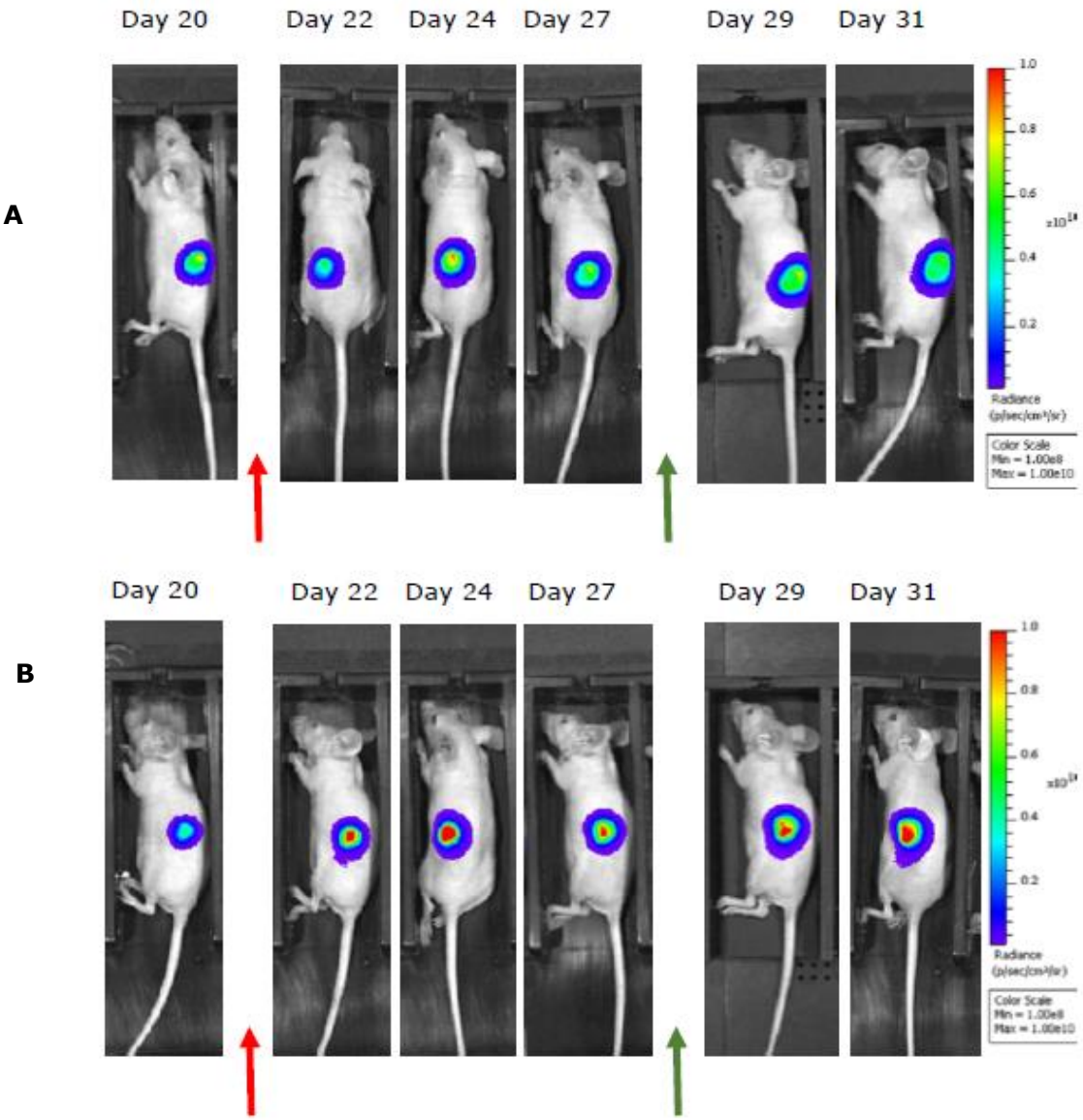


Figure 32. Representative images of 1 mouse treated with oligonucleotides with PEI formulation over time are shown with timing of the intravenous (red arrow) and intertumoral (green arrow) injections indicated. (**A**) Treated with ss-siRNA (**165**) and PEI. (**B**) Treated with negative control (**166**) and PEI

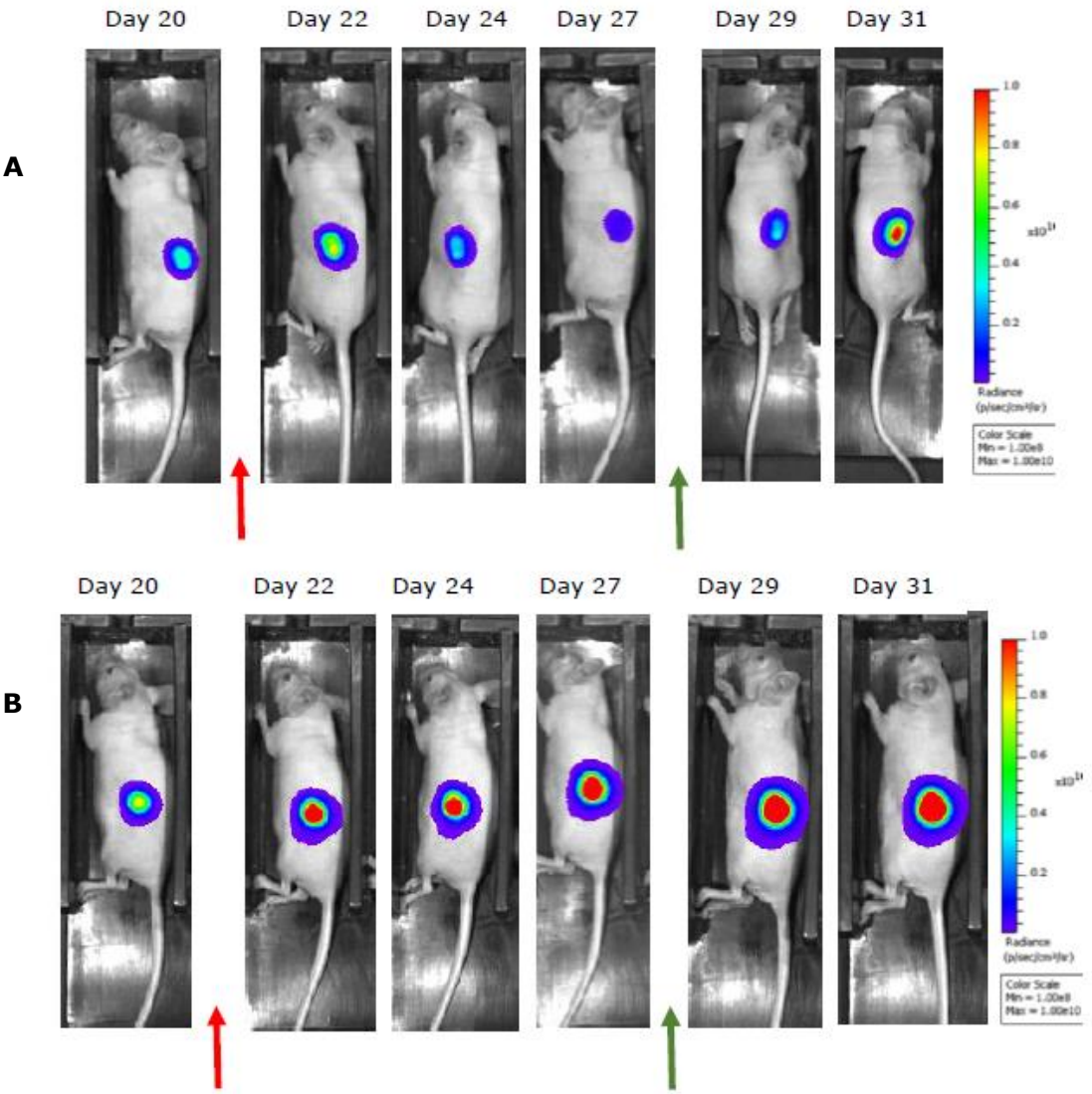


Figure 33. Representative images of 1 mouse treated with oligonucleotides without PEI formulation over time are shown with timing of the intravenous (red arrow) and intertumoral (green arrow) injections indicated. (**A**) Treated with ss-siRNA (**165**) only. (**B**) Treated with negative control (**166**) only.

To investigate efficacy *in vivo*, the luciferase-targeting ss-siRNA **165** or a control oligonucleotide **166** were administered, with or without the delivery agent, PEI, initially *via* the intravenous (IV) route and then *via* the intra-tumoural (IT) route in mice with established MDA-MB-231-fluc xenografts. General animal condition was monitored daily and there was no apparent toxicity associated with delivery of oligonucleotides to the animals with body weights maintained in all 4 groups. The results for gene silencing experiments were processed using GraphPad Prism 6 software, which also included statistical

calculations. The data were analysed using one-way ANOVA test and were corrected for multiple comparisons by using Sidak's test.[106] The values in figures with individual samples and grouped controls were presented as the mean with SD. Every assay was performed in triplicate. Following IV or IT administration of the PEI-complexed oligonucleotides, whilst luminescence increased over time due to tumour growth, there was lower bioluminescence in the luciferase-targeted ss-siRNA-treated **165** group compared with the control oligonucleotide **166** group which was significant for the IV administration ($p<0.05$, Two-way ANOVA) and more marked effect at 24 than at 72 h. For the non-PEI complexed ss-siRNA **165**, there was no effect at 24 h when administered IV but shown significant effect at 72 h (**Figure 34**).

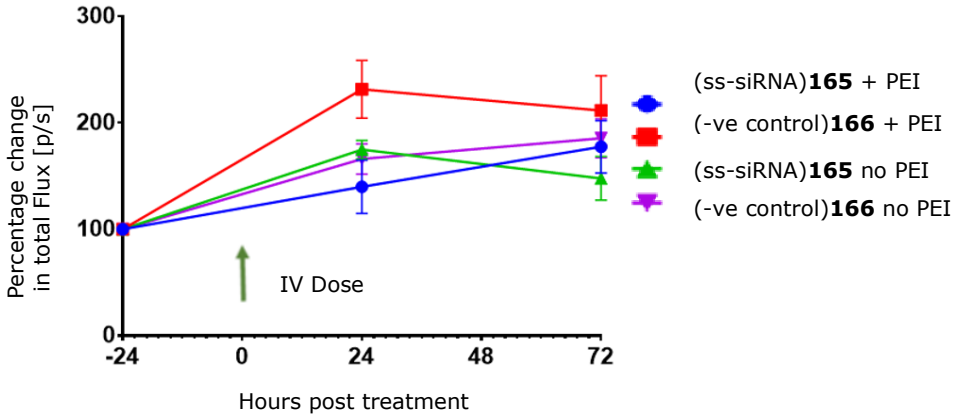


Figure 34. The efficacy of ss-siRNA *in vivo*. MDA-MB-231-fluc cells expressing luciferase were used to establish sub-cutaneous xenografts in nude mice and imaged 24 h before, and 48 and 72 h after intravenous injection of a luciferase-targeting (**165**) or control oligonucleotide (**166**) complexed with PEI or without PEI. Percentage change in bioluminescence (Total flux in photons per second) at 24 and 72 h post-injection is shown.

In contrast, the effect of ss-siRNA **165** was more marked at 24 h than 72 h when administered IT with and without using the PEI formulation. However, the effect of ss-siRNA was greater when used without PEI when compred to ss-siRNA-PEI formualtion (**Figure 35**).

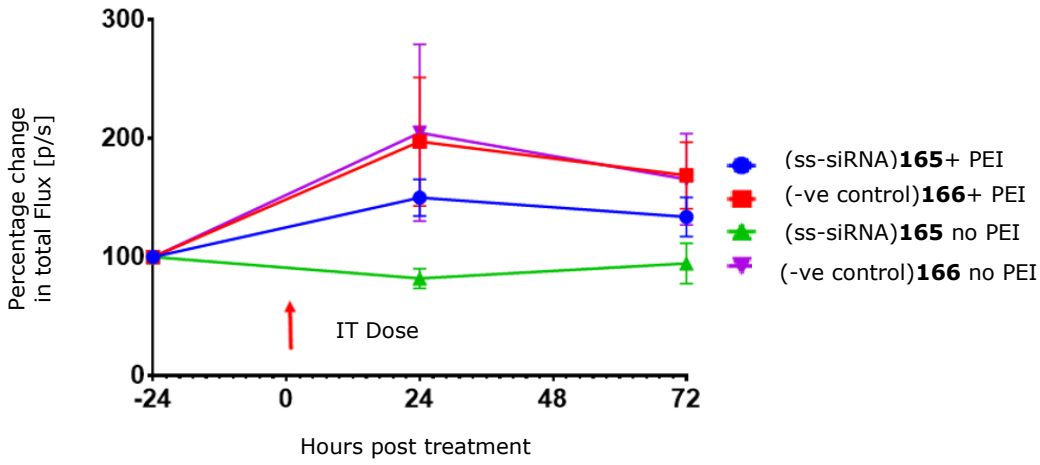


Figure 35. The efficacy of ss-siRNA *in vivo*. MDA-MB-231-fluc cells expressing luciferase were used to establish sub-cutaneous xenografts in nude mice and imaged 24 h before, and 48 and 72 h after intra-tumoral injection of a luciferase-targeting (**165**) or control oligonucleotide (**166**) complexed with PEI or without PEI. Percentage change in bioluminescence (Total flux in photons per second) at 24 and 72 h post-injection is shown.

3.7 Synthesis of (*E*)-VP-containing ssRNAs at 3′-terminus

We show previously that (*E*)-VP can be incorporated in the 5′-terminus of the oligonucleotide to support the oligonucleotide resistance towards nuclease hydrolysis. However, an oligonucleotide carrying an (*E*)-vinylphosphonate at 3′-terminus has not been previously made **170**. The standard solid support (**156**, **Figure 36)** already has the first nucleoside attached, and the produced oligonucleotide **167** would have first unmodified nucleotide at 3′ end. While, the universal column **168** does not contain any nucleoside in it is structure. Therefore, the universal column (**168**, **Figure 36)** is suitable to make an oligonucleotide that has modification at 3′- end **170**.

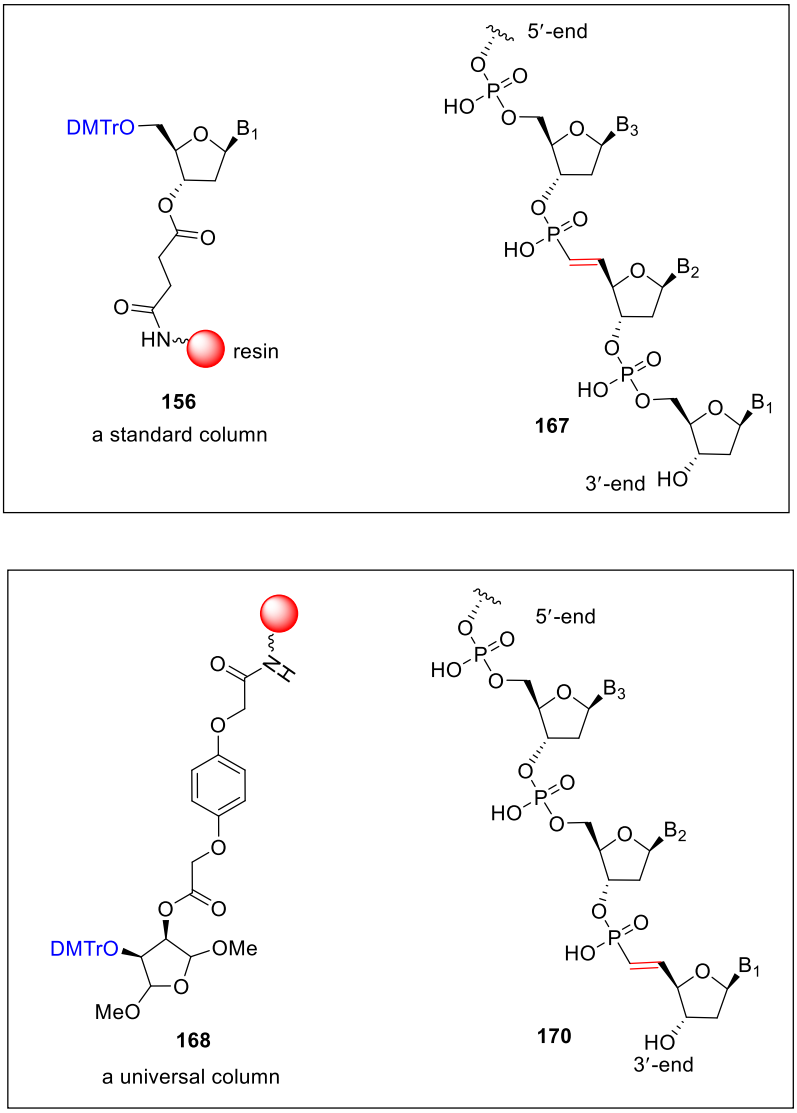
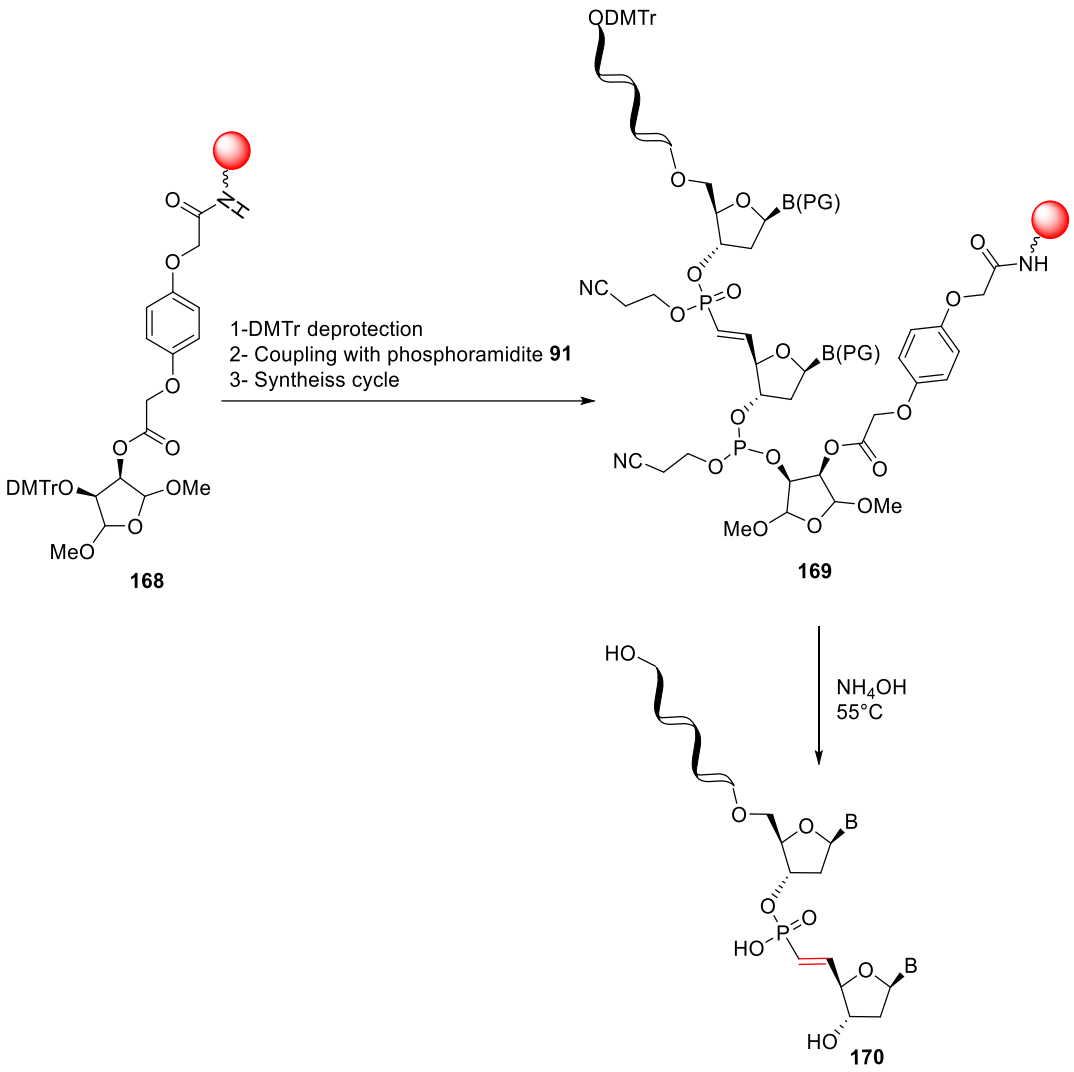


Figure 36. The difference between chemical structure of a standard column **156** and a universal column **168.**

We have used the universal solid support **168**, which is a polystyrene resin, to synthesise ssRNAs that has vinylphosphonate dinucleotides attached at the 3′-termius **170**. The first step is required to remove the DMTr from the solid support **168** and then the modified base **91** can be coupled to the activated solid support, when the synthesis is finished, the solid support can be removed easily to give the oligonucleotide with a 3′-OH **170** (**Scheme 42**).



Scheme 42. Cleaving the oligonucleotide **170** from the universal solid support **168**.

Having developed a method to incorporate (*E*)-VP at the 3′ terminus, we will then be able to examine if the need for phosphorathioate modifications can be removed, since vinylphosphonate can increase the nuclease resistance to the 3′terminus. Thus, we have incorporated the (*E*)-VP at various locations along the oligonucleotide backbone (**Table 3**). We first synthesized poly T DNA sequence that has one (*E*)-VP at 3′-terminus **171**, this sequence was made due to the available material to optimise the condition before we start using the material with limited availability. After the successful synthesis of **171**, we then synthesised a fully oxidised backbone of ssRNA **172** that has (*E*)-VP modifications at 3′ and 5′ ends following the same sequence of nucleotides in

ssRNA **166**. In addition, in order to compare the activity of (*E*)-VP at 3'terminus **172**, we have synthesized **173** ssRNA that has a fully oxidised backbone and only one (*E*)-VP at 5'-terminus. Furthermore, we have also synthesized ssRNA **174** that has (*E*)-VP incorporated between two nucleotides and this will give our vinylphosphonate reagent the option to be incorporated at any place of the oligonucleotide backbone (**Table 4**).

Table 4. The synthesised single-stranded RNAs with the introduction of DNA and fluorinated and methylated nucleotides. Green stands for 2'-F and blue - 2'-OMe modified nucleotides, underlined – AA overhangs, DNA nucleotides are marked in red, DNA dinucleotide linked by the (*E*)-vinylphosphonate is labelled with * (T*A), (A*A).

ID	**Structure 5'→3'**	Length
171	TTT TTT TTT TTT TTT TTT TT*T	23
172	T*AA UGU UUU UGG CAU CUU CCA A*A	23
173	T*AA UGU UUU UGG CAU CUU CCA AA	23
174	T*AA UGU UUU UGG CAU CUU CC A*AA	23

From the last several examples we have synthesized (**165**, **171**, **172**, **173** and **174**), we have shown that we can incorporated our vinylphosphonate dinucleotide at 5'-terminus (nt 2) **173**, 3'-terminus **171** and internal position **174** of the oligonucleotide sequence and that will give us more flexibility to when ssRNA sequence is designed

3.8 MS data

Electro-spray negative ionisation (ESI, M-) mass spectrometry data was obtained using the Thermo LTQ FT Ultra to confirm masses of synthesised ssRNAs. The yield percentage were obtained from the DNA/RNA synthesiser (**Table 5**).

Table 5. MS data and the yield percentage for some synthesised oligonucleotides.

ID	Calculated	Observed	Yield % (synthesizer)
165	7544.13	7544.04	100%
166	7593.20	7594.14	99%
171	6930.52	6930.09	99%
172	7367.66	7367.20	98%
173	7431.71	7431.17	97%
174	7367.66	7367.09	98%

3.9 Synthesis of consecutive (*E*)-vinylphosphonates

We have shown that ss-siRNA contained (*E*)-VP at the second nucleotide of 5′-terminus poses an effective gene silencing *in vitro* and *in vivo*. Making an oligonucleotide that have two or three consecutive (*E*)-VP at 5′-terminus was our concern to examine how that can affect the activity of the ss-siRNA for gene silencing. As we think that the presence of that unique structure at 5′-end can enhance the stability of AGO-ss-siRNA complex and result in an effective gene silencing and also protecting the oligonucleotide from nuclease degradation. Therefore, in order to test our hypothesis, we decided to make a mono **177**, double **178** and triple **179** consecutive 5′-(*E*)-VP (**Figure 37**).

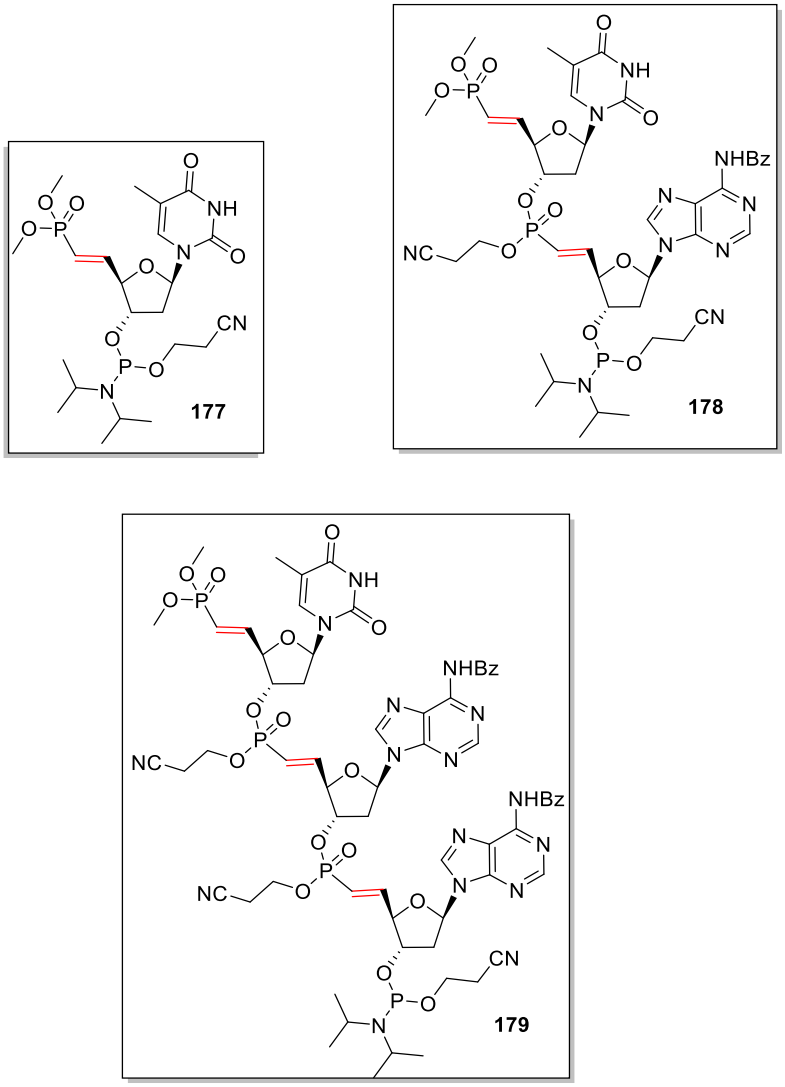
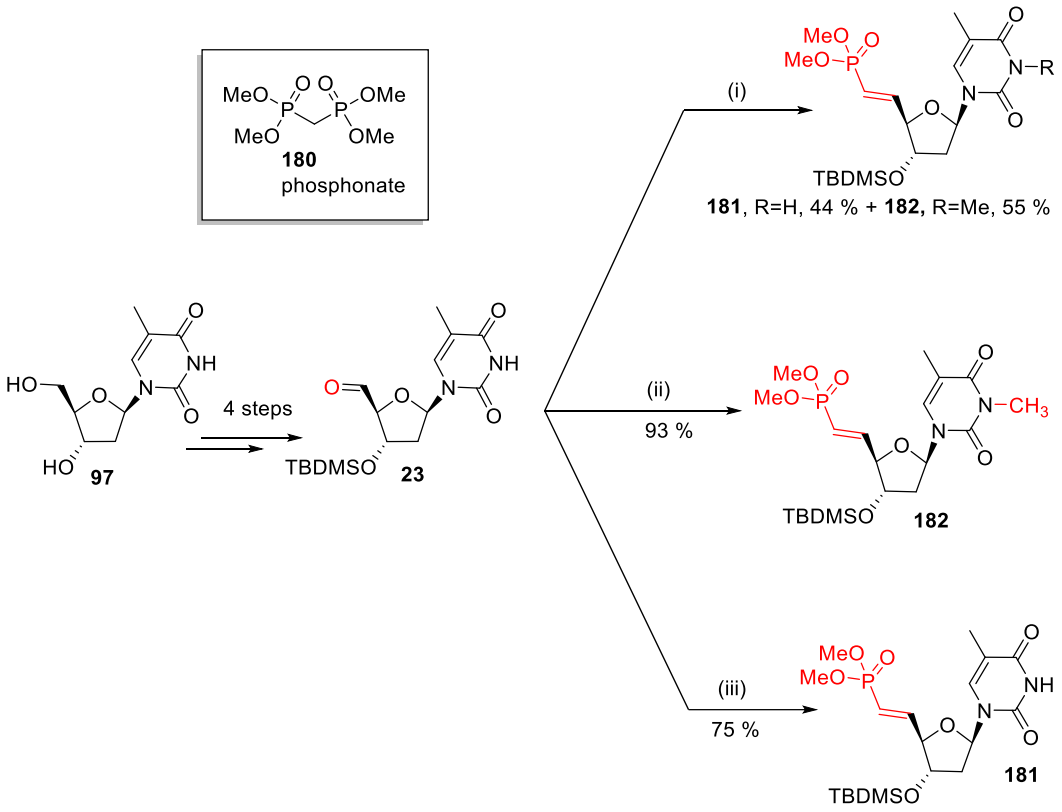


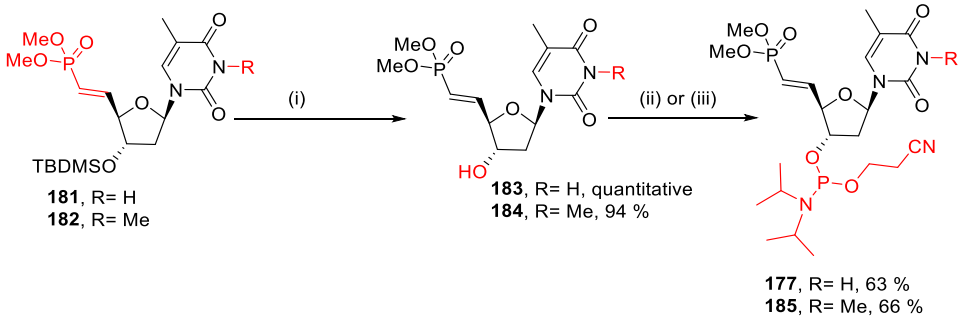
Figure 37.Terminal 5′-(*E*)-VP phosphoramidites.

Starting from thymidine **97**, aldehyde **23** was made following the same procedures that shown priviously in **Scheme 21**. Aldehyde **23** was then reacted with phosphonate **180** in the presence of sodium hydride at room temperature the desired product **181** was obtained in 44% and *N*-methylated product **182** in 55%. The reaction was repeated and left overnight, only *N*-methylated product **182** was obtained in 93% from the reaction. Another attempt was carried out in an ice bath for 3 h, and that provide the desired product **181** in 75% (**Scheme 43**).



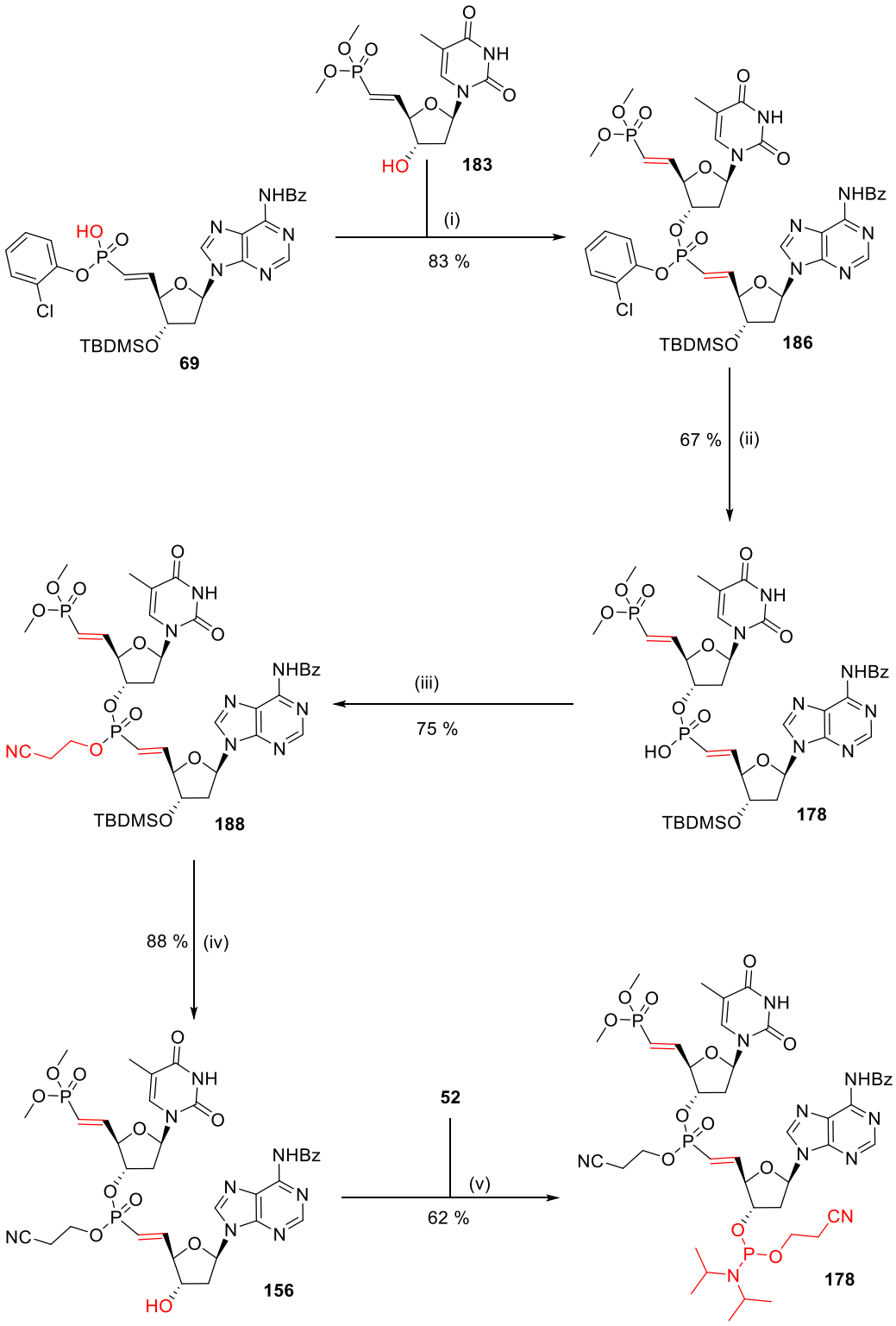
Scheme 43. Synthesis of (*E*)-VP **181** and **182**. Reagent: (i) 2.0 eq. phosphonate, Potassium *tert*. Butoxide, THF, RT, 3h; (ii) 2.0 eq. phosphonate, 1.8 eq. NaH, RT, 19 h; (iii) 2.0 eq. phosphonate **180**, 1.8 eq. NaH, 0 °C, 3 h.

TBDMS groups were deprotected from both the desired product **181** and *N*-methylated **182** to provide the corresponding alcohols **183** and **184** respectively. Phosphoramidites **177** and **185** were then obtained as mixtures of two diastereoisomers from the reaction of alcohols **183** and **184** with phosphoramidite **52** (**Scheme 44**).



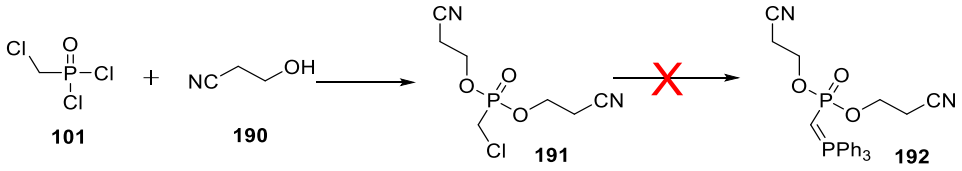
Scheme 44. Synthesis of phosphoramidites **177** and **185**. Reagents: (i) 5.0 eq. $Et_3N.3HF$, THF, RT, 5h; (ii) 1.1 eq. phosphoramidite **21**, 1.1 eq. 5-methyl-1-*H*-tetrazole, MeCN, RT, 16h; (iii) 1.2 eq. phosphoramidite **30**, 4.0 eq. diisopropyl ethylamine, dichloromethane, RT, 22h.

The double consecutive (*E*)-VP **186** was obtained in 83% as a mixture of two diastereoisomers from the coupling reaction between alcohol **183** and phosphonic acid **69**. The chlorophenyl group was removed from the consecutive dinucleotide **186** to give the phosphonic acid **187** which was then protected with cyanoethyl alcohol to provide the dinucleotide **188** as a mixture of two diastereoisomers. The TBDMS group was then removed from the dinucleotide **188** to give alcohol **189** in 62%. The phosphoramidite **178** was finally generated as a mixture of four diastereoisomers (**Scheme 45**).



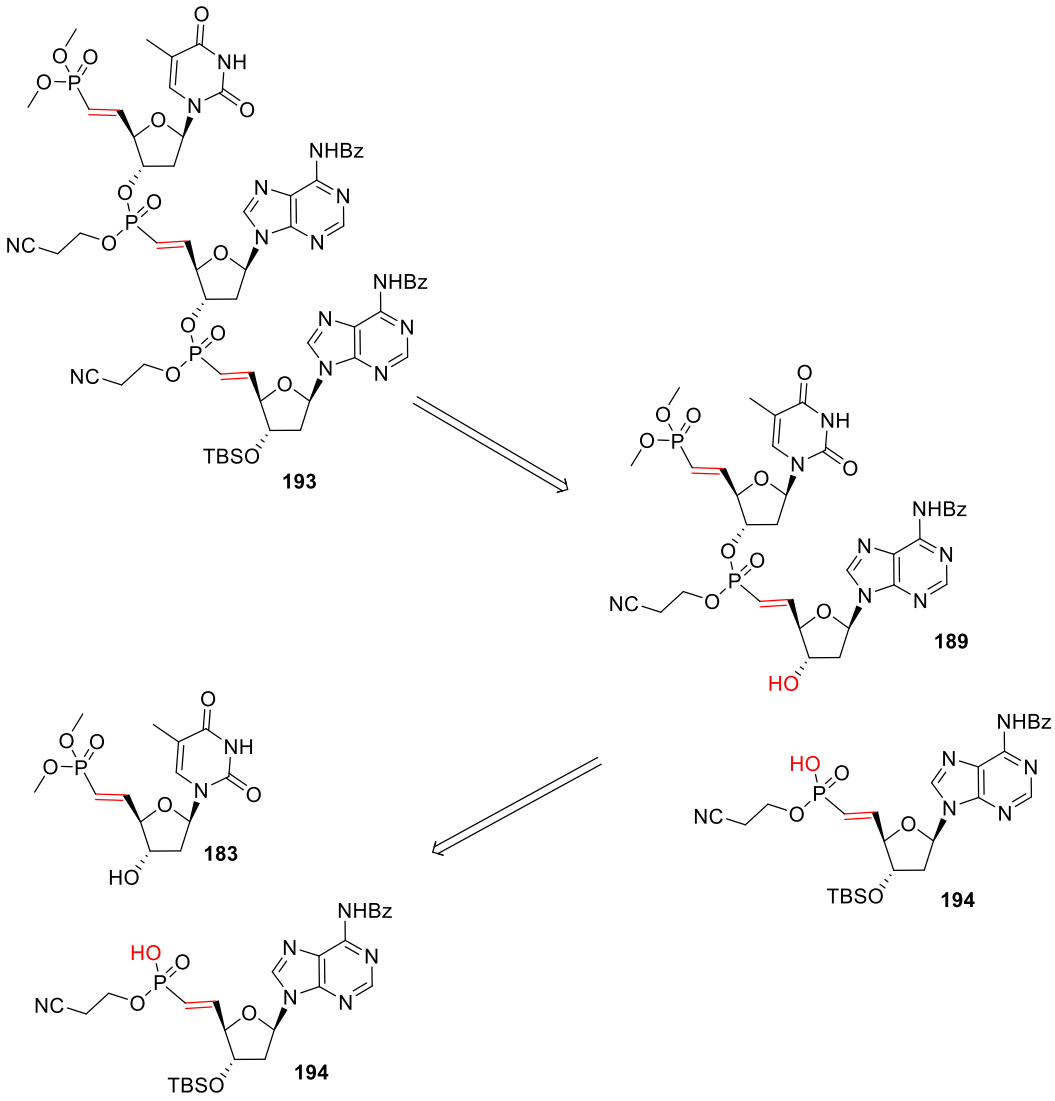
Scheme 45. Synthesis of double consecutive (*E*)-VP **178**. reagents: **Reagents**: (i) 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, RT, 21 h; (ii) 1.5 eq. 2-nitrobenzaldoxime, 1.5 eq. tetramethylguanidine, 6.5 eq. triethylamine, dioxane, RT, 20 h; (iii) 5.0 eq. 3-hydroxypropionitrile, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, RT, 18 h; (iv) 5.0 eq. $Et_3N{\cdot}3HF$, THF, RT, 5 h; (v) 2.0 eq. phosphoramidite **52**, 2.0 eq**.** 5-methyl-1*H*-tetrazole, DMF:dichloromethane (1:3), RT, 1 h.

Trimer **193** with three consecutive (*E*)-VP can be made *via* the same route that used to make the double consecutive (*E*)-VP, but we need to swap the chlorophenyl group with cyanoethyl alcohol twice, as it cannot be done in one step. Therefore, we investigated to make another ylide **192** which contain cyanoethoxy instead of chlorophenoxy group. Using the same procedure of making ylide **24** the first step was successfully made, and the phosphonate **191** was obtained in a good yield (quantitative). However, it was not possible to make the ylide **192** during the second step. It seems the high temperature has removed the cyanoethyl group, we tried to reduce heating to RT, but no product was observed after three days of reaction. Adding solvent to the reaction and heating the reaction mixture to 40 °C was also unsuccessful (**Scheme 46**).



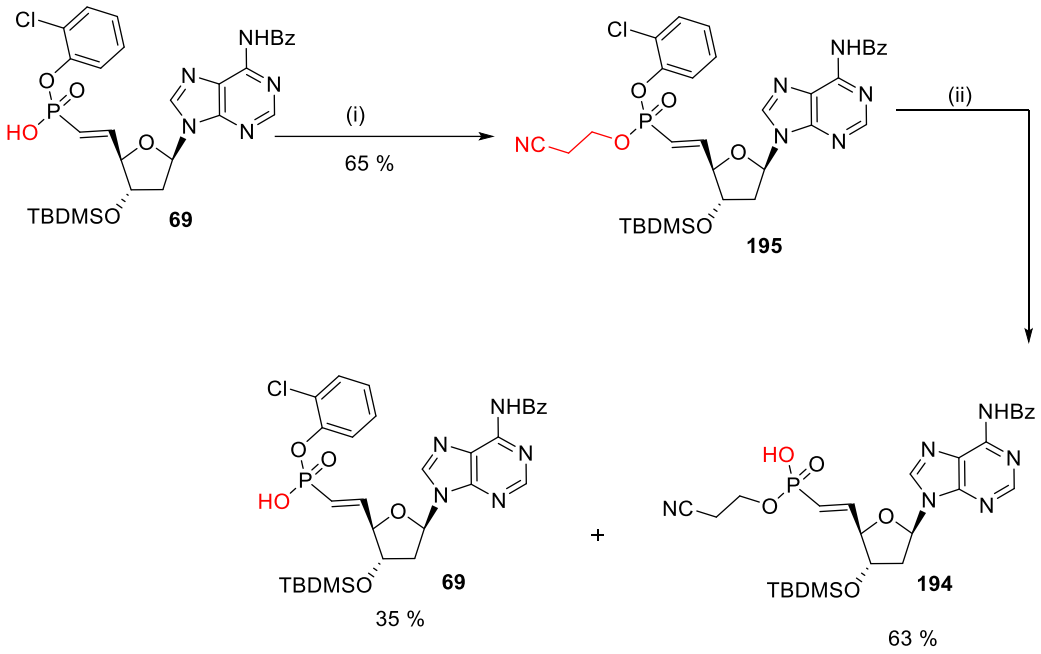
Scheme 46. Our attempt of making ylide **192**.

Therefore, we decided to make the cyanoethyl phosphonic acid **194**, and that can be coupled with alcohol **183** then TBDMS deprotection for the resulted dinucleotide **188** to give alcohol **189** which was coupled with another cyanoethyl phosphonic acid **194** to provide the trinucleotide **193** (**Scheme 47**).



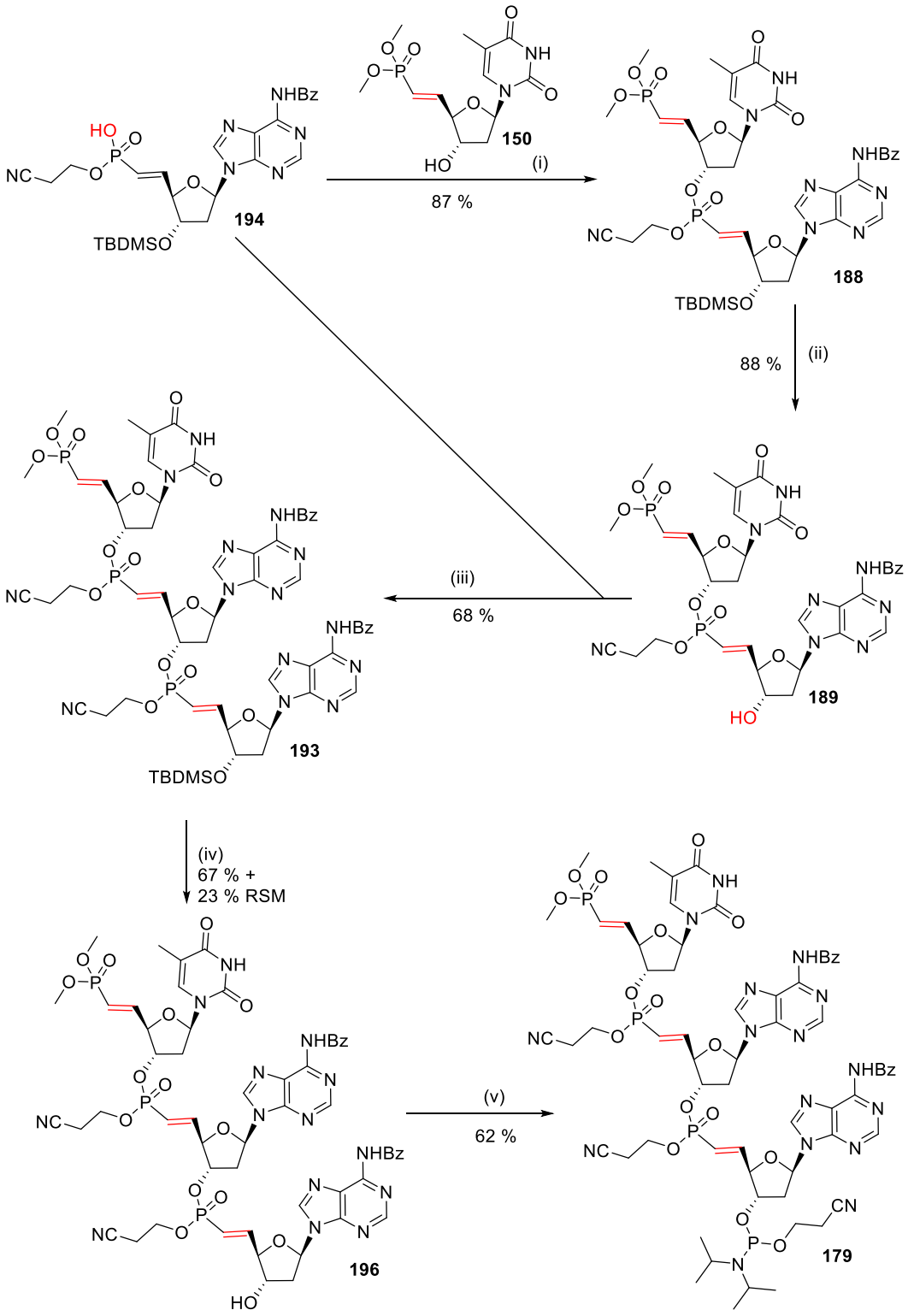
Scheme 47. The retrosynthesis of three consecutive (*E*)-VP.

Cyanoethyl phosphonic acid **194** was synthesized by protection of phosphonic acid **69** with cyanoethyl alcohol, followed by deprotection of the chlorophenyl group. Thus, **194** was obtained in 63%, and 35% as a by-product **69** due to the competition from cyanoethyl group for deprotection (**Scheme 48**).



Scheme 48. Synthesis of phosphonic acid **194**. Reagents: (i) 5.0 eq. 3-hydroxypropionitrile, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.5 eq. *N*-methylimidazole, pyridine, RT, 22 h; (ii) 1.0 eq. 2-nitrobenzaldoxime, 1.0 eq. tetramethylguandinine, 1.0 eq. triethylamine, dioxane, RT, 19 h.

The cyanoethylphosphonic acid **194** was coupled with alcohol **183** to give dinucleotide **188** as a mixture of two diastereoisomers. The TBDMS group was then selectively deprotected to provide alcohol dinucleotide **189**. The resulting alcohol was then coupled with another molecule of cyanoethyl phosphonic acid **194** to give the trimer **193** as a mixture of four diastereoisomers. The TBDMS was deprotected to provide alcohol trimer **196** which was reacted with phosphoramidite reagent **52** to provide the final product **179** as a mixture of eight diastereoisomers (**Scheme 49**).



Scheme 49. Synthesis of double consecutive (*E*)-VP **179**. Reagents: (i) 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, RT, 19 h; (ii) 5.0 eq. $Et_3N{\cdot}3HF$, THF, RT, 5 h; (iii) 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, RT, 18 h; (iv) 1.0 eq. $Et_3N{\cdot}3HF$, THF, RT, 6 h; (v) 2.0 eq. phosphoramidite **52**, 2.0 eq**.** 5-methyl-1*H*-tetrazole, DMF:dichloromethane (1:3), RT, 1 h.

3.10 Synthesis of ss-siRNA containing 5′- consecutive (*E*)-VP

Phosphoramidites **177**, **178**, and **179** were incorporated into solid-phase synthesis to make the oligomers **197**, **198** and **199** (**Table 6**).

Table 6. The synthesised ss-siRNA with the introduction of DNA and fluorinated and methylated nucleotides**.** Green stand for 2′-F and blue - 2′-OMe modified nucleotides, underlined – AA overhangs, "s" phosphorothioate linkage, DNA nucleotides are marked in red, DNA dinucleotide linked by the (*E*)-vinylphosphonate is labelled with * (T*A), (A*A).

ID	**Structure 5′→3′**	Length
197	*TAA UGU UUU UGG CAU CsUsUs CsCsAs AsA	23
198	Me*TAA UGU UUU UGG CAU CsUsUs CsCsAs AsA	23
199	*T*AA UGU UUU UGG CAU CsUsUs CsCsAs AsA	23

The two-methyl group at 5′ terminus can be removed from the resulted oligomers by using the same condition that is reported in Haraszti *et al*. research.[25]

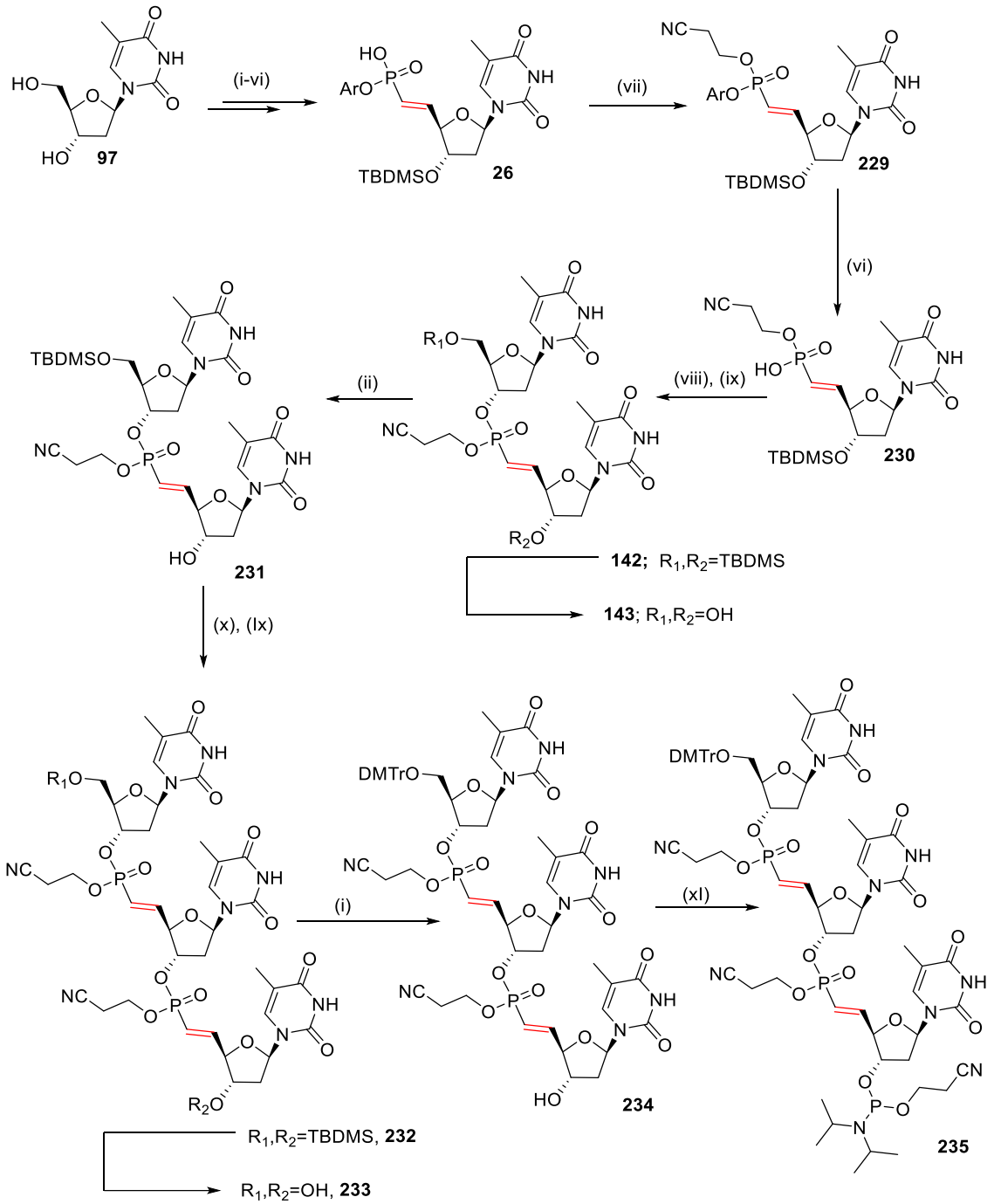
4 Conclusion and future work

In conclusion, we have developed a reliable, and scalable synthetic route to vinylphosphonate from readily available DNA nucleosides, using an (*E*)-selective Wittig reaction as a key step to create the vinylphosphonate. This method turned out to be a reliable route towards all the vinylphosphonate internucleotide-linkage combinations. All dinucleotides were obtained in accepted yield, as shown previously in (**Table 2**).

This route produces a phosphoramidite **91** that contains standard protecting group, 5′-hydroxyl (DMT) and the vinylphosphonate (cyanoethyl), which simplifies oligonucleotide synthesis, as standard coupling and deprotection protocols can be used. We next prepared an oligonucleotide with a 5′-terminus (*E*)-VP at nt 2 position and contained a variety of chemical modifications, including 2′-F and 2′-OMe groups. *In vitro* study showed that the (*E*)-vinylphosphonate-containing oligomer **165** performed very well as a single-stranded RNA (95% knockdown, 3 days), and this oligomer continued to be the best performing after 6-day exposure (82% knockdown). These results indicate that the oligomers were effective and stable. In addition, the *in vivo* study confirmed the activity of our *E*-VP containing ss-siRNA **165**, which has shown significant activity in comparison to the negative control. The activity and stability indicate that the presence of 5′-(*E*)-VP at nt 2 in corporation with other modification has led to increase the nuclease stability of the ss-siRNA. Interestingly, the activity of ss-siRNA was more significant when injected IT than IV. In addition, ss-siRNA was more effective when used without PEI in the IT injection. This could be related to the serum-nuclease activity in case of intravenous injection, we suggest increasing the number of VP in the oligonucleotide chain to increase their stability or we could incorporate VP modification at both 3′ and 5′ ends of the oligonucleotide sequence.

We have also demonstrated that our (*E*)-vinylphosphonate are well tolerated not only at 5'terminus but also at 3'terminus of the oligomer. Additionally, we shown that our phosphoramidite reagents could be coupled from both 3' and 5' ends of the oligonucleotide backbone. We suggest that the synthesized oligonucleotide that has VP modification at both 3' and 5' ends can be compared *in vitro* and *in vivo* with the one that have VP modification at 5' end only, this will allow us to compare the activity and to investigate whether this can improve their nuclease stability *in vivo*.

Our group has previously demonstrated that we can incorporate DNA nucleosides in the ss-RNAs without loss of potency. Therefore, we decided to modify Lima vinylphosphonate into a DNA nucleoside, and we were able to synthesize nucleoside with one (*E*)-VP, and this will enable us in the future to compare the silencing efficiency of the oligomer that has an (*E*)-VP at position nt 1 with the one that has an (*E*)-VP at nt 2 position. Furthermore, we developed a synthetic route to make dinucleotide and trinucleotide that have two and three consecutive vinylphosphonate, respectively. However, these phosphoramidite reagents can only be incorporated at 5' terminus of the oligomer. In future investigations, it might be possible to extend this study. Therefore, we suggested a synthetic route to make a consecutive vinylphosphonate that can be incorporated at any position of the sequence. This route produces a phosphoramidite reagent **235** that contains standard protection of the 5'-hydroxyl (DMTr) and the vinylphosphonate (cyanoethyl), which simplifies oligonucleotide synthesis, as standard coupling and deprotection protocols can be used. The presence of the DMTr protecting group at the 5'-terminus of the growing oligomer allows for (if required) quantification of the degree of incorporation of the vinylphosphonate-containing phosphoramidite **235**. This multiple incorporation is not possible with the (*E*)-VP that reported in the literature (**Scheme 50**).



Scheme 50. Our suggested synthetic route to make a consecutive phosphoramidite **235**. Reagents; (i) 1.0 eq. DMTrCl, pyridine, rt; (ii) 1.5 eq. TBDMSCl, 3.0 eq. imidazole, DMF, rt; (iii) dichloroacetic acid/ CH_2Cl_2 (3%, v/v), RT, 20 min; (iv) 1.5 eq. DMP, CH_2Cl_2, RT, 3h; (v) 1.5 eq. ylide **24**, CH_2Cl_2, rt; (vi) 1.2 eq. 2-nitrobenzaldoxime, 1.2 eq. tetramethylguanidine, 6.0 eq. triethylamine, dioxane, rt; (vii) 5.0 eq. 3-hydroxyprotpiononitrile 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, rt; (viii) 1.0 eq. alcohol **71**, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, rt; (ix) 5.0 eq. $Et_3N{\cdot}3HF$, THF, RT, 5 h; (x) phosphonate **230**, 2.5 eq. 2,4,6-triisopropylbenzenesulfonyl chloride, 6.0 eq. *N*-methylimidazole, pyridine, rt; (xi) 2.0 eq. phosphoramidite **52**, 2.0 eq**.** 5-methyl-1*H*-tetrazole, DMF:dichloromethane (1:3), RT, 1 h.

Once phosphoramidite reagent **235** becomes available, this study can be extended further by incorporating this trimer **235** in the solid-phase synthesis to make an oligonucleotide with a consecutive vinylphosphonate. Additionally, probably it could be possible to make an oligonucleotide contains more than consecutive VP, and that can open the way to make a fully (*E*)-VP modified oligonucleotide in addition with using VP at 3' end, that can be used as a therapeutic tool to knockdown any disease-relevant target e.g. Asthma, cancer, diabetes, etc.

We did not look at the mechanism by which the gene inhibition was triggered, however some publications suggest that not only RNAi but also other non-RNAi related mechanisms are involved.[107] It would be very interesting to perform similar experiments in our laboratory. Establishing which mechanism is activated by presented here ASOs is important and therefore it will be one of future experiments performed in the Hayes and collaborators laboratories.

A different project addressed in this thesis demonstrated that the alkylation of commercially available phosphoramidite reagents is possible in one step by means of phase transfer catalysis. This approach presents a powerful tool for future research.

5 Experimental

5.1 General consideration

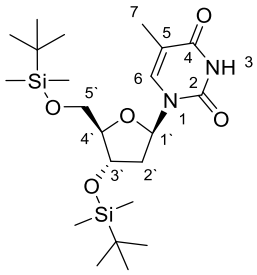
Starting materials were obtained from commercial suppliers and used without further purification unless stated otherwise. Reactions were performed in flame or oven-dried apparatus under argon atmosphere using distilled solvents. dichloromethane and pyridine were distilled over calcium hydride and DMF was distilled over calcium sulphate. Reactions were monitored by thin layer chromatography (TLC) using aluminium plates precoated with Merck silica gel, they were visualised using ultraviolet light (λmax = 254 nm), and then stained using basic potassium permanganate solution. Solvents were removed using a Büchi rotary evaporator at 35 °C. Flash column chromatography was carried out using Merk silica gel 60, 35-70 µm, as the stationary phase and solvents were of analytical purity. 'Ether' refers to diethyl ether and 'petrol' to the fraction bp 40-60 °C. Optical rotations were measured on Stanley ADP440 polarimeter and concentrations are recorded in g/100 mL using chloroform or methanol as a solvent. Infrared spectra were acquired on Bruker FTIR spectrometer; all data were obtained for solid compounds using ATR method. NMR spectra were obtained at 298K, using either a Bruker AV(III)400 spectrometer equipped with a BBFO probe or Bruker AV(III)400HD spectrometer equipped with a nitrogen cryoprobe operating at frequencies of 400 MHz (^{1}H) and 101 MHz (^{13}C) or a Bruker AV(III)500 spectrometer equipped with a helium cryoprobe operating at frequencies of 500 MHz (^{1}H) and 126 MHz (^{13}C). The spectra were measured as dilute solutions in either $CDCl_3$ or CD_3OD. Shifts are expressed in parts per million (ppm) downfield with the solvent residual peak ($CDCl_3$ δ_H 7.26, δ_C 77.16, CD_3OD δ_H 3.31, δ_C 49.0) as the internal standard. ^{31}P and ^{19}F NMR spectra were recorded with H_3PO_4 and $CFCl_3$ respectively, as external standards. All coupling constants are reported in Hertz (Hz) and multiplicities are labelled s (singlet), d (doublet), t (triplet), q

(quartet), m (multiplet), br (broad), app (apparent) or some combination thereof. Hydrogen atoms of the sugar ring are designated by the superscript prime mark (H-1', H-2' etc.) to distinguish them from those of the base (H-1, H-2 etc.). Where there are two hydrogen atoms attached to a sugar ring carbon, the atoms are arbitrarily designated H_A and H_B. In dinucleotides, individual nucleoside units are designated by the base abbreviation and if necessary numbered sequentially in the 3'(O)→5'(C) chain direction (e.g. T_1, T_2). Assignments in the ^1H spectra were made by using two-dimensional NMR spectroscopy; COSY, HSQC, HMBC. Mass spectra were recorded on a Bruker MicroTOF system, using electrospray (ESI) techniques.

All oligonucleotides were synthesised on 394 and 3400 ABI (Applied Biosystems) DNA/RNA synthesizers. CPG Solid Supports; standard and universal (SynBaseTM by Link Technologies), the 2'-OMe, RNA, DNA and 2'-F-phosporamidites and reagents for the synthesizer were purchased from Link Technologies Ltd. NH_4OH (35%) was purchased from Fischer, $Et_3N.3HF$ and *N*-methylpyrrolidinone (NMP) was purchased from Sigma-Aldrich, Oligonucleotide Purification Cartridge (OPC®) were obtained from Applied Biosystems, illustra Nap-5™ columns were purchased from GE Healthcare Europe GmbH. Water used for oligonucleotides analysis and purification was a commercial nuclease-free water. MeCN HPLC grade was used for oligos synthesis and purification. Mass spectra were recorded on a Bruker Apex IV using electrospray (ES) ionisation techniques with negative (-) ion detection.

5.2 Synthesis of phosphonic acid (T) **26**

1-[(2*R*,4*S*,5R)-4-[(*tert*-butyldimethylsilyl)oxy]-5-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione **98**

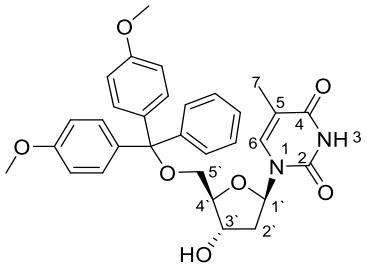


Chemical Formula: $C_{22}H_{42}N_2O_5Si_2$
Exact Mass: 470.2632

Thymidine **97** (2.00 g, 8.26 mmol), *tert*-butyldimethylsilyl chloride (3.12 g, 20.7 mmol) and imidazole (2.82 g, 41.7 mmol) were mixed in 16 mL anhydrous DMF under argon atmosphere and stirred at room temperature for 2h. The mixture was then partitioned between water (12 mL) and dichloromethane (12 mL). The organic layer was further washed with water (4 mL). The aqueous layer was extracted with dichloromethane (3 × 4 mL), and the combined organic layers were dried over anhydrous sodium sulphate and filtrated. The filtrate was concentrated under reduced pressure to afford a sticky oil, which co-evaporated with toluene (3 × 6 mL) to provide the crude product. The crude product was then purified by column chromatography using ethyl acetate : petrol (1:4) to afford the desired product **98** (3.88 g, quantitative). m.p. 146-148 °C; $[\alpha]_D^{23.9}$ + 13.50 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max}/cm^{-1} 2952, 2857, 1702, 1664, 1471, 1252, 829; δ_H (500 MHz, $CDCl_3$): 8.66 (1H, br. s, NH), 7.47 (1H, q, *J* 1.2, H-6), 6.34 (1H, dd, *J* 8.0, 5.8, H-1′), 4.4 (1H, dt, *J* 6.0, 2.5, H-3′), 3.93 (1H, q, *J* 2.5, H-4′), 3.87 (1H, dd, *J* 11.4, 2.5, H-5′), 3.76 (1H dd, *J* 11.4, 2.5, H-5′), 2.24 (1H, ddd, *J* 13.1, 5.8, 2.5, H_A-2′), 2.00 (1H ddd, *J* 13.1, 8.0, 6.0, H_B-2′), 1.91 (3H d, *J* 1.2, H-7), 0.92 (9H, $SiC(CH_3)_3$), 0.89 (9H, $SiC(CH_3)_3$), 0.11 (3H, $Si(CH_3)_2$), 0.11 (3H, $Si(CH_3)_2$), 0.08 (3H, $Si(CH_3)_2$), 0.07 (3H, $Si(CH_3)_2$); δ_C

(126 MHz, $CDCl_3$): 164.0 (C-4), 150.4 (C-2), 135.6 (CH-6), 110.9 (C-5), 87.9 (CH-4′), 84.0 (CH-1′), 72.0 (CH-3′), 63.0 (CH_2-5′), 41.5 (CH_2-2′), 26.0 (3 × SiC(CH_3)$_3$), 25.8 (3 × SiC(CH_3)$_3$), 18.5 (SiC(CH_3)$_3$), 18.1 (SiC(CH_3)$_3$), 12.7 (CH_3-7), -4.5 (2 × Si(CH_3)$_2$), -5.3 (2 × Si(CH_3)$_2$); *m/z* (ESI$^+$) 471.2703 (M+H. $C_{22}H_{43}N_2O_5Si_2$ requires 471.2705), 493.2527 (M+Na. $C_{22}H_{42}N_2NaO_5Si_2$ requires 493.2524).

1-((2*R*,4*S*,5*R*)-5-(2,2-bis(4-methoxyphenyl)-2-phenylethyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **22**

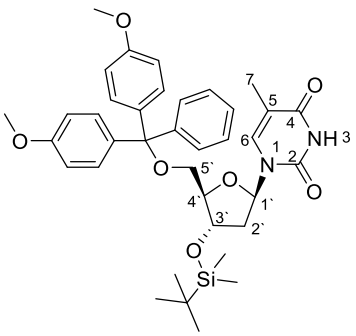


Chemical Formula: $C_{31}H_{32}N_2O_7$
Exact Mass: 544.2210

Thymidine **97** (3.00 g, 12.4 mmol) was dissolved in pyridine (10 mL), and the water was removed by azeotropic distillation with pyridine (3 × 10 mL). The residue was dissolved in pyridine (15 mL), dimethxytrityl chloride (4.60 g, 13.6 mmol) was added in portions at 0 °C and the reaction mixture was stirred at this temperature for 10 min then allowed to warm up to room temperature. The resultant mixture was stirred at RT for 22 h. When the reaction is complete, pyridine was removed by reduced pressure, and the residue was dissolved in ethyl acetate (200 mL), washed with water (100 mL). The separated aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over anhydrous sodium sulphate. After filtration, the volatiles were removed on reduced pressure and the residue was purified by column chromatography using ethyl acetate : petrol (1:4 → 1:1) to provide the desired product **22** as a white foam (6.74 g, quantitative). FTIR (ATR) ν_{max}/cm^{-1} 3484, 3049, 3002, 2948, 2834, 1673, 1606, 1508, 1250, 826; δ_H (400 MHz, $CDCl_3$):

9.00 (1H, br. s, NH), 7.59 (1H, q, *J* 1.3, H-6), 7.43-7.38 (2H, m, ArH), 7.34-7.22 (7H, m, ArH), 6.86-6.80 (4H, m, ArH), 6.35 (1H, dd, *J* 7.8, 5.8, H-1′), 4.61-4.52 (1H, m, H-3′), 3.97 (1H, q, *J* 3.0, H-4′), 3.79 (6H, 2 × OCH_3), 3.47 (1H, dd, *J* 10.5, 3.0, H_A-5′), 3.27 (1H, dd, *J* 10.5, 3.0, H_B-5′), 2.68 (1H, br., OH-3′), 2.37-2.30 (1H, m, H_A-2′), 2.25-2.19 (1H, m, H_B-2′), 1.50 (3H, d, *J* 1.3, H-7); δ_C (101 MHz, $CDCl_3$): 163.9 (C-4), 158.8 (2 × ArC), 150.6 (C-2), 144.4 (ArC), 135.8 (CH-6), 135.5 (ArC), 135.46 (ArC), 130.2 (4 × ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.3 (ArCH), 113.4 (4 × ArCH), 111.2 (C-5), 87.1 (CAr_3), 86.3 (CH-4′), 84.8 (CH-1′), 72.67 (CH-3′), 63.7 (CH_2-5′), 55.4 (2 × OCH_3), 41.1 (CH_2-2′), 12.0 (CH_3-7); *m/z* (ESI^+) 567.2110 (M+Na. $C_{31}H_{32}N_2NaO_7$ requires 567.2102).

1-((2*R*,4*S*,5*R*)-5-(2,2-bis(4-methoxyphenyl)-2-phenylethyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **100**

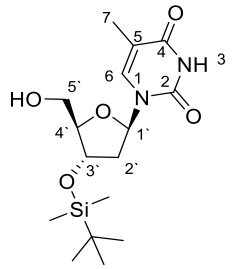


Chemical Formula: $C_{37}H_{46}N_2O_7Si$
Exact Mass: 658.3074

Imidazole (3.27 g, 48.0 mmol) was added in one portion to a stirring solution of alcohol **22** (7.00 g, 13.7 mmol) in DMF (15 mL) at 0 °C and the resulting solution was stirred at this temperature for 30 min. TBDMSCl (3.07 g, 20.5 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 3 days at this temperature. DMF was removed *via* azeotrope with toluene (5 × 100 mL) to afford a sticky oily residue, diethyl ether (100 mL) and water (50 mL) were added and the separated aqueous layer

was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo* to afford the desired product **100** (9.50 g, quantitative) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2951, 2928, 2854, 1684, 1604, 1462, 1247, 827;δ_H (500 MHz, $CDCl_3$): 8.78 (1H, br. s, NH), 7.66 (1H, q, *J* 1.3, H-6), 7.43-7.38 (2H, m, ArH), 7.34-7.27 (6H, m, ArH), 7.25-7.23 (1H, m, ArH), 6.86-6.8 (4H, m, ArH), 6.35 (1H, t, *J* 6.6, H-1'), 4.52 (1H, dt, *J* 6.6, 3.4, H-3'), 3.97 (1H, q, *J* 2.8, H-4'), 3.79 (6H, 2 × OCH_3), 3.47 (1H, dd, *J* 10.7, 2.8, H_A-5'), 3.27 (1H, dd, *J* 10.7, 2.8, H_B-5'), 2.37-2.30 (1H, m, H_A-2'), 2.25-2.19 (1H, m, H_B-2'), 1.50 (3H, d, *J* 1.3, H-7), 0.83 (9H, $SiC(CH_3)_3$), 0.02 (3H, $Si(CH_3)_2$), -0.03 (3H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 163.9 (C-4), 158.8 (2 × ArC), 150.4 (C-2), 144.44 (ArC), 135.7 (CH-6), 135.6 (ArC), 135.5 (ArC), 130.2 (4 × ArCH), 129.3 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.3 (ArCH), 113.4 (4 × ArCH), 111.2 (C-5), 86.9 (CH-4' + C-Ar_3), 85.0 (CH-1'), 72.2 (CH-3'), 63.01 (CH_2-5'), 55.4 (2 × OCH_3), 41.7 (CH_2-2'), 25.7 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), 12.0 (CH_3-7), -4.5 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); *m/z* (ESI^+) 681.2949 (M+Na. $C_{37}H_{46}N_2NaO_7Si$ requires 681.2966).

1-[(2*R*,4*S*,5R)-4-[(*tert*-butyldimethylsilyl)oxy]-5-(hydroxymethyl)oxolan-2-yl]-5 methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione **99**



Chemical Formula: $C_{16}H_{28}N_2O_5Si$
Exact Mass: 356.1767

Method A

To a solution of compound **98** (4.00 g, 8.49 mmol) in dichloromethane (44 mL) was added a mixture of trifluoracetic acid and water (10:1, v/v, 4.4 mL). The resulting mixture was stirred at RT for 45 min, then in an ice bath for 30 min.

The solution was diluted with dichloromethane (8.0 ml), washed with iced-cold water (40 ml), saturated solution of sodium bicarbonate (80 mL) and brine (50 mL), the mixture was stirred for 30 minutes and then separated, the organic layer was washed with water (5.0 mL) and the aqueous layer was extracted with dichloromethane (3 × 8.0 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated and the resides were purified by column chromatography using ethyl acetate : petrol (1:1) to afford the desired product **99** as white foam (2.30 g, 76%) and 15% was recovered of starting material **98**.

Method B

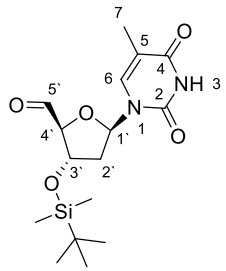
A mixture of $CuSO_4.5H_2O$ (25.0 mg, 100 µmol), 3',5'-bis(OTBDMS) thymidine **97** (235 mg, 500 µmol) in methanol (6.00 mL) was stirred at 50 °C for 22 h. After which time the mixture was filtrated, the volatiles were removed by vacuum and the residue was purified by column chromatography ethyl acetate : petrol (1:1) to afford the desired product **99** (98 mg, 55%)

Method C

Compound **100** (9.00 g, 13.6 mmol) was dissolved in 3% (v/v) dichloroacetic acid in dichloromethane (225 mL) and stirred for 15 min at room temperature. The mixture was neutralized by saturated solution of sodium bicarbonate and stirred for an additional 10 min, brine (50 mL), dichloromethane (50 mL) were added and after separation the organic layer, the aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate : petrol (1:4 → 1:1) to afford the desired product as a white foam **99** (4.35 g, 88%) and (1.00 g, 11%) was recovered of starting material **100**. m.p 116-117 °C; $[\alpha]_D^{24}$ + 44.0 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max} /cm^{-1} 3474, 3176, 2955, 2857, 1692, 1664, 1483, 1228, 920, 776; δ_H (400 MHz, $CDCl_3$): 9.02 (1H, br. s, NH),

7.37 (1H, q, *J* 1.2, H-6), 6.14 (1H, t, *J* 6.8, H-1′), 4.48 (1H, dt, *J* 6.9, 3.8, H-3′), 3.91 (2H, m, H-4′ + H-5′), 3.74 (1H, dd, *J* 12.6, 3.8, H-5′), 2.79 (1H, br s, OH), 2.34 (1H, dt, *J* 13.4, 6.8, H_A-2′), 2.21 (1H, ddd, *J* 13.4, 6.8, 3.8, H_B-2′), 1.90 (3H, d, *J* 1.2, H-7), 0.88 (9H, $SiC(CH_3)_3$), 0.08 (6H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 164.0 (C-4), 150.5 (C-2), 137.2 (CH-6), 111.1 (C-5), 87.7 (CH-4′), 87.04 (CH-1′), 71.7 (CH-3′), 62.1 (CH_2-5′), 40.6 (CH_2-2′), 25.9 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), 12.6 (CH_3-7), -4.6 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); *m/z* (ESI^+) 379.1661 (M+Na. $C_{16}H_{28}N_2NaO_5Si$ requires 379.1660).

(2*S*,3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5- methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1- yl)oxolane-2-carbaldehyde **23**



Chemical Formula: $C_{16}H_{26}N_2O_5Si$
Exact Mass: 354.1611

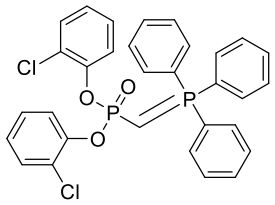
Method A

To a solution of 3′-O-(*tert*-butyldimethylsilyl) thymidine **99** (1.50 g, 4.2 mmol) in acetonitrile (40 mL), IBX (3.54 g 12.6 mmol) was added and the resultant suspension was heated under reflux for 3 h. After cooling to room temperature, the suspension was filtered. The insoluble residue was washed with ethyl acetate (3 × 30 mL) and the solvent of the combined filtrates was removed under reduced pressure. The solid residue was dissolved in dichloromethane (100 mL) and washed with sodium bicarbonate (25 mL) and the separated aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried over magnesium sulphate and the volatiles were removed under reduced pressure to yield (1.25 g, quantitative) of **23** as a white foam which was used in the subsequent reaction without further purification.

Method B

To a stirred solution of 3′-O-(*tert*-butyldimethylsilyl) thymidine **99** (1.00 g, 2.80 mmol) in dichloromethane (2.5 mL), Dess-martin periodinane (1.90 g, 3.76 mmol) was added in one portion and the reaction mixture was stirred at room temperature for 4 h. After which time, saturated solution of sodium bicarbonate (50 mL), saturated solution of sodium thiosulphate (50 mL) and brine (50 mL) were added and the mixture was stirred for 1 h, diluted with dichloromethane (80 mL). The mixture was separated, the aqueous layer was extracted with dichloromethane (4 × 40 ml) and the combined organic layers were dried over anhydrous $MgSO_4$, the volatiles were evaporated to afford the desired aldehyde **23** as white foam (990 mg, quantitative). The freshly prepared aldehyde **23** was used without any further purification. m.p. 99-102 $^{\circ}C$; $[\alpha]_D^{24.2}$ + 8.76 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max} /cm^{-1} 3187, 3068, 2952, 2856, 1682, 1464, 1133, 830, 757; δ_H (400 MHz, $CDCl_3$): 9.76 (1H, s, H-5′), 8.30 (1H, br. s, NH), 7.57 (1H, q, *J* 1.2, H-6), 6.32 (1H, dd, *J* 8.2, 5.8, H-1′), 4.67 (1H, dt, *J* 5.6, 2.0, H-3′), 4.48 (1H, d, *J* 2.0, H-4′), 2.32 (1H, ddd, *J* 13.5, 5.8, 2.0, H_B-2′), 2.04 (1H, ddd, *J* 13.5, 8.2, 5.6, H_B-2′), 1.96 (3H, d, *J* 1.2, H-7), 0.92 (9H, $SiC(CH_3)_3$), 0.14 (6H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 198.74 (CH-5′), 164.0 (C-4), 150.3 (C-2), 136.5 (CH-6), 111.4 (C-5), 91.7 (CH-4′), 88.2 (CH-1′), 77.5 (CH-3′), 39.8 (CH_2-2′), 25.8 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), 12.7 (CH_3-7), -4.7 ($Si(CH_3)_2$), -4.8 ($Si(CH_3)_2$); *m/z* (ESI^+) 377.1502 (M+Na. $C_{16}H_{26}N_2NaO_5Si$) requires 377.1503.

bis(2-chlorophenyl) [(triphenyl-λ⁵- phosphanylidene)methyl]phosphonate **24**

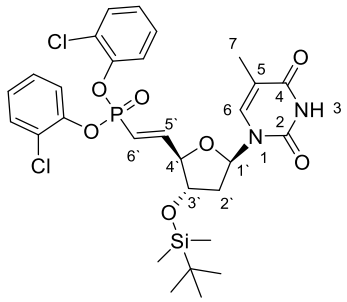


Chemical Formula: $C_{31}H_{24}Cl_2O_3P_2$
Exact Mass: 576.0578

Triethyl amine (11.4 mL, 82.1 mmol) was added dropwise to a stirring solution of chloromethylphosphonic dichloride **101** (4.0 mL, 39.2 mmol) and *o*-chlorophenol (8.4 mL, 81.0 mmol) in diethyl ether (120 mL), the reaction mixture was allowd to warmup and then stirred at RT for 2 d. After which time water (100 mL) and dichloromethane (100 mL) were added, the aqueous layer was extracted with dichloromethane (3 × 100 mL), the combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to leave a yellow oil residue **102**. The crude compound was used for the next step without any purification. Triphenylphosphine (9.57 g, 36.5 mmol) was added to the residue **102** (12.8 g, 36.4 mmol) and the mixture was stirred at 160 °C for 18 h. After which time the reaction was allowed to cool to RT the resulting solid was dissolved in dichloromethane (160 mL) followed by addition of sodium hydroxide solution (2M, 160 mL) and stirred for 30 min. The aqueous layer was extracted three times with dichloromethane (160 mL), the combined organic layers were washed with brine (5 mL) and dried over anhydrous magnesium sulphate. The volatiles were evaporated, and the desired product was obtained as a white powder **24** (17.8 g, 79%, over two steps). m.p 135-137 °C; FTIR (ATR) ν_{max} /cm^{-1}; 1584, 1475, 1435, 1335, 1220, 1058, 919; δ_H (400 MHz, $CDCl_3$): 7.56-7.44 (12H, m, ArH), 7.39-7.31 (8H, m, ArH), 7.07 (2H, td, *J* 7.8, 1.8, ArH), 7.03-6.97 (2H, m, ArH); δ_C (101MHz, $CDCl_3$): 148.3 (2 × d, *J* 6.3, ArC), 133.2 (6 × ArCH), 133.1 (3 × ArCH), 132.2 (2 × d, *J* 6.3, ArC), 131.9 (6 × ArCH), 130.1 (2 × ArCH), 128.7 (2 × ArCH), 128.6 (2 × ArCH), 127.4 (2 × ArCH), 126.3 (3 × ArC), 124.5 (2 × ArCH), 123.2 (2 × d, *J* 3.1, ArCH); δ_P (162 MHz,

$CDCl_3$): 28.6 (d, $^2J_{P\text{-}P}$ 47.2), 21.5 (d, $^2J_{P\text{-}P}$ 47.2); *m/z* (ES^+) 577.0641 (M+H. $C_{31}H_{25}Cl_2O_3P_2$ requires 577.0650), 599.0435 (M+Na. $C_{31}H_{24}Cl_2NaO_3P_2$ requires 599.0470).

bis(2-chlorophenyl) [(*E*)-2-[(3*S*,5R)-3-[(*tert*- butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo- 1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **25**

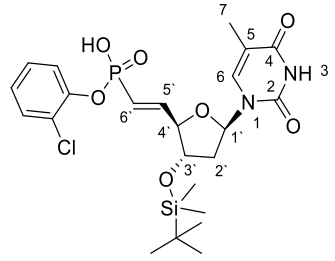


Chemical Formula: $C_{29}H_{35}Cl_2N_2O_7PSi$
Exact Mass: 652.1328

The ylide **24** (2.30 g, 4.03 mmol) was added in one portion to a stirring solution of aldehyde **23** (0.95 g, 2.68 mmol) in dichloromethane (24 mL) and the resulting solution was stirred at RT for 18 h. The solvent was evaporated, and the crude product was dry-loaded onto silica gel and purified by column chromatography using ethyl acetate: petrol (2:3) to afford the desired product **25** as white foam (1.78 g, 68%). $[\alpha]_D^{24.4}$ + 57.83 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max} /cm^{-1} 3180, 3069, 2953, 2856, 1686, 1583, 1475, 1364, 1211, 1033, 925 (s); δ_H (500 MHz, $CDCl_3$): 8.87 (1H, br. NH), 7.46-7.33 (4H, m, ArH), 7.26-7.20 (2H, m, ArH), 7.19-7.02 (4H, m, 2 × ArH + H-6 + H-5′), 6.4-6.2 (2H, m, H-6′, H-1′), 4.45-4.39 (1H, m, H-4′), 4.23-4.15 (1H, m, H-3′), 2.31-2.23 (1H, m, H_A-2′), 2.09-1.99 (1H, m, H_B-2′), 1.93 (3H, d, *J* 1.6, H-7), 0.88 (3H, $SiC(CH_3)_3$), 0.88 (3H, $SiC(CH_3)_3$), 0.87 (3H, $SiC(CH_3)_3$), 0.05 (3H, $Si(CH_3)_2$), 0.05 (3H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 163.5 (C-4), 151.5 (d, *J* 6.1, CH-5′), 150.3 (C-2), 146.2 (d, *J* 7.6, ArC), 146.1 (dd, *J* 7.7, ArC), 134.9 (CH-6), 130.8 (ArCH), 130.7 (ArCH), 128.2 (d, *J* 1.3, ArCH), 128.1 (d, *J* 1.3, ArCH), 126.6 (d,

J 1.1, ArCH), 126.5 (d, *J* 1.1, ArCH), 125.9 (d, *J* 6.3, ArC), 125.8 (d, *J* 6.1, ArC), 122.7 (d, *J* 2.7, ArCH), 122.4 (d, *J* 2.7, ArCH), 116.4 (d, *J* 192.7, CH-6'), 111.9 (C-5), 85.9 (d, *J* 22.8, CH-4'), 85 (CH-1'), 74.75 (CH-3'), 39.9 (CH_2-2'), 25.7 (3 × Si(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 12.8 (CH_3-7), -4.5 (Si(CH_3)$_2$), -4.7 (Si(CH_3)$_2$); δ_p (121 MHz, $CDCl_3$): 11.1; *m/z* (ESI$^+$) 653.1411 (M+H. $C_{29}H_{36}Cl_2N_2O_7PSi$ requires 653.1401), 675.1243 (M+Na. $C_{29}H_{35}Cl_2N_2NaO_7PSi$ requires 675.1220), 670.1662 (M+NH_4. $C_{29}H_{39}Cl_2N_3O_7PSi$ requires 670.1666).

[(*E*)-2-[(3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5- (5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1- yl)oxolan-2-yl]ethenyl](2-chlorophenoxy)phosphinic acid **26**



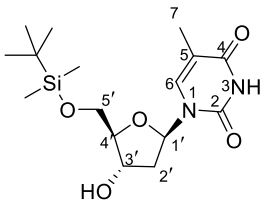
Chemical Formula: $C_{23}H_{32}ClN_2O_7PSi$
Exact Mass: 542.1405

To a solution of vinylphosphonate **25** (1.10 g, 1.68 mmol) in dioxane (50 mL) was added in one portion a solution of tetramethylguanidine (253 µL, 2.02 mmol), triethyl amine (1.42 mL, 10.1 mmol) and 2-nitrobenzaldoxime (334 mg, 2.02 mmol) in dioxane (15 mL) and the resulting mixture was stirred at room temperature for 3 days. The volatiles were evaporated and the residue was purified by column chromatography using methanol : dichloromethane (1:9) to afford the desired product as white powder **26** (910 mg, quantitative). m.p. 125-127 °C; $[\alpha]_D^{23}$ + 11.43 (C=1 methanol); FTIR (ATR) ν_{max}/cm^{-1} 3500-3000, 2951, 1686, 1474, 1228, 1054, 831, 760; δ_H (400 MHz, CD_3OD): 7.55 (1H, dt, *J* 8.2, 1.4, ArH), 7.38 (1H, q, *J* 1.2, H-6), 7.36 (1H, dd, *J* 7.9, 1.0, ArH), 7.20 (1H, ddd, *J* 8.2, 7.4, 1.6, ArH), 7.05-7.00 (1H, ddd, *J* 9.5, 8.2, 1.6, ArH), 6.57 (1H, ddd, *J* 20.9, 17.1, 5.4, H-5'), 6.26 (1H, dd, *J* 7.8, 6.1, H-1'),

6.12 (1H, ddd, *J* 17.9, 17.1, 1.6, H-6′), 4.33-4.25 (1H, m, H-4′), 4.25 (1H, dt, *J* 6.2, 3.2, H-3′), 2.20-2.03 (2H, m, H_A-2′ + H_B-2′), 1.87 (3H, d, *J* 1.2, H-7), 0.89 (9H, $SiC(CH_3)_3$), 0.08 (3H, $Si(CH_3)_2$), 0.07 (3H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 166.2 (C-4), 152.3 (C-2), 150.0 (d, *J* 6.6, ArC), 144.6 (d, *J* 4.8, CH-5′), 137.5 (CH-6), 131.1 (ArCH), 128. 7(ArCH), 126.6 (d, *J* 6.4, ArC), 124.9 (d, *J* 183, CH-6′), 125.2 (ArCH), 123.8 (d, *J* 2.8, ArCH), 112.0 (C-5), 88.3 (d, *J* 21.9, CH-4′), 86.4 (CH-1′), 76.9 (CH-3′), 40.3 (CH_2-2′), 26.2 (3 × $SiC(CH_3)_3$), 18.8 ($SiC(CH_3)_3$), 12.6 (CH_3-7), -4.6 (2 × $Si(CH_3)_2$); δ_p (162 MHz, CD_3OD): 7.6; *m/z* (ESI^+) 565.127031 (M+Na. $C_{23}H_{32}ClN_2NaO_7PSi$ requires 565.129713).

5.2.1 Synthesis of T*T phosphoramidite **41**

1-[(2*R*,4*S*,5R)-5-{[(*tert*-butyldimethylsilyl)oxy]methyl}-4-hydroxyoxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4- dione **70**

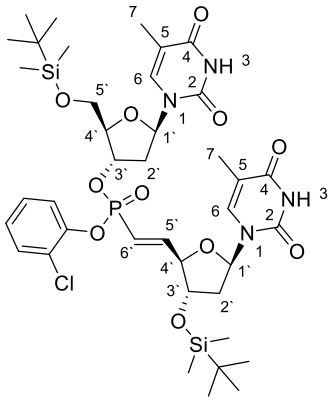


Chemical Formula: $C_{16}H_{28}N_2O_5Si$
Exact Mass: 356.1767

To a solution of thymidine **97** (3.00 g, 12.4 mmol) in anhydrous DMF (20 mL) at 0 °C was added imidazole (2.04 g, 29.8 mmol) followed by gradual addition of *tert*-butyldimethylsilyl chloride (2.25 g, 14.9 mmol) over period of 10 min. The cooling bath was removed, and the reaction mixture was stirred at room temperature for 3 d. Iced water (100 mL) was added to the reaction mixture, and the white solid was collected by vacuumed filtration. The white solid was applied on azeotropic distillation with toluene (3 × 20 mL) to remove the remining DMF. The crude product was purified by column chromatography using ethyl acetate : petrol (1:1→ 4:1) to afford the desired product **70** as white solid (3.82 g, 86%). m.p 190-192 °C; $[\alpha]_D^{24.5}$ – 10.04 (C=1.00 $CHCl_3$); FTIR (ATR)

ν_{max}/cm^{-1}: 3547, 3164, 2950, 2928, 2855, 1680, 1469, 1257, 830; δ_H (400 MHz, $CDCl_3$): 8.93 (1H, br. s, NH), 7.51 (1H, q, *J* 1.2, H-6), 6.39 (1H, dd, *J* 8.2, 5.7, H-1′), 4.46 (1H, dt, *J* 5.9, 2.5, H-3′), 4.05 (1H, q, *J* 2.5, H-4′), 3.89 (1H, dd, *J* 11.3, 2.8, H_A-5′), 3.83 (1H, dd, *J* 11.3, 2.6, H_B-5′), 3.42-2.45 (1H, br s, OH), 2.37 (1H, ddd, *J* 13.7, 5.7, 2.5, H_A-2′), 2.09 (1H ddd, *J* 13.7, 8.2, 5.9, H_B-2′), 1.91 (3H, d, *J* 1.2, H-7), 0.92 (9H, $SiC(CH_3)_3$), 0.11 (3H, $Si(CH_3)_2$), 0.11 (3H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 163.9 (C-4), 150.6 (C-2), 135.6 (CH-6), 111.0 (C-5), 87.4 (CH-4′), 87.1 (CH-1′), 72.7 (CH-3′), 63.7 (CH_2-5′), 41.3 (CH_2-2′), 26.1 (3 × $SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), 12.67 (CH_3-7), -5.26 (2 × $Si(CH_3)_2$); *m/z* (ESI^+) 379.1668 (M+Na. $C_{16}H_{28}N_2NaO_5Si$ requires 379.1660), 357.1848 (M+H. $C_{16}H_{29}N_2O_5Si$ requires 357.1840).

(2*R*,3*S*,5R)-2-{[(*tert*- butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2- chlorophenyl [(*E*)-2-[(3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **105**



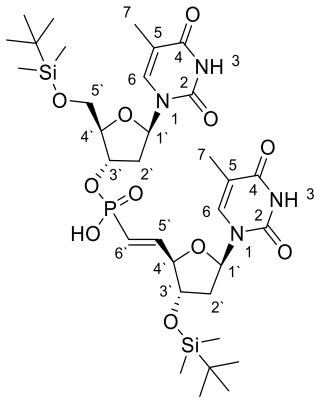
Chemical Formula: $C_{39}H_{58}ClN_4O_{11}PSi_2$
Exact Mass: 880.3067

Phosphonate **26** (1.00 g, 1.84 mmol), alcohol **70** (674 m, 1.90 mmol) and *N*-methylimidazole (900 µL, 11.4 mmol) were dissolved in pyridine (6 ml), evaporated three times. The residue was dissolved in pyridine (6 mL), Triisopropylbenzenesulfonyl chloride (3.10 g, 10.5 mmol) was added, the resulting mixture was then stirred at room temperature for 20 h. The solvent

was evaporated and the residue was dry-loaded onto silica gel and purified by column chromatography using petrol : ethyl acetate (1:4) to afford the desired product **105** (1.44 g, 89%, 1:1 mixture of diastereoisomers) as a white foam. m.p 118-120 °C; $[\alpha]_D^{24.6}$ + 18.35 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max}/cm^{-1} :3180, 3058, 2952, 2856, 1683, 1470, 1251, 831, 775 ; δ_H (400 MHz, $CDCl_3$): 8.94 (0.4H, br. N-H), 8.73 (1H, br. N-H), 8.64 (0.6H, br. N-H), 7.48 (1H, m, H-6), 7.45-7.41 (1H, m, ArH), 7.40 (0.4H, t, *J* 1.5, ArH), 7.38 (0.6H, t, *J* 1.5, ArH),7.28-7.21 (1H, m, ArH), 7.18-7.11 (1H, m, ArH), 7.09 (0.4H, q, *J* 1.2, H-6), 7.05 (0.6H, q, *J* 1.2, H-6), 7.04-6.92 (1H, m, T_2- H-5′), 6.42-6.36 (1H, m, T_1-H1′), 6.29-6.12 (2H, m, T_2-1′ + T_2-H-6′), 5.27-5.20 (1H, m, T_1-H-3′), 4.43-4.34 (1.4H, m, T_2-H-4′ + T_2-H-3′), 4.38 (0.6H, q, *J* 1.6, T_1-H-4′), 4.23-4.17 (1H, m, T_1-H-4′ + T_2-H-3′), 3.90-3.72 (2H, m, T_1-H_A-5′ + T_1-H_B-5′), 2.64 (0.4H, dd, *J* 13.6, 5.0, T_1-H_A-2′), (0.6H, dd, *J* 13.6, 5.0, T_1-H_A-2′), 2.40-2.06 (3H, m, T_1-H_B-2′ + T_2-H_A-2′ + T_2-H_B-2′), 1.96-1.86 (6H, m, 2 × H-7), 0.90 (18H, 2 × $SiC(CH_3)_3$), 0.09 (12H, 2 × $(Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 163.8 (2 × C-4), 163.7 (2 × C-4), 150.9 (d, *J* 6.4, T_2-CH-5′), 150.7 (d, *J* 6.4, T_2-CH-5′), 150.5 (C-2), 150.4 (C-2), 150.3 (2 × C-2), 146.3 (d, *J* 6.6, ArC), 146.3 (d, *J* 6.6, ArC), 136.2 (CH-6), 135.5 (CH-6), 135.0 (2 × CH-6), 130.9 (2 × ArCH), 128.3 (ArCH), 128.1 (ArCH), 126.4 (ArCH), 126.3 (ArCH), 125.9 (d, *J* 5.9, ArC), 125.8 (d, *J* 5.8, ArC), 122.4 (d, *J* 2.6, ArCH), 122.2 (d, *J* 2.2, ArCH), 117.0 (d, *J* 192, T_2-CH-6′), 116.9 (d, *J* 192, T_2-CH-6′), 111.8 (C-5), 111.7 (C-5), 111.5 (C-5), 111.4 (C-5), 86.5 (T_2-CH-1′), 86.2 (d, *J* 4.1, T_1-CH-4′), 86.1 (d, *J* 4.7, T_1-CH-4′), 85.8 (d, *J* 22.8, T_2-CH-4′), 85.6 (T_2-CH-1′), 84.8 (T_1-CH-1′), 84.7 (T_1-CH-1′), 78.3 (d, *J* 6.3, T_1-CH-3′), 78.1 (d, *J* 6.5, T_1-CH-3′), 75.0 (d, *J* 1.2, T_2-CH-3′), 74.90 (d, *J* 1.3, T_2-CH-3′), 63.4 (T_1-CH_2-5′), 63.3 (T_1-CH_2-5′), 39.9 (T_2-CH_2-2′), 39.8 (d, *J* 4.3, T_1-CH_2-2′), 39.4 (T_2-CH_2-2′), 39.6 (d, *J* 4.6, T_1-CH_2-2′), 26.1 ($SiC(CH_3)_3$), 25.8 ($SiC(CH_3)_3$), 18.4 ($SiC(CH_3)_3$), 18.0 ($SiC(CH_3)_3$), 12.8 (CH_3-7), 12.7 (CH_3-7), 12.6 (2 × CH_3-7), -4.6 (2 × $Si(CH_3)_2$), -4.7 (2 ×

Si(CH_3)$_2$), -5.3 (2 × Si(CH_3)$_2$), -5.4 (2 × Si(CH_3)$_2$); δ_P (162MHz, $CDCl_3$):15.0, 14.9*m/z* (ESI$^+$) 903.2937 (M+Na. $C_{39}H_{58}ClN_4NaO_{11}PSi_2$ requires 903.2959).

[(*E*)-2-[(3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]({[(2*R*,3*S*,5R)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl]oxy})phosphinic acid **141**

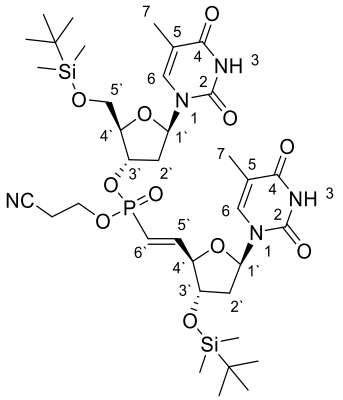


Chemical Formula: $C_{33}H_{55}N_4O_{11}PSi_2$
Exact Mass: 770.3143

To a mixture of dinucleotide **105** (1.40 g, 1.56 mmol) in dioxane (60 mL) was added in one portion a solution of tetramethylguanidine (300 µL, 2.39 mmol), triethylamine (1.6 mL, 11.4 mmol), 2-nitrobenzaldoxime (400 mg, 2.41 mmol) in dioxane (20 mL). The resulting mixture was stirred at room temperature for 3 days. The volatiles were removed by vacuum and the residue was purified using column chromatography using solvent system dichloromethane : methanol (9:1) to afford the desired product **141** as white powder (1.20 g, quantitative). m.p 134-136 °C; $[\alpha]_D^{24.7}$ + 49.9 (C=1.00 MeOH); FTIR (ATR) ν_{max}/cm^{-1}: 2952, 2856, 1682, 1603, 1463, 1056, 832; δ_H (CD_3OD, 400 MHz): 7.63 (1H, q, *J* 1.2, H-6), 7.47 (1H, q, *J* 1.2, H-6), 6.57 (1H, ddd, *J* 20.3, 17.2, 5.5, T_2-H-5′), 6.31- 6.20 (2H, m, T_1-H-1′ + T_2-H-1′), 6.05 (1H, td, *J* 17.2, 1.4, T_2-H-6′), 4.77 (1H, t, *J* 6.6, T_1-H-3′), 4.40 (1H, dt, *J* 6.8, 4.1, T_2-H-3′), 4.32-4.26 (1H, m, T_2-H-4′), 4.25-4.19 (1H, m, T_1-H-4′), 3.95-3.8 (2H, m, T_1-H_A-5′

+ T_1-H_B-5′), 2.49 (1H, dd, *J* 13.4, 5.6, T_1-H_A-2′), 2.36 (1H, dt, *J* 13.6, 6.8, T_2-H_A-2′), 2.26-2.17 (1H, m, T_2-H_B-2′), 2.16-2.05 (1H, m, T_1-H_B-2′), 1.90 (3H, d, *J* 1.2, H-7), 1.88 (3H, d, *J* 1.2, H-7), 0.92 (9H, SiC(CH_3)$_3$), 0.92 (9H, SiC(CH_3)$_3$), 0.14-0.05 (12H, Si(CH_3)$_2$); δ_C (CD_3OD, 128 MHz): 166.3 (2 × C-4), 152.3 (C-2), 152.2 (C-2), 143.7 (d, *J* 4.4, T_2-CH-5′), 138.4 (CH-6), 137.2 (CH-6), 126.6 (d, *J* 180.2, T_2-CH-6′), 112.0 (C-5), 111.5 (C-5), 88.3 (d, *J* 21.1, T_2-CH-4′), 88.23 (d, *J* 5.1, T_1-CH-4′), 86.80 (T_2-CH-1′), 86.52 (T_1-CH-1′), 76.8 (T_1-CH-3′), 76.7 (T_2-CH-3′), 64.8 (T_1-CH_2-5′), 40. 9(d, *J* 3.6, T_1-CH_2-2′), 40.37 (T_2-CH_2-2′), 26.5 (SiC(*C*H_3)$_3$), 26.2 (SiC(*C*H_3)$_3$), 19.2 (Si*C*(CH_3)$_3$), 18.8 (Si*C*(CH_3)$_3$), 12. 7(CH_3-7), 12.5 (CH_3-7), -4.4 (2 × Si(*C*H_3)$_2$), -5.1 (2 × Si(*C*H_3)$_2$); δ_P (162MHz, CD_3OD): 9.5; *m/z* (ESI$^+$) 793.3003 (M+Na. $C_{33}H_{55}N_4NaO_{11}PSi_2$ requires 793.3036).

(2*R*,3*S*,5R)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **142**



Chemical Formula: $C_{36}H_{58}N_5O_{11}PSi_2$
Exact Mass: 823.3409

Method A

Phosphonate **230** (950 g, 1.96 mmol), alcohol **70** (656 mg, 1.96 mmol) and *N*-methylimidazole (1 mL, 12.7 mmol) were dissolved in pyridine (3.0 ml), and

the water was removed by azeotropic with pyridine (3 × 6 mL), Triisopropylbenzenesulfonyl chloride (712 mg, 2.35 mmol) was added, the resulting mixture was then stirred at room temperature for 16 h. The solvent was evaporated and the residue was dry-loaded onto silica gel and purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1)→ ethyl acetate) to afford the desired product **142** (1.10 g, 68%, 1:1 mixture of diastereoisomers) as a white foam.

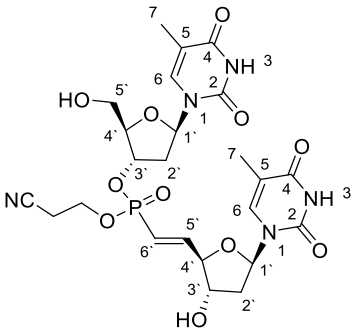
Method B

3-hydroxypropionitrile (500 µL, 7.40 mmol), *N*-methylimidazole (1.8 mL, 22.5 mmol), phosphonate dimer **149** (1.20 g, 1.55 mmol) were dissolved in pyridine (10 mL) and supplied for azeotropic distillation (3 × 10 mL), the residue was dissolved in pyridine (8 mL). Triisopropylbenzenesulphonyl chloride (2.24 g, 7.40 mmol) was added to residues, the mixture was then stirred at room temperature for 20 h. After which time the solvent was evaporated and the residue was dry loaded onto silica gel and purified by column chromatography using ethyl acetate to afford the desired product **142** (763 mg, 64%, 1:1 mixture of diastereoisomers) as a as white foam. m.p 107-108 $^{\circ}$C; $[\alpha]_D^{24.8}$ + 30.8 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max}/cm^{-1} : 3171, 3045, 2953, 2856, 1682, 1465, 1249, 831, 777; δ_H (400MHz, $CDCl_3$): 9.43 (0.5H, s, NH), 9.34 (0.5H, s, NH), 9.31 (0.5H, s, NH), 9.30 (0.5H, s, NH), 7.49 (0.5H, q, *J* 1.2, H-6), 7.46 (0.5H, q, *J* 1.2, H-6), 7.13 (0.5H, q, *J* 1.2, H-6), 7.11 (0.5H, q, *J* 1.2, H-6), 7.04-6.84 (1H, m, T_2-H-5′), 6.41-6.32 (1H, m, T_1-H-1′), 6.25 (0.5H, t, *J* 6.8, T_2-H-1′), 6.16-5.97 (1.5H, m, T_2-H-1′ + T_2-H6′), 5.08 (1H, q, *J* 6.9, T_1-H-3′), 4.41-4.31 (2H, m, T_2-H-4′ + T_2-H-3′), 4.30-4.13 (3H, m, T_1-H-4′ + OC*H*$_2$CH$_2$CN), 3.94-3.81 (2H, m, T_1-H_A-5′ + T_1-H_B-5′), 2.83-2.72 (2H, m, OCH$_2$C*H*$_2$CN), 2.63-2.54 (1H, m, T_1-H_A-2′), 2.46-2.38 (0.5H, m, T_2-H_A-2′), 2.34-2.20 (1.5H, m, T_2-H_A-2′ + T_2-H_B-2′), 2.18-2.05 (1H, m, T_1-H_B-2′), 1.97-1.85 (6H, m, H-7), 10.91 (4.5H, s, SiC(CH_3)$_3$), 0.91 (4.5H, s, SiC(CH_3)$_3$), 0.89

(4.5H, s, $SiC(CH_3)_3$), 0.88 (4.5H, s, $SiC(CH_3)_3$), 0.12 (1.5H, s, $Si(CH_3)_2$), 0.11(1.5H, s, $Si(CH_3)_2$), 0.11 (1.5H, s, $Si(CH_3)_2$), 0.11 (1.5H, s, $Si(CH_3)_2$), 0.09 (1.5H, s, $Si(CH_3)_2$), 0.08 (1.5H, s, $Si(CH_3)_2$), 0.08 (1.5H, s, $Si(CH_3)_2$), 0.07 (1.5H, s, $Si(CH_3)_2$); δ_C (101MHz, $CDCl_3$): 164.0 (C-4), 164.0 (C-4), 163.9 (C-4), 163.8 (C-4), 150.7 (C-2), 150.6 (C-2), 150.5 (d, 6.5, T_2-CH-5′), 150.4 (C-2), 150.3 (C-2), 150.1 (d, *J* 6.7, T_2-CH-5′), 136.8 (CH-6), 136.2 (CH-6), 135.0 (CH-6), 135.0 (CH-6), 117.9 (d, *J* 191.4, T_2-CH-6′), 116.8 (d, *J* 189.0, T_2-CH-6′), 117.0 (CN), 116.5 (CN), 111.8 (C-5), 111.5 (2 × C-5), 111.4 (C-5), 87.2 (T_2-CH-1′), 86.2 (T_1-CH-4′), 86.1 (T_1-CH-4′), 86.0 (T_2-CH-1′), 85.9 (2 × d, *J* 22.0, T_2-CH-4′), 84.8 (T_1-CH-1′), 84.7 (T_1-CH-1′), 77.9 (d, *J* 5.3, T_1-CH-3′), 77.5 (d, *J* 5.3, T_1-CH-3′), 75.0 (d, *J* 1.2, T_2-CH-3′), 74.9 (d, *J* 1.2, T_2-CH-3′), 63.5 (T_1-CH_2-5′), 63.3 (T_1-CH_2-5′), 60.9 (d, *J* 4.5, *C*H_2CH_2CN), 60.4 (d, *J* 4.8, *C*H_2CH_2CN), 39.7 (d, *J* 3.5, T_1-CH_2-2′), 39.64 (T_2-CH_2-2′), 39.61 (T_2-CH_2-2′), 39.6 (d, *J* 3.5, T_1-CH_2-2′), 26.1 (3 × $SiC(CH_3)_3$), 26.0 (3 × $SiC(CH_3)_3$), 25.8 (6 × $SiC(CH_3)_3$), 20.1 (d, *J* 7.0 CH_2CH_2CN), 20.0 (d, *J* 7.0, CH_2CH_2CN), 18.4 (2 × $SiC(CH_3)_3$), 18.03 (2 × $SiC(CH_3)_3$), 12.6 (4 × CH_3-7), -4.5 (2 × $Si(CH_3)_2$), -4.6 (2 × $Si(CH_3)_2$), -5.3 (2 × $Si(CH_3)_2$), -5.3 ($Si(CH_3)_2$), -5.4 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 18.83, 18.75; *m/z* (ESI$^+$) 846.3291 (M+Na. $C_{36}H_{58}N_5NaO_{11}PSi_2$ requires 846.3301).

2-cyanoethyl (2*R*,3*S*,5R)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl [(*E*)-2-[(3*S*,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate

143

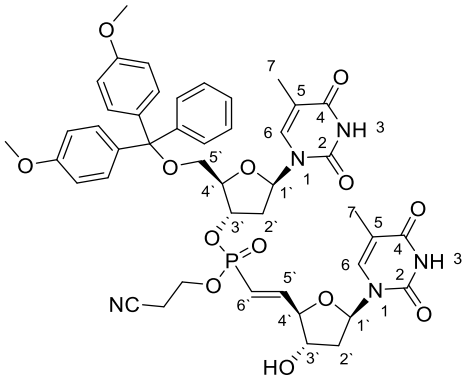


Chemical Formula: $C_{24}H_{30}N_5O_{11}P$
Exact Mass: 595.1679

Triethylamine trihydrofluoride (750 µL, 4.60 mmol) was added in one portion to a stirring solution of dinucleotide **142** (380 mg, 460 µmol) in THF (9 mL) and the resulting mixture was stirred at RT for 5 hours. The solvent was evaporated, and the residue was dry-loaded onto silica gel and purified by column chromatography using methanol: dichloromethane (1:9) to afford the desired product **143** (280 mg, quantitative, 1:1 mixture of diastereoisomers) as white powder. m.p 144-146 °C; $[\alpha]_D^{24.9}$ -7.62 (C=1.00 MeOH); FTIR (ATR) ν_{max}/cm^{-1} : 3400, 3182, 2980, 2822, 1680, 1470, 1271, 1040, 998; δ_H (400MHz, CD_3OD): 7.80 (0.5H, q, *J* 1.3, H-6), 7.78 (0.5H, q, *J* 1.3, H-6), 7.43 (0.5H, q, *J* 1.3, H-6), 7.41 (0.5H, q, *J* 1.3, H-6), 7.04 (1H, ddd, *J* 23.4, 17.2, 4.2, T_2-H-5′), 6.36-6.10 (3H, m, T_1-H-1′ + T_2-H-1′ + T_2-H-6′), 5.20-5.11 (1H, m, T_1-H-3′), 4.45-4.36 (2H, m, T_2-H-3′ + T_2-H-4′), 4.29 (2H, dt, *J* 12.7, 6.1, C*H*$_2$CH$_2$CN), 4.23 (0.5H, q, *J* 3.0, T_1-H-4′), 4.18 (0.5H, q, *J* 3.0, T_1-H-4′), 3.82 (1H, d, *J* 3.2, T_1-H_A-5′), 3.79 (1H, d, *J* 3.1, T_1-H_B-5′), 2.91 (2H, q, *J* 5.7, CH$_2$*C*H$_2$CN), 2.60-2.49 (1H, m, T_1-H_A-2′), 2.47-2.35 (2H, m, T_2-H_A-2′ + T_2-H_B-2′), 2.31-2.23 (1H, m, T_1-H_B-2′), 1.90 (1.5H, d, *J* 1.2, H-7), 1.89 (1.5H, d, *J* 1.2, H-7), 1.88 (3H, H-7); δ_C (101MHz, CD_3OD): 166.3 (4 × C-4), 152.7 (2 × d, *J*, 6.3, T_2-CH-5′), 152.4 (2 × C-2), 152.3 (C-2), 152.2 (C-2), 138.5 (CH-6),

138.3 (CH-6), 137.9 (CH-6), 137.8 (CH-6), 118.8 (CN), 118.7 (CN), 118. 3(d, *J* 189.2, T_2-CH-6′), 117.0 (d, *J* 189.5, T_2-CH-6′), 112.1 (C-5), 112.0 (C-5), 112.0 (2 × C-5), 87.3 (2 × T_2- CH-1′), 87.2 (d, *J* 5.8, T_1-CH-4′), 87.1 (d, *J* 22.5 T_2-CH-4′), 87.0 (d, *J* 5.7, T_1-CH-4′), 86.9 (d, *J* 23, T_2-CH-4′), 86.1 (2 × T_1-CH-1′), 78.7 (d, *J* 5.8, T_1-CH-3′), 78.4 (d, *J* 5.9, T_1-CH-3′), 75.02 (2 × T_2-CH-3′), 62.7 (d, *J* 5.1, *C*$H_2$$CH_2$CN), 62.6 (d, *J* 5.2, *C*$H_2$$CH_2$CN), 62.44 ($T_1$-$CH_2$-5′), 62.35 ($T_1$-$CH_2$-5′), 39.8 (d, *J* 3.2, T_1-CH_2-2′), (d, *J* 3.2, T_1-CH_2-2′), 39.5 (2 × T_2-CH_2-2′), 20.3 (d, *J* 6.8, CH_2*C*H_2CN), 20.3 (d, *J* 7.3, CH_2*C*H_2CN), 12.5 (4 × CH_3-7); δ_P (162 MHz, CD_3OD): 18.9, 18.8; *m/z* (ESI^+) 618.1553 (M+Na. $C_{24}H_{30}N_5NaO_{11}P$ requires 618.1572).

(2*R*,3*S*,5R)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(3*S*,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **51**



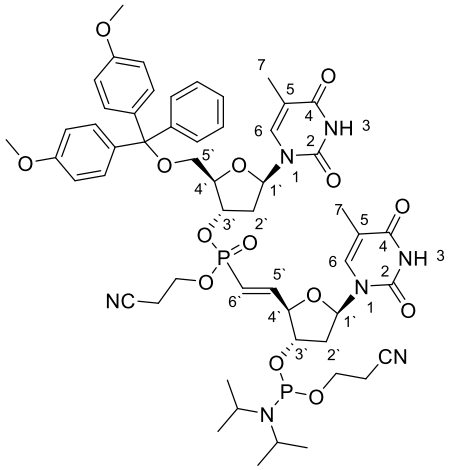
Chemical Formula: $C_{45}H_{48}N_5O_{13}P$
Exact Mass: 897.2986

3′,5′-diol dinucleotide **143** (200 mg, 360 µmol) was dissolved in pyridine (3.0 mL), and the water was removed by azeotropic distillation (3 ×3.0 mL). The residue was then dissolved in pyridine (800 µL), dimethoxytrityl chloride (140 mg, 400 µmol) was added dropwise at 0 °C, the resulting mixture was stirred at RT for 19 h. Sodium bicarbonate (saturated solution, 1.5 mL) was added, the mixture was stirred for 30 minutes then dichloromethane (2.5 mL) was added

and the mixture was stirred for further 10 minutes. The mixture was then separated; the aqueous layer was extracted with dichloromethane (4 × 1.5 mL) and the combined organic layers were washed with water (1 mL) and brine (1 mL). The solvent was evaporated and the residue was purified by column chromatography using dichloromethane : methanol (9:1) to afford the desired product **51** (229 mg, 71%, 1:1 mixture of diastereoisomers) as white foam. m.p 138-140 °C ; $[\alpha]_D^{24.9}$ + 24.2 (C=1.00 $CHCl_3$); FTIR (ATR) ν_{max}/cm^{-1}: 3172.7, 3054.9, 2930, 2836, 1681.7, 1606, 1597, 1464, 1247, 1029, 998, 725; δ_H (400 MHz, $CDCl_3$): 9.98 (0.5H, s, NH), 9.89 (0.5H, s, NH), 9.85 (0.5H, s, NH), 9.82 (0.5H, s, NH), 7.56 (0.5H, H-6), 7.54 (0.5H, H-6),7.39-7.32 (2H, m, ArH), 7.31-7.18 (7H, m, ArH), 7.14 (0.5H, H-6), 7.12 (0.5H, H-6), 7.09-6.95 (1H, m, T_2-H-5′), 6.87-6.77 (4H, m, ArH), 6.4 (1H, dt, *J* 9.8, 5.0, T_1-H-1′), 6.24 (0.5H, t, *J* 6.6, T_2-H-1′), 6.18 (0.5H, t, *J* 6.5, T_2-H-1′), 6.13-5.93 (1H, m, T_2-H-6′), 5.24 (0.5H, m, T_1-H-3′), 5.18 (0.5H, m, T_1-H-3′), 4.48-4.40 (1H, m, T_2-H-3′ + T_2-H-4′), 4.39-4.34 (1H, m, T_2-H-3′ + T_2-H-4′), 4.30-4.00 (3H, m, T_1-H-4′ + C*H*$_2$CH$_2$CN), 3.77 (6H, 2 × OMe), 3.54-3.45 (1H, m, T_1-H_A-5′), 3.41-3.00 (1H, m, T_1-H_B-5′), 2.74 (1H, t, *J* 5.9, CH_2C*H*$_2$CN), 2.69-2.54 (2H, m, T_1-H_A-2′ + CH_2C*H*$_2$CN), 2.51-2.22 (3H, m, T_1-H_B-2′ + T_2-H_A-2′ + T_2-H_B-2′), 1.88 (3H, s, H-7), 1.39 (3H, s, H-7); δ_c (126 MHz, $CDCl_3$): 164.3 (2 × C-4), 164.2 (2 × C-4), 158.8 (4 × ArC), 151.2 (d, *J* 7.3, CH-5′), 151.7 (d, *J* 7.3, CH-5′), 151.1 (2 × C-2), 150.8 (2 × C-2), 144.2 (2 × ArC), 136.7 (CH-6), 136.4 (CH-6), 135.4 (2 × CH-6), 135.2 (ArC), 135.13 (ArC), 135.1 (2 × ArC), 130.3 (8 × ArCH), 128.2 (8 × ArCH), 127.3 (2 × ArCH), 117.2 (CN), 115.9 (CN), (d, *J* 190.5, CH-6′), 116.8 (d, *J* 198.5, CH-6′), 113.4 (8 × ArCH), 112 (C-5), 111.9 (C-5), 111.7 (C-5), 111.5 (C-5), 87.4 (2 × CAr_3), 86.0 (d, *J* 22.2, T_2-CH-4′), 85.8 (d, *J* 22.0, T_2-CH-4′), 85.5 (d, *J* 5.5, T_1-CH-4′), 85.4 (d, *J* 5.7, T_1-CH-4′), 84.8 (2 × T_2-CH-1′), 84.54 (T_1-CH-1′), 85.47 (T_1-CH-1′), 77.8 (2 × d, *J* 5.6, T_1-CH-3′), 74.1 (2 × T_2-CH-3′), 63.3 (T_1-CH-5′), 61.03 (d, *J* 4.8, *CH*$_2$CH$_2$CN),

60.6 (d, *J* 4.8, *CH*$_2$CH$_2$CN), 55.4 (4 × OCH$_3$), 39.4 (T$_1$-CH-2′), 39.3 (T$_1$-CH-2′), 38.9 (2 × T$_2$-CH-2′), 20.0 (d, *J* 6.5, CH$_2$*C*H$_2$CN), 19.9 (d, *J* 6.8, CH$_2$*C*H$_2$CN), 12.7 (2 × CH$_3$-7), 11.9 (2 × CH$_3$-7); δ_p (162 MHz, CDCl$_3$): 18.8, 18.7; *m/z* (ESI$^+$) 920.2871 (M+Na. $C_{45}H_{48}N_5NaO_{13}P$ requires 920.2878).

(2*R*,3*S*,5R)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(3*S*,5R)-3-({[bis(propan-2-yl)amino](2-cyanoethoxy)phosphanyl}oxy)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **41**



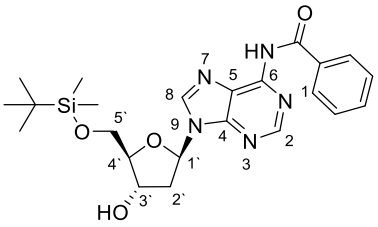
Chemical Formula: $C_{54}H_{65}N_7O_{14}P_2$
Exact Mass: 1097.4065

5-Methyl-1*H*-tetrazole (123 mg, 1.47 mmol) and phosphordiamidite **52** (465 µL, 1.47 mmol) were added in one portion to a stirring solution of dinucleotide **51** (1.10 g, 1.23 mmol) in dry dichloromethane (14 mL) and the reaction mixture was stirred at RT for 20 h. The reaction mixture was diluted with acetonitrile (50 mL), quenched with triethylamine (1 mL) and water (1 mL) and washed with n-hexane (100 mL). After separation the dichloromethane /acetonitrile layer, was diluted with ether (50 mL), washed with water (60 mL) and brine (50 mL), dried over Na_2SO_4 and the volatiles were evaporated, the residue was purified by column chromatography using ethyl acetate to afford the desired phosphoramidite **41** (863 mg, 64%, mixture of 4 diastereoisomers)

as a white foam. $[\alpha]_D^{25}$ + 22.85 (C=1 $CHCl_3$); FTIR (ATR) ν_{max}/cm^{-1}: 3176, 3055, 2967, 2931, 1683, 1607, 1508, 1463, 1120, 1029, 976, 902; δ_H (400 MHz, $CDCl_3$): 8.85 (0.5H, s, NH), 8.50 (1.5H, s, NH), 7.59-7.54 (1H, m, H-6), 7.39-7.33 (2H, m, ArH), 7.33-7.21 (7H, m, ArH), 7.14-7.07 (1H, m, H-6), 7.14-6.91 (2H, m, H-6 + T_2-H-5′), 6.87-6.81 (4H, m, ArH), 6.49-6.42 (1H, m, H-1′), 6.31-6.23 (0.5H, m, H-1′), 6.18-5.96 (1.5H, m, H-1′ + H-6′), 6.29-5.17 (1H, m, H-3′), 4.65-4.42 (2H, m, H-3′ + H-4′), 4.31-4.00 (3H, m, H-4′ + OC*H*$_2$$CH_2CN$), 3.93-3.83 (1H, m, OC*H*$_2$$CH_2CN$), 3.81-3.78 (6H, m, 2 × ^{DMT}OMe), 3.75-3.67 (1H, m, OC*H*$_2$$CH_2CN$), 3.65-3.47 (3H, m, 2 × C*H*$(CH_3)_2$, T_1-H_A-5′), 3.42-3.34 (1H, m, T_1-H_B-5′), 2.77-2.33 (8H, m, 2 × CH_2C*H*$_2$CN + T_1-H_A-2′ + T_1-H_B-2′ + T_2-H_A-2′ + T_2-H_B-2′), 1.36-1.91 (3H, s, H-7), 1.88 (3H, s, H-7), 1.41-1.36 (3H, s, H-7), 1.22-1.15 (12H, m, 2 × CH(C*H*$_3$)$_2$); δ_c (126 MHz, $CDCl_3$): 163.7-163.2 (8 × C-4), 158.9 (8 × ArC), 150.6-149.8 (8 × C-2 + 4 × T_2-CH-5′), 144.3-144.0 (4 × ArC), 137.2-134.9 (6 × CH-6 + 8 × ArC), 130.3 (16 × ArCH), 128.4-128.1 (16 × ArCH), 127.4 (4 × ArCH), 118.2-115.7 (8 × CN + 4 × T_2-CH-6′), 113.4 (16 × ArCH), 112.0-111.9 (4 × C-5), 111.8 (2 × C-5), 111.7 (2 × C-5), 87.5-87.4 (2 × CH-1′ + 4 × ^{DMT}CAr_3), 85.8-84.4 (4 × T_1-CH-4′ + 4 × T_2-CH-4′ + 6 × CH-1′), 77.40 (4 × CH-3′), 76.3-76.1 (4 × CH-3′), 63.4 (T_1-CH_2-5′), 61.0-60.3 (4 × OC$H_2$$CH_2CN$), 58.3-57.8 (4 × OC$H_2$$CH_2CN$), 55.4 (8 × ^{DMT}OMe), 43.5 (4 × d, *J* 12.4, C*H*$(CH_3)_2$), 43.4 (4 × d, *J* 12.4, C*H*$(CH_3)_2$), 39.6-39.2 (4 × CH_2-2′), 38.4-38.0 (4 × CH_2-2′), 24.88-24.60 (8 × CH(C*H*$_3$)$_2$), 20.7-20.5 (6 × CH_2C*H*$_2$CN), 20.1 (3 × d, *J* 6.8, CH_2C*H*$_2$CN), 12.6 (2 × CH_3-7), 11.8 (2 × CH_3-7); δ_p (121 MHz, $CDCl_3$): 149.3 (2 × P), 149.0 (2 × P), 19.0, 18.8, 18.7, 18.5; *m/z* (ESI^+) 1120.3930 (M+Na. $C_{54}H_{65}N_7NaO_{14}P_2$ requires 1120.3956).

5.2.2 Synthesis of A*T phosphoramidite **83**

N-{9-[(2*R*,4*S*,5R)-5-{[(*tert*-butyldimethylsilyl)oxy]methyl}-4-hydroxyoxolan-2-yl]-9H-purin-6-yl}benzamide **71**

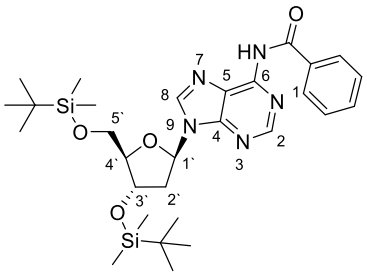


Chemical Formula: $C_{23}H_{31}N_5O_4Si$
Exact Mass: 469.2145

6-*N*-Benzoyl-2′-deoxyadenosine **108** (500 mg, 1.41 mmol) was dried by azeotropic removal of water with pyridine (3 × 10 mL). The residue was dissolved in DMF (4.5 mL) and cooled down to 0°C. Imidazole (210 mg, 3.1 mmol) and activated molecular sieves were added and the mixture was stirred at this temperature for 15 min. TBDMSCl (2.34 g, 1.55 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 2 days. Most of DMF was removed by azeotropic distillation with toluene (3 × 50 mL). and the residue was dissolved in ethyl acetate (100 mL) and washed with water (100 mL), After separation the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and the volatiles removed *in vacuo*. Purification of the residue by column chromatography using gradient elution; petrol : ethyl acetate (1:1)→ ethyl acetate) provided the desired product **71** (600 mg, 91%) as a white solid. And **111** (80 mg, 9%) as a by-product. FTIR (ATR) ν_{max}/cm^{-1} : 3270, 2951, 2927, 2855, 1698, 1609, 1597, 1402, 1249, 1086; δH (400MHz, $CDCl_3$): 9.19 (1H, s, N-H), 8.77 (1H, s, H-2), 8.35 (1H, s, H-8), 8.06-7.97 (2H, m, ArH), 7.62-7.55 (1H, m, ArH), 7.54-7.45 (2H, m, ArH), 6.54 (1H, t, *J* 6.4 H-1′), 4.73-4.65 (1H, m, H-3′), 4.10 (1H, q, *J* 4.0, H-4′), 3.91-3.80 (2H, m, H_A-5′ + H_B-5′), 3.47 (1H, OH), 2.74-2.64 (1H, m, H_A-2′), 2.58 (1H, ddd, *J* 13.0, 6.4, 4.0, H_B-2′), 0.88 (9H, $SiC(CH_3)_3$), 0.07 (3H, s, $Si(CH_3)_2$), 0.06 (3H, s, $Si(CH_3)_2$); δ_C

(101 MHz, $CDCl_3$): 164.9 (^{Bz}CO), 152.7 (CH-2), 151.4 (C-4), 149.5 (C-6), 141.5 (CH-8), 133.8 (ArC), 132.9 (ArH), 128.9 (2 × ArH), 128.0 (2 × ArH), 123.2 (C-5), 87.5 (CH-4′), 84.8 (CH-1′), 72.2 (CH-3′), 63.5 (CH_2-5′), 41.5 (CH_2-2′), 26.1 (3 × SiC(CH_3)$_3$), 18.5 (SiC(CH_3)$_3$), -5.3 (Si(CH_3)$_2$), -5.4 (Si(CH_3)$_2$); *m/z* (ESI^+) 470.2224 (M+H. $C_{23}H_{32}N_5O_4Si$ requires 470.2218), 492.2040 (M+Na. $C_{23}H_{31}N_5NaO_4Si$ requires 492.2038).

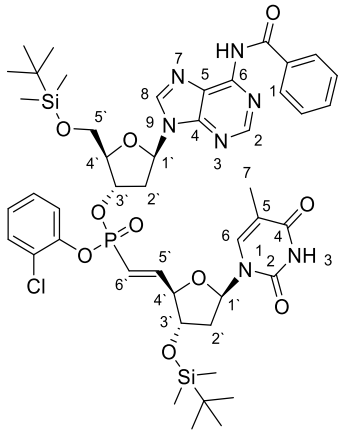
N-{9-[(2*R*,4*S*,5R)-4-[(*tert*-butyldimethylsilyl)oxy]-5-{[(tert butyldimethylsilyl)oxy]methyl}oxolan-2-yl]- 9H-purin-6-yl}benzamide **111**



Chemical Formula: $C_{29}H_{45}N_5O_4Si_2$
Exact Mass: 583.3010

FTIR (ATR) ν_{max}/cm^{-1} : 2952, 2928, 2856, 1696, 1608, 1578, 1389, 1219, 832; δH (400MHz, $CDCl_3$): 9.18 (1H, s, N-H), 8.79 (1H, s, H-2), 8.32 (1H, s, H-8), 8.03-8.00 (2H, m, ArH), 7.66-7.55 (1H, m, ArH), 7.53-7.47 (2H, m, ArH), 6.36 (1H, t, *J* 6.4 H-1′), 4.62 (1H, dt, *J* 5.9, 3.3 H-3′), 4.04 (1H, q, *J* 3.3, H-4′), 3.88 (1H, dd, *J* 11.2, 4.1, H_A-5′), 3.78 (1H, dd, *J* 11.2, 3.3, H_B-5′), 2.67 (1H, ddd, *J* 13, 6.4, 5.7, H_A-2′), 2.47 (1H, ddd, *J* 13, 6.1, 3.8, H_B-2′), 0.91 (9H, SiC(CH_3)$_3$), 0.90 (9H, SiC(CH_3)$_3$), 0.10 (6H, s, Si(CH_3)$_2$), 0.08 (6H, s, Si(CH_3)$_2$); (101 MHz, $CDCl_3$): 164.8 (CO), 152.7 (CH-2), 151.5 (C-4), 149.6 (C-6), 141.6 (CH-8), 133.9 (ArC), 132.8 (ArH), 128.9 (2 × ArH), 128.0 (2 × ArH), 123.4 (C-5), 88.2 (CH-4′), 84.8 (CH-1′), 72.0 (CH-3′), 62.9 (CH_2-5′), 41.5 (CH_2-2′), 26.1 (3 × SiC(CH$_3$)$_3$), 25.9 (3 × SiC(CH$_3$)$_3$), 18.6 (SiC(CH_3)$_3$), 18.1 (SiC(CH_3)$_3$), -4.5 (Si(CH_3)$_2$), -4.7 (Si(CH_3)$_2$), -5.2 (Si(CH_3)$_2$), -5.3 (Si(CH_3)$_2$); *m/z* (ESI^+) 584.3084 (M+H. $C_{29}H_{46}N_5O_4Si_2$ requires 584.3083), 606.2900 (M+Na. $C_{29}H_{45}N_5NaO_4Si_2$ requires 606.2902).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2{[(tertbutyldimethylsilyl)oxy]methyl}oxolan-3-yl 2-chlorophenyl [(*E*)-2-[(2*R*,3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **72**

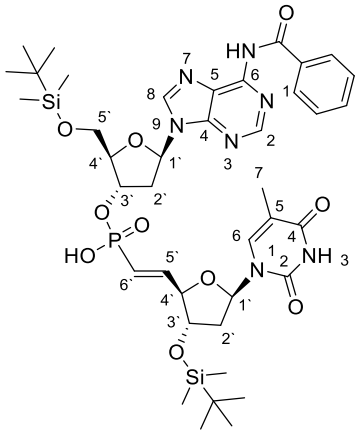


Chemical Formula: $C_{46}H_{61}ClN_7O_{10}PSi_2$
Exact Mass: 993.3445

A mixture of phosphonate **26** (600 mg, 1.10 mmol), alcohol **79** (564 mg, 1.20 mmol) and *N*-methylimidazole (567 µL, 7.15 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (3 mL), 2,4,6-triisopropylbenzenesulfonyl chloride (834 mg, 2.75 mmol) was added. The reaction mixture was stirred at RT for 16 h, the volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:4) → ethyl acetate) to afford the desired product **72** (900 mg, 82%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2952, 2928, 2855, 1690, 1608, 1476, 1251, 1070, 1007, 834, 777; δH (400 MHz, $CDCl_3$): 9.50 (0.4H, bs, NH), 9.22 (1H, bs, NH), 9.06 (0.6H, bs, NH), 8.89 (0.4H, s, A-H-2), 8.82 (0.6H, s, AH- 2), 8.39 (0.4H, s, A-H-8), 8.34 (0.6H, s, A-H-8), 8.11-7.98 (2H, m, ArH), 7.63-7.57 (1H, m, ArH), 7.55-7.49 (2H, m, ArH), 7.46-7.41 (2H, m, ArH), 7.32-7.23 (1H, m, ArH), 7.18-7.12 (1H, m, ArH), 7.11-6.96 (2H, m, T-H-6 + T-H-5′), 6.67 (0.4H, dd, *J* 8.6, 5.5, A-H-1′), 6.58 (0.6H, dd, *J* 8.0, 6.0, A-H-1′), 6.31-6.16 (1.5, m, T-H-1′ + T-H-6′), 6.11 (0.4H, t, *J* 6.7, T-H-1′), 5.47-5.38 (1H, m, A-

H-3′), 4.45-4.38 (1.6H, m, T-H-4′ + A-H-4′), 4.37-4.29 (0.8H, m, A-H-4′ + T-H-3′), 4.21 (0.6H, dt, *J* 6.8, 4.4, T-H-3′), 3.95-3.84 (1.6H, m, A-H_A-5′ + A-H_B-5′), 3.79 (0.4H, dd, *J* 11.4, 2.8, A-H_B-5′), 2.96-2.88 (0.4H, m, A-H_A-2′), 2.82-2.70 (1.6H, m, A-H_A-2′ + A-H_B-2′), 2.45-2.36 (0.4H, m, T-H_A-2′), 2.30-2.18 (1.6H, m, T-H_A-2′ + T-H_B-2′), 1.95 (1.2H, d, *J* 1.2, T-H-7), 1.93 (1.8H, d, *J* 1.2, T-H-7), 0.90 (6H, s, SiC(CH_3)$_3$), 0.89 (12H, s, SiC(CH_3)$_3$), 0.10 (3H, s, Si(CH_3)$_2$), 0.05 (12H, s, Si(CH_3)$_2$); *δ*C (101 MHz, $CDCl_3$): 164.9 (2 × CO), 163.7 (T-C-4), 163.5 (T-C-4), 152.8 (2 × A-CH-2), 151.7 (2 × A-C- 4), 150.8 (2 × d, *J* 5.9, T-CH-5′), 150.3 (2 × T-C-2), 149.9 (2 × A-C-6), 146.3 (2 × d, *J* 6.8, ArC), 141.3 (A-CH-8), 141.2 (A-CH-8), 136.7 (T-CH-6), 135.5 (T-CH-6), 133.8 (2 × ArC), 132.9 (2 × ArCH), 130.95 (ArCH), 130.90 (ArCH), 128.9 (4 × ArCH), 128.3 (1 × ArCH), 128.2 (5 × ArCH), 126.5 (ArCH), 126.4 (ArCH), 125.8 (d, *J* 5.8, ArC), 125.7 (d, *J* 6.1, ArC), 123.4 (2 × A-C-5), 122.5 (d, *J* 2.8, ArCH), 122.3 (d, *J* 2.8, ArCH), 117.1 (d, *J* 192.0, T-CH-6′), 116.8 (d, *J* 192.2, T-CH-6′), 111.8 (T-C-5), 111.6 (T-C-5), 87.6 (T CH-1′), 86.6 (d, *J* 5.0, A-CH-4′), 86.5 (d, *J* 6.9, A-CH-4′), 86.2 (d, *J* 22.8, T-CH-4′), 85.7 (d, *J* 22.8, T-CH-4′), 85.4 (T-CH-1′), 84.4 (2 × A-CH-1′), 78.2 (d, *J* 6.2, A-CH-3′), 78.1 (d, *J* 6.4, A-CH-3′), 75.3 (T-CH-3′), 74.7 (T-CH-3′), 63.4 (A-CH_2-5′), 63.2 (A-CH_2-5′), 40.6 (d, *J* 3.5, A-CH_2-2′), 40.2 (d, *J* 3.7, A-CH_2-2′), 39.8 (T-CH_2-2′), 39.5 (T-CH_2-2′), 26.1 (6 × SiC(*C*H_3)$_3$), 25.8 (6 × SiC(*C*H_3)$_3$), 18.5 (2 × Si*C*(CH_3)$_3$), 18.0 (2 × Si*C*(CH_3)$_3$), 12.8 (T-CH_3-7), 12.7 (T-CH_3-7), -4.5 (2 × Si(CH_3)$_2$), -4.6 (2 × Si(CH_3)$_2$), -5.2 (2 × Si(CH_3)$_2$), -5.3 (2 × Si(CH_3)$_2$); *δ*P (162 MHz, $CDCl_3$): 14.89, 14.81; *m*/*z* (ESI$^+$) 994.3492 (M+H. $C_{46}H_{62}ClN_7O_{10}PSi_2$ requires 994.3517), 1016.3301 (M+Na. $C_{46}H_{61}ClN_7NaO_{10}PSi_2$ requires 1016.3337).

{[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-3-yl]oxy}[(*E*)-2-[(2*R*,3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphinic acid **75**

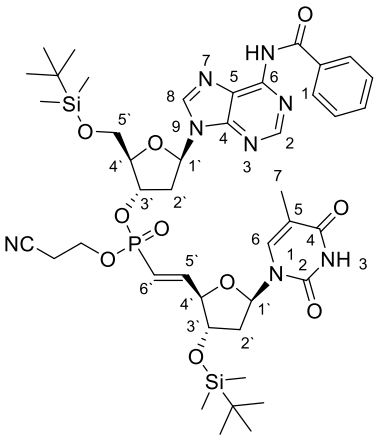


Chemical Formula: $C_{40}H_{58}N_7O_{10}PSi_2$
Exact Mass: 883.3521

Dinucleotide **72** (200 mg, 201 µmol), 2-nitrobenzaldoxime (51 mg, 302 µmol), tetramethylguanidine (38 µL, 302 µmol), triethylamine (170 µL, 1.21 mmol) and dioxane (12 mL). The reaction mixture was stirred at RT for 22 h, then dry loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane : methanol (9: 1 → 4:1) to afford the desired product **75** (140 mg, 79%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 2951, 2928, 2855, 1685, 1581, 1523, 1460, 1250, 1063, 832; δH (400 MHz, CD_3OD): 8.80 (1H, bs, A-H-2), 8.62 (1H, s, A-H-8), 8.20-7.98 (2H, m, ArH), 7.71-7.62 (1H, m, ArH), 7.61-7.51 (2H, m, ArH), 7.47-7.37 (1H, m, T-H-6), 6.70-6.64 (2H, m, A-H-1′ + T-H-5′), 6.19 (1H, t, *J* 6.2, T-H-1′) 6.11 (1H, t, *J* 17.2, T-H-6′), 5.01-4.90 (1H, m, A-H-3′), 4.42-4.33 (1H, bs, T-H-3′), 4.32-4.20 (2H, m, T-H-4′ + A-H-4′), 3.97-3.78 (2H, m, A-H-5′ + A-H_B-5′), 2.92-2.63 (2H, m, A-H_A-2′ + A-H_B-2′), 2.39-2.26 (1H, m, T-H_A-2′), 2.24-2.16 (1H, m, T-H_B-2′), 1.91-1.78 (3H, bs, T-H-7), 0.89 (9H, s, $SiC(CH_3)_3$), 0.85 (9H, s, $SiC(CH_3)_3$), 0.13-0.01 (12H, m, $Si(CH_3)_2$); δc (126 MHz, CD_3OD): 166.2 (2 × C), 153.6-153.4 (2 × C), 152.9 (A-CH-2), 152.2 (C), 144.1-143.4 (T-CH-5′ + A-CH-8), 138.4 (T-CH-6), 134.2

(ArCH), 134.1 (C), 129.8 (2 × ArCH), 129.6 (ArCH), 129.5 (ArCH), 126.4 (d, *J* 181.6, T-CH-6′), 123.5 (C), 111.9 (C), 88.9-88.1 (T-CH-4′ + T-CH-4′), 87.0 (T-CH-1′), 86.4 (A-CH-1′), 76.7 (A-CH-3′), 76.5 (d, *J* 4.6, T-CH-3′), 64.8 (A-CH_2-5′), 41.6 (d, *J* 4.0, A-CH_2-2′), 40.3 (T-CH_2-2′), 26.5 (3 × SiC(CH_3)$_3$), 26.3 (3 × SiC(CH_3)$_3$), 19.3 (SiC(CH_3)$_3$), 18.8 (SiC(CH_3)$_3$), 12.6 (T-CH_3-7), -4.40 (Si(CH_3)$_2$), -4.43 (Si(CH_3)$_2$), -5.17 (Si(CH_3)$_2$), -5.19 (Si(CH_3)$_2$); δP (162 MHz, CD_3OD): 9.57; *m/z* (ESI$^+$) 884.3558 (M+H. $C_{40}H_{59}N_7O_{10}PSi_2$ requires 884.3594), 906.3395 (M+Na. $C_{40}H_{58}N_7NaO_{10}PSi_2$ requires 906.3414).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[(tertbutyldimethylsilyl)oxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **77**



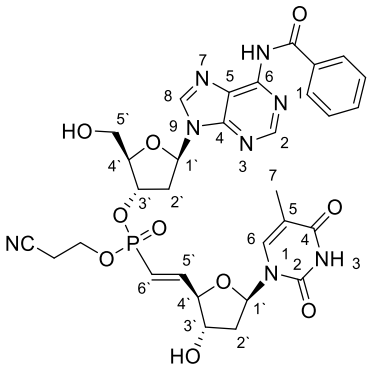
Chemical Formula: $C_{43}H_{61}N_8O_{10}PSi_2$
Exact Mass: 936.3787

A mixture of dinucleotide **75** (600 mg, 680 µmol), 3-hydroxypropionitrile (232 µL, 3.40 mmol) and *N*-methylimidazole (350 µL, 4.42 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (4 mL). 2,4,6-triisopropylbenzenesulfonyl chloride (515 mg, 1.70 mmol) was added. The reaction mixture was stirred at RT for 19 h, the volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; dichloromethane → dichloromethane: methanol (19:1)

to afford the desired product **77** (500 mg, 79%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2953, 2929, 2857, 1687, 1610, 1581, 1460, 1250, 1072, 1002, 833, 777; δH (400 MHz, $CDCl_3$): 9.74 (0.5H, br. s, NH), 9.37-9.13 (1.5H, m, NH), 8.89 (0.5H, s, A-H-2), 8.81 (0.5H, s, A-H-2), 8.41 (0.5H, s, A-H-8), 8.34 (0.5H, s, A-H-8), 8.04 (2H, d, *J* 7.2, ArH), 7.64-7.55 (1H, m, ArH), 7.51 (2H, t, *J* 7.5, ArH), 7.14 (0.5H, q, *J* 1.2, T-H-6), 7.07 (0.5H, q, *J* 1.2, T-H-6), 7.02-6.97 (0.5H, m, T-H-5′), 6.96-6.86 (0.5H, m, T-H-5′), 6.64 (0.5H, dd, *J* 8.6, 5.6, A-H-1′), 6.59 (0.5H, t, *J* 7.0, A-H-1′), 6.26 (0.5H, app t, *J* 6.8, T-H-1′), 6.14-6.01 (1.5H, m, T-H-1′ + T-H-6′), 5.34-5.22 (1H, m, A-H-3′), 4.42-4.22 (5H, m, T-H-3′ + T-H-4′ + A-H-4′ + C*H*$_2$CH$_2$CN), 3.95-3.83 (2H, m, A-H$_A$-5′ + A-H$_B$-5′), 2.90-2.70 (4H, m, A-H$_A$-2′ + A-H$_B$-2′ + CH$_2$C*H*$_2$CN), 2.33-2.17 (2H, m, T-H$_A$-2 + T-H$_B$-2′), 1.95 (1.5H, d, *J* 1.2, T-H-7), 1.94 (1.5H, d, *J* 1.2, T-H-7), 0.92-0.87 (18H, m, SiC(CH$_3$)$_3$), 0.12-0.07 (12H, m, (Si(CH$_3$)$_2$); δC (101 MHz, $CDCl_3$): 164.9 (2 × CO), 163.8 (T-C-4), 163.7 (T-C-4), 152.9 (A-CH-2), 152.8 (A-CH-2), 151.6 (2 × A-C-4), 150.4 (T-C-2), 150.3 (T-C-2), 150.2 (d, *J* 6.2, T-CH-5′), 150.3 (d, *J* 6.3, T-CH-5′), 149.8 (A-C-6), 149.7 (A-C-6), 141.4 (A-CH-8), 141.3 (A-CH-8), 137.2 (T-CH-6), 136.0 (T-CH-6), 133.7 (2 × ArC), 132.9 (2 × ArCH), 128.9 (4 × ArCH), 128.2 (4 × ArCH), 123.4 (2 × A-C-5), 116.9 (d, *J* 190.1, T-CH-6′), 116.9 (CN), 116.8 (d, *J* 190.7, T-CH-6′), 116.5 (CN), 111.8 (T-C-5), 111.5 (T-C-5), 87.7 (T-CH-1′), 86.7 (d, *J* 5.3, A-CH-4′), 86.5 (d, *J* 5.0, A-CH-4′), 86.2 (d, *J* 22.3, T-CH-4′), 85.7 (d, *J* 22.3, T-CH-4′), 85.5 (T-CH-1′), 84.4 (A-CH-1′), 84.4 (A-CH-1′), 77.8 (d, *J* 5.5, A-CH-3′), 77.3 (d, *J* 5.9, A-CH-3′), 75.2 (T-CH-3′), 74.9 (T-CH-3′), 63.5 (A-CH$_2$-5′), 63.2 (A-CH$_2$-5′), 60.9 (d, *J* 4.7, *C*H$_2$CH$_2$CN), 60.5 (d, *J* 4.8, *C*H$_2$CH$_2$CN), 40.2 (d, *J* 2.6, A-CH$_2$-2′), 40.1 (d, *J* 3.5, A-CH$_2$-2′), 39.6 (T-CH$_2$-2′), 39.4 (T-CH$_2$-2′), 26.1 (2 × SiC(*C*H$_3$)$_3$), 26.0 (4 × SiC(*C*H$_3$)$_3$), 25.8 (6 × SiC(*C*H$_3$)$_3$), 20.2 (d, *J* 6.5, CH$_2$*C*H$_2$CN), 20.1 (d, *J* 6.7, CH$_2$*C*H$_2$CN), 18.5 (Si*C*(CH$_3$)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 18.1 (2 × Si*C*(CH$_3$)$_3$), 12.7 (T-CH$_3$-7), 12.6 (T-

CH_3-7), -4.5 (2 × $Si(CH_3)_2$), -4.6 (2 × $Si(CH_3)_2$), -5.2 ($Si(CH_3)_2$), -5.3 ($Si(CH_3)_2$), -5.3 ($Si(CH_3)_2$), -5.4 ($Si(CH_3)_2$); δP (162 MHz, $CDCl_3$): 18.87, 18.66; *m/z* (ESI^+) 937.3844 (M+H. $C_{43}H_{62}N_8O_{10}PSi_2$ requires 937.3860), 959.3648 (M+Na. $C_{43}H_{61}N_8NaO_{10}PSi_2$ requires 959.3679).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **79**

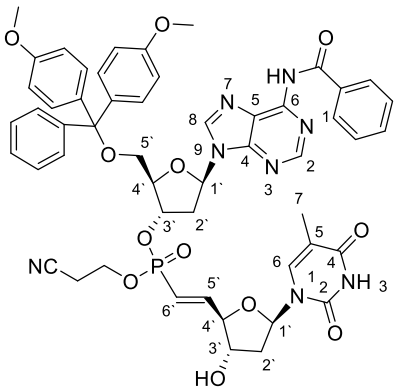


Chemical Formula: $C_{31}H_{33}N_8O_{10}P$
Exact Mass: 708.2057

Triethylamine trihydrofluoride (122 µL, 750 µmol) was added in one portion to a stirring solution of dinucleotide **77** (70 mg, 75.0 µmol, 1:1 mixture of diastereoisomers) in THF (1.5 mL) and the resulting reaction mixture was stirred at RT for 4 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using gradient elution; ethyl acetate : methanol (9:1 → 4:1) to afford the desired product **79** (40 mg, 76%, 1:1 mixture of diastereoisomers) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 3323, 2925, 1664, 1613, 1452, 1235, 997; δH (400 MHz, CD_3OD): 8.70 (1H, s, A-H-2), 8.62 (0.5H, s, A-H-8), 8.34 (0.5H, s, A-H-8), 8.08 (2H, d (obscured), *J* 7.1, ArH), 7.65 (1H, tt (obscured), *J* 7.4, 1.6, ArH), 7.55 (2H, t (obscured), *J* 7.6, ArH), 7.43-7.39 (1H, m, T-H-6), 7.15-7.01 (1H, m, T-H-5'), 6.61 (1H, m, A-H-1'), 6.26-6.12 (2H, m, T-H-1' + T-H-6'), 5.41-5.33 (1H, m, A-H-3'), 4.44-4.30 (5H, m, T-H-3' + T-H-4' + A-H-4' + C*H*$_2$$CH_2CN$), 3.89-3.80 (2H, m, A-$H_A$-5' + A-$H_B$-

5′), 3.15-3.03 (1H, m, A-H_A-2′), 2.97-2.91 (2H, m, CH_2CH_2CN), 2.86-2.77 (1H, m, A-H_B-2′), 2.48-2.37 (1H, m, T-H_A-2′), 2.32-2.23 (1H, m, T-H_B-2′), 1.89 (1.5H, d, *J* 1.2, T-H-7), 1.87 (1.5H, d, *J* 1.2, T-H-7); *δ*C (101 MHz, CD_3OD): 168.1 (2 × ^{Bz}CO), 166.3 (2 × T-C-4), 153.1 (2 × A-CH-2), 153.0 (A-C-4), 152.9 (A-C-4), 152.8 (2 × d, *J* 4.6, T-CH-5′), 152.3 (T-C-2), 152.2 (T-C-2), 151.3 (2 × A-C-6), 144.6 (2 × A-CH-8), 138.6 (T-CH-6), 138.3 (T-CH-6), 134.9 (2 × ArC), 133.9 (2 × ArCH), 129.8 (4 × ArCH), 129.5 (4 × ArCH), 125.5 (A-C-5), 125.4 (A-C-5), 118.9 (CN), 118.8 (CN), 117.4 (d, *J* 188.8, T-CH-6′), 117.2 (d, *J* 188.9, T-CH-6′), 112.1 (T-C-5), 111.9 (T-C-5), 88.2 (d, *J* 5.8, A-CH-4′), 88.1 (d, *J* 6.0, A-CH-4′), 87.6 (T-CH-1′), 87.1 (T-CH-1′), 87.1 (d, *J* 23.0, T-CH-4′), 87.0 (d, *J* 22.8, T-CH-4′), 86.5 (2 × A-CH-1′), 78.9 (d, *J* 6.0, A-CH-3′), 78.8 (d, *J* 5.1, A-CH-3′), 75.1 (T-CH-3′), 75.0 (T-CH-3′), 62.8 (d, *J* 4.6, CH_2CH_2CN), 62.8 (A-CH_2-5′), 62.7 (A-CH_2-5′), 62.7 (d, *J* 5.1, CH_2CH_2CN), 40.0 (2 × A-CH_2-2′), 39.5 (2 × T-CH_2-2′), 20.4 (d, *J* 4.9, CH_2CH_2CN), 20.3 (d, *J* 5.7, CH_2CH_2CN), 12.5 (2 × T-CH_3-7); *δ*P (162 MHz, CD_3OD): 19.04, 18.88; *m*/*z* (ESI^+) 709.2110 (M+H. $C_{31}H_{34}N_8O_{10}P$ requires 709.2130).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **81**

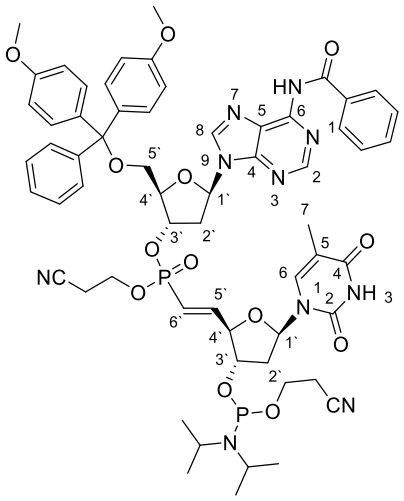


Chemical Formula: $C_{52}H_{51}N_8O_{12}P$
Exact Mass: 1010.3364

Dinucleotide **79** (450 mg, 635 µmol, 1:1 mixture of diastereoisomers) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (2 mL). Molecular sives and DMTrCl (237 mg, 698 µmol) were added in one portion under argon gas, the reaction mixture was stirred at RT for 4 d. The volatiles were evaporated and the residue was purified by column chromatography dichloromethane : methanol (1:1) to afford the desired product **81** (480 mg, 75%, 1:2 mixture of diastereoisomers) as a white foam and recovered starting material **79** (100 mg, 22%) as white powder. FTIR (ATR) ν_{max}/cm^{-1} : 2930, 2835, 1686, 1607, 1508, 1296, 1246 1029; δH (500 MHz, $CDCl_3$): 10.07 (0.5H, s, NH), 9.91 (0.5H, s, NH), 9.47 (1H, NH), 8.73 (0.5H, s, A-H-2), 8.70 (0.5H, s, A-H-2), 8.22 (0.5H, s, A-H-8), 8.20 (0.5H, s, A-H-8), 8.00-7.09 (2H, m, ArH), 7.61-7.55 (1H, m, ArH), 7.53-7.47 (2H, m, ArH), 7.40-7.32 (2H, m, ArH), 7.28-7.15 (7H, m, T-H-6 + ArH), 7.12-7.00 (2H, m, T-H-5′ + ArH), 6.78 (4H, td, *J* 8.8, 2.4, ArH), 6.55-6.46 (1H, m, A-H-1′), 6.22 (0.5H, t, *J* 6.7, T-H-1′), 6.14-5.99 (1.5H, m, T-H-1′ + T-H-6′), 5.40-5.30 (1H, m, A-H-3′), 4.40-4.10 (5H, m, T-H-3′ + T-H-4′ + A-H-4′ + C*H*$_2$CH$_2$CN), 3.75 (3H, s, $ArOCH_3$), 3.74 (3H, s, $ArOCH_3$), 3.50-3.33 (2H, m, A-H_A-5′ + A-

H_B-5′), 3.18-3.00 (1H, m, A-H_A-2′), 2.86-2.76 (1H, m, A-H_B-2′), 2.71 (1H, t, *J* 6.0, CH_2CH_2CN), 2.62-2.54 (1H, m, CH_2CH_2CN), 2.40-2.20 (2H, m, T-H_A-2′ + T-H_B-2′), 1.88 (3H, s, T-H-7); *δ*C (126 MHz, $CDCl_3$): 165.1 (2 × ^{Bz}CO), 164.1 (T-C-4), 164.05 (T-C-4), 158.7 (2 × ArC), 158.6 (ArC), 158.6 (ArC), 152.6 (2 × A-CH-2 + 2 × A-C-4), 151.6 (d, *J* 5.8, T-CH-5′), 151.3 (d, *J* 5.7, T-CH-5′), 151.5 (T-C-2), 151.4 (T-C-2), 150.9 (2 × A-C-6), 144.4 (ArC), 144.4 (ArC), 141.9 (A-CH-8), 141.8 (A-CH-8), 136.6 (T-CH-6), 136.2 (T-CH-6), 135.5 (ArC), 135.5 (ArC), 135.4 (2 × ArC), 135.4 (2 × ArC), 133.6 (2 × ArCH), 133.0 (4 × ArCH), 130.9 (2 × ArCH), 130.9 (2 × ArCH), 128.9 (4 × ArCH), 128.2 (4 × ArCH), 128.2 (2 × ArCH), 128.0 (2 × ArCH), 128.0 (2 × ArCH), 127.2 (2 × ArCH), 127.2 (2 × ArCH), 123.6 (A-C-5), 123.6 (A-C-5), 117.5 (2 × CN), 117.0 (d, *J* 187.1, T-CH-6′), 117.0 (d, *J* 187.0, T-CH-6′), 113.3 (8 × ArH), 111.7 (T-C-5), 111.5 (T-C-5), 86.9 (CAr_3), 86.9 (CAr_3), 85.5 (d, *J* 22.6, T-CH-4′), 85.3 (d, *J* 22.0, T-CH-4′), 85.2 (T-CH-1′), 85.2 (T-CH-1′), 85.1 (d, *J* 6.0, A-CH-4′), 85.1 (d, *J* 6.1, A-CH-4′), 84.6 (A-CH-1′), 84.5 (A-CH-1′), 77.3 (A-CH-3′), 74.7 (T-CH-3′), 63.1 (A-CH_2-5′), 63.1 (A-CH_2-5′), 60.8 (d, *J* 5.5, CH_2CH_2CN), 55.4 (4 × $ArOCH_3$), 39.1 (T-CH_2-2′), 39.0 (T-CH_2-2′), 38.8 (A-CH_2-2′), 38.7 (A-CH_2-2′), 20.1 (d, *J* 7.4, CH_2CH_2CN), 20.0 (d, *J* 7.8, CH_2CH_2CN), 12.7 (T-CH_3-7), 12.6 (T-CH_3-7); *δ*P (162 MHz, $CDCl_3$): 19.02, 18.68; *m/z* (ESI^+) 1011.3427 (M+H. $C_{52}H_{52}N_8O_{12}P$ requires 1011.3437), 1033.3244 (M+Na. $C_{52}H_{51}N_8NaO_{12}P$ requires 1033.3256).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-3-({[bis(propan-2-yl)amino](2-cyanoethoxy)phosphanyl}oxy)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **83**



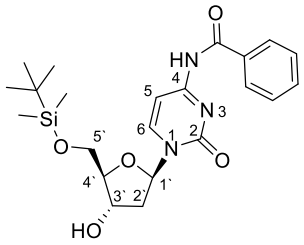
Chemical Formula: $C_{61}H_{68}N_{10}O_{13}P_2$
Exact Mass: 1210.44

5-Methyl-1*H*-tetrazole (20.1 mg, 240 µmol) was added in one portion to a stirring solution of dinucleotide **81** (230 mg, 227 µmol) and phosphordiamidite **52** (70 µL, 227 mmol) in dry dichloromethane (3.5 mL) and the reaction mixture was stirred at RT for 22 h. The solution was diluted with acetonitrile (7 mL), quenched with TEA (113 µL) and water (113 µL) and washed with n-hexane (11.5 mL). After separation the dichloromethane/acetonitrile layer was diluted with ether (23 mL), washed with water (35 mL) and brine (23 mL), dried over Na_2SO_4 and the volatiles were removed. The residue was purified by column chromatography using ethyl acetate to afford the desired phosphoramidite **83** (200 mg, 72%, mixture of 4 diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2966, 2931, 1688, 1606, 1508, 1245, 1176, 1029, 828; δ_H (400 MHz, $CDCl_3$): 9.06 (1H, br, s, NH), 8.82-8.77 (0.6H, A-H-2),8.74-8.70 (0.4H, A-H-2), 8.20-8.17 (0.6H, A-H-8), 8.16 (0.4H, A-H-8), 8.06-7.99 (2H, ArH), 7.64-7.57 (1H, m, ArH), 7.56-7.49 (2H, m, ArH), 7.42-7.34 (2H, m,

ArH), 7.32-7.19 (7H, m, ArH), 7.17-6.93 (2H, T-H-6 + T-CH-5′), 6.85-6.76 (4H, m, ArH), 6.63-6.50 (1H, m, H-1′), 6.33-6.25 (0.4H, m, H-1′), 6.17-6.02 (1.6H, m, H-1′ + T-CH-6′), 5.43-5.29 (1H, m, A-H-3′), 4.64-4.36 (1H, m, T-H-3′ + A-H-4′ + T-H-4′), 4.34-4.15 (1.4H, m, OC*H*$_2$CH$_2$CN), 3.92-3.81 (1H, m, OC*H*$_2$CH$_2$CN), 3.80-3.67 (6H, m, 2 × ^{DMT}OMe), 3.75-3.66 (1.6H, m, OC*H*$_2$CH$_2$CN), 3.65-3.58 (2H, m, 2 × C*H*(CH$_3$)$_2$), 3.50-3.37 (2H, m, A-H$_A$-5′ + A-H$_B$-5′), 3.15-2.95 (1H, m, H-2′), 3.16-2.95 (1H, m, H-2′), 2.92-2.79 (1H, m, H-2′), 2.77-2.58 (2 × OCH$_2$*C*H$_2$CN), 2.54-2.36 (2H, m, H-2′), 1.96-1.91 (3H, s, H-7), 1.22-1.16 (12H, m, 2 × CH(C*H*$_3$)$_2$); δ_c (126 MHz, CDCl$_3$): 164.7 (4 × ^{Bz}CO), 163.60 (T-C-4), 163.56 (T-C-4), 163.44 (T-C-4), 163.41 (T-C-4), 158.81 (4 × ^{DMT}ArC), 158.76 (4 × ^{DMT}ArC), 153.0-152.7 (4 × A-CH-2), 151.70 (4 × A-C-4), 150.3-149.7 (4 × T-CH-5′ +4 × A-C-6 + 4 × T-C-2), 144.5-144.3 (4 × ^{DMT}ArC), 141.8-141.4 (4 × A-CH-8), 137.1 (T-CH-6), 136.8 (T-CH-6), 136.2 (T-CH-6), 136.0 (T-CH-6), 135.5-135.3 (8 × ^{DMT}ArC), 133.7 (4 × ^{Bz}ArC), 132.9 (4 × ArCH), 130.2 (16 × ArCH), 129.0 (8 × ArCH), 128.4-127.9 (24 × ArCH), 127.3-127.1 (4 × ArCH), 123.6 (4 × A-C-5), 118.17-115.7 (4 × T-CH-6′ + 8 × CN), 113.5-113.2 (16 × ArCH), 119.0 (2 × T-C-5), 11.7 (2 × T-C-5), 87.9-84.4 (4 × T-CH-1′ + 4 × A-CH-1′ + 4 × ^{DMT}CAr$_3$ + 4 × A-CH-4′ + 4 × T-CH-4′), 77.6-77.4 (4 × A-CH-3′), 76.2-75.2 (4 × T-CH-3′), 63.3 (A-CH-5′), 60.9-60.4 (4 × O*C*H$_2$CH$_2$CN),58.3-57.8 (4 × O*C*H$_2$CH$_2$CN), 55.4 (8 × ^{DMT}OMe), 43.6-43.3 (8 × *C*H(CH$_3$)$_2$), 39.3-37.9 (4 × T-CH2-2′ + 4 × A-CH2-2′), 25.0-24.5 (8 × CH(*C*H$_3$)$_2$), 20.64 (2 × d, *J* 7.0, OCH$_2$*C*H$_2$CN), 20.61 (2 × d, *J* 7.0, OCH$_2$*C*H$_2$CN), 20.15 (2 × d, *J* 6.5, OCH$_2$*C*H$_2$CN), 19.9 (2 × d, *J* 6.5, OCH$_2$*C*H$_2$CN), 12.66-12.5 (4 × T-CH$_3$-7); δP (162 MHz, CDCl$_3$): 149.32 (2 × P), 149.05, 149.02, 19.00, 18.69, 18.68, 18.41; *m/z* (ESI$^+$) 1211.4487 (M+H. $C_{61}H_{69}N_{10}O_{13}P_2$ requires 1211.4515), 1233.4299 (M+Na. $C_{61}H_{68}N_{10}NaO_{13}P_2$ requires 1233.4335).

5.2.3 Synthesis of C*T dinucleotide 239

N-{1-[(2*R*,4*S*,5R)-5-{[(*tert*-butyldimethylsilyl)oxy]methyl}-4-hydroxyoxolan-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide **103**

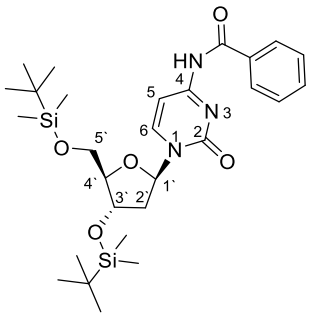


Chemical Formula: $C_{22}H_{31}N_3O_5Si$
Exact Mass: 445.2033

Imidazole (904 mg, 13.2 mmol) was added at 0 °C to a solution of 4-*N*-Benzoyl-2′-deoxycytidine **110** (2.00 g, 6.04 mmol) in DMF (16 mL), *tert*-Butyldimethylsilyl chloride (1.00 g, 6.64 mmol) was added at this temperature and the reaction mixture was allowed to warmup to RT and stirred for 20 h. DMF was removed by azeotropic distillation with toluene (3 × 20 mL), and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate : methanol (9:1) to afford the desired product **103** (2.30 g, 85%) as a white foam, and **236** (350 mg, 10%) as a by-product. FTIR (ATR) ν_{max}/cm^{-1} : 3270, 2950, 2927, 2855, 1697, 1646, 1555, 1481, 1390, 1310, 1249, 1090, 780; δH (400MHz, $CDCl_3$): 8.88 (1H, br s, N-H), 8.43 (1H, d, *J* 7.5, H-6), 7.95-7.82 (2H, m, ArH), 7.64-7.65 (1H, m, ArH), 7.54-7.51 (3H, m, ArH + H-5), 6.38 (1H, t, *J* 6.2, H-1′), 4.50-4.44 (1H, m, H-3′), 4.15 (1H, q, *J* 2.7, H-4′), 4.48 3.95 (1H, dd, *J* 11.5, 2.7, H_A-5′), 3.85 (1H, dd, *J* 11.5, 2.7, H_B-5′), 3.63 (1H, br s, OH), 2.37 2.37 (1H, ddd, *J* 13.6, 6.2, 3.9, H_A-2′), 2.23-2.12 (1H, m, H_B-2′), 0.9 (9H, s, $SiC(CH_3)_3$), 0.11 (3H, s, $Si(CH_3)_2$), 0.10 (3H, s, $Si(CH_3)_2$); δc (101 MHz, $CDCl_3$): 166.8 (^{Bz}CO), 162.4 (C4), 155.3 (C-2), 145.1 (CH-6), 133.3 (ArH), 133.2 (ArC), 129.2 (2 × ArH), 127.8 (ArH), 96.6 (CH-5), 88.0 (CH-4′), 87.6 (CH-1′), 71.6 (CH-3′), 63.10 (CH-5′), 42.6 (CH-2′), 26.0 ($SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), -5.28 ($Si(CH_3)_2$), -5.39

(Si(CH_3)$_2$); *m/z* (ESI$^+$) 446.2108 (M+H. $C_{22}H_{32}N_3O_5Si$ requires 446.2106), 468.1930 (M+Na. $C_{22}H_{31}N_3NaO_5Si$ requires 468.1925).

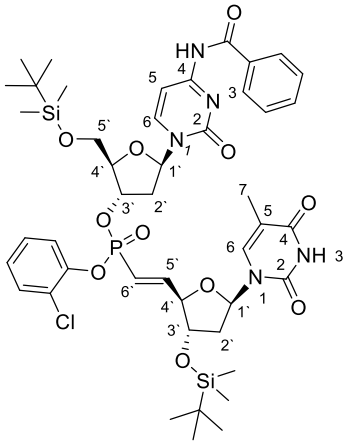
N-{1-[(2*R*,4*S*,5R)-4-[(*tert*-butyldimethylsilyl)oxy]-5-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide **236**



Chemical Formula: $C_{28}H_{45}N_3O_5Si_2$
Exact Mass: 559.2898

By-product, FTIR (ATR) ν_{max}/cm^{-1} : 2953, 2928, 2855, 1693, 1665, 1483, 1392, 1312, 1250, 1092, 832, 775; δH (500MHz, $CDCl_3$): 8.67 (1H, br s, N-H), 8.45 (1H, d, *J* 7.5, H-6), 7.89 (2H, d, *J* 7.7, ArH), 7.64-7.57 (1H, m, ArH), 7.55-7.38 (3H, m, ArH + H-5), 6.28 (1H, dd, *J* 6.5, 4.7, H-1'), 4.44-4.38 (1H, m, H-3'), 4.00-3.93 (2H, m, H-4' + H_A-5'), 3.79 (1H, dd, *J* 12.1, 2.9, H_B-5'), 2.58-2.49 (1H, m, H_A-2'), 2.16 (1H, ddd, *J* 13.5, 6.5, 4.7, H_B-2), 0.94 (9H, SiC(CH_3)$_3$), 0.88 (9H, SiC(CH_3)$_3$), 0.13 (6H, Si(CH_3)$_2$). 0.06 (6H, Si(CH_3)$_2$); δc (101 MHz, $CDCl_3$): 166.3 (^{Bz}CO), 162.1 (C-4), 155.1 (C-2), 145.1 (CH-6), 133.3 (ArH), 129.2 (ArC), 127.6 (2 × ArH), 127.8 (ArH), 96.0 (CH-5), 88.0 (CH-4'), 87.0 (CH-1'), 70.13 (CH-3'), 61.9 (CH-5'), 42.5 (CH-2'), 26.1 (SiC(CH_3)$_3$), 25.9 (SiC(CH_3)$_3$), 18.5 (SiC(CH_3)$_3$), 18.1 (SiC(CH_3)$_3$), -4.4 (Si(CH_3)$_2$), -4.8 (Si(CH_3)$_2$), -5.29 (Si(CH_3)$_2$), -5.34 (Si(CH_3)$_2$); *m/z* (ESI$^+$) 560.2971 (M+H. $C_{28}H_{46}N_3O_5Si_2$ requires 560.2971), 582.2789 (M+Na. $C_{28}H_{45}N_3NaO_5Si_2$ requires 582.2790).

(2*R*,3*S*,5R)-5-(4-benzamido-2-oxo-1,2-dihydropyrimidin-1-yl)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-3-yl 2-chlorophenyl [(*E*)-2-[(2*R*,3*S*,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]ethenyl]phosphonate **106**

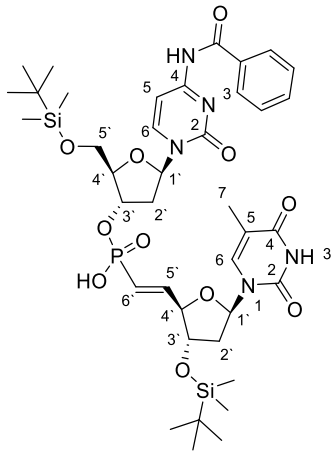


Chemical Formula: $C_{45}H_{61}ClN_5O_{11}PSi_2$
Exact Mass: 969.3332

A mixture of phosphonate **125** (600 mg, 1.10 mmol), alcohol **103** (535 mg, 1.2 mmol) and *N*-methylimidazole (872 µL, 11.0 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (3 mL), molecular sieves and 2,4,6-triisopropylbenzenesulfonyl chloride (1.66 g, 5.5 mmol) were added under argon. The reaction mixture was stirred at RT for 23 h, the volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol:ethyl acetate (1:1) → ethyl acetate) to afford the desired product **106** (640 mg, 60%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2951, 2927, 2855, 1487, 1299, 1250, 922, 832, 777; δH (400MHz, $CDCl_3$): 9.12 (0.4H, br s, NH), 9.01 (0.6H, br s, NH), 8.96-8.75 (1H, br s, NH), 8.30 (1H, d, *J* 7.4, C-H-6), 7.91 (2H, d, *J* 7.2, ArH), 7.64-7.56 (1H, m, ArH), 7.55-7.45 (3H, m, 2 × ArH + C-H-5), 7.44-7.35 (2H, m, ArH), 7.29-7.21 (1H, m, ArH), 7.18-7.10 (1.4H, m, 0.4 × T-H-6 + ArH), 7.07 (0.6H, q, *J* 1.2, T-H-6), 7.04-6.90 (1H, m, T-H-5'), 6.43-6.36 (1H, m, C-H-1'), 6.32-6.11 (2H, m, T-H-1' + T-H-6'), 5.30-5.21

(1H, m, C-H-3′), 4.44-4.34 (2H, m, T-H-4′ + 0.6 × C-H-4′ + 0.4 × T-H-3′), 4.30 (0.4H, q, *J* 2.0, C-H-4′), 4.2 (0.6H, dt, *J* 6.8, 4.4, T-H-3′), 3.98-3.86 (1.6H, m, C-H-5′), 3.78 (0.4H, dd, *J* 11.6, 2.1, C-H-5′), 2.94 (0.4H, ddd, *J* 14.1, 5.5, 1.8, C-H_A-2′), 2.86 (0.6H, ddd, *J* 14.1, 5.5, 1.8, C-H_A-2′), 2.36-2.11 (3H, m, T-H_A-2′ + T-H_B-2′ + C-H_B-2′), 1.94-1.91 (3H, m, T-H-7), 0.89 (6H, s, $SiC(CH_3)_3$), 0.88 (12H, s, $SiC(CH_3)_3$), 0.1 (2H, s, $Si(CH_3)_2$), 0.09 (3H, s, $Si(CH_3)_2$), 0.07 (3.3H, s, $Si(CH_3)_2$), 0.07 (3.7H, s, $Si(CH_3)_2$); *δ*c (121 MHz, $CDCl_3$): 163.7 (T-C-4), 163.6 (T-C-4), 162.4 (2 × C-C-4), 154.8 (2 × C-C-2), 151.5 (d, *J* 6.4, T-CH-5′), 150. 6 (d, *J* 6.2, T-CH-5′), 150.4 (T-C-2), 150.3 (T-C-2), 146.2 (d, *J* 6.3, ArC), 146.1 (d, *J* 6.3, ArC), 144.5 (2 × C-CH-6), 136.0 (T-CH-6), 135.3 (T-CH-6), 133.3 (2 × ArCH), 133.1 (2 × ArC), 130.9 (4 × ArCH), 129.1 (6 × ArCH), 128.3 (ArCH), 128.1 (ArCH), 127.7 (4 × ArCH), 126.4 (ArCH), 126.3 (ArCH), 125.9 (d, *J* 6.0 ArC), 125.8 (d, *J* 6.0, ArC), 122.3 (d, *J* 2.8, ArCH), 122.2 (d, *J* 2.5, ArCH), 116.9 (d, *J* 192, T-CH-6′), 116.8 (d, *J* 192, T-CH-6′), 111.8 (T-C-5), 111.7 (T-C-5), 96.7 (2 × C-CH-5), 87.2 (2 × C-CH-1′), 86.9 (d, *J* 5.5, C-CH-4′), 86.8 (d, *J* 6.0, C-CH-4′), 85.8 (T-CH-1′), 85.8 (d, *J* 22.0, T-CH-4′), 85.6 (d, *J* 22.0, T-CH-4′), 85.4 (T-CH-1′), 78.1 (d, *J* 5.9, C-CH-3′), 77.7 (d, *J* 6.6, C-CH-3′), 74.8 (2 × T-CH-3′), 63.1 (C-C-H-5′), 62.9 (C-CH-5′), 41.2 (d, 3.6, C-CH-2′), 41.1 (d, 4.3, C-CH-2′), 39.9 (T-CH-2′), 39.8 (T-CH-2′), 26.0 ($SiC(CH_3)_3$), 25.79 ($SiC(CH_3)_3$), 25.77 ($SiC(CH_3)_3$), 18.35 (2 × $SiC(CH_3)_3$), 18.02 (2 × $SiC(CH_3)_3$), 12.8 (T-CH_3-7), 12.7 (T-CH_3-7), -4.55 ($Si(CH_3)_2$), -4.57 ($Si(CH_3)_2$), -4.66 ($Si(CH_3)_2$), -5.36 ($Si(CH_3)_2$), -5.40 ($Si(CH_3)_2$), -5.46 ($Si(CH_3)_2$); *δ*P (162 MHz, $CDCl_3$): 15.09, 14.73; *m/z* (ESI^+) 970.3376 (M+H. $C_{45}H_{62}ClN_5O_{11}PSi_2$ requires 970.3405), 992.3178 (M+Na. $C_{45}H_{61}ClN_5NaO_{11}PSi_2$ requires 992.3224), 1038.3786 (M + $C_3H_5N_2$. $C_{48}H_{66}ClN_7O_{11}PSi_2$ Requires 1038.3779).

(2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **237**

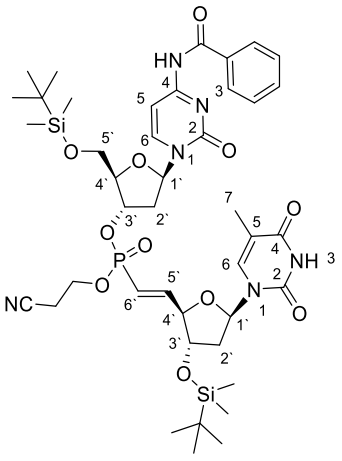


Chemical Formula: $C_{39}H_{58}N_5O_{11}PSi_2$
Exact Mass: 859.3409

To a solution of dinucleotide **106** (480 mg, 490 µmol) in dioxane (20 mL), a mixture of 2-nitrobenzaldoxime (123 mg, 740 µmol), tetramethylguanidine (93 µL, 740 µmol), triethylamine (416 µL, 3.00 mmol) in dioxane (5 mL) was added. The reaction mixture was stirred at RT for 3 d, then dry-loaded with silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **237** (375 mg, 88%) as a white powder. *δ*H (500 MHz, CD_3OD): 8.80 (1H, d, *J* 7.5, C-H-6), 8.01-7.95 (2H, m, ArH), 7.67-7.61 (1H, m, ArH), 7.53 (2H, d, *J* 7.8, ArH), 7.50 (1H, br d, *J* 7.5, C-H-5), 7.46 (1H, q, *J* 1.3, T-H-6), 6.57 (1H, ddd, *J* 20.1, 17.0, 5.7, T-H-5′), 6.30-6.22 (2H, m, C-H-1′ + T-H-1′), 6.05 (1H, t, *J* 17.0, T-H-6′), 4.82-4.76 (1H, m, C-H-3′), 4.46 (1H, dt, *J* 4.7, 3.9, T-H-3′), 4.36-4.32 (1H, bs, C-H-4′), 4.31-4.26 (1H, br s, T-H-4′), 3.95 (1H, dd, *J* 11.6, 2.4, C-H_A-5′), 3.88 (1H, dd, *J* 11.6, 2.6, C-H_B-5′), 2.80 (1H, dd, J 13.9, 5.6, C-H_A-2′), 2.38-2.30 (1H, m, T-H_A-2′), 2.23-2.12 (2H, m, C-H_B-2′ + T-H_B-2′), 1.90 (3H, d, *J* 1.3, T-H-7), 0.91 (9H, s, $SiC(CH_3)_3$), 0.90 (9H, s, $SiC(CH_3)_3$), 0.13 (9H, s,

$Si(CH_3)_2$), 0.11 (9H, s, $Si(CH_3)_2$); δc (126 MHz, CD_3OD): 169.1 (^{Bz}CO), 166.3 (T-C-4), 164.6 (C-C-4), 157.7 (C-C-2), 152.3 (T-C-2), 146.0 (C-CH-6), 143.7 (br s, T-CH-5′), 138.3 (T-CH-6), 134.5 (ArC), 133.2 (ArCH), 129.9 (2 × ArCH), 129.3 (2 × ArCH), 126.6 (d, *J* 180, T-CH-6′), 112.0 (T-C-5), 98.4 (C-CH-5), 89.2 (C-CH-1′ + C-CH-4′), 89.3 (d, *J* 21.2, T-CH-4′), 86.7 (T-CH-1′), 86.7 (T-CH-1′), 76.7 (T-CH-3′), 76.3 (d, *J* 4.6, C-CH-3′), 64.5 (C-CH_2-5′), 42.5 (d, *J* 2.4, C-CH_2-2′), 40.3 (T-CH_2-2′), 26.5 (3 × SiC(CH_3)$_3$), 26.3 (3 × SiC(CH_3)$_3$), 19.2 (Si*C*$(CH_3)_3$), 18.8 (Si*C*$(CH_3)_3$), 12.6 (T-CH_3-7), -4.41 ($Si(CH_3)_2$), -4.46 ($Si(CH_3)_2$), -5.25 ($Si(CH_3)_2$), -5.29 ($Si(CH_3)_2$); δP (162 MHz, CD_3OD): 9.65; *m/z* (ESI^+) 860.3461 (M+H. $C_{39}H_{59}N_5O_{11}PSi_2$ requires 860.3482), 882.3273 (M+Na. $C_{39}H_{58}N_5NaO_{11}PSi2$ requires 882.3301).

(2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-cyanoethyl) ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **238**



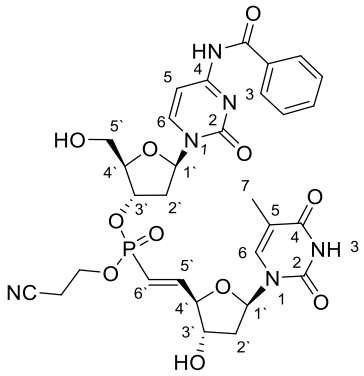
Chemical Formula: $C_{42}H_{61}N_6O_{11}PSi_2$
Exact Mass: 912.3674

A mixture of phosphonate **237** (375 mg, 440 µmol), 3-hydroxypropionitrile (150 µL, 2.20 mmol) and *N*-methylimidazole (230 µL, 2.90 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved

in pyridine (1.8 mL), molecular sieves and 2,4,6-triisopropylbenzenesulfonyl chloride (333 mg, 1.10 mmol) were added under argon. The reaction mixture was stirred at RT for 24 h, the volatiles were removed *in vacuo* and the residue was purified by column using dichloromethane : methanol (19:1) to afford the desired product **238** (325 mg, 80%, 1:1 mixture of diastereoisomers) as a white foam. δH (400MHz, $CDCl_3$): 9.75-9.47 (2H, br s, ^{Bz}NH + T-NH), 8.36-8.26 (1H, m, C-H-6), 7.91 (2H, d, *J* 7.2, ArH), 7.64-7.58 (1H, m, ArH), 7.55-7.47 (3H, m, 2 × ArH + C-H-5), 7.44-7.35 (2H, m, ArH), 7.19 (0.4H, q, *J* 1.3, T-H-6), 7.15 (0.4H, q, *J* 1.3, T-H-6), 7.06-6.88 (1H, m, T-H-5′), 6.39-6.33 (1H, m, C-H-1′), 6.28 (0.6H, t, *J* 6.8, T-H-1′), 6.15 (0.4H, t, *J* 6.8, T-H-1′), 6.12-5.99 (1H, m, T-H-6′), 5.14-5.16 (1H, m, C-H-3′), 4.45-4.40 (0.4H, m, T-H-3′), 4.39-4.19 (4.6H, m, OC*H*$_2$CH$_2$CN + C-H-4′ + T-H-4′ + 0.6 × T-H-3′), 4.00-3.82 (2H, m, C-H$_A$-5′ + C-H$_B$-5′), 2.94-2.86 (1H, m, C-H$_A$-2′), 2.83-2.73 (2H, m, OCH$_2$C*H*$_2$CN), 2.52-2.42 (0.4H, m, T-H$_A$-2′), 2.28-2.10 (2.6H, m, C-H$_B$-2′ + 1.6 × T-H$_B$-2′), 1.95 (1.8H, d, *J* 1.3, T-H-7), 1.92 (1.8H, d, *J* 1.3, T-H-7), 0.19-0.87 (18H, s, SiC(CH$_3$)$_3$), 0.14-0.07 (12H, s, Si(CH$_3$)$_2$; δc (121 MHz, $CDCl_3$): 166.6 (2 × ^{Bz}ArC), 163.7 (T-C-4), 136.6 (T-C-4), 162.5 (C-C-4), 162.4 (C-C-4), 155.0 (2 × C-C-2), 150.7 (d, *J* 6.7, T-CH-5′), 150.3 (2 × T-C-2), 49.5 (d, *J* 6.7, T-CH-5′), 144.5 (2 × C-CH-6), 136.8 (T-CH-6), 135.9 (T-CH-6), 133.4 (2 × ArCH), 133.0 (2 × ArC), 129.2 (4 × ArCH), 127.7 (4 × ArCH), 116.9 (d, *J* 192, T-CH-6′), 116.9 (CN), 116.54 (CN), 116.5 (d, *J* 190, T-CH-6′), 111.8 (T-C-5), 111.6 (T-C-5), 96.7 (2 × C-CH-5), 87.3 (C-CH-1′), 87.2 (C-CH-1′), 86.9 (d, *J* 5.5, C-CH-4′), 86.8-86.7 (C-CH-4′ + T-CH-1′), 85.9 (d, *J* 22.0, T-CH-4′), 85.8 (d, *J* 22.0, T-CH-4′), 85.6 (T-CH-1′), 77.8 (d, *J* 5.3, C-CH-3′), 77.0 (d, *J* 6.6, C-CH-3′), 74.9 (d, *J* 1.5, T-CH-3′), 74.8 (d, *J* 1.5, T-CH-3′), 63.3 (C-C-H-5′), 63.0 (C-CH-5′), 60.9 (d, *J* 5.0, O*C*H$_2$CH$_2$CN), 60.3 (d, *J* 5.0, O*C*H$_2$CH$_2$CN), 41.2 (d, *J* 3.0, C-CH-2′), 41.1 (d, *J* 3.0, C-CH-2′), 39.7 (T-CH-2′), 39.6 (T-CH-2′), 26.0 (2 × SiC(*C*H$_3$)$_3$), 25.8 (2 × SiC(*C*H$_3$)$_3$), 20.14 (d, *J* 7.0, OCH$_2$*C*H$_2$CN),

20.0 (d, *J* 7.0, OCH_2CH_2CN), 18.4 (2 × $SiC(CH_3)_3$), 18.05 (2 × $SiC(CH_3)_3$), 12.66 (T-CH_3-7), 12.64 (T-CH_3-7), -4.5 ($Si(CH_3)_2$), -4.6 ($Si(CH_3)_2$), -5.35 ($Si(CH_3)_2$), -5.39 ($Si(CH_3)_2$), -5.41 ($Si(CH_3)_2$); δP (162 MHz, $CDCl_3$): 18.85, 18.70; *m/z* (ESI^+) 913.3747 (M+H. $C_{42}H_{62}N_6O_{11}PSi_2$ requires 913.3742), 935.3555 (M+Na. $C_{42}H_{61}N_6$ Na $O_{11}PSi_2$ requires 935.3567).

(2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl (2-cyanoethyl) ((*E*)-2-((2*R*,3*S*,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **239**

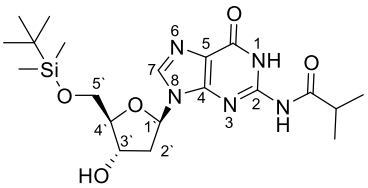


Triethylamine trihydrofluoride (718 µL, 4.40 mmol) was added in one portion to a stirring solution of dinucleotide **238** (400 mg, 440 µmol) in THF (8 mL) and the resulting reaction mixture was stirred at RT for 4 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (19:1 → 9:1) to afford the desired product **239** (180 mg, 60%, 3:2 mixture of diastereoisomers) as a white powder. δH (400MHz, CD_3OD): 8.47 (0.7H, d, *J* 7.5, C-H-6), 8.46 (0.7H, d, *J* 7.5, C-H-6), 7.99-7.93 (2H, m, ArH), 7.66-7.57 (1H, m, ArH), 7.60 (1H, br d, *J* 7.5, C-H-5), 7.56-7.50 (2H, m, ArH), 7.44-7.39 (1H, m, T-H-6), 7.12-6.98 (1H, m, T-H-5'), 6.32-6.12 (3H, m, T-H-1' + C-H-1' + T-H-6'), 5.21-5.13 (1H, m, C-H-3'), 4.45-4.39 (2H, m, T-H-3' + T-H-4'), 4.36 (0.7H, q, *J* 3.1, C-H-4'), 4.33-4.26 (2.3H, m, 0.3 × C-H-4' + 2 × CH_2CH_2CN), 3.91-3.81 (2H, m, C-H-

5′), 2.96-2.89 (2H, m, CH_2CH_2CN), 2.88-2.77 (1H, m, C-H_A-2′), 2.48-2.35 (2H, m, C-H_B-2′ + T-H_A-2′), 2.33-2.22 (1H, m, T-H_B-2′), 1.90-1.86 (1H, m, T-H-7); δc (121 MHz, CD_3OD): 169.1 (2 × ^{Bz}ArC), 166.2 (2 × T-C-4), 164.9 (2 × C-C-4), 157.7 (2 × C-C-2), 152.8 (d, *J* 6.0, T-CH-5′), 152.7 (d, *J* 6.0, T-CH-5′), 152.2 (2 × T-C-2), 146.3 (2 × C-CH-6), 138.4 (T-CH-6), 138.2 (T-CH-6), 134.7 (ArCH), 134.1 (ArCH), 129.8 (2 × ArCH), 129.6 (ArC), 129.5 (ArC), 129.1 (2 × ArCH), 118.8 (CN), 118.7 (CN), 117.3 (d, *J* 189, T-CH-6′), 117.2 (d, *J* 189, T-CH-6′), 112.1 (T-C-5), 112.0 (T-C-5), 98.5 (2 × C-CH-5), 88.6 (2 × C-CH-1′), 88.2 (d, *J* 5.3, C-CH-4′), 88.0 (d, *J* 5.6, C-CH-4′), 87.2 (2 × T-CH-1′), 87.1 (d, *J* 22.5, T-CH-4′), 87.0 (d, *J* 22.8, T-CH-4′), 78.3 (d, *J* 5.6, C-CH-3′), 78.0 (d, *J* 5.7, C-CH-3′), 75.0 (2 × d, *J* 2.0, T-CH-3′), 62.7 (d, *J* 5.1, CH_2CH_2CN), 62.6 (d, *J* 5.4, CH_2CH_2CN), 62.1 (C-CH_2-5′), 62.0 (C-CH_2-5′), 41.2 (d, 3.2, C-CH_2-2′), 41.1 (d, 3.4, C-CH_2-2′), 39.5 (2 × T-CH_2-2′), 20.4 (d, *J* 6.5, CH_2CH_2CN), 20.3 (d, *J* 6.7, CH_2CH_2CN), 12.5 (2 × T-CH_3-7), δP (162 MHz, $CDCl_3$): 18.98, 18.92; *m/z* (ESI$^+$) 685.2032 (M+H. $C_{30}H_{34}N_6O_{11}P$ requires 685.2018), 707.1839 (M+Na. $C_{30}H_{33}N_6NaO_{11}P$ requires 707.1837).

5.2.4 Synthesis of G*T dinucleotide 107

synthesis of *N*-(9-((2*R*,4*S*,5*R*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide **104**

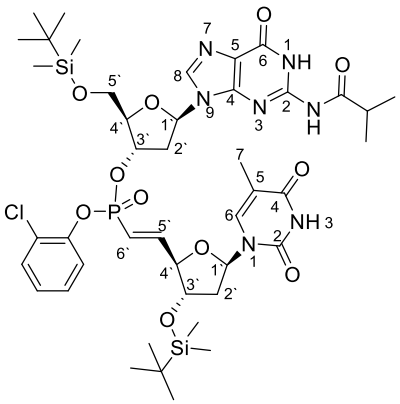


Chemical Formula: $C_{20}H_{33}N_5O_5Si$
Exact Mass: 451.2251

2-*N*-^{i}Bu-deoxyguanosine **111** (2.00 g, 5.06 mmol) was dried by azeotropic removal of water with pyridine (3 × 10mL). The residue was dissolved in DMF (12 mL) and stirred at 0°C for 15 min. Imidazole (962 mg, 14.1 mmol),

TBDMSCl (1.26 g, 6.80 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 22 h. DMF was removed *via* azeotrope with toluene (5 × 50 mL) to afford a sticky oily residues. Water (100 mL), dichloromethane (100 mL) and ethyl acetate (100 mL) was added to the residues. After separation the aqueous layer was extracted with a mixture of CH_2Cl_2 and ethyl acetate (1:1, 3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4 and the volatiles removed *in vacuo*. Purification of the residue by column chromatography using gradient elution; petrol : ethyl acetate (4:1) → ethyl acetate) provided the desired product **104** (1.98 g, 76%) as a white solid. FTIR (ATR) ν_{max}/cm^{-1} 3435, 3169, 2953, 2929, 2857, 1678, 1607, 1560, 1471, 1400, 1251, 1092, 833, 776; δH (400MHz, $CDCl_3$): 12.44 (1H, br s, N-H), 11.94 (1H, br s, N-H), 8.02 (1H, s, H-8), 6.03 (1H, t, *J* 6.6, H-1′), 4.69 (1H, br s, OH), 4.56 (1H, m, H-3′), 4.08 (1H, q, *J* 3.12, H-4′), 3.79 (2H, s, H_A-5′, H_B-5′), 3.02-2.93 (1H, m, C*H*$(CH_3)_2$)), 2.42-2.29 (2H, m, H_A-2′, H_B-2′), 1.27 (6H, dd, *J* 9.3,6.8, CH(C*H*$_3)_2$), 0.84 (9H, s, SiC$(CH_3)_3$), 0.03 (6H, s, Si$(CH_3)_2$); δc (101 MHz, $CDCl_3$): 180.7 (CO), 156.4 (C-6), 148.8 (C-2), 148.4 (C-4), 137.5 (CH-8), 120.6 (C-5), 88.0 (CH-4′), 84.4 (CH-1′), 71.8 (CH-3′), 63.9 (CH-5′), 41.76 (CH-2′), 36.24 (*C*H$(CH_3)_2$), 26.6 (SiC(*C*H$_3)_3$), 19.24 (CH(*C*H$_3)_2$), 19.18 (CH(*C*H$_3)_2$), 18.51 (Si*C*$(CH_3)_3$), -5.29 (Si(*C*H$_3)_2$), -5.37 (Si(*C*H$_3)_2$); *m/z* (ESI$^+$) 452.2336 (M+H. $C_20H34N5O5Si$ requires 452.2324), 474.2149 (M+Na. $C_{20}H_{33}N_5NaO_5Si$ requires 474.2143), 520.2667 (M + $C_3H_5N_2$. $C_{23}H_{38}N_7O_5Si$ requires 520.2698).

(2*R*,3*S*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **107**



Chemical Formula: $C_{43}H_{63}ClN_7O_{11}PSi_2$
Exact Mass: 975.3550

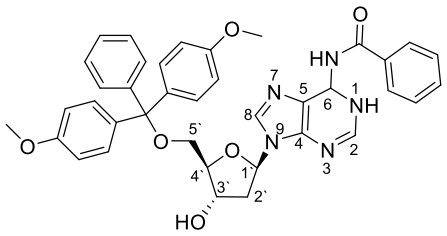
A mixture of phosphonate **26** (200 mg, 0.368 mmol), alcohol **104** (166 mg, 0.368 mmol) and *N*-methylimidazole (190 µL, 2.39 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (2 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (279 mg, 0.92 mmol) were added. The reaction mixture was stirred at RT for 18 h, the volatiles were removed *via* reduced pressure and the residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **107** (265 mg, 73%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2952, 2929, 2857, 1681, 1606, 1555, 1478, 1361, 1251, 833, 777; δH (400MHz, $CDCl_3$): 12.4 (0.5H, s, G-N-H), 12.15 (0.5H, s, G-N-H), 10.73 (0.5H, s, ^{iBu}N-H), 10.2 (0.5H, s, T-N-H), 9.94 (0.5H, br s, ^{iBu}N-H), 9.25 (0.5H, br s, T-N-H,), 8.08 (0.5H, s, G-H-8), 7.94 (0.5H, s, G-H-8), 7.48-7.37 (2H, m, ArH), 7.29-7.22 (1H, m, ArH), 7.18-7.09 (2H, m, ArH, T-H-6), 7.08-6.91 (1H, m, T-H-5'), 6.35 (0.5H, dd, *J* 9.0, 5.6, G-H-1'), 6.26 (0.5H, t, *J* 7.0, G-H-1'), 6.18 (0.5H, ddd, *J* 22.0, 17.0, 2.0, T-H-6'), 6.16 (0.5H, ddd, *J* 21.0, 17.0, 2.0, T-H-6'), 6.06 (0.5H, t, *J* 6.6, T-H-1'), 5.73 (0.5H, dd, *J* 7.9, 4.6, T-H-1'), 5.60-5.52 (0.5H, m, G-H-3'),

5.4 (0.5H, dd, *J* 7.8, 5.4, G-H-3′), 4.60 (0.5H, dt, *J* 7.1, 4.9, T-H-3′), 4.49-4.44 (0.5H, m, T-H-4′), 4.42-4.37 (0.5H, m, T-H-4′), 4.35 (0.5H, dt, *J* 6.7, 4.4, T-H-3′), 4.31 (0.5H, q, *J* 2.6, G-H-4′), 4.16 (0.5H, q, *J* 2.3, G-H-4′), 3.85-3.65 (2H, m, T-H_A-5′ + T-H_B-5′), 2.98-2.57 (3.5H, m, G-H_A-2′ + G-H_B-2′ + $^{iBu}CH(CH_3)_2$) + 0.5 × T-H_A-2′), 2.41 (0.5H, dt, *J* 13.1, 6.5, T-H_A-2′), 2.34-2.22 (1H, m, T-H_B-2′), 1.97 (1.5H, d, *J* 1.4, T-H-7), 1.94 (1.5H, d, *J* 1.4, T-H-7), 1.26 (3H, 2 × d, *J* 6.8, $^{iBu}CH(CH_3)_2$), 1.22 (1.7H, d, *J* 7.0, $^{iBu}CH(CH_3)_2$), 1.16 (1.3H, d, *J* 6.7, $^{iBu}CH(CH_3)_2$), 0.91 (4H, $SiC(CH_3)_3$), 0.88 (9H, $SiC(CH_3)_3$), 0.86 (5H, $SiC(CH_3)_3$), 0.10 (1.3H, $Si(CH_3)_2$), 0.08 (1.3H, $Si(CH_3)_2$), 0.08 (1.3H, $Si(CH_3)_2$)), 0.06 (2.8H, $Si(CH_3)_2$), 0.05 (2H, $Si(CH_3)_2$), 0.04 (1.6H, $Si(CH_3)_2$), 0.03 (1.8H, $Si(CH_3)_2$); *δ*c (121 MHz, $CDCl_3$): 180.13 (^{iBu}CO), 179.42 (^{iBu}CO), 165.88 (T-C-4), 164.35 (T-C-4), 155.58 (G-C-6), 155.47 (G-C-6), 150.15 (d, *J* 6.2, T-CH-5′), 150.10 (d, *J* 6.5, T-CH-5′), 149.96 (T-C-2), 149.4 (T-C-2), 149.3 (G-C-2), 148.36 (G-C-2), 148.12 (G-C-4), 147.87 (G-C-4), 146.33 (d, *J* 7.0, ArC), 146.14 (d, *J* 7.4, ArC), 141.53 (CH-6, T), 137.64 (T-CH-6), 137.21 (2 × G-CH-8), 130.86 (2 × ArCH), 128.33 (ArCH), 127.98 (ArCH), 126.45 (ArCH), 126.25 (ArCH), 126.01 (d, *J* 5.9, ArC), 125.43 (d, *J* 6.1, ArC), 122.41 (d, *J* 2.9, ArCH), 122.21 (d, *J* 2.6, ArCH), 121.60 (G-C-5), 121.53 (G-C-5), 116.64 (d, *J* 193, T-CH-6′), 111.3 (T-C-5), 110.4 (T-C-5), 92.93 (T-CH-1′), 88.29 (T-CH-1′), 87.3 (d, *J* 21.7, T-CH-4′), 86.65 (d, *J* 6.7, G-CH-4′), 86.33 (d, *J* 22.0, T-CH-4′), 86.13 (d, *J* 6.7, G-CH-4′), 83.72 (G-CH-1′), 83.66 (G-CH-1′), 78.42 (d, *J* 5.3, G-CH-3′), 77.74 (d, *J* 6.5, G-CH-3′), 75.65 (d, *J* 1.2, T-CH-3′), 75.24 (d, *J* 1.2, T-CH-3′), 63.81 (G-CH-5′), 63.03 (G-CH-5′), 39.98 (G-CH_2-2′), 39.78 (d, *J* 4.5, G-H_2-2′), 39.5 (T-CH-2′), 39.2 (T-CH-2′), 36.24 ($^{iBu}CH(CH_3)_2$), 36.00 ($^{iBu}CH(CH_3)_2$), 26.14 ($SiC(CH_3)_3$), 26.01 ($SiC(CH_3)_3$), 25.82 ($SiC(CH_3)_3$), 25.78 ($SiC(CH_3)_3$), 19.25 ($^{iBu}CH(CH_3)_2$), 19.16 ($^{iBu}CH(CH_3)_2$), 19.06 ($^{iBu}CH(CH_3)_2$), 19.03 ($^{iBu}CH(CH_3)_2$), 18.53 ($SiC(CH_3)_3$), 18.45 ($SiC(CH_3)_3$), 18.03 (2 × $SiC(CH_3)_3$), 12.61 (CH_3-7), 12.32 (CH_3-7), -4.52 ($Si(CH_3)_2$), -4.56 ($Si(CH_3)_2$), -

4.72 (2 × $Si(CH_3)_2$), -5.27 ($Si(CH_3)_2$), -5.33 ($Si(CH_3)_2$), -5.41 ($Si(CH_3)_2$), -5.49 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 15.83, 14.18; *m/z* (ESI^+) 976.3666 (M+H. $C_{43}H_{64}ClN_7O_{11}PSi_2$ requires 976.3623).

5.3 Synthesis of phosphonic acid (A) **69**

N-(9-((2*R*,4*S*,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-6,9-dihydro-1H-purin-6-yl)benzamide **113**

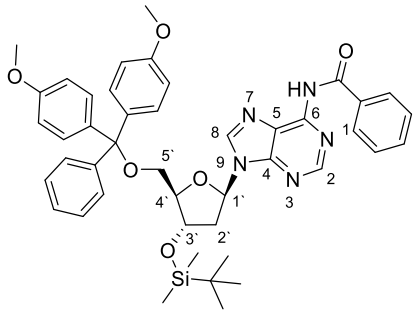


Chemical Formula: $C_{38}H_{37}N_5O_6$
Exact Mass: 659.2744

N-benzoyldeoxadenosine **108** (10.0 g, 28.1 mmol) was dissolved in pyridine (10 mL), and the water was removed by azeotropic distillation with pyridine (3 × 10 mL). The residue was dissolved in pyridine (25 mL), dimethoxytrityl chloride (9.52 g, 28.1 mmol) was added in portions at 0 °C and the reaction mixture was stirred this temperature for 10 min then allowed to warm up to room temperature. The resultant mixture was stirred at RT for 16 h. when the reaction is complete, pyridine was removed by reduced pressure and the residue was dissolved in ethyl acetate (200 mL), washed with water (100 mL) and the separated aqueous layer was extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were dried over anhydrous sodium sulphate. The volatiles were removed by reduced pressure and the residue was purified by column chromatography using petrol : ethyl acetate (4:1) → ethyl acetate to afford the desired product **193** as a white foam (18.5 g, quantitative). 3336, 2948, 2926, 2856, 1699, 1607, 1579, 1507, 1489, 1453, 1297, 1174, 1031, 906, 827, 793, 725; δH (400MHz, $CDCl_3$): 9.05 (1H, s, N-H), 8.75 (1H, s, H-2), 8.17 (1H, s, H-8), 8.08-7.99 (2H, m, ArH), 7.66-7.60

(2H, m, ArH), 7.33-7.20 (7H, m, ArH), 6.86-6.77 (4H, m, ArH), 6.51 (1H, dd, *J* 6.4, H-1′), 4.74 (1H, dt, *J* 6.2, 4.0, H-3′), 4.21-4.16 (1H, m, H-4′), 3.80 (6H, s, 2 × OCH_3), 3.49-3.39 (2H, m, H_A-5′ + H_B-5′), 2.97-2.87 (1H, m, H_A-2′), 2.64-2.56 (1H, m, H_B-2′); δc (101 MHz, $CDCl_3$): 164.7 (^{Bz}CO), 158.7 (2 × ^{DMT}ArC), 152.7 (CH-2), 151.5 (C-4), 149.6 (C-6), 144.6 (^{DMT}ArC), 141.6 (CH-8), 135.7 (2 × ^{DMT}ArC), 133.8 (^{Bz}ArC), 132.9 (ArCH), 130.1 (4 × ArH), 129.02 (2 × ArCH), 128.2 (2 × ArCH), 128.0 (2 × ArCH), 127.98 (2 × ArCH), 127.1 (ArCH), 123.5 (C-5), 113.3 (4 × ArCH), 86.8 ($^{DMT}CAr_3$), 86.3 (CH-4′), 84.8 (CH-1′), 72.75 (CH-3′), 63.8 (CH-5′), 55.38 (2 × OCH_3), 40.4 (CH-2′); *m/z* (ESI^+) 658.2678 (M+H. $C_{38}H_{36}N_5O_6$ requires 658.2660), 680.2493 (M+Na. $C_{38}H_{35}N_5NaO_6$ requires 680.2480).

N-{9-[(2*R*,4*S*,5R)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]-9H-purin-6-yl}benzamide **114**

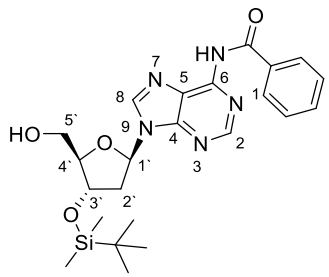


Chemical Formula: $C_{44}H_{49}N_5O_6Si$
Exact Mass: 771.3452

Imidazole (170 mg, 2.50 mmol) was added in one portion to a stirring solution of alcohol **113** (657 mg, 1.00 mmol) in DMF (2 mL) at 0 °C and the resulting solution was stirred at this temperature for 30 min. TBDMSCl (181 mg, 2.50 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 3 days at this temperature. Diethyl ether (5 mL) and water (5mL) were added and the separated aqueous layer was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by

column chromatography using: petrol : ethyl acetate (1:1) to afford the desired product **114** (710 mg, 92%) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2951, 2928, 2854, 1698, 1607, 1578, 1507, 1545, 1412, 1326, 1297, 1247,1097, 830; δH (400MHz, $CDCl_3$): 9.04 (1H, bs, NH), 8.76 (1H, s, H-2), 8.22 (1H, s, H-8), 8.05- 7.97 (2H, m, ArH), 7.64-7.57 (1H, m, ArH), 7.55-7.49 (2H, m, ArH), 7.43-7.36 (2H, m, ArH), 7.33-7.17 (7H, m, ArH), 6.84-6.75 (4H, m, ArH), 6.49 (1H, dd, *J* 6.5, 6.1, H-1′), 4.64-4.59 (1H, m, H-3′), 4.13 (1H, q, *J* 4.4, H-4′), 3.77 (6H, s, 2 × OCH_3), 3.41 (1H, dd, *J* 10.4, 4.5, H_A-5′), 3.30 (1H, dd, *J* 10.4, 4.4, H_B-5′), 2.80 (1H, ddd, *J* 13, 6.5, 5.8, H_A-2′), 2.46 (1H, ddd, *J* 13, 6.1, 3.6, H_B-2′), 0.88 (9H, $SiC(CH_3)_3$), 0.07 (3H, $Si(CH_3)_2$), 0.03 (3H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 164.7 (^{Bz}CO), 158.7 (2 × ArC), 152.7 (CH-2), 151.6 (C-4), 149.6 (C-6), 144.6 (ArC), 141.7 (CH-8), 135.8 (2 × ArC), 133.9 (ArC), 132.9 (ArCH), 130.1 (4 × ArCH), 129.0 (2 × ArCH), 128.3 (2 × ArCH), 128.0 (4 × ArCH), 127.1 (ArCH), 123.6 (C-5), 113.3 (4 × ArCH), 87.2 (CH-4′), 86.6 (CAr_3), 85.0 (CH-1′), 72.7 (CH-3′), 63.3 (CH_2-5′), 55.4 (2 × OCH_3), 40.9 (CH_2-2′), 25.9 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.5 ($Si(CH_3)_2$), -4.7($Si(CH_3)_2$); *m/z* (ESI$^+$) 772.3519 (M+H. $C_{44}H_{50}N_5O_6Si$ requires 792.3525), 794.3341 (M+Na. $C_{44}H_{49}N_5NaO_6Si$ requires 794.3344).

N-{9-[(2*R*,4*S*,5R)-4-[(*tert*-butyldimethylsilyl)oxy]-5-(hydroxymethyl)oxolan-2-yl]-9H-purin-6-yl}benzamide **115**



Chemical Formula: $C_{23}H_{31}N_5O_4Si$
Exact Mass: 469.2145

Method A

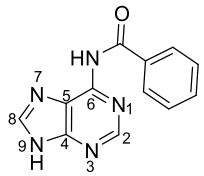
Compound **114** (1.80 g, 1.95 mmol) was dissolved in 5% (v/v) dichloroacetic acid in dichloromethane (40 mL) and stirred for 15 min at room temperature. The mixture was neutralized by saturated solution of sodium bicarbonate and stirred for an additional 10 min, brine (20mL) was added and after separation the organic layer, the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate : methanol (9:1) to afford the desired product **115** (980 mg, 89%).

Method B

A solution of TFA/H_2O (24 mL, 1:1, v/v) was added in one portion to a stirred solution of Compound **114** (3.00 g, 3.9 mmol) in THF (48 mL) at 0°C. the reaction mixture was stirred at this temperature for 2 h. The mixture was then neutralized by saturated solution of sodium bicarbonate and stirred for an additional 30 min, ethyl acetate (500 mL) was added and after separation the organic layer, the aqueous layer was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using gradient elution; petrol : ethyl acetate (1:1)

→ ethyl acetate : methanol (9:1) to afford the desired product **115** (1.17 g, 64%). FTIR (ATR) ν_{max}/cm^{-1} : 3272, 2951, 2928, 2855,1697, 1609, 1580, 1407,1250, 1095; δH (400MHz, $CDCl_3$): 9.13 (1H, s, N-H), 8.76 (1H, s, H-2), 8.09 (1H, s, H-8), 8.03-8.00 (2H, m, ArH), 7.63-7.58 (1H, m, ArH), 7.54-7.50 (2H, m, ArH), 6.36 (1H, dd, *J* 9.4, 5.5, H-1'), 5.77 (1H, dd, *J* 11.3, 2.2 OH), 4.71 (1H, d, *J* 5.1 H-3'), 4.17-4.14 (1H, m, H-4'), 3.94 (1H, dt, *J* 13, 1.9, H_A-5'), 3.75 (1H, ddd, *J* 13, 11.3, 1.9 H_B-5'), 3.03 (1H, ddd, *J* 13, 9.4, 5.1, H_A-2'), 2.26 (1H, ddd, *J* 13, 5.5, 1.3, H_B-2'), 0.93 (9H, s, $SiC(CH_3)_3$), 0.12 (6H, s, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 164.8 (CO), 152.2 (CH-2), 150.8 (C-4), 150.4 (C-6), 142.7 (CH-8), 133.5 (ArC), 133.0 (ArH), 129 (2 × ArH), 128 (2 × ArH), 124.6 (C-5), 90.5 (CH-4'), 88.0 (CH-1'), 73.9 (CH-3'), 63.3 (CH_2-5'), 41.5 (CH_2-2'), 25.9 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.6 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); *m/z* (ESI^+) 470.2217 (M+H. $C_{23}H_{32}N_5O_4Si$ requires 470.2218), 492.2032 (M+Na. $C_{23}H_{31}N_5NaO_4Si$ requires 492.2038).

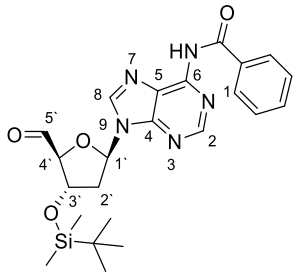
N-(9H-2-purin-6-yl)benzamide**112**



Chemical Formula: $C_{12}H_9N_5O$
Exact Mass: 239.0807

FTIR (ATR) ν_{max}/cm^{-1}: 3027, 2951, 1684, 1619, 1592, 1518, 1254; (400MHz, CD_3OD): 8.71 (1H, s, H-2), 8.47 (1H, s, H-8), 8.14-8.07 (2H, m, ArH), 7.69-7.63 (1H, m, ArH), 7.60-7.53 (2H, m, ArH); δ_C (101 MHz, $CDCl_3$): 168.8 (CO), 161.8 (C-4), 152.9 (CH-2), 146.8 (C-6), 146.4 (CH-8), 134.2 (ArC), 134.1 (ArH), 129.8 (2 × ArH), 129.5 (2 × ArH), 115.4 (C-5); *m/z* (ESI^+) 262.0699 (M+Na. $C_{12}H_9N_5NaO$ requires 262.0699).

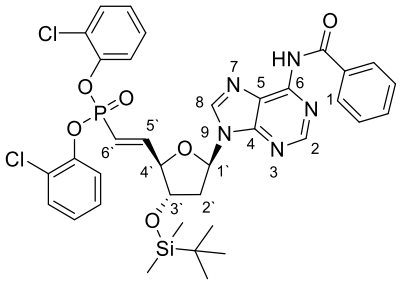
N-{9-[(2*R*,4*S*,5S)-4-[(*tert*-butyldimethylsilyl)oxy]-5-formyloxolan-2-yl]-9H-purin-6-yl}benzamide **67**



Chemical Formula: $C_{23}H_{29}N_5O_4Si$
Exact Mass: 467.1989

3′-O-(TBDMS)-*N*-Benzoyldeoxyadenosine **115** (3.80 g, 8.08 mmol) was dissolved in dry CH_2Cl_2 (45 mL) and stirred at 0 °C in an ice bath. To this solution was added Dess-Martin periodinane (3.43 g, 8.08 mmol) in one portion and the reaction stirred at 0 °C for 3 h. The reaction mixture was diluted with dichloromethane (50 mL), and stirred with a saturated solution of sodium thiosulphate (50 mL) and sodium bicarbonate (50 mL) for 30 min. The reaction mixture was extracted with dichloromethane (5 × 100 mL). The organic layers were pooled and washed with brine (50 mL), dried over $MgSO_4$ and concentrated to give the crude aldehyde **67** (3.5 g, 93%) as a white foam. δH (400MHz, $CDCl_3$): 9.80 (1H, d, *J* 0.8, H-5′), 9.0 (1H, s, N-H), 8.77 (1H, s, H-2), 8.28 (1H, s, H-8), 8.05-8.01 (2H, m, ArH), 7.65-7.99 (1H, m, ArH), 7.56-7.50 (2H, m, ArH), 6.59 (1H, dd, *J* 7.9, 6.0, H-1′), 4.92 (1H, dt, *J* 4.9, 2.2, H-3′), 4.49 (1H, d, *J* 2.2, H-4′), 2.97-2.87 (1H, m, H_A-2′), 2.50 (1H, ddd, *J* 13.5, 6.1, 2.4, H_B-2′), 0.95 (9H, s, $SiC(CH_3)_3$), 0.17 (6H, s, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 200.4 (CH-5′), 164.7 (CO), 152.7 (CH-2), 142.7 (C), 150.6 (C), 142.2 (CH-8), 133.0 (ArC), 132.9 (ArH), 129.0 (2 × ArH), 128.0 (2 × ArH), 124.0 (C-5), 92.0 (CH-4′), 86.8 (CH-1′), 73.7 (CH-3′), 40.0 (CH_2-2′), 25.8 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.6 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); *m/z* (ESI^+) 468.2062 (M+H. $C_{23}H_{30}N_5O_4Si$ requires 468.2062).

bis(2-chlorophenyl) [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2- yl]ethenyl]phosphonate **68**

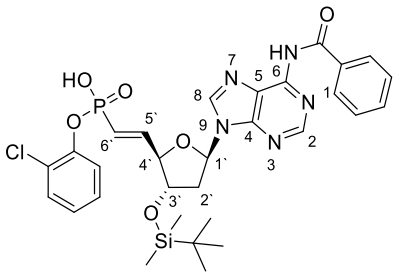


Chemical Formula: $C_{36}H_{38}Cl_2N_5O_6PSi$
Exact Mass: 765.1706

Ylide **24** (1.29 g, 2.24 mmol) was added in one portion to a stirring solution of aldehyde **67** (700 mg, 1.50 mmol) in dichloromethane (8 mL) and the resulting solution was stirred at RT for 21 h. The volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate : methanol (19:1) to afford the desired product **68** (800 mg, 70%) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 3061, 2952, 2928, 2855, 1693, 1607, 1579, 1476, 1233, 1086, 997, 924; δH (400MHz, $CDCl_3$): 9.04 (1H, s, N-H), 8.78 (1H, s, H-2), 8.10 (1H, s, H-8), 8.06-8.00 (2H, m, ArH), 7.65-7.57 (1H, m, ArH), 7.56-7.50 (2H, m, ArH), 7.44-7.36 (4H, m, ArH), 7.25-7.08 (5H, m, ArH, H-5'), 6.50 (1H, app dd, *J* 7.1, 5.9, H-1'), 6.35 (1H, ddd, *J* 22.6, 17.1, 1.80, H-6'), 4.61-4.56 (1H, m, H-4'), 4.55-4.51 (1H, m, H-3'), 2.87 (1H, ddd, *J* 13, 7.1, 6.0, H_A-2'), 2.44 (1H, ddd, *J* 13, 5.9, 3.7, H_B-2'), 0.92 (9H, $SiC(CH_3)_3$), 0.11 (6H, s, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 164.7 (CO), 153.0 (CH-2), 151.7 (C-4), 151.7 (d, *J* 5.9, CH-5'), 149.8 (C-6), 146.3 (d, *J* 7.6, ArC), 146.2 (d, *J* 7.8, ArC), 141.7 (CH-8), 133.8 (ArC), 133.0 (ArCH), 130.8 (ArCH), 130.8 (ArCH), 129.0 (2 × ArCH), 128.1 (ArCH), 128.0 (ArCH), 128.0 (2 × ArCH), 126.44 (ArCH), 126.39 (ArCH), 125.9 (d, *J* 5.5, ArC), 125.9 (d, *J* 5.1, ArC), 123.8 (C-5), 122.6 (d, *J* 2.9, ArCH), 122.5 (d, *J* 2.8, ArCH), 116.6 (d, *J* 192.0, CH-6'), 86.9 (d, *J* 23.0, CH-4'), 85.2 (CH-1'), 75.3 (CH-3'), 39.4 (CH_2-2'), 25.9 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.58

($Si(CH_3)_2$), -4.63 ($Si(CH_3)_2$); δP (162 MHz, $CDCl_3$): 11.52; *m/z* (ESI^+) 766.1784 (M+H. $C_{36}H_{39}Cl_2N_5O_6PSi$ requires 766.1779), 788.1621 (M+Na. $C_{36}H_{38}Cl_2N_5NaO_6PSi$ requires 788.1598).

[(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl](2-chlorophenoxy)phosphinic acid **69**



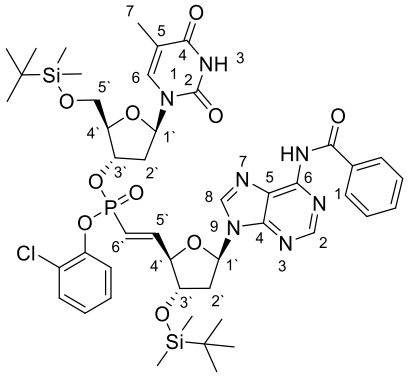
Chemical Formula: $C_{30}H_{35}ClN_5O_6PSi$
Exact Mass: 655.1783

Vinylphosphonate **68** (1.25 g, 1.63 mmol), 2-nitrobenzaldoxime (326 mg, 1.96 mmol), tetramethylguanidine (247 µL, 1.96 mmol), triethylamine (1.4 mL, 9.80 mmol) and dioxane (80 mL). The reaction mixture was stirred at RT for 3 d, then dry loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 7:3) to afford the desired product **69** (820 g, 78%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 2927, 2854, 1689, 1614, 1582, 1476, 1232, 1084, 1056, 939, 902; δH (400 MHz, CD_3OD): 8.71 (1H, s, H-2), 8.42 (1H, s, H-8), 8.07 (2H, d, *J* 7.7, ArH), 7.73-7.39 (4H, m, ArH), 7.30-7.20 (1H, m, ArH), 7.03 (1H, br, ArH), 6.95-6.83 (1H, m, ArH), 6.70-6.50 (1H, m, H-5'), 6.44 (1H, br, H-1'), 6.06-5.82 (1H, m, H-6'), 4.33 (2H, br, H-3', H-4'), 2.71 (1H, s, br, H_A-2'), 2.30 (1H, s, br, H_B-2'), 0.88 (9H, s, $SiC(CH_3)_3$), 0.05 (3H, s, $Si(CH_3)_2$), 0.02 (3H, s, $Si(CH_3)_2$); δ_C (101 MHz, CD_3OD): 168.2 (^{Bz}CO), 153.3 (CH-2), 153.1 (C-4), 151.0 (C-6), 150.1 (d, *J* 6.0, ArC), 144.7 (d, *J* 6.5, CH-5'), 141.7 (CH-8), 135.0 (ArC), 133.9 (ArCH), 133.1 (ArCH), 129.8 (ArCH), 129.5 (2 × ArCH), 128.6 (2 × ArCH), 126.7 (d, *J* 5.3,

ArC), 125.8 (d, *J* 180, CH-6′), 125.2 (ArCH), 123.6 (ArCH + C-5), 89.9 (d, *J* 21.6, CH-4′), 86.1 (CH-1′), 77.2 (CH-3′), 36.3 (CH_2-2′), 18.9 (2 × SiC(CH_3)$_3$), -4.6 (Si(CH_3)$_2$); *δ*P (162 MHz, CD_3OD): 5.94; *m/z* (ESI$^+$) 656.1855 (M+H. $C_{30}H_{36}ClN_5O_6PSi$ requires 656.1856).

5.3.1 Synthesis of T*A phosphoramidite **84**

(2*R*,3*S*,5R)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-chlorophenyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3[(tertbutyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]phosphonate **73**



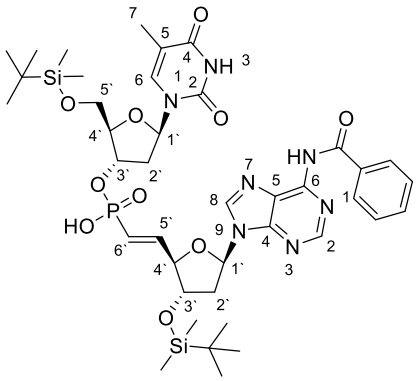
Chemical Formula: $C_{46}H_{61}ClN_7O_{10}PSi_2$
Exact Mass: 993.3445

A mixture of phosphonate **69** (820 mg, 1.25 mmol), alcohol **71** (490 mg, 1.38 mmol) and *N*-methylimidazole (640 µL, 8.12 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (3 mL). molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (947 mg, 3.13 mmol) were added. The reaction mixture was stirred at RT for 18 h, the volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1 → 1:4) to afford the desired product **73** (950 mg, 76%, 3:2 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2954, 2924, 2853, 1687, 1611, 1455, 1249, 1041, 1001; *δ*H (500 MHz, $CDCl_3$): 9.71 (0.3H, bs, NH), 9.57 (0.7H, bs, NH), 9.53 (0.7H, bs, NH), 9.50 (0.3H, b, NH), 8.82 (0.3H, s, A-H-2), 8.75

(0.7H, s, A-H-2), 8.20 (0.3H, s, A-H-8), 8.14 (0.7H, s, A-H-8), 8.09-7.05 (2H, m, ArH), 7.59-7.52 (1H, m, ArH), 7.52-7.43 (3H, m, ArH + T-H-6), 7.42-7.31 (2H, m, ArH), 7.25-7.20 (1H, m, ArH), 7.12 (1H, t, *J* 7.6, ArH), 7.10–6.99 (1H, m, A-H-5′), 6.52 (0.3H, t, *J* 6.6, A-H-1′), 6.47 (0.7H, t, *J* 6.6, A-H-1′), 6.39 (0.3H, dd, *J* 9.3, 5.2, T-H-1′), 6.32 (0.7H, dd, *J* 9.0, 5.2, T-H-1′), 6.19 (0.7H, ddd, *J* 21.4, 16.0, 1.3, A-H-6′), 6.13 (0.3H, ddd, *J* 21.5, 17.1, 1.8, A-H-6′), 5.27-5.17 (1H, m, T-H-3′), 4.63 (0.4H, dt, *J* 5.7, 3.6, A-H-3′), 4.59-4.51 (1.7H, m, A-H-4′ + A-H-3′), 4.2 8(0.7H, d, *J* 1.6, T-H-4′), 4.2 2(0.3H, d, *J* 1.5, T-H-4′), 3.88-3.7 2(2H, m, T-H_A-5′ + T-H_B-5′), 3.03-2.9 2(1H, m, A-H_A-2′), 2.56 (0.3H, dd, *J* 13.6, 5.3, A-H_B-2′), 2.52-2.39 (1.7H, m, A-H_B-2′ + T-H_A-2′), 2.15-2.04 (1H, m T-H_B-2′), 1.87 (3H, s, T-H-7), 0.90 (18H, SiC(CH_3)$_3$), 0.09 (12H, Si(CH_3)$_2$); δ_C (126 MHz, $CDCl_3$): 165.4 (CO), 165.2 (CO), 163.9 (T-C-4), 163.85 (T-C-4), 152.8 (A-CH-2), 152.7 (A-CH-2), 151.8 (2 × A-C-4), 151.2 (d, *J* 6.7, A-CH-5′), 150.8 (d, *J* 6.5, A-CH-5′), 150.4 (2 × T-C-2), 150.2 (A-C-6), 150.1 (A-C-6), 146.3 (d, *J* 6.8, ArC), 146.2 (d, *J* 6.7, ArC), 142.1 (A-CH-8), 141.9 (A-CH-8), 135.1 (T-CH-6), 135.0 (T-CH-6), 133.7 (ArC), 133.6 (ArC), 132.83 (ArCH), 132.76 (ArCH), 130.9 (2 × ArCH), 128.8 (2 × ArCH), 128.7 (2 × ArCH), 128.4 (2 × ArCH), 128.3 (2 × ArCH), 128.2 (ArCH), 128.1 (ArCH), 126.4 (ArCH), 126.3 (ArCH), 125.8 (2 × d, *J* 5.8, ArC), 124.0 (2 × A-C-5), 122.2 (d, *J* 2.3, ArCH), 122.1 (d, *J* 2.2, ArCH), 117.2 (d, *J* 192, T-CH-6′), 117.1 (d, *J* 192, T-CH-6′), 111.3 (T-C-5), 111.27 (T-C-5), 87.0 (d, *J* 22.6, A-CH-4′), 86.7 (d, *J* 22.5, A-CH-4′), 86.1 (d, *J* 4.6, T-CH-4′), 85.9 (d, *J* 4.5, T-CH-4′), 85.2 (A-CH-1′), 85.0 (A-CH-1′), 84.6 (2 × T-CH-1′), 78.1 (d, *J* 6.4, T-CH-3′), 78.0 (d, *J* 6.2, T-CH-3′), 75.3 (A-CH-3′), 75.2 (A-CH-3′), 63.3 (T-CH_2-5′), 63.2 (T-CH_2-5′), 39.9 (d, *J* 4.1, T-CH_2-2′), 39.7 (d, *J* 4.6, T-CH_2-2′), 39.2 (A-CH_2-2′), 39.0 (A-CH_2-2′), 26.0 (6 × SiC(*C*H_3)$_3$), 25.8 (6 × SiC(*C*H_3)$_3$), 18.4 (2 × Si*C*(CH_3)$_3$), 18.1 (2 × Si*C*(CH_3)$_3$), 12.6 (2 × T-CH_3-5), -4.5 (2 × Si(CH_3)$_2$), -4.6 (2 × Si(CH_3)$_2$), -5.3 (2 × Si(CH_3)$_2$), -5.4 (2 × Si(CH_3)$_2$); δP (162 MHz, $CDCl_3$): 15.26,

15.00; *m/z* (ESI$^+$) 994.3499 (M+H. $C_{46}H_{62}ClN_7O_{10}PSi_2$ requires 994.3517), 1016.3310 (M+Na. $C_{46}H_{61}ClN_7NaO_{10}PSi_2$ requires 1016.3337).

[(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]({[(2*R*,3*S*,5R)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl]oxy})phosphinic acid **76**

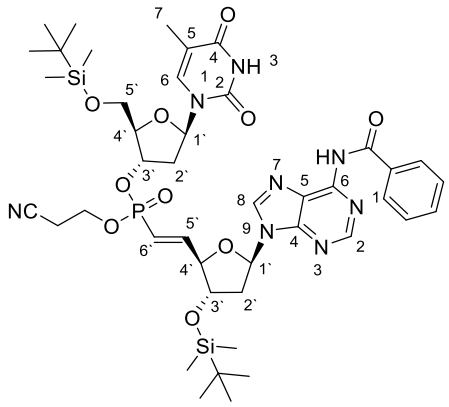


Chemical Formula: $C_{40}H_{58}N_7O_{10}PSi_2$
Exact Mass: 883.3521

Dinucleotide **73** (700 mg, 704 mmol), 2-nitrobenzaldoxime (176 mg, 1.06 mmol), tetramethylguanidine (133µL, 1.06 mmol), triethylamine (5.93 µL, 4.23 mmol) and dioxane (36 mL). The reaction mixture was stirred at RT for 4 d, then dry loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **76** (528 mg, 85%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 2951, 2928, 2855, 1685, 1581, 1517, 1461, 1250, 1061, 831; δH (400 MHz, CD_3OD): 8.74 (1H, s, A-H-2), 8.57 (1H, s, A-H-8), 8.15-8.03 (2H, m, ArH), 7.69-7.62 (1H, m, ArH), 7.60-7.51 (3H, m, T-H-6, ArH), 6.70-6.52 (2H, m, A-H-5′ + A-H-1′), 6.26-6.18 (1H, m, T-H-1′), 5.96 (1H, t, *J* 17.1, A-H-6′), 4.74-4.66 (2H, m, A-H-3′ + T-H-3′), 4.62-4.55 (1H, s br, A-H-4′), 4.51-4.43 (1H, br, T-H-4′), 3.84-3.72 (2H, m, T-H_A-5′ + T-H_B-5′), 3.07-2.94 (1H, m, A-H_A-2′), 2.55-2.45 (1H, m, A-H_B-2′), 2.42 (1H, dd, *J* 13.3, 5.3, T-H_B-2), 2.06 (1H, ddd, *J* 13.9, 8.9, 5.7, T-H_A-2′), 1.84 (3H, d, *J* 1.2, T-H-7), 0.96 (9H, s, SiC(CH_3)$_3$), 0.87

(9H, s, SiC(CH_3)$_3$), 0.17 (6H, s, Si(CH_3)$_2$), 0.06 (3H, s, Si(CH_3)$_2$), 0.05 (3H, s, Si(CH_3)$_2$); δc (126 MHz, CD_3OD): 168.3 (CO), 166.2 (T-C-4), 153.5 (A-CH-2), 153.3 (A-C-4), 152.1 (T-C-2), 150.8 (A-C-6), 145.0 (A-CH-8), 143.5 (d, *J* 4.6, A-CH-5′), 137.2 (T-CH-6), 135.0 (ArC), 133.9 (ArCH), 129.7 (2 × ArCH), 129.4 (2 × ArCH), 127.0 (d, *J* 177.2, A-CH-6′), 125.1 (A-C-5), 111.3 (T-C-5), 89.2 (d, *J* 20.8, A-CH-4′), 88.3 (d, *J* 4.5, T-CH-4′), 86.4 (T-CH-1′), 85.9 (A-CH-1′), 77.1 (A-CH-3′), 76.1 (d, *J* 4.7, T-CH-3′), 64.7 (T-CH_2-5′), 41.0 (d, *J* 3.6, T-CH_2-2′), 40.4 (A-CH_2-2′), 26.5 (3 × SiC(*CH_3*)$_3$), 26.3 (3 × SiC(*CH_3*)$_3$), 19.2 (Si*C*(CH_3)$_3$), 18.9 (Si*C*(CH_3)$_3$), 12.7 (T-CH_3-7), -4.5 (Si(CH_3)$_2$), -4.5 (Si(CH_3)$_2$), -5.2 (Si(CH_3)$_2$), -5.3 (Si(CH_3)$_2$); δP (162 MHz, CD_3OD): 9.28; *m/z* (ESI$^+$) 884.3582 (M+H. $C_{40}H_{59}N_7O_{10}PSi_2$ requires 884.3594), 906.3371 (M+Na. $C_{40}H_{58}N_7NaO_{10}PSi_2$ requires 906.3414).

(2*R*,3*S*,5R)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]phosphonate **78**

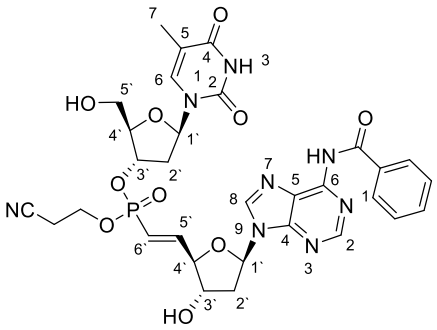


Chemical Formula: $C_{43}H_{61}N_8O_{10}PSi_2$
Exact Mass: 936.3787

A mixture of dinucleotide **76** (800 mg, 905 µmol), 3-hydroxypropionitrile (310µL, 4.53 mmol) and *N*-methylimidazole (466 µL, 5.88 mmol) was dried by azeotropic removal of water with pyridine (3 × 8 mL). The residue was dissolved in pyridine (4.6 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (686 mg, 2.26 mmol) were added. The reaction mixture was stirred at RT for 19 h, the volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1)→ ethyl acetate → ethyl acetate : methanol (9:1) to afford the desired product **78** (655 mg, 77%,1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2951, 2928, 2855, 1686, 1607, 1511, 1248, 1000, 831; δH (400 MHz, $CDCl_3$) 9.89 (0.5H, bs, NH), 9.81 (0.5H, bs, NH), 9.75 (0.5H, bs, NH), 9.68 (0.5H, bs, NH), 8.88 (1H, s, A-H-2), 8.24 (0.5H, s, A-H-8), 8.23 (0.5H, s, AH- 8), 8.11-7.97 (2H, m, ArH), 7.61-7.52 (1H, m, ArH), 7.50-7.41 (3H, m, ArH + T-H-6), 7.03 (0.5H, ddd, *J* 23.3, 17.0, 4.4, A-H-5′), 6.97 (0.5H, ddd, *J* 23.1, 17.0, 4.3, A-H-5′), 6.55-6.48 (1H, m A-H-1′), 6.33 (0.5H, dd, *J* 9.1, 5.2, T-H-1′), 6.28 (0.5H, dd, *J* 9.0, 5.3, T-H-1′), 6.00 (0.5H, ddd, *J* 20.8, 17.0, 1.8,

A-H-6′), 5.97 (0.5H, ddd, *J* 20.8, 17.0, 1.8, A-H-6′), 5.10-4.09 (1H, m, T-H-3′), 4.69-4.63 (1H, m, A-H-3′), 4.59-4.54 (1H, m, A-H-4′), 4.27-4.11 (3H, m, T-H-4′ + C*H*$_2$CH$_2$CN), 3.88-3.76 (2H, m, T-H$_A$-5′ + T-H$_B$-5′), 3.07-2.96 (1H, m, A-H$_A$-2′), 2.82-2.68 (2H, m, CH$_2$C*H*$_2$CN), 2.55-2.39 (2H, m, A-H$_B$-2′ + T-H$_A$-2′), 2.14-2.07 (1H, m, T-H$_B$-2′), 1.87 (3H, T-H-7), 0.90 (18H, SiC(CH$_3$)$_3$), 0.10 (12H, Si(CH$_3$)$_2$); *δ*c (126 MHz, CDCl$_3$): 165.4 (CO), 165.3 (CO), 164.0 (2 × T-C-4), 152.7 (A-CH-2), 152.6 (A-CH-2), 152.0 (2 × A-C-4), 150.8 (d, *J* 6.8, A-CH-5′), 150.6 (2 × T-C-2), 150.1 (d, *J* 6.3, A-CH-5′), 150.2 (2 × A-C-6), 142.2 (A-CH-8), 142.1 (A-CH-8), 135.1 (2 × T-CH-6), 133.7 (ArC), 133.6 (ArC), 132.9 (ArCH), 132.8 (ArCH), 128.73 (2 × ArCH), 128.67 (2 × ArCH), 128.4 (2 × ArCH), 128.36 (2 × ArCH), 124.1 (A-C-5), 124.0 (A-C-5), 116.9 (2 × d, *J* 190, T-CH-6′), 116.8 (CN), 116.5 (CN), 111.3 (2 × T-C-5), 87.0 (d, *J* 22.3, 2 × A-CH-4′), 86.0 (d, *J* 4.6, T-CH-4′), 85.9 (d, *J* 5.4, T-CH-4′), 85.1 (A-CH-1′), 85.0 (A-CH-1′), 84.7 (T-CH-1′), 84.6 (T-CH-1′), 77.7 (d, *J* 5.6, T-CH-3′), 77.3 (d, *J* 6.0, T-CH-3′), 75.24 (A-CH-3′), 75.2 (A-CH-3′), 63.4 (T-CH$_2$-5′), 63.2 (T-CH$_2$-5′), 60.7 (d, *J* 4.9, *CH*$_2$CH$_2$CN), 60.4 (d, *J* 4.6, *CH*$_2$CH$_2$CN), 39.7 (d, app *J* 3.8, T-CH$_2$-2′), 39.7 (d, app *J* 3.8, T-CH$_2$-2′), 39.1 (A-CH$_2$-2′), 39.0 (A-CH$_2$-2′), 26.0 (6 × SiC(*C*H$_3$)$_3$), 25.8 (6 × SiC(*C*H$_3$)$_3$), 20.1 (d, app, *J* 7.0, 2 × CH$_2$*C*H$_2$CN), 18.4 (2 × Si*C*(CH$_3$)$_3$), 18.1 (2 × Si*C*(CH$_3$)$_3$), 12.6 (2 × T-CH$_3$-7), -4.5 (2 × Si(CH$_3$)$_2$), -4.6 (2 × Si(CH$_3$)$_2$), -5.3 (2 × Si(CH$_3$)$_2$), -5.4 (2 × Si(CH$_3$)$_2$); *δ*P (162 MHz, CDCl$_3$): 18.82, 18.70; *m/z* (ESI$^+$) 937.3845 (M+H. $C_{43}H_{62}N_8O_{10}PSi_2$ requires 937.3860), 959.3655 (M+Na. $C_{43}H_{61}N_8NaO_{10}PSi_2$ requires 959.3679).

2-cyanoethyl (2*R*,3*S*,5R)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxyoxolan-2-yl]ethenyl]phosphonate **80**

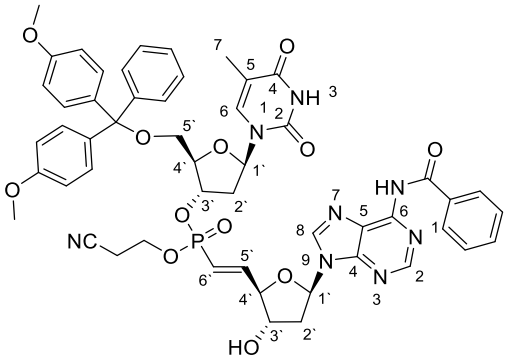


Chemical Formula: $C_{31}H_{33}N_8O_{10}P$
Exact Mass: 708.2057

Triethylamine trihydrofluoride (1.1 mL, 6.94 mmol) was added in one portion to a stirring solution of dinucleotide **78** (650 mg, 694 µmol, 3:2 mixture of diastereoisomers) in THF (11.5 mL) and the resulting reaction mixture was stirred at RT for 4 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane : methanol (9:1 → 4:1) to afford the desired product **80** (365 mg, 74%, 3:2 mixture of diastereoisomers) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 3324, 2952, 1682, 1610, 1453, 1239, 997; δH (400 MHz, CD_3OD): 8.7 5(0.4H, s, A-H-2), 8.7 4 (0.6H, s, A-H-2), 8.5 5(0.6H, s, A-H-8), 8.5 4(0.4H, s, A-H-8), 8.10-8.0 3(2H, m, ArH), 7.76-7.7 2(1H, m T-H-6), 7.67-7.6 1(1H, m, ArH), 7.57-7.5 0(2H, m, ArH), 7.17-7.1 2(1H, m, A-H-5′), 6.64-6.5 7(1H, m, A-H-1′), 6.32-6.2 3(1H, m, T-H-1′), 6.12-5.9 6(1H, A-H-6′), 5.14-5.05 (1H, m, T-H-3′), 4.77-4.70 (1H, m, A-H-3′), 4.63-4.5 8(1H, m, A-H-4′), 4.27-4.19 (2H, m, C*H*$_2$CH$_2$CN), 4.17 (0.6H, q, *J* 3.0, T-H-4′), 4.11 (0.4H, q, *J* 3.0, T-H-4′), 3.79-3.72 (2H, m, T-H$_A$-5′ + T-H$_B$-5′), 3.15-3.04 (1H, m, A-H$_A$-2′), 2.92-2.84 (2H, m, CH$_2$C*H*$_2$CN), 2.59-2.30 (3H, m, A-H$_B$-2′ + T-H$_A$-2′ + T-H$_B$-2′), 1.84 (1.4H, d, *J* 1.2, T-H-7), 1.83 (1.6H, d, *J* 1.2, T-H-7); δc (101 MHz, CD_3OD): 168.1 (2 × CO), 166.3 (2 × T-C-4), 153.3 (2 × A-CH-2), 153.2 (2 × A-C-4), 153. 1(d, *J* 6.0, A-CH-5′), 152.9 (d, *J* 5.9, A-CH-5′), 152.3 (2 × A-C-6), 151.2 (2 × T-C-2), 144.8 3(A-CH-8), 144.78

(A-CH-8), 137.9 (2 × T-CH-6), 134.9 (2 × ArC), 133.9 (2 × ArCH), 129.7 (4 × ArCH), 129.4 (4 × ArCH), 125.6 (2 × A-C-5), 118.7 (CN), 118.6 (CN), 117.2 (2 × d, *J* 189.0, A-CH-6′), 111.80 (T-C-5), 111.78 (T-C-5), 88.0 (2 × d, *J* 22.0, A-CH-4′), 87.2 (d, *J* 5.5, T-CH-4′), 87.2 (d, *J* 5.6, T-CH-4′), 86.5 (2 × A-CH-1′), 86.13 (T-CH-1′), 86.08 (T-CH-1′), 78.5 (d, *J* 5.7, T-CH-3′), 78.3 (d, *J* 5.8, T-CH-3′), 75.4 (2 × A-CH-3′), 62.6 (d, *J* 5.1, *C*$H_2$$CH_2$CN), 62.5 (d, *J* 5.1, *C*$H_2$$CH_2$CN), 62.4 (T-$CH_2$-5′), 63.3 (T-$CH_2$-5′), 39.71 (app d, *J* 3.5, T-CH_2-2′), 39.7 (app d, *J* 3.4, T-CH_2-2′), 39.3 (2 × A-CH_2-2′), 20.3 (d, *J* 7.0, CH_2*C*H_2CN), 20.3 (d, *J* 6.5, CH_2*C*H_2CN), 12.5 (2 × T-CH_3-7); *δ*P (162 MHz, CD_3OD): 18.93, 18.92; *m/z* (ESI$^+$) 709.2134 (M+H. $C_{31}H_{34}N_8O_{10}P$ requires 709.2130), 731.1958 (M+Na. $C_{31}H_{33}N_8NaO_{10}P$ requires 731.1949).

(2*R*,3*S*,5R)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxyoxolan-2-yl]ethenyl]phosphonate **82**



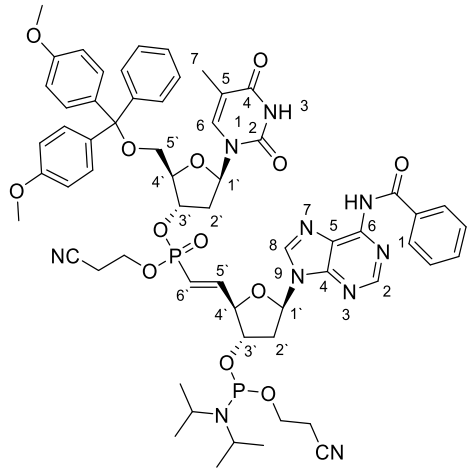
Chemical Formula: $C_{52}H_{51}N_8O_{12}P$
Exact Mass: 1010.3364

Dinucleotide **80** (400 mg, 564 µmol, 3:2 mixture of diastereoisomers) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.7 mL). Molecular sives and DMTrCl (230 mg, 677 µmol) were added in one portion under argon gas and the reaction mixture was stirred at RT for 23 h. The volatiles were removed *in vacuo* and the residue purified by

column chromatography dichloromethane : methanol (9:1) to afford the desired product **82** (380 mg, 67%, 3:2 mixture of diastereoisomers) and recovered starting material **80** (100 mg, 22%) as white powder. FTIR (ATR) $\nu_{max/}cm^{-1}$: 2960, 2930, 1685, 1606, 1508, 1294, 999; δH (400 MHz, $CDCl_3$): 10.5 (0.6H, bs, NH), 10.3 (0.4H, bs, NH), 9.72 (1H, bs, NH), 8.77 (1H, s, A-H-2), 8.24 (0.6H, s, A-H-8), 8.23 (0.4H, s, A-H-8), 8.06-7.98 (2H, m, ArH), 7.58-7.51 (2H, m, ArH + T-H-6), 7.46 (2H, q, *J* 8.0 ArH), 7.38-7.28 (3H, m, ArH), 7.25-7.17 (7H, m, ArH), 7.13-6.96 (1H, m, A-H-5′), 6.86-6.78 (4H, m, ArH), 6.57-6.50 (1H, m, A-H-1′), 6.40-6.32 (1H, m, T-H-1′), 6.02-5.88 (1H, m, A-H-6′), 5.21- 5.08 (1H, m, T-H-3′), 4.83-4.76 (0.6H, m, A-H-3′), 4.69-4.66 (0.4H, m, A-H-3′), 4.62-4.58 (0.6H, m, A-H-4′), 4.55-4.50 (0.4H, m, A-H-4′), 4.21-3.96 (3H, m, T-H-4′ + C*H*$_2$CH$_2$CN), 3.75 (6H, s, ArOCH$_3$), 3.54-3.42 (1H, m, T-H$_A$-5′), 3.36-3.28 (1H, m, T-H$_B$-5′), 2.99-2.85 (1H, m, A-H$_A$-2′), 2.75 (1H, t, *J* 6.0, CH$_2$C*H*$_2$CN), 2.63-2.50 (3H, m, CH$_2$C*H*$_2$CN + T-H$_A$-2′ + A-H$_B$-2′), 2.46-2.33 (1H, m, T-H$_B$-2′), 1.38 (1.6H, br s, T-H-7), 1.36 (1.4H, br s, T-H-7); δc (121 MHz, $CDCl_3$): 166.55 (CO), 166.51 (CO), 164.3 (T-C-4), 164.2 (T-C-4), 158.9 (2 × ArC), 158.86 (ArC), 158.84 (ArC), 152.6 (2 × A-CH-2), 152.0 (A-C-4), 151.9 (A-C-4), 151.1 (d, *J* 6.0, A-CH-5′), 150.5 (d, *J* 5.7, A-CH-5′), 150.9 (T-C-2), 150.8 (T-C-2), 150.0 (2 × A-C-6), 144.2 (ArC), 144.1 (ArC), 142.3 (A-CH-8), 142.2 (A-CH-8), 135.5 (2 × T-CH-6), 135.3 (2 × ArC), 135.26 (ArC), 135.1 (ArC), 133.6 (ArC), 133.6 (ArC), 132.9 (2 × ArCH), 130.3 (8 × ArCH), 128.8 (4 × ArCH), 128.4 (2 × ArCH), 128.3 (2 × ArCH), 128.2 (2 × ArCH), 128.2 (2 × ArCH), 127.4 (2 × ArCH), 123.9 (2 × A-C-5), 117.1 (CN), 116.9 (d, *J* 190.7, A-CH-6′), 116.7 (CN), 116.4 (d, *J* 189.7, A-CH-6′), 113.5 (8 × ArCH), 111.8 (2 × T-C-5), 87.4 (CAr$_3$), 87.3 (CAr$_3$), 86.4 (2 × d, *J* 22.4, A-CH-4′), 84.8 (2 × A-CH-1′), 84.7 (2 × T-CH-1′), 85.6 (2 × T-CH-4′), 77.5 (2 × d, *J* 5.5, T-CH-3′), 74.2 (2 × A-CH-3′), 63.3 (2 × T-CH$_2$-5′), 60.9 (d, *J* 5.3, *C*H$_2$CH$_2$CN), 61.6 (d, *J* 5.3, *C*H$_2$CH$_2$CN), 55.4 (4 × ArOCH$_3$), 39.4 (T-CH$_2$-2′),

38.7 (A-CH_2-2′), 20.0 (d, *J* 6.5, CH_2CH_2CN), 19.9 (d, *J* 6.5, CH_2CH_2CN), 11.8 (T-CH_3-7), 11.77 (T-CH_3-7); δP (162 MHz, $CDCl_3$): 18.8, 18.7; *m/z* (ESI^+) 1011.3437 (M+H. $C_{52}H_{52}N_8O_{12}P$ requires 1011.3437), 1033.3246 (M+Na. $C_{52}H_{51}N_8NaO_{12}P$ requires 1033.3256).

(2*R*,3*S*,5R)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-({[bis(propan-2-yl)amino](2-cyanoethoxy)phosphanyl}oxy)oxolan-2-yl]ethenyl]phosphonate **84**



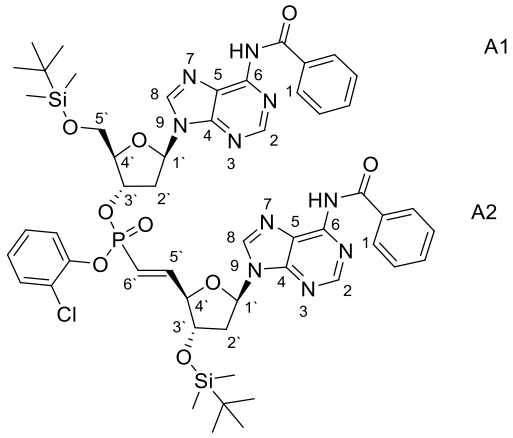
Chemical Formula: $C_{61}H_{68}N_{10}O_{13}P_2$
Exact Mass: 1210.4443

To a mixture of compound **84** (480 mg, 475 µmol) and phosphordiamidite **52** (181 µL, 570 µmol) in dry dichloromethane (7 mL), a solution of (5-Methyl-1*H*-tetrazole 48 mg, 570 µmol in dry acetonitrile) (1 mL) was added in one portion and the resultant mixture was stirred at room temperature for 20 h. The solution was diluted with acetonitrile (12 mL), quenched with TEA (240 µL) and water (240 µL) and washed with n-hexane (24 mL). After separation the dichloromethane/acetonitrile layer was diluted with ether (50 mL), washed with water (70 mL) and brine (50 mL), dried over Na_2SO_4 and the volatiles were removed and the crude product was triturated with pentane to afford phosphoramidite **84** (387 mg, 67%) was obtained as a mixture of 4 diastereoisomers. FTIR (ATR) ν_{max}/cm^{-1}: 2964, 2930, 1686, 1605, 1507,

1246,1176, 999, 791; δ_H (400 MHz, $CDCl_3$): 9.52-9.26 (1H, br.s, NH), 9.04-8.50 (2H, m, br.s, NH, A-H-2), 8.26-8.13 (1H, A-H-2), 8.07-7.93 (2H, ArH), 7.64-7.33 (4H, m, 3 × ArH + T-H-6), 7.38-7.32 (2H, m, ArH), 7.30-7.19 (7H, m, ArH), 7.15-6.94 (1H, m, A-H-5′), 6.87-6.78 (4H, m, ArH), 6.59-6.49 (1H, m, H-1′), 6.46-6.34 (1H, m, H-1′), 6.08-5.82 (1H, m, A-CH-6′), 5.25-5.12 (1H, m, T-H-3′), 5.01-4.58 (2H, m, A-H-3′ + A-H-4′), 4.25-3.87 (4H, m, T-H-4′ + 3 × O*C*H_2CH_2CN), 3.81-3.73 (7H, m, O*C*H_2CH_2CN + 2 × ^{DMT}OMe), 3.70-3.31 (4H, m, 2 × C*H*$(CH_3)_2$ + A-H_A-5′ + A-H_B-5′), 3.17-2.98 (1H, m, OC*H*$_2CH_2CN$), 2.83-2.33 (7H, m, 4 × H-2′ + 3 × OC*H*$_2CH_2CN$), 1.88-1.64 (3H, s, H-7), 1.26-1.15 (12H, m, 2 × CH(C*H*$_3)_2$); δ_c (126 MHz, $CDCl_3$): 165.3 (C), 165.2 (C), 165.0 (2 × C), 163.6-163.5 (4 × C), 159.0-158.8 (8 × ^{DMT}ArC), 153.0-152.7 (4 × A-CH-2), 151.99-151.88 (4 × C), 150.7-149.9 (4 × A-CH-5′ + 8 × C), 144.3-144.0 (4 × ^{DMT}ArC), 142.1-141.9 (4 × A-CH-8), 135.6-135.3 (4 × T-CH-6), 135.3-135.0 (8 × ^{DMT}ArC), 133.8-133.6 (4 × ^{Bz}ArC), 133.0-132.8 (4 × ArCH), 130.4-130.2 (16 × ArCH), 129.0-128.7 (8 × ArCH), 128.4-128.0 (24 × ArCH), 127.5-127.3 (4 × ArCH), 124.0-123.8 (4 × A-C-5), 118.1-115.8 (4 × A-CH-6′ + 8 × CN), 113.46 (16 × ArCH), 111.8-111.7 (4 × T-C-5), 87.4 (4 × ^{DMT}CAr_3), 86.5-84.2 (4 × T-CH-1′ + 4 × A-CH-1′ + 4 × A-CH-4′ + 4 × T-CH-4′), 77.5-77.0 (4 × T-CH-3′), 76.5-75.7 (4 × A-CH-3′), 63.4-63.2 (T-CH-5′), 60.9-60.4 (4 × O*C*H_2CH_2CN), 5.82 (4 × d, *J* 19.7, O*C*H_2CH_2CN), 55.5-55.3 (8 × ^{DMT}OMe), 43.7-43.4 (8 × *C*H$(CH_3)_2$), 39.6-39.3 (4 × CH_2-2′), 38.2-37.9 (4 × CH_2-2′), 24.9-24.6 (8 × CH(*C*$H_3)_2$), 20.7-20.5 (4 × OCH$_2$*C*H_2CN), 20.0 (2 × d, *J* 6.0, OCH$_2$*C*H_2CN), 19.8 (2 × d, *J* 6.0, OCH$_2$*C*H_2CN), 11.88-11.67 (4 × T-CH_3-7); δP (162 MHz, $CDCl_3$): 149.50, 149.38, 149.01, 148.96, 18.86, 18.84, 18.70, 18.60; *m/z* (ESI$^+$) 1211.4468 (M+H. $C_{61}H_{69}N_{10}O_{13}P_2$ requires 1211.4515), 1233.4289 (M+Na. $C_{61}H_{68}N_{10}NaO_{13}P_2$ requires 1233.4335).

5.3.2 Synthesis of A*A phosphoramidite **140**

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[(tertbutyldimethylsilyl)oxy]methyl}oxolan-3-yl 2-chlorophenyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]phosphonate **119**



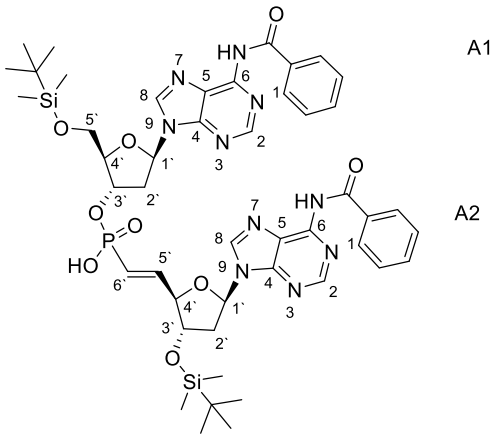
Chemical Formula: $C_{53}H_{64}ClN_{10}O_9PSi_2$

A mixture of phosphonate **69** (600 mg, 920 µmol), alcohol **71** (432 mg, 920 µmol) and *N*-methylimidazole (474 µL, 5.98 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (2 mL). molecular sieves and 2,4,6-triisopropylbenzenesulfonyl chloride (332 mg, 1.10 mmol) were added under argon. The reaction mixture was stirred at RT for 16 h, then it was diluted with ethyl acetate (100 mL) and quenched with 10% citric acid (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 100), and the combined organic layers were dried over anhydrous Na_2SO_4, the volatiles were removed by reduced pressure. The residue was then purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate) to afford the desired product **119** (755 g, 75%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2952, 2928, 2855, 1695, 1607, 1579, 1510, 1477, 1453, 1404, 1249, 1068, 833; δH (400 MHz, $CDCl_3$): 9.26 (0.4H, s, N-H), 9.17 (0.6H, s, NH), 9.15 (1H, s, NH), 8.76 (0.4H, s, A-H-2), 8.74 (0.6H, s, A-H-2), 8.72 (1H, s, A-H-2), 8.33 (0.4H, s, A_1-H-8),

8.29 (0.6H, s, A_1-H-8), 8.14 (0.4H, s, A_2-H-8), 8.10 (0.6H, s, A_2-H-8), 8.02-7.97 (4H, m, ArH), 7.61-7.53 (2H, m, ArH), 7.52-7.54 (2H, m, ArH), 7.43-7.35 (4H, m, ArH), 7.25-7.19 (1H, m, ArH), 7.15-6.99 (2H, m, ArH + A_2-H-5′), 6.63 (0.4H, dd, *J* 8.3, 5.8, A_1-H-1′), 6.55-6.44 (16H, m, 0.6 × A_1-H-1′ + A_2-H-1′), 6.28-6.10 (1H, m, A_2-H-6′), 5.48-5.33 (1H, m, A_1-H-3′), 4.64 (0.4H, dt, *J* 5.8, 3.7, A_2-H-3′), 4.60-4.53 (1.6H, m, 0.5 × A_2-H-3′ + A_2-H-4′), 4.37 (0.6H, q, *J* 2.9, A_1-H-4′), 4.33 (0.4H, q, *J* 2.6, A_1-H-4′), 3.90-3.75 (2H, m, A_1-H-5′), 3.07-2.73 (3H, m, A_1-H_A-2′, A_1-H_B-2′, A_2-H_A-2′), 2.50-2.39 (1H, m, A_2-H_B-2′), 0.93-0.90 (9H, m, $SiC(CH_3)_3$), 0.86-0.82 (9H, m, $SiC(CH_3)_3$), 0.14-0.10 (6H, m, $(Si(CH_3)_2$), 0.06-0.03 (6H, m, $(Si(CH_3)_2$); *δ*c (126 MHz, $CDCl_3$): 164.9 (^{Bz}CO), 164.8 (3 × ^{Bz}CO), 152.8 (4 × CH-2), 151.8 (C-4), 151.7 (C-4), 151.61 (2 × C-4), 151.58 (2 × C-4), 151.1 (d, *J* 6.5, A_2-CH-5′), 150.9 (d, *J* 6.5, A_2-CH-5′), 149.93 (C-6), 149.89 (C-6), 149.62 (C-6), 149.59 (C-6), 146.25 (d, *J* 6.7, ArC), 146.21 (d, *J* 6.6, ArC), 141.96 (A_2-CH-8), 141.92 (A_2-CH-8), 141.25 (A_1-CH-8), 141.22 (A_1-CH-8), 133.8 (2 × ArC), 133.6 (2 × ArC), 132.9 (2 × ArCH), 132.8 (2 × ArCH), 130.9 (2 × ArCH), 129.0 (8 × ArCH), 128.0 (8 × ArCH), 126.4 (ArCH), 126.3 (ArCH), 125. 80 (d, *J* 5.8, ArC), 125.75 (d, *J* 5.8, ArC), 124.04 (A_2-C-5), 123.97 (A_2-C-5), 123.3 (2 × A_1-C-5), 122.2 (d, *J* 2.7, ArCH), 122.1 (d, *J* 2.5, ArCH), 117.3 (d, *J* 191.6, A_2-CH-6′), 117.0 (d, *J* 191.3, A_2-CH-6′), 87.1 (d, *J* 22.7, A_2-CH-4′), 86.6 (d, *J* 23.0, A_2-CH-4′), 86.6 (2 × A_1-CH-4′), 85.22 (A_2-CH-1′), 85.14 (A_2-CH-1′), 84.5 (A_1-CH-1′), 84.4 (A_1-CH-1′), 77.9 (d, *J* 6.2, A_1-CH-3′), 77.8 (d, *J* 6.3, A_1-CH-3′), 75.3 (d, *J* 1.7, A_2-CH-3′), 75.2 (d, *J* 1.7, A_2-CH-3′), 63.2 (A_1-CH_2-5′), 63.16 (A_1-CH_2-5′), 40.5 (d, *J* 3.9, A_1-CH_2-2′), 40.2 (d, *J* 3.9, A_1-CH_2-2′), 39.3 (A_2-CH_2-2′), 39.1 (A_2-CH_2-2′), 26.0 (2 × $SiC(CH_3)_3$), 25.8 (2 × $SiC(CH_3)_3$), 18.4 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.57 ($Si(CH_3)_2$), -4.6 ($Si(CH_3)_2$), -4.64 (2 × $Si(CH_3)_2$), -5.31 ($Si(CH_3)_2$), -5.33 ($Si(CH_3)_2$), -5.44 (2 × $Si(CH_3)_2$); *δ*P (162 MHz, $CDCl_3$): 15.34, 14.89; *m/z*

(ESI$^+$) 1107.3867 (M+H. $C_{53}H_{65}ClN_{10}O_9PSi_2$ requires 1107.3895), 1129.3663 (M+Na. $C_{53}H_{65}ClN_{10}NaO_9PSi_2$ requires 1129.3715).

{[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-3-yl]oxy}[(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]phosphinic acid **144**

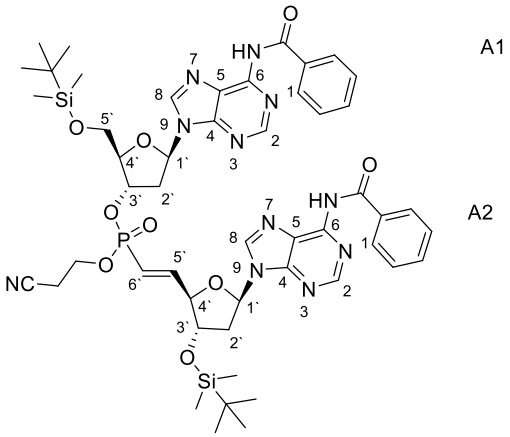


Chemical Formula: $C_{47}H_{61}N_{10}O_9PSi_2$

Dinucleotide **119** (1.50 g, 1.36 mmol), 2-nitrobenzaldoxime (337 mg, 2.03 mmol), tetramethylguanidine (254 µL, 2.03 mmol), triethylamine (1.15 mL, 8.16 mmol) and dioxane (75 mL). The reaction mixture was stirred at RT for 4 d, then dry loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **144** (1.16 g, 86%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1}: 2951, 2928, 2855, 1655, 1609, 1459, 1249, 1404, 1216, 1063; δH (400 MHz, CD_3OD): 8.80-8.76 (2H, m, A-H-2), 8.6-8.47 (2H, m, A-H-8), 8.05 (4H, t, *J* 8.9, ArH), 7.68-7.57 (2H, m, ArH), 7.56-7.45 (4H, m, ArH), 6.75-6.42 (3H, m, A_2-H-5′ + A_1-H-1′ + A_2-H-1′), 6.00 (1H, t, *J* 17.0, A_2-6′), 4.92-4.82 (1H, br. s, A_1-H-3′), 4.73-4.62 (1H, br s, A_2-H-3′), 4.51-4.39 (1H, br s, A_2-H-4′), 4.29-4.11 (1H, br s, A_1-H-4′), 3.91-3.74 (2H, m, A_1-H-5′), 3.05-2.91 (1H, m, A_2-H_A-2′), 2.80-2.62 (2H, m, A_1-H_A-2′ + A_1-H_B-2′), 2.51-2.38 (1H, m, A_2-H_B-2′), 0.93

(9H, s, $SiC(CH_3)_3$), 0.81 (9H, s, $SiC(CH_3)_3$), 0.14 (6H, ($Si(CH_3)_2$), 0.01 (6H, ($Si(CH_3)_2$); δc (126 MHz, $CDCl_3$): 168.0 (2 × ^{Bz}CO), 153.3 (2 × CH-2), 151.61 (2 × C-4), 151.1 (C-6), 150.9 (C-6), 144.6 (CH-8), 143.8 (CH-8),143.6 (d, *J* 5.0, A_2-CH-5′), 150.9 (d, *J* 6.5, A_2-CH-5′), 134.9 (2 × ArC), 133.8 (2 × ArCH), 129.7 (4 × ArCH), 129.4 (4 × ArCH), 126.9 (d, *J* 177.5, A_2-CH-6′), 125.2 (C-5), 125.0 (C-5), 89.2 (d, *J* 20.0, A_2-CH-4′), 88.7 (d, *J* 5.4, A_1-CH-4′), 88.3 (CH-1′), 86.1 (CH-1′), 77.1 (A_2-CH-3′), 75.7 (d, *J* 4.8, A_1-CH-3′), 64.6 (A_1-CH_2-5′), 41.3 (d, *J* 2.4, A_1-CH_2-2′), 40.4 (A_2-CH_2-2′), 26.5 ($SiC(CH_3)_3$), 26.3 ($SiC(CH_3)_3$), 19.2 ($SiC(CH_3)_3$), 19.9 ($SiC(CH_3)_3$), -4.46 ($Si(CH_3)_2$), -4.51 ($Si(CH_3)_2$), -5.26 ($Si(CH_3)_2$), -5.21 ($Si(CH_3)_2$; δP (162 MHz, CD_3OD): 9.51; *m/z* (ESI^+) 997.3942 (M+H. $C_{47}H_{62}N_{10}O_9PSi_2$ requires 997.3972), 1019.3732 (M+Na. $C_{47}H_{61}N_{10}NaO_9PSi_2$ requires 1019.3791).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-[(*tert*-butyldimethylsilyl)oxy]oxolan-2-yl]ethenyl]phosphonate **145**



Chemical Formula: $C_{50}H_{64}N_{11}O_9PSi_2$
Exact Mass: 1049.4165

Method A

Phosphonate **69** (300 g, 500 µmol), alcohol **71** (235 mg, 500 µmol) and *N*-methylimidazole (257 µL, 3.25 mmol) were dissolved in pyridine (1 ml), and

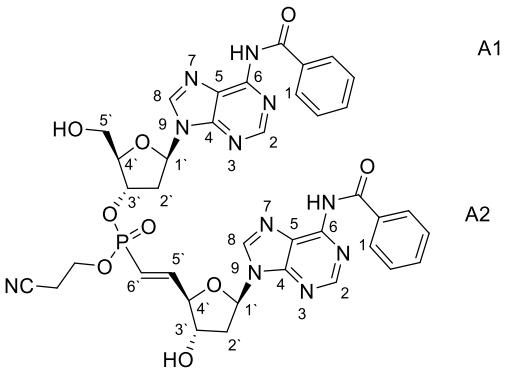
the water was removed by azeotropic with pyridine (3 × 3 mL), Triisopropylbenzenesulfonyl chloride (151 mg, 500 µmol) was added, the resulting mixture was then stirred at room temperature for 26 h. The solvent was evaporated and the residue was dry-loaded onto silica gel and purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1)→ ethyl acetate) to afford the desired product **145** (530 mg, quantitative, 1:1 mixture of diastereoisomers) as a white foam.

Method B

A mixture of dinucleotide **144** (500 mg, 747 µmol), 3-hydroxypropionitrile (255 µL, 3.74 mmol) and *N*-methylimidazole (384 µL, 4.85 mmol) was dried by azeotropic removal of water with pyridine (3 × 3 mL). The residue was dissolved in pyridine (1.5 mL), molecular sieves and 2,4,6-triisopropylbenzenesulfonyl chloride (566 mg, 1.87 mmol) were added under argon. The reaction mixture was stirred at RT for 2 d, then it was diluted with ethyl acetate (50 mL) and quenched with 5% citric acid (50 mL). The aqueous layer was extracted with ethylactetate (3 × 50 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, the volatiles were removed by reduced pressure and the residue was purified by column chromatography using dichloromethane : methanol (19:1) to afford the desired product **144** (450 mg, 85%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) $\nu_{max/}cm^{-1}$: 2952, 2924, 2854, 1697, 1608, 1578, 1455, 1248, 1071, 937, 834; δH (400 MHz, $CDCl_3$): 9.60-8.98 (2H, br, NH), 8.77 (0.5H, s, A_2-H-2), 8.76 (0.5H, s, A_2-H-2), 8.74 (0.5H, s, A_1-H-2), 8.71 (0.5H, s, A_1-H-2), 8.33 (0.5H, s, A_1-H-8), 8.29 (0.5H, s, A_1-H2-8), 8.20 (0.5H, s, A_2-H-8), 8.16 (0.5H, s, A_2-H-8), 8.04-7.97 (4H, m, ArH), 7.62-7.54 (2H, m, ArH), 7.52-7.44 (2H, m, ArH), 7.10-6.94 (1H, ddd, *J* 23.2, 17.1, 4.2, A_2-H-5′), 6.60 (0.5H, dd, *J* 8.0, 6.1, A_1-H-1′), 6.56-6.46 (1.5H, m, 0.5 × A_1-H-1′ + A_2-H-1′), 6.02 (0.5H, ddd, *J* 21.1, 17.0, 1.7, A_2-H-6′), 6.02 (0.5H, ddd, *J* 21.0, 17.0, 1.7, A_2-H-6′), 5.29-5.18 (1H, m, A_1-H-3′), 4.70 (0.5H,

td, *J* 5.8, 3.9, A_2-H-3′), 4.66 (0.5H, td, *J* 5.8, 3.8, A_2-H-3′), 4.61-4.55 (1H, m, A_2-H-4′), 4.36 (0.5H, q, *J* 3.8, A_1-H-4′), 4.29 (0.5H, q, *J* 3.8, A_1-H-4′), 4.27-4.12 (2H, m, C*H*$_2$CH$_2$CN), 3.92-3.77 (2H, m, A_1-H-5′), 3.10-2.95 (1H, m, A_2-H_A-2′), 2.86-2.68 (4H, m, A_1-H_A-2′ + A_1-H_B-2′ + CH$_2$C*H*$_2$CN), 2.51-2.42 (1H, m, A_2-H_B-2′), 0.96-0.90 (9.5H, SiC(CH$_3$)$_3$), 0.89-0.84 (8.5H, SiC(CH$_3$)$_3$), 0.18-0.12 (6.5H, (Si(CH$_3$)$_2$), 0.08-0.03 (5.5H, (Si(CH$_3$)$_2$); *δ*c (101 MHz, CDCl$_3$): 164.9 (2 × ^{Bz}CO), 152.7 (A_1-CH-2 + A_2-CH-2), 151.9 (A_2-C-4), 151.8 (A_2-C-4), 151.6 (2 × A_1-C-4), 150.6 (d, *J* 6.6, A_2-CH-5′), 150.5 (d, *J* 6.7, A_2-CH-5′), 150.0 (A_2-C-6), 149.9 (A_2-C-6), 159.7 (2 × A_1-C-6), 142.0 (2 × A_2-CH-2), 141.34 (A_1-CH-2), 141.28 (A_1-CH-2), 133.8 (2 × ArC), 133.6 (2 × ArC), 133.0 (2 × ArCH), 132.9 (2 × ArCH), 129.0 (8 × ArCH), 128.1 (8 × ArCH), 124.1 (A_2-C-5), 123.99 (A_2-C-5), 123.4 (2 × A_1-C-5), 117.2 (d, *J* 190.1, A_2-CH-6′), 117.1 (d, *J* 190.2, A_2-CH-6′), 116.8 (CN), 116.5 (CN), 87.0 (d, *J* 22.3, A_2-CH-4′), 86.9 (d, *J* 22.1, A_2-CH-4′), 86.6 (d, *J* 5.0, A_1-CH-4′), 86.5 (d, *J* 5.2, A_1-CH-4′), 85.2 (A_2-CH-1′), 84.9 (A_2-CH-1′), 84.6 (A_1-CH-1′), 84.5 (A_1-CH-1′), 77.5 (d, *J* 5.7, A_1-CH-3′), 77.1 (d, *J* 5.8, A_1-CH-3′), 75.2 (A_2-CH-3′), 63.3 (A_1-CH$_2$-5′), 63.1 (A_1-CH$_2$-5′), 60.7 (d, *J* 4.9, *C*H$_2$CH$_2$CN), 60.6 (d, *J* 4.6, *C*H$_2$CH$_2$CN), 40.12 (d, *J* 3.9, A_1-CH-2′), 40.05 (d, *J* 3.8, A_1-CH-2′), 39.12 (A_2-CH-2′), 39.06 (A_2-CH-2′), 26.1 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 20.1 (d, *J* 6.4, CH$_2$*C*H$_2$CN), 20.0 (d, *J* 6.6, CH$_2$*C*H$_2$CN), 18.4 (Si*C*(CH$_3$)$_3$), 18.1 (Si*C*(CH$_3$)$_3$), -4.53 (Si(CH$_3$)$_2$), -4.55 (Si(CH$_3$)$_2$), -4.6 (2 × Si(CH$_3$)$_2$), -5.29 (Si(CH$_3$)$_2$), -5.32 (Si(CH$_3$)$_2$), -5.38 (Si(CH$_3$)$_2$), -5.41 (Si(CH$_3$)$_2$); *δ*P (162 MHz, CDCl$_3$): 18.93, 18.66; *m/z* (ESI$^+$) 1050.4275 (M+H. $C_{50}H_{65}N_{11}O_9PSi_2$ requires 1050.4237), 1072.4078 (M+Na. $C_{50}H_{64}N_{11}NaO_9PSi_2$ requires 1072.4057).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxyoxolan-2-yl]ethenyl]phosphonate **146**

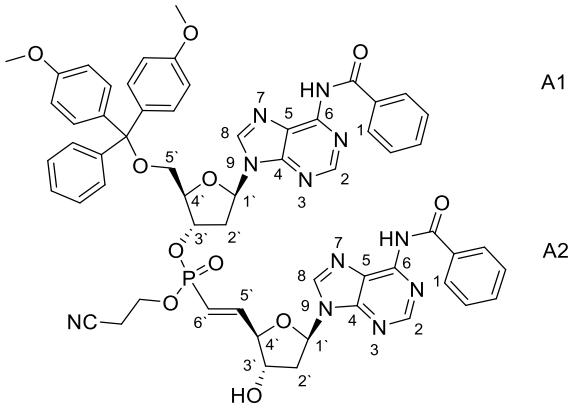


Chemical Formula: $C_{38}H_{36}N_{11}O_9P$

Triethylamine trihydrofluoride (700 µl, 4.30 mmol) was added in one portion to a stirring solution of dinucleotide **145** (450 mg, 430 µmol, 1:1 mixture of diastereoisomers) in THF (6 mL) and the resulting reaction mixture was stirred at RT for 5 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using dichloromethane : methanol (9:1) to afford the desired product **146** (250 mg, 71%, 1:1 mixture of diastereoisomers) as a white powder. FTIR (ATR) ν_{max}/cm^{-1}: 3252, 2936, 1693, 1608, 1697, 1579, 1509, 1453, 1242, 1220, 998, 897; δH (400 MHz, CD_3OD): 8.72 (0.5H, s, A_2-H-2), 8.70 (0.5H, s, A_2-H-2), 8.63 (0.5H, s, A_1-H-2), 8.62 (0.5H, s, A_1-H-2), 8.58 (0.5H, s, A_1-H-8), 8.57 (0.5H, s, A_1-H-8), 8.52 (0.5H, s, A_2-H-8), 8.51 (0.5H, s, A_2-H-8), 7.66-7.43 (6H, m, ArH), 7.21-7.05 (1H, m, A_2-H-5′), 6.64-6.50 (2H, m, A_1-H-1′, A_2-H-1′), 6.09 (0.5H, ddd, *J* 22.0, 17.1, 1.7, A_2-H-6′), 6.06 (0.5H, ddd, *J* 22.0, 17.1, 1.7, A_2-H-6′), 5.35-5.24 (1H, m, A_1-H-3′), 4.79-4.71 (1H, m, A_2-H-3′), 4.67-4.59 (1H, m, A_2-H-4′), 4.33-4.19 (3H, m, A_1-H-4′ + C*H*$_2$CH$_2$CN), 3.88-3.71 (2H, m, A_1-H_A-5′ + A_1-H_B-5′), 3.19-3.07 (1H, m, A_2-H_A-2′), 3.06-2.96 (1H, m, A_1-H_A-2′), 2.95-2.85 (2H, m, CH$_2$C*H*$_2$CN), 2.79-2.65 (1H, m, A_1-H_B-2′), 2.60-2.49 (1H, m, A_2-H_B-2′); δc (101 MHz, CD_3OD): 168.0 (4 × ^{Bz}CO), 153.2 (2 × A_2-CH-5′ + 2 × A_2-C-4), 152.9 (2 × A_1-CH-2 + 2 × A_2-

CH-2), 152.8 (2 × A_1-C-4), 151.23 (2 × A_1-C-6 + 2 × A_2-C-6), 144.84 (2 XA_2-CH-8), 144.78 (2 × A_2-CH-8), 144.6 (2 × A_1-CH-8), 134.9 (2 × ArC), 134.8 (2 × ArC), 133.9 (2 × ArCH), 133.8 (2 × ArCH), 129.7 (8 × ArCH), 129.4 (8 × ArCH), 125.6 (2 × A_2-C-5), 125.4 (2 × A_1-C-5), 118.8 (CN), 118.7 (CN), 117.2 (2 × d, *J* 189.2, A_2-CH-6′), 88.3-87.8 (2 × A_1-CH-4′ + 2 × A_2-CH-4′), 86.7 (2 × A_2-CH-1′), 86.5 (2 × A_1-CH-1′), 78.9 (d, *J* 5.5, A_1-CH-3′), 78.7 (d, *J* 5.7, A_1-CH-3′), 75.4 (2 × d, *J* 1.8, A_2-CH-3′), 62.82 (A_1-CH_2-5′), 62.77 (A_1-CH_2-5′), 62.7 (d, *J* 5.1, *C*H_2CH_2CN), 62.6 (d, *J* 5.1, *C*H_2CH_2CN), 39.9 (2 × A_1-CH_2), 39.3 (2 × A_2-CH_2), 20.4 (d, *J* 6.5, CH_2*C*H_2CN), 20.3 (d, *J* 6.5, CH_2*C*H_2CN); *δ*P (162 MHz, CD_3OD): 19.03, 19.00; *m/z* (ESI^+) 822.2494 (M+H. $C_{38}H_{37}N_{11}O_9P$ requires 822.2508), 844.2329 (M+Na. $C_{38}H_{36}N_{11}NaO_9P$ requires 844.2327).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxyoxolan-2-yl]ethenyl]phosphonate **147**



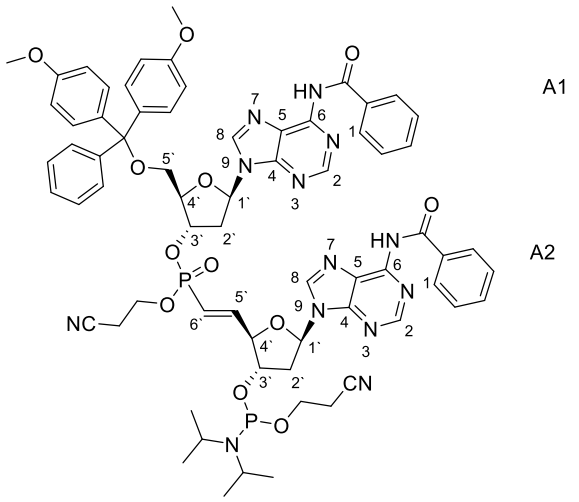
Chemical Formula: $C_{59}H_{54}N_{11}O_{11}P$
Exact Mass: 1123.3742

Dinucleotide **147** (250 mg, 300 µmol) was dried by azeotropic removal of water with pyridine (3 × 3 mL). The residue was dissolved in pyridine (300 µL). Molecular sives and DMTrCl (103 mg, 300 µmol) were added in one portion under argon gas, the reaction mixture was stirred at RT for 24 h. The volatiles

were removed *in vacuo* and the residue were purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **147** (250 mg, 73%, 1:2 mixture of diastereoisomers) as a white foam and recovered starting material **146** (20 mg, 8%) as white powder. ν_{max}/cm^{-1}: 2931, 2836, 1695, 1607, 1579, 1507, 1486, 1453, 1245, 1175, 1031, 725; δH (400 MHz, $CDCl_3$): 9.28 (0.5H, s, A_2-N-H), 9.24 (0.5H, s, A_2-N-H), 9.17 (0.5H, s, A_1-N-H), 9.14 (0.5H, s, A_1-N-H), 8.74 (0.5H, s, A_2-H-2), 8.72 (0.5H, s, A_2-H-2), 8.64 (0.5H, s, A_1-H-2), 8.61 (0.5H, s, A_1-H-2), 8.19 (0.5H, s, A_1-H-8), 8.16 (0.5H, s, A_1-H-8), 8.14 (0.5H, s, A_2-H-8), 8.13 (0.5H, s, A_2-H-8), 8.03-7.93 (4H, m, ArH), 7.60-7.42 (6H, m, ArH), 7.37-7.31 (2H, m, ArH), 7.28-7.00 (8H, m, 7 × ArH + 1H, m, A_2-H-5′), 6.80-6.73 (4H, m, ArH), 6.54-6.42 (2H, m, A_1-H-1′, A_2-H-1′), 6.06-5.91 (1H, m, A_2-H-6′), 5.37-5.25 (1H, m, A_1-H-3′), 4.76 (0.5H, q, *J* 5.3, A_2-H-3′), 4.68 (0.5H, q, *J* 4.5, A_2-H-3′), 4.62-4.57 (0.5H, m, A_2-H-4′), 4.53-4.49 (0.5H, m, A_2-H-4′), 4.38-4.30 (1H, A_1-H-4′), 4.26-4.09 (1.5H, m, C*H*$_2$CH$_2$CN), 4.06-3.98 (0.5H, m, C*H*$_2$CH$_2$CN), 3.76-3.71 (6H, 2 × ArOCH$_3$), 3.49-3.29 (2H, m, A_1-H_A-5′ + A_1-H_B-5′), 3.18-3.05 (1H, m, A_1-H_A-2′), 3.04-3.20 (1H, m, A_2-H_A-2′), 2.80-2.66 (2H, m, A_1-H_B-2′ + CH$_2$C*H*$_2$CN), 2.61-2.50 (2H, m, A_2-H_B-2′ + CH$_2$C*H*$_2$CN); δC (101 MHz, $CDCl_3$): 165.0 (2 × ^{Bz}CO), 164.8 (2 × ^{Bz}CO), 158.7 (2 × ^{DMT}ArC), 158.6 (2 × ^{DMT}ArC), 152.7 (2 × A_1-CH-2 + 2 × A_2-CH-2), 151.8 (A_2-C-4), 151.7 (A_2-C-4), 151.5 (2 × A_1-C-4), 151.1 (d, *J* 6.6, A_2-CH-5′), 150.5 (d, *J* 6.6, A_2-CH-5′), 149.8 (2 × A_2-C-6), 149.6 (2 × A_1-C-6), 144.2 (^{DMT}ArC), 144.39 (^{DMT}ArC), 142.04 (A_1-CH-8), 142.0 (A_1-CH-8), 141.91 (A_2-CH-8), 141.87 (A_2-CH-8), 135.6 (^{DMT}ArC), 135.5 (^{DMT}ArC), 135.4 (2 × ^{DMT}ArC), 133.7 (2 × ^{Bz}ArC), 133.5 (2 × ^{Bz}ArC), 133.0 (4 × ArCH), 130.2 (8 × ArCH), 129.0 (9 × ArCH), 128.2 (4 × ArCH), 128.0 (14 × ArCH), 127.2 (2 × ArCH), 123.93 (A_2-C-5), 123.85 (A_2-C-5), 123.6 (A_1-C-5), 123.58 (A_1-C-5), 117.96 (d, *J* 191, A_2-CH-6′), 117.0 (CN), 116.8 (CN), 116.6 (d, *J* 190, A_2-CH-6′), 113.4 (8 × ArCH), 86.8 (CAr$_3$), 86.8 (CAr$_3$), 86.5 (d, *J* 22.7, A_2-CH-4′),

86.9 (d, *J* 22.1, A_2-CH-4′), 86.4 (d, *J* 22.3, A_2-CH-4′), 85.3 (d, *J* 6.5, A_1-CH-4′), 85.2 (d, *J* 6.6, A_1-CH-4′), 84.9 (A_2-CH-1′), 84.8 (A_2-CH-1′), 84.76 (A_1-CH-1′), 84.71 (A_1-CH-1′), 77.26 (d, *J* 5.3, A_1-CH-3′), 77.1 (d, *J* 5.8, A_1-CH-3′), 74.2 (A_2-CH-3′), 63.1 (2 × A_1-CH_2-5′), 60.8 (2 × d, *J* 4.8, *CH_2*CH_2CN), 55.4 (4 × ArOMe), 38.7 (2 × A_2-CH-2′), 38.6 (2 × A_1-CH-2′), 20.1 (d, *J* 6.3, CH_2*CH_2*CN), 20.0 (d, *J* 6.5, CH_2*CH_2*CN); *δ*P (162 MHz, $CDCl_3$): 18.95, 18.69; *m/z* (ESI$^+$) 1124.3825 (M+H. $C_{59}H_{55}N_{11}O_{11}P$ requires 1124.3815), 1146.3610 (M+Na. $C_{59}H_{54}N_{11}NaO_{11}P$ requires 1146.3634).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}oxolan-3-yl 2-cyanoethyl [(*E*)-2-[(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-({[bis(propan-2-yl)amino](2-cyanoethoxy)phosphanyl}oxy)oxolan-2-yl]ethenyl]phosphonate **140**



Chemical Formula: $C_{68}H_{71}N_{13}O_{12}P_2$
Exact Mass: 1323.4820

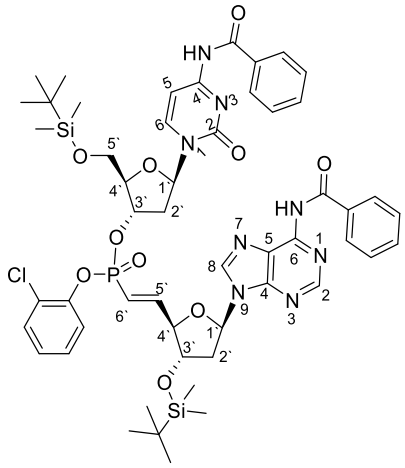
To a mixture of compound **147** (290 mg, 258 µmol) and phosphordiamidite **52** (100 µL, 310 µmol) in dry dichloromethane (3.5 mL), a solution of (5-Methyl-1*H*-tetrazole 26 mg, 310 µmol in dry acetonitrile) (500 µL) was added in one portion and the resultant mixture was stirred at room temperature for 19 h. The solution was diluted with acetonitrile (2 mL), quenched with TEA (240 µL) and water (240 µL) and washed with n-hexane (24 mL). After separation the

dichloromethane/acetonitrile layer was diluted with ether (50 mL), washed with water (70 mL) and brine (50 mL), dried over Na_2SO_4 and the volatiles were removed and the crude product was triturated with pentane to afford phosphoramidite **140** (130 mg, 40%) was obtained as a mixture of 4 diastereoisomers. ν_{max}/cm^{-1}: 2964, 2930, 1694, 1606, 1578, 1507, 1486, 1452, 1245, 1176, 1031, 725; δH (400 MHz, $CDCl_3$): 9.45-8.95 (2H, m, NH), 8.82-8.70 (1H, m, A-H-2), 8.68-8.58 (1H, m, A-H-2), 8.24-8.10 (2H, m, A-H-8), 8.04-7.94 (4H, m, ArH), 7.64-7.30 (9H, m, ArH), 7.25-6.99 (7H, m, ArH, A_2-H-5′), 6.82-6.72 (4H, m, ArH), 6.59-6.44 (2H, m, A-H-1′), 6.08-5.05 (1H, m, A-H-6′), 5.40-5.23 (1H, m, A_1-H-3′), 4.94-4.61 (2H, m, A_2-H-3′ + A_2-H-4′), 4.41-4.32 (1H, m, A_1-H-4′), 4.27-4.00 (2H, m, CH_2CH_2CN), 3.96-3.84 (1H, m, CH_2CH_2CN), 3.95-3.84 (1H, m, CH_2CH_2CN), 3.77-3.74 (6H, $ArOCH_3$), 3.73-3.70 (1H, s, CH_2CH_2CN), 3.69-3.60 (2H, m, CH-NR_2), 3.40-3.33 (2H, m, A_1-H-5′), 3.20-2.95 (2H, m, A_1-H_A-2′ + A_1-H_B-2′), 2.82-2.51 (6H, m, CH_2CH_2CN + A_2-H_A-2′ + A_2-H_B-2′), 1.33-1.55 (12H, m, CH_3); δ_c (126 MHz, $CDCl_3$): 164.9-164.5 (8 × ^{Bz}CO), 158.72 (4 × ^{DMT}ArC), 158.68 (4 × ^{DMT}ArC), 152.9-152.5 (4 × A_1-CH-2 + 4 × A_2-CH-2), 151.9-151.5 (8 × C), 150.5-149.8 (4 × A_2-CH-5′), 149.6 (8 × C), 144.4-144.3 (4 × ^{DMT}ArC), 142.1-141.6 (4 × A_1-CH-8 + 4 × A_2-CH-8), 135.5-135.3 (12 × ^{DMT}ArC), 133.8-133.5 (8 × ^{Bz}ArC), 133.0 (8 × ArCH), 130.3-130.0 (16 × ArCH), 129.0-128.8 (16 × ArCH), 128.4-128.1 (8 × ArCH), 128.1-127.8 (24 × ArCH), 127.25-127.04 (4 × ArCH), 124.0-123.6 (4 × A_1-C-5 + 4 × A_2-C-5), 118.4-115.6 (4 × A_2-CH-6′ + 8 × CN), 113.4-113.1 (16 × ArCH), 86.9-84.7 (4 × A_1-CH-1′ + 4 × A_2-CH-1′ + 4 × ^{DMT}CAr_3 + 4 × A_1-CH-4′ + 4 × A_2-CH-4′), 77.6-77.4 (4 × A_1-CH-3′), 76.4-75.7 (4 × A_2-CH-3′), 63.2 (A_1-CH-5′), 60.8-60.4 (4 × OCH_2CH_2CN), 58.3-58.0 (4 × OCH_2CH_2CN), 55.4 (8 × ^{DMT}OMe), 43.7-43.3 (8 × $CH(CH_3)_2$), 38.8-37.8 (4 × A_1-CH2-2′ + 4 × A_2-CH2 2′), 24.8-24.6 (8 × CH(CH_3)$_2$), 20.7-19.6 (8 OCH_2CH_2CN); δP (162 MHz,

$CDCl_3$): 149.45, 149.37, 149.03, 148.91, 18.89, 18.71 (2 × P), 18.50; *m/z* (ESI^+) 1346.4670 (M+Na. $C_{68}H_{71}N_{13}NaO_{12}P_2$ requires 1346.4712).

5.3.3 Synthesis of C*A dinucleotide **120**

(2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate



Chemical Formula: $C_{52}H_{64}ClN_8O_{10}PSi_2$
Exact Mass: 1082.3710

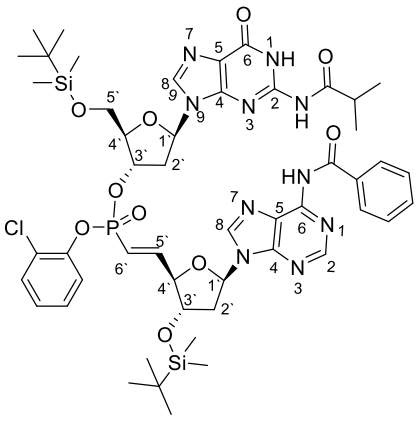
A mixture of phosphonate **69** (420 mg, 760 µmol), alcohol **103** (374 mg, 840 µmol) and *N*-methylimidazole (603 µL, 7.62 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (3 mL). Molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (1.15 g, 3.81 mmol) were added. The reaction mixture was stirred at RT for 20 h, the reaction mixture was poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **69** (370 mg, 53%, 1:1 mixture

of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2952, 2928, 2856, 1693, 1662, 1579, 1479, 1395, 1249, 833, 779; δH (400MHz, $CDCl_3$): 9.24 (0.3H, br s, N-H), 9.11 (0.7H, br s, N-H), 8.83-8.60 (1H, brs, N-H), 8.79 (0.3H, s, A-H-2), 8.73 (0.7H, s, A-H-2), 8.3 (0.7H, d, *J* 7.5, C-H-6), 8.28 (0.3H, d, *J* 7.4, C-H-6), 8.24 (0.3H, s, A-H-8), 8.10 (0.7H, s, A-H-8), 8.06-8.00 (2H, m, ArH), 7.91-7.84 (2H, m, ArH) 7.64-7.55 (2H, m, ArH), 7.54-7.46 (4.7H, m, 4 × ArH + 0.7 × C-H-5), 7.42-7.31 (2.3H, m, 0.3 × C-H-5 + 2 × ArH), 7.25-7.17 (1H, m, ArH), 7.14-6.95 (2H, m, A-H-5′ + ArH), 6.54 (0.3H, dd, *J* 7.5, 6.0, C-H-1′), 6.48 (0.7H, dd, *J* 7.0, 6.6, C-H-1′), 6.4 (0.3H, dd, *J* 7.9, 5.5, A-H-1′), 6.35 (0.7H, dd, *J* 7.4, 5.7, A-H-1′), 6.23-6.09 (1H, m, A-H-6′), 5.28-5.20 (1H, m, C-H-3′), 4.63 (0.3H, dt, *J* 6.0, 4.4, A-H-3′), 4.61-4.53 (1.7H, m, 0.7 × A-H-3′ + A-H-4′), 4.38 (0.7H, q, *J* 2.2, C-H-4′), 4.32 (0.3H, q, *J* 2.0, C-H-4′), 3.96-3.75 (2H, m, C-$H_{A,B}$-5′), 3.05-2.78 (2H, m, A-H_A-2′ + C-H_A-2′), 2.50-2.40 (1H, m, A-H_B-2′), 2.22-2.11 (1H, m, C-H_B-2′), 0.94-0.91 (9H, $SiC(CH_3)_3$), 0.88-0.85 (9H, $SiC(CH_3)_3$), 0.13 (2H, $Si(CH_3)_2$), 0.11 (4H, $Si(CH_3)_2$), 0.09 (2H, $Si(CH_3)_2$), 0.07 (3H, $Si(CH_3)_2$), 0.05 (1H, $Si(CH_3)_2$); δc (101 MHz, $CDCl_3$): 166.44 (2 × ^{Bz}CO), 164.77 (2 × ^{Bz}CO), 162.23 (2 × C-C-4), 154.8 (2 × C-C-2), 152.82 (2 × A-CH-2), 151.9 (A-C-4), 151.7 (A-C-4), 151. 2 (d, *J* 6.7, A-CH-5′), 150. 8 (d, *J* 6.5, A-CH-5′), 149.9 (2 × A-C-6), 146.3 (d, *J* 6.8, ArC), 146.2 (d, *J* 7.0, ArC), 144.6 (2 × C-CH-6), 141.8 (2 × A-CH-8), 133.8 (2 × ArC), 133.3 (4 × ArCH), 133.2 (2 × ArC), 132.89 (4 × ArCH), 130.9 (4 × ArCH), 129.2 (4 × ArCH), 129.0 (4 × ArCH), 128.1 (6 × ArCH), 127.6 (4 × ArCH), 126.34 (2 × ArCH), 125.9 (d, *J* 6.0 ArC), 125.80 (d, *J* 6.0 ArC), 123.99 (A-C-5-A), 122.2 (2 × d, *J* 2.5, ArCH), 117.2 (d, *J* 192, A-CH-6′), 96.5 (2 × C-CH-5), 87.34-86.51 (combined, A-CH-1′+ A-CH-4′+ C-CH-4′), 85.1 (C-CH-1′), 84.9 (C-CH-1′), 77.9 (d, *J* 6.0, C-CH-3′), 78.14 (d, *J* 6.5, C-CH-3′), 75.23 (2 × A-CH-3′), 63.1 (C-CH-5′), 62.9 (C-CH-5′), 41.3 (d, *J* 3.5, C-CH_2-2′), 41.1 (d, *J* 4.3, C-CH_2-2′), 39.2 (A-CH_2-2′), 25.97 (6 × $SiC(CH_3)_3$), 25.83 (6 × $SiC(CH_3)_3$),

18.34 (2 × Si*C*(CH_3)$_3$), 18.09 (2 × Si*C*(CH_3)$_3$), -4.55 (Si(CH_3)$_2$), -4.59 (Si(CH_3)$_2$), -4.60 (Si(CH_3)$_2$), -4.62 (Si(CH_3)$_2$), -5.39 (Si(CH_3)$_2$), -5.41 (Si(CH_3)$_2$), -5.48 (Si(CH_3)$_2$); δ_P (162 MHz, $CDCl_3$): 15.13, 15.1; *m/z* (ESI$^+$) 1083.3773 (M+H. $C_{52}H_{65}ClN_8O_{10}PSi_2$ requires 1083.3783).

5.3.4 Synthesis of G*A dinucleotide **121**

(2*R*,3*S*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate



Chemical Formula: $C_{50}H_{66}ClN_{10}O_{10}PSi_2$
Exact Mass: 1088.3928

A mixture of phosphonate **69** (300 mg, 460 µmol), alcohol **104** (227 mg, 500 µmol) and *N*-methylimidazole (237 µL, 3.00 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (348 mg, 1.15 mmol) were added. The reaction mixture was stirred at RT for 23 h, and was poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried over Na_2SO_4. The volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the

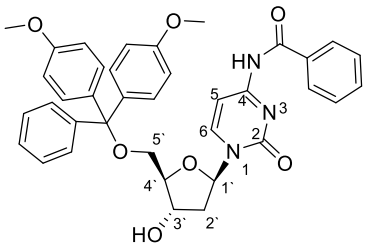
desired product **121** (380 mg, 76%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2952, 2929, 2856, 1685, 1606, 1560, 1477, 1250, 833, 778; δH (400MHz, $CDCl_3$): 12.11 (0.4H, s, G-N-H), 12.01 (0.6H, s, G-N-H), 10.95 (0.4H, s, ^{iBu}N-H), 10.31 (0.6H, s, ^{iBu}N-H), 9.82 (0.4H, br s, A-N-H), 9.78 (0.6H, br s, A-N-H), 8.90 (0.6H, s, A-H-2), 8.80 (0.4H, s, A-H-2), 8.22 (0.4H, s, A-H-8), 8.15 (0.6H, s, A-H-8), 8.09-8.01 (2H, m, ArH), 7.86 (0.4H, s, G-H-8), 7.84 (0.6H, s, G-H-8), 7.61-7.54 (1H, m, ArH), 7.50-7.44 (2H, m, ArH), 7.42-7.36 (1.6, m, ArH), 7.33-7.30 (0.4H, dt, *J* 8.1, 1.5, ArH), 7.25-7.17 (1H, m, ArH), 7.15-6.95 (2H, m, ArH + A-H-5′), 6.48-6.30 (1.6H, m, A-H-1′ + 0.6 × A-H-6′), 6.21-6.05 (1.4H, m, G-H-1′ + 0.4 × A-H-6′), 5.55-5.48 (0.6H, m, G-H-3′), 5.47-5.41 (0.4H, m, G-H-3′), 4.81-4.75 (0.4H, m, A-H-3′), 4.61-4.51 (1H, m, A-H-4′), 4.49-4.44 (0.6H, m, A-H-3′), 4.26 (0.6H, q, *J* 2.5, G-H-4′), 4.23 (0.4H, q, *J* 2.3, G-H-4′), 3.82-3.65 (2H, m, G-$H_{A,B}$-5′), 3.44-3.34 (0.4H, dt, *J* 13.0, 6.3, A-H_A-2′), 3.21-3.10 (0.6H, ddd, *J* 13.0, 7.6, 5.6, A-H_A-2′), 2.73-2.48 (3H, m, 2 × G-$H_{A,B}$-2′ + ^{iBu}C*H*$(CH_3)_2$), 2.45-2.32 (1H, m, H_B-2′-A), 1.17–1.00 (6H, m, ^{iBu}CH(C*H*$_3)_2$), 0.91-0.88 (9H, SiC$(CH_3)_3$), 0.85-0.75 (9H, SiC$(CH_3)_3$), 0.14-0.15 (6H, Si$(CH_3)_2$), 0.03-(-)0.04 (6H, Si$(CH_3)_2$); δc (101 MHz, $CDCl_3$): 179.8 (^{iBu}CO), 179.4 (^{iBu}CO), 165.3 (^{Bz}CO), 165.2 (^{Bz}CO), 155.6 (2 × G-C-6), 152.35 (2 × A-CH-2), 152.0 (2 × A-C-4), 150.76 (d, *J* 7.5, A-CH-5′), 150.65 (d, *J* 6.6, A-CH-5′), 150.2 (A-C-6), 150.1 (A-C-6), 148.5 (G-C-2), 148.2 (G-C-2), 148.0 (G-C-4), 147.9 (G-C-4), 146.4 (d, *J* 6.6, ArC), 146.2 (d, *J* 6.7, ArC), 143.8 (A-CH-8), 143.2 (A-CH-8), 136.7 (G-CH-8), 136.5 (G-CH-8), 133.2-133.0 (2 × ArCH + 2 × ArC), 130.87 (2 × ArCH), 128.9 (4 × ArCH), 128.10 (6 × ArCH), 126.4 (ArCH), 126.2 (ArCH), 125.9 (d, *J* 5.9 ArC), 125.8 (d, *J* 5.9 ArC), 125.1 (2 × A-C-5), 122.1 (2 × d, *J* 2.1, ArCH), 121.2 (G-C-5), 121.1 (G-C-5), 117.0 (d, *J* 192, A-CH-6′), 117.0 (d, *J* 192, A-CH-6′), 116.6 (d, *J* 193, A-CH-6′), 87.0 (d, *J* 22.3, A-CH-4′), 86.7 (d, *J* 22.5, A-CH-4′), 86.30 (A-CH-1′), 86.11 (A-CH-1′), 86.0-85.8 (2 × G-CH-4′), 83.6 (G-CH-1′), 83.55 (G-

CH-1′), 77.6 (d, *J* 6.3, G-CH-3′), 77.5 (d, *J* 6.8, G-CH-3′), 75.5 (d, *J* 1.6, A-CH-3′), 75.3 (d, *J* 1.6, A-CH-3′), 63.2 (G-CH-5′), 63.0 (G-CH-5′), 41.2 (d, *J* 4.1, G-CH-2′), 40.5 (d, *J* 3.2, G-CH-2′), 38.3 (A-CH-2′), 38.1 (A-CH-2′), 36.3 (^{iBu}*C*H(CH$_3$)$_2$), 36.2 (^{iBu}*C*H(CH$_3$)$_2$), 26.0 (4 × SiC(*C*H$_3$)$_3$), 25.96 (4 × SiC(*C*H$_3$)$_3$), 25.8 (4 × SiC(*C*H$_3$)$_3$), 19.7 (^{iBu}CH(*C*H$_3$)$_2$), 19.5 (^{iBu}CH(*C*H$_3$)$_2$), 18.7 (^{iBu}CH(*C*H$_3$)$_2$), 18.6 (^{iBu}CH(*C*H$_3$)$_2$), 18.4 (2 × Si*C*(CH$_3$)$_3$), 18.1 (2 × Si*C*(CH$_3$)$_3$), -4.56 (Si(CH$_3$)$_2$), -4.62 (Si(CH$_3$)$_2$), -4.64 (Si(CH$_3$)$_2$), -4.70 (Si(CH$_3$)$_2$), -5.40 (2 × Si(CH$_3$)$_2$), -5.51 (Si(CH$_3$)$_2$), -5.53 (Si(CH$_3$)$_2$); δ_P (162 MHz, CDCl$_3$): 16.04, 15.65; *m/z* (ESI$^+$) 1089.3988 (M+H. C$_{50}$H$_{67}$ClN$_{10}$O$_{10}$PSi$_2$ requires 1089.4001), 1111.3791 (M+Na. C$_{50}$H$_{66}$ClN$_{10}$NaO$_{10}$PSi$_2$ requires 1111.3820).

5.4 Synthesis of phosphonic acid (C) **126**

N-(1-((2*R*,4*S*,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide

240

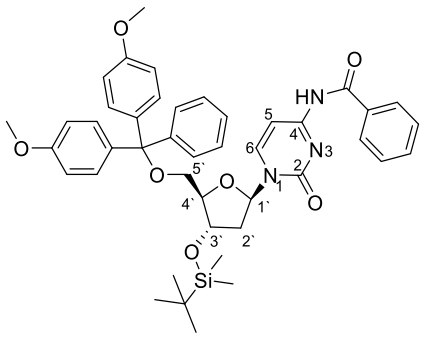


Chemical Formula: C$_{37}$H$_{35}$N$_3$O$_7$
Exact Mass: 633.2475

4-N-benzoyl-2′-deoxycytidine **110** (1.00 g, 3.02 mmol) was dissolved in pyridine (10 mL), and the water was removed by azeotropic distillation with pyridine (3 × 10 mL). The residue was dissolved in pyridine (10 mL), dimethoxytrityl chloride (1.22 g, 3.62 mmol) was added in portions at 0 °C and the reaction mixture was stirred this temperature for 10 min then allowed to warm up to room temperature. The resultant mixture was stirred at RT for 25 h. Pyridine was removed by reduced pressure, and the residue was dissolved in ethyl acetate (200 mL) and washed with water (100 mL) the separated

aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over anhydrous sodium sulphate. The volatiles were removed on reduced pressure and the residue was purified by column chromatography using; petrol : ethyl acetate (4:1 → 1:1) to afford the desired product **240** as a white foam (1.90, quantitative). FTIR (ATR) $\nu_{max/}cm^{-1}$ 3379, 3214, 3083, 2949, 2835, 1701, 1639, 1607, 1485, 1246, 1033; δ_H (400 MHz, $CDCl_3$): 8.84 (1H, br. s, NH), 8.31 (1H, d, *J* 7.7, H-6), 7.86 (2H, d, 7.5, ArH), 7.62-7.55 (1H, m, ArH), 7.51-7.45 (2H, m, ArH), 7.43-7.38 (2H, m, ArH), 7.37-7.21 (8H, m, ArH + H-5), 6.92-6.79 (1H, m, ArH), 6.3 (1H, t, *J* 5.8, H-1'), 4.58-4.45 (1H, m, H-3'), 4.13 (1H, q, *J* 3.8, H-4'), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.50 (1H, dd, *J* 10.8, 3.1, H_A-5'), 3.27 (1H, dd, *J* 10.8, 3.8, H_B-5'), 2.81-2.70 (1H, m, H_A-2'), 2.35-2.23 (1H, m, H_B-2'); δ_C (101 MHz, $CDCl_3$): 166.3 (^{Bz}CO), 162.4 (C-4), 158.8 (2 × ^{DMTr}ArC), 155.3 (C-2), 144.9 (CH-6), 135.6 (ArC), 135.5 (ArC), 133.2 (ArCH), 130.2 (2 × ArCH), 130.1 (2 × ArCH), 129.1 (2 × ArCH), 128.3 (2 × ArCH), 128.2 (2 × ArCH), 127.7 (ArCH), 127.3 (ArCH), 113.5 (4 × ArCH), 96.7 (CH-5), 87.4 (CAr_3), 87.1 (CH-1'), 86.6 (CH-4'), 70.93 (CH-3'), 62.8 (CH_2-5'), 55.4 (2 × OCH_3), 41.2 (CH_2-2'); *m/z* (ESI^+): 634.2560 (M+H. $C_{37}H_{36}N_3O_7$ requires 634.2548); 656.2367 (M+Na. $C_{37}H_{35}N_3NaO_7$ requires 656.2373).

N-(1-((2*R*,4*S*,5*R*)-5-((bis(4 methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert* butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide **122**

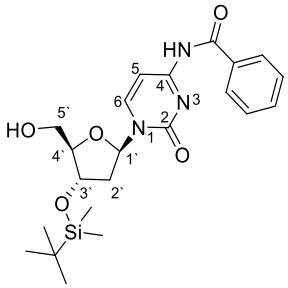


Chemical Formula: $C_{43}H_{49}N_3O_7Si$
Exact Mass: 747.3340

Imidazole (1.35 g, 15.6 mmol) was added in one portion to a stirring solution of alcohol **240** (3.30 g, 5.20 mmol) in DMF (9.0 mL) at 0 °C, the resulting solution was stirred at this temperature for 5 min. TBDMSCl (1.18 g, 7.82 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 2 days at this temperature. DMF was removed *via* azeotrope with toluene (5 × 100 mL) to afford a sticky oily residue. Diethyl ether (100 mL) and water (100 mL) were added and the separated aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo* to afford the desired product **122** (4.00 g, 100%) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2951, 2927, 2854, 1694, 1660, 1482, 1247, 829; δH (400MHz, $CDCl_3$): 8.75 (1H, br s, N-H), 8.44 (1H, d, *J* 7.5, H-6), 7.88 (2H, d, *J* 7.7, ArH), 7.64-7.65 (1H, m, ArH), 7.54-7.48 (2H, m, ArH), 7.43-7.38 (2H, m, ArH), 7.35-7.23 (7H, m, ArH + H-5), 6.80 (4H, d, *J* 8.5, ArH), 6.26 (1H, dd, *J* 6.0, 4.4, H-1'), 4.46 (1H, q, *J* 6.0, H-3'), 4.0 (1H, dt, *J* 6.0, 3.0, H-4'), 3.80 (6H, 2 × OCH_3), 3.55 (1H, dd, *J* 10.8, 3.0, H_A-5'), 3.35 (1H, dd, *J* 10.8, 3.0, H_B-5'), 2.60-2.53 (1H, m, H_A-2'), 2.23 (1H, ddd, *J* 13.7, 6.0, 4.4, H_B-2'), 0.82 (9H, s, $SiC(CH_3)_3$), 0.01 (3H, s, $Si(CH_3)_2$), -0.05 (3H, s, $Si(CH_3)_2$); δc (101 MHz, $CDCl_3$): 162.2 (C-4), 158.9 (2 × ArC), 155.1 (C-2), 144.9 (CH-6), 144.1 (ArC),

135.5 (ArC), 135.4 (ArC), 133.3 (ArC + ArH), 130.3 (ArH), 130.2 (ArH), 129.2 (2 × ArH), 128.4 (2 × ArH), 128.1 (2 × ArH), 127.6 (2 × ArH), 127.3 (2 × ArH), 113.4 (4 × ArCH), 96.5 (CH-5), 87.2 (CH-1'), 87.0 (CAr3), 86.4 (CH-4'), 70.5 (CH-3'), 62.0 (CH-5'), 56.4 (2 × OCH_3), 41.74 (CH-2'), 25.80 (SiC(CH_3)$_3$), 18.1 (SiC(CH_3)$_3$), -4.5 (Si(CH_3)$_2$), -4.9 (Si(CH_3)$_2$); *m/z* (ESI$^+$) 748.3298 (M+H. $C_{43}H_{50}N_3O_7Si$ requires 748.3418), 770.3118 (M+Na. $C_{43}H_{49}N_3NaO_7Si$ requires 770.3237).

N-(1-((2*R*,4*S*,5*R*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide **123**

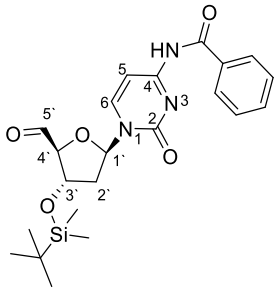


Chemical Formula: $C_{22}H_{31}N_3O_5Si$
Exact Mass: 445.2033

Compound **122** (4.00 g, 5.35 mmol) was dissolved in 5% (v/v) dichloroacetic acid in dichloromethane (100 mL) and stirred for 22 min at room temperature. The mixture was neutralized by saturated solution of sodium bicarbonate and stirred for an additional 10 min, brine (80 mL), dichloromethane (80 mL) were added and after separation the organic layer, the aqueous layer was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using gradient elution; petrol : ethyl acetate (1:1→ 1:4) to afford the desired product **123** (2.11 g, 89%). FTIR (ATR) ν_{max}/cm^{-1} 3351, 2928, 2855, 1695, 1645, 1483, 1248, 832; δH (400MHz, $CDCl_3$): 8.77 (1H, br s, N-H), 8.30 (1H, d, *J* 7.5, H-6), 7.85 (2H, m, ArH), 7.62-7.44 (4H, m, 3 × ArH, H-5), 6.18 (1H, t, *J* 6.1, H-1'), 4.54-4.45 (1H, m, H-3'),

4.04-3.97 (1H, m, H-4′, H_A-5′), 3.83-3.77 (1H, m, H_B-5′), 2.96 (1H, br s, OH), 2.52-2.43 (1H, m, H_A-2′), 2.40-2.32 (1H, m, H_B-2′), 0.88 (9H, s, SiC(CH_3)$_3$), 0.07 (6H, s, Si(CH_3)$_2$); δc (101 MHz, $CDCl_3$): 166.6 (^{Bz}CO), 162.3 (C-4), 155.3 (C-2), 145.9 (CH-6), 133.3 (ArH + ArC), 129.1 (2 × ArH), 127.7 (2 × ArH), 96.6 (CH-5), 88.8 (CH-1′), 88.1 (CH-4′), 70.95 (CH-3′), 61.67 (CH-5′), 41.74 (CH-2′), 25.85 (SiC(*C*H_3)$_3$), 18.1 (Si*C*(CH_3)$_3$), -4.5 (Si(*C*H_3)$_2$), -4.8(Si(*C*H_3)$_2$); *m/z* (ESI$^+$) 446.2107 (M+H. $C_{22}H_{32}N_3O_5Si$ requires 446.2106), 468.1915 (M+Na. $C_{22}H_{31}N_3NaO_5Si$ requires 468.1925).

N-(1-((2*R*,4*S*,5S)-4-((*tert*-butyldimethylsilyl)oxy)-5-formyltetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide **124**

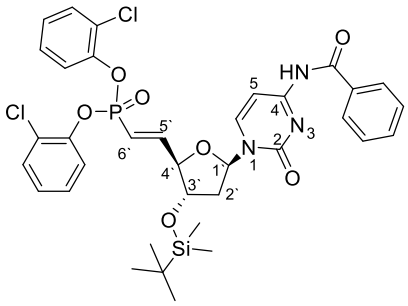


Chemical Formula: $C_{22}H_{29}N_3O_5Si$
Exact Mass: 443.1876

To a solution of alcohol **123** (2.10 g, 4.72 mmol) in acetonitrile (60 mL), stabilized IBX (3.69 g 14.1 mmol) was added and the resultant suspension was heated under reflux (80 °C) for 3 h. After cooling to the room temperature, the suspension was filtered, and the insoluble residue was washed with ethyl acetate (3 × 30 mL). The solvent of the combined filtrates was removed under reduced pressure. The solid residue was dissolved in ethyl acetate (100 mL) and washed with sodium bicarbonate (50 mL), the separated aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over magnesium sulphate and the volatiles were removed under reduced pressure to yield (1.81 g, 87%) of **124** as a white foam which was used in the subsequent reaction without further purification. δ_H (400 MHz, $CDCl_3$): 9.79

(1H, s, H-5′), 8.47 (1H, d, *J* 7.5, H-6), 7.95-7.90 (2H, m, ArH), 7.65-7.59 (1H, m, ArH), 7.55-7.48 (3H, m, ArH), 6.34 (1H, dd, *J* 7.2, 5.9, H-1′), 4.67 (1H, dt, *J* 5.3, 2.7, H-3′), 4.62 (1H, d, *J* 2.7, H-4′), 2.76 (1H, ddd, *J* 13.7, 5.9, 2.7, H_B-2′), 2.00 (1H, ddd, *J* 13.7, 7.2, 5.5, H_B-2′), 0.92 (9H, $SiC(CH_3)_3$), 0.14 (6H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 198.1 (CH-5′), 162.0 (C), 154.9 (C-2), 145.34 (CH-6), 133.4 (ArCH), 133.2 (C), 129.2 (2 × ArCH), 127.8 (2 × ArCH), 96.9 (CH-5), 92.3 (CH-4′), 89.9 (CH-1′), 72.5 (CH-3′), 41.1 (CH_2-2′), 25.8 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.7 ($Si(CH_3)_2$), -4.8 ($Si(CH_3)_2$); *m/z* (ESI^+) 444.1949 (M+H. $C_{22}H_{30}N_3O_5Si$) requires 444.1949.

synthesis of bis(2-chlorophenyl)((*E*)-2-((2*R*,3*S*,5*R*)-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate 125

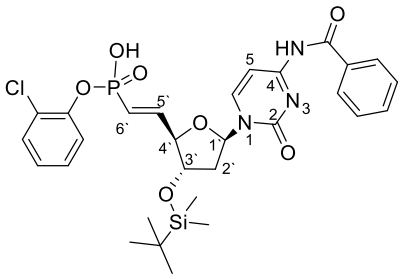


Chemical Formula: $C_{35}H_{38}Cl_2N_3O_7PSi$
Exact Mass: 741.1594

Ylide **24** (3.50 g, 6.09 mmol) was added in one portion to a stirring solution of aldehyde **124** (1.80 g, 4.08 mmol) in dichloromethane (15 mL) and the resulting solution was stirred at RT for 24 h. The volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (4:1 → 1:1), to afford the desired product **125** (2.00 g, 66%) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2952, 2928, 2855, 1660, 1621, 1475, 1254, 925, 833; δH (400MHz, $CDCl_3$): 8.75 (1H, br s, N-H), 7.91 (2H, d, *J* 7.6, ArH), 7.79 (1H, d, *J* 7.5, H-6), 7.65-7.59 (1H, m, ArH), 7.58-7.49 (3H, m, ArH, H-5), 7.48-7.41 (4H, m, ArH), 7.29-7.23 (2H, m, ArH), 7.21-7.04

(3H, m, ArH, H-5′), 6.42-6.25 (2H, m, H-1′, H-6′), 4.53-4.45 (1H, m, *J* 6.0, H-4′), 4.10 (1H, q, *J* 5.4, H-3′), 2.63-2.52 (1H, m, H_A-2′), 2.11-2.00 (1H, m, H_B-2′), 0.87 (9H, s, SiC(CH_3)$_3$), 0.04 (6H, d, Si(CH_3)$_2$); δc (101 MHz, $CDCl_3$): 162.4 (C-4), 151.2 (d, *J* 5.7, H-5′), 146.2 (2 × d, *J* 7.4, ArC), 143.5 (CH-6), 133.4 (ArH), 130.90 (2 × ArH), 130.86 (2 × ArH), 129.2 (ArH), 128.25 (ArH), 128.2 (ArH), 127.72 (ArH), 126.65 (ArH), 126.0 (ArH), 125.9 (d, *J* 6.3, ArC), 125.8 (d, *J* 6.5, ArC), 122.7 (d, *J* 2.8, ArH), 122.6 (d, *J* 2.8, ArH), 116.7 (d, *J* 191, H-6′), 96.9 (CH-5), 87.23 (CH-1′), 86.2 (d, *J* 23, CH-4′), 74.4 (d, *J* 2.3, CH-3′), 41.5 (CH-2′), 25.80 (SiC(*C*H_3)$_3$), 18.0 (Si*C*(CH_3)$_3$), -4.5 (Si(*C*H_3)$_2$), -4.8 (Si(*C*H_3)$_2$); δ_P (162 MHz, $CDCl_3$): 10.8; *m/z* (ESI$^+$) 742.1537 (M+H. $C_{35}H_{39}Cl_2N_3O_7PSi$ requires 742.1672), 764.1368 (M+Na. $C_{35}H_{38}Cl_2N_3NaO_7PSi$ requires 764.1491).

2-chlorophenyl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **126**



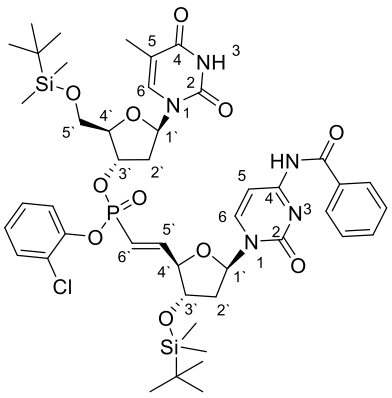
Chemical Formula: $C_{29}H_{35}ClN_3O_7PSi$
Exact Mass: 631.1670

To a solution of vinylphosphonate **125** (1.90 g, 2.56 mmol) in dioxane (70 mL) was added in one portion a solution of tetramethylguanidine (287 µL, 3.07 mmol), triethyl amine (2.15 mL, 15.1 mmol) and 2-nitrobenzaldoxime (510 mg, 3.07 mmol) in dioxane (15 mL) and the resulting mixture was stirred at room temperature for 2 days. The volatiles were evaporated, and the residue was purified by column chromatography using; methanol : dichloromethane

(1:9) to afford the desired product **126** as pale-yellow powder (1.37 g, 84%). δ_H (400 MHz, CD_3OD): 8.01-7.94 (2H, m, ArH + H-6), 7.67-7.58 (1H, m, ArH), 7.56-7.50 (2H, m, ArH), 7.47 (1H, d, *J* 7.5, H-5), 7.35 (1H, dd, *J* 7.9, 1.6, ArH), 7.20 (1H, td, *J* 7.9, 1.7, ArH), 7.01 (1H, td, *J* 6.5, 1.4, ArH), 6.59 (1H, ddd, *J* 21.0, 17.1, 5.6, H-5′), 6.25-6.06 (1H, m, H-1′ + H-6′), 4.41-4.35 (1H, m, H-4′), 4.17 (1H, dt, *J* 5.8, 3.7, H-3′), 2.46-2.37 (1H, m, H_A-2′), 2.10-1.99 (1H, m, H_B-2′), 0.87 (9H, $SiC(CH_3)_3$), 0.05 (3H, $Si(CH_3)_2$), 0.03 (3H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 164.5 (C), 157.8 (C), 150.0 (d, *J* 5.5, ArC), 145.8 (CH-6), 144.2 (d, *J* 4.4, CH-5′), 134.6 (ArC), 134.2 (ArCH), 131.2 (ArCH), 129.9 (2 × ArCH), 129.3 (2 × ArCH), 128.8 (ArCH), 126.6 (d, *J* 6.3, ArC), 124.9 (d, *J* 183, CH-6′), 125.4 (ArCH), 124.5 (d, *J* 183, CH-6′), 123.8 (d, *J* 2.0, ArCH), 98.9 (CH-5), 89.1 (d, *J* 21.9, CH-4′), 89.0 (CH-1′), 76.6 (CH-3′), 41.9 (CH_2-2′), 26.5 ($SiC(CH_3)_3$), 18.8 ($SiC(CH_3)_3$), -4.6 ($Si(CH_3)_2$); δ_p (162 MHz, CD_3OD): 6.5; *m/z* (ESI^+) 632.1742 (M+H. $C_{29}H_{36}ClN_3O_7PSi$ requires 632.1743).

5.4.1 Synthesis of T*C dinucleotide **128**

(2*R*,3*S*,5*R*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5*R*)-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **128**



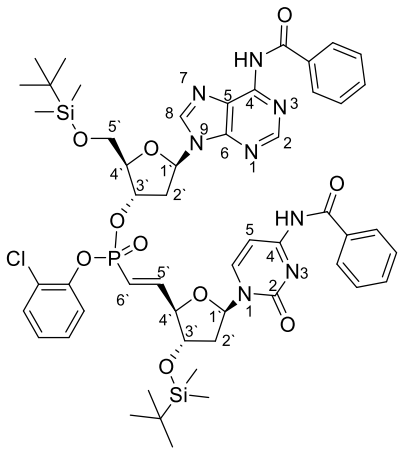
Chemical Formula: $C_{45}H_{61}ClN_5O_{11}PSi_2$
Exact Mass: 969.3332

A mixture of phosphonate **126** (100 mg, 0.16 mmol), alcohol **71** (56 mg, 0.16 mmol) and *N*-methylimidazole (76 µL, 0.96 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (0.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (58.2 mg, 0.192 mmol) were added. The reaction mixture was stirred at RT for 16 h, the reaction mixture was poured into citric acid solution (10%, 20 mL), ethyl acetate (50 mL) and stirred for 30 min. The separated aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **128** (120 mg, 78%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2951, 2928, 2855, 1686, 1664, 1623, 1556, 1478, 1399, 1251, 832, 777; δH (400MHz, $CDCl_3$): 8.90 (2H, br s, N-H), 7.99-7.84 (2.4H, m, ArH + C-H-6), 8.37-8.27 (0.6H, d, *J* 7.5, C-H-6), 7.64-7.38 (7H, m, 5 × ArH + T-H-6 + C-H-5), 7.3-7.22 (1H, m,

ArH), 7.16 (1H, q, *J* 8.0, ArH), 7.07-6.90 (1H, m, C-H-5′), 6.43-6.35 (1H, m, T-H-1′), 6.30-6.14 (2H, m, C-H-1′ + C-H-6′), 5.26 (1H, dt, *J* 12.8, 6.7, T-H-3′), 4.51-4.43 (1H, m, C-H-4′), 4.43 (0.5H, app q, *J* 2.0, T-H-4′), 4.27-4.18 (1H, m, C-H-3′ + T-H-4′), 4.13-4.06 (0.5H, m, C-H-3′), 3.91 (1H, d, *J* 2.0, T-H_A-5′), 3.86 (1H, dd, *J* 11.5, 2.2, T-H_B-5′), 3.78 (0.4H, dd, *J* 11.5, 2.2, T-H_A-5′), 2.65-2.52 (2H, m, C-H_A-2′ + T-H_A-2′), 2.24-2.10 (2H, m, C-H_B-2′ + T-H_B-2′), 1.90 (3H, s, T-H-7), 0.90 (4.25H, $SiC(CH_3)_3$), 0.89 0.90 (4.75H, $SiC(CH_3)_3$), 0.87 0.90 (9H, $SiC(CH_3)_3$), 0.11 (1.5H, $Si(CH_3)_2$), 0.10 (1.5H, $Si(CH_3)_2$), 0.09 (1.5H, $Si(CH_3)_2$), 0.08 (1.5H, $Si(CH_3)_2$), 0.05 (1.5H, $Si(CH_3)_2$), 0.04 (1.5H, $Si(CH_3)_2$), 0.03 (3H, $Si(CH_3)_2$); δc (121 MHz, $CDCl_3$): 166.7 (2 × ^{Bz}CO), 163.8 (2 × T-C-4), 162.5 (2 × C-C-4), 154.8 (2 × C-C-2), 150.5 (d, *J* 6.5, C-CH-5′), 150.40 (d, *J* 6.5, C-CH-5′), 150.35 (T-C-2), 150.3 (T-C-2), 146.2 (d, *J* 6.3, ArC), 146.1 (d, *J* 6.3, ArC), 143.9 (C-CH-6), 143.6 (C-CH-6), 135.0 (2 × T-CH-6), 133.4 (2 × ArCH), 130.9 (2 × ArCH), 129.1 (4 × ArCH), 128.3 (ArCH), 128.2 (ArCH), 127.8 (2 × ArCH), 125.84 (d, *J* 6.0, ArC), 125.76 (d, *J* 5.8, ArC), 122.4 (d, *J* 2.5, ArCH), 122.2 (d, *J* 2.7, ArCH), 117.56 (d, *J* 91.4, C-CH-6′), 117.32 (d, *J* 191.0, C-CH-6′), 111.3 (2 × T-C-5), 97.1 (2 × C-CH-5), 87.3 (2 × C-CH-1′), 86.39 (T-CH-4′), 86.2 (d, *J* 4.9, T-CH-4′), 86.09 (d, *J* 22.2, C-CH-4′), 86.05 (d, *J* 22.0, C-CH-4′), 84.75 (2 × T-CH-1′), 78.3 (d, *J* 5.9, T-CH-3′), 78.2 (d, *J* 6.0, T-CH-3′), 74.5 (d, *J* 1.7, C-CH-3′), 74.3 (d, *J* 1.5, C-CH-3′), 63.3 (2 × T-CH-5′), 41.5 (C-CH-2′), 41.4 (C-CH-2′), 39.8 (d, *J* 4.2, T-CH-2′), 39.7 (d, *J* 4.2, T-CH-2′), 26.03 ($SiC(CH_3)_3$), 25.77 ($SiC(CH_3)_3$), 25.76 ($SiC(CH_3)_3$), 18.4 (2 × $SiC(CH_3)_3$), 18.0 (2 × $SiC(CH_3)_3$), 12.6 (T-CH_3-7), -4.53 ($Si(CH_3)_2$), -4.55 ($Si(CH_3)_2$), -4.73 ($Si(CH_3)_2$), -5.26 ($Si(CH_3)_2$), -5.3 ($Si(CH_3)_2$), -5.37 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 14.6, 14.4 *m/z* (ESI^+) 970.3397 (M+H. $C_{45}H_{62}ClN_5O_{11}PSi_2$ requires 970.3405), 992.3218 (M+Na. $C_{45}H_{61}ClN_5NaO_{11}PSi_2$ requires 992.3224).

5.4.2 Synthesis of A*C dinucleotide **127**

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **127**



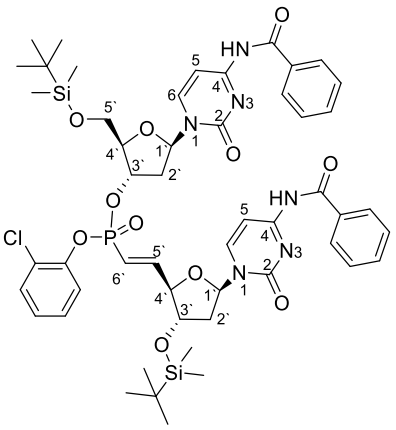
Chemical Formula: $C_{52}H_{64}ClN_8O_{10}PSi_2$
Exact Mass: 1082.3710

A mixture of phosphonate **126** (300 mg, 470 µmol), alcohol **70** (247 mg, 520 µmol) and *N*-methylimidazole (242 µL, 3.06 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL). Molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (356 mg, 1.18 mmol) were added. The reaction mixture was stirred at RT for 23 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography (5% → 10% methanol in dichloromethane) to afford the desired product **127** (420 mg, 81%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2952, 2928, 2856, 1664, 1556, 1478, 1225, 834, 778; δH (400MHz, $CDCl_3$): 9.04 (1H, br s, A-N-H), 8.78 (1H, m, A-H-2), 8.35 (0.35H, s, A-H-8), 8.33 (0.65H, s, A-H-8), 8.06-7.97 (2H, m, C-H-5 + ArH), 7.94-7.85 (2H, m, ArH + 0.35 × C-H-6), 7.79 (0.65H, d, *J* 7.5, H-6),

7.69-7.38 (9H, m, ArH + C-H-5), 7.32-7.23 (1H, m, ArH), 7.21-7.13 (1H, m, ArH), 7.09-6.95 (1H, m, C-H-5′), 6.67-6.57 (1H, m, A-H-1′), 6.32-6.17 (2H, m, C-H-1′ + C-H-6′), 5.31-5.43 (1H, m, H-3′, A), 4.52-4.46 (1H, m, C-H-4′), 4.45 (0.65H, app q, *J* 2.5, A-H-4), 4.38 (0.36H, q, *J* 2.2, A-H-3′), 4.10 (0.35H, app a *J* 6.0, C-H-3′), 3.97-3.87 (1.65H, m, A-$H_{A,B}$-5′), 3.82 (0.35H, dd, *J* 11.4, 2.9, A-H_B-5′), 2.91-2.76 (2H, m, A-H_A-2′ + A-H_B-2′), 2.63-2.51 (1H, m, C-H_A-2′), 2.26-2.18 (0.35H, m, C-H_B-2′), 2.18-2.10 (0.65H, m, C-H_B-2′), 0.91-0.86 (18H, $SiC(CH_3)_3$), 0.11-0.02 (12H, $Si(CH_3)_2$); *δ*c (101 MHz, $CDCl_3$): 166.45 (2 × ^{Bz}CO), 165.72 (2 × ^{Bz}CO), 162.4 (2 × C-C-4), 154.46 (2 × C-C-2), 152.82 (2 × A-CH-2), 151.66 (2 × A-C-4), 150. 64 (d, *J* 6.3, C-CH-5′), 150. 50 (d, *J* 5.8, C-CH-5′), 149.64 (2 × A-C-6), 146.19 (2 × d, *J* 6.3, ArC), 143.89 (C-CH-6), 143.63 (C-CH-6), 141.28 (A-CH-8), 141.21 (A-CH-8), 133.81 (2 × ArC), 133.42 (2 × ArCH), 133.0 (2 × ArC), 132.89 (2 × ArCH), 130.96 (2 × ArCH), 129.22 (2 × ArCH), 128.98 (2 × ArCH), 128.33 (ArCH), 128.26 (ArCH), 127.99 (2 × ArCH), 127.68 (2 × ArCH), 126.60 (ArCH), 126.47 (ArCH), 125.80 (d, *J* 6.0, ArC), 125.70 (d, *J* 5.8, ArC), 123.36 (2 × A-C-5), 122.74 (d, *J* 2.5, ArCH), 122.22 (d, *J* 2.6, ArCH), 117.65 (d, *J* 192, C-CH-6′), 117.16 (d, *J* 191, C-CH-6′), 96.96 (2 × C-CH-5), 87.54 (C-CH-1′), 87.27 (C-CH-1′), 86.68 (d, *J* 5.1, A-CH-4′), 86.56 (d, *J* 5.0, A-CH-4′), 86.35 (d, *J* 23.4, C-CH-4′), 86.03 (d, *J* 23.0, C-CH-4′), 84.46 (A-CH-1′), 78.18 (d, *J* 6.0, A-CH-3′), 78.14 (d, *J* 6.2, A-CH-3′), 74.53 (d, *J* 1.5, C-CH-3′), 74.31 (d, *J* 1.5, C-CH-3′), 63.25 (2 × A-CH-5′), 41.46 (C-CH_2-2′), 41.41 (C-CH_2-2′), 40.39 (d, *J* 4.2, A-CH_2-2′), 40.29 (d, *J* 4.2, A-CH_2-2′), 26.07 ($SiC(CH_3)_3$), 26.58 ($SiC(CH_3)_3$), 18.47 ($SiC(CH_3)_3$), 18.04 ($SiC(CH_3)_3$), -4.49 ($Si(CH_3)_2$), -4.52 ($Si(CH_3)_2$), -4.69 ($Si(CH_3)_2$), -5.23 ($Si(CH_3)_2$), -5.26 ($Si(CH_3)_2$), -5.37 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 14.72, 14.23; *m/z* (ESI^+) 1083.3773 (M+H. $C_{52}H_{65}ClN_8O_{10}PSi_2$ requires 1083.3783), 1105.3563 (M+Na. $C_{52}H_{64}ClN_8NaO_{10}PSi_2$ requires 1105.3602).

5.4.3 Synthesis of C*C dinucleotide **129**

synthesis of (2*R*,3*S*,5*R*)-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5*R*)-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **129**



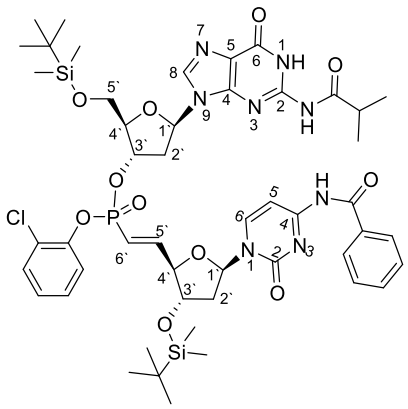
Chemical Formula: $C_{51}H_{64}ClN_6O_{11}PSi_2$
Exact Mass: 1058.3598

A mixture of phosphonate **126** (300 mg, 0.47 mmol), alcohol **103** (233 mg, 0.52 mmol) and *N*-methylimidazole (245 µL, 3.09 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (360 mg, 1.19 mmol) were added. The reaction mixture was stirred at RT for 20 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **129** (380 mg, 76%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2953, 2929, 2857, 1661, 1622, 1556, 1479, 1395, 1250, 834, 779; δH (400MHz, $CDCl_3$): 8.77 (2H, br s, N-H), 8.37-8.27 (1H, m, H-6), 7.99 (0.4H, d, *J* 7.5, H-6), 7.93-7.85 (4H, m, ArH), 7.81 (0.4H, d, *J* 7.5, H-6), 7.64-7.55 (3H, m, ArH + H-5), 7.53-

7.45 (5.4H, m, ArH + H-5), 7.45-7.41 (1H, m, ArH), 7.4 (0.6H, q, *J* 1.6, ArH), 7.30-7.22 (1H, m, ArH), 7.20-7.12 (1H, m, ArH), 7.07-6.90 (1H, m, C_2-H-5′), 6.43-6.36 (1H, m, C_1-H-1′), 6.31-6.15 (2H, m, C_2-H-1′ + C_2-H-6′), 5.35-5.22 (1H, m, C_1-H-3′), 4.51-4.42 (1.6H, m, C_2-H-4′ + 0.6 × C_1-H-4′), 4.32 (0.4H, q, *J* 2.2, C_1-H-4′), 4.26 (0.4H, app td, *J* 6.9, 5.2, C_2-H-3′), 4.10 (0.4H, app td, *J* 6.8, 5.6, C_2-H-3′), 3.97 (0.6H, dd, *J* 11.6, 2.2, C_1-H_A-5′), 3.92 (1H, dd, *J* 11.5, 2.3, C_1-H_B-5′), 3.81 (0.4H, dd, *J* 11.5, 2.2, C_1-HA-5′), 2.98-2.87 (1H, m, C_1-H_A-2′), 2.62-2.51 (1H, m, C_2-H_A-2′), 2.27-2.28 (1.4H, m, C_1-H_B-2′ + 0.4 × C_2-H_B-2′), 2.05-2.19 (0.6H, m, C_2-H_B-2′), 0.93-0.83 (18H, $SiC(CH_3)_3$), 0.14-0.02 (12H, $Si(CH_3)_2$); *δ*c (101 MHz, $CDCl_3$): 166.53 (4 × ^{Bz}CO), 162.4 (4 × C-4), 154.8 (4 × C-2), 150. 42 (d, *J* 6.0, C_2-CH-5′), 150.42 (d, *J* 5.8, C_2-CH-5′), 146.17 (d, *J* 6.7, ArC), 146.14 (d, *J* 6.9, ArC), 144.49 (3 × CH-6), 144.49 (CH-6), 133.32 (6 × ArCH), 130.95 (ArCH), 130.92 (ArCH), 129.16 (6 × ArCH), 128.19 (ArCH), 128.16 (ArCH), 127.66 (8 × ArCH), 126.55 (ArCH), 126.40 (ArCH), 125.84 (d, *J* 6.0, ArC), 125.76 (d, *J* 5.8, ArC), 122.35 (d, *J* 2.5, ArCH), 122.28 (d, *J* 2.7, ArCH), 117.56 (d, *J* 191.4, C_2-CH-6′), 117.32 (d, *J* 191.0, C_2-CH-6′), 96.93 (4 × CH-5), 87.24 (2 × C_1-CH-1′ + 2 × C_2-CH-1′), 86.91 (d, *J* 4.6, C_1-CH-4′), 86.69 (d, *J* 4.9, C_1-CH-4′), 86.30 (d, *J* 23.5, C_2-CH-4′), 86.10 (d, *J* 23.1, C_2-CH-4′), 79.99 (d, *J* 5.9, C_1-CH-3′), 79.79 (d, *J* 6.6, C_1-CH-3′), 74.47 (d, *J* 1.7, C_2-CH-3′), 74.32 (d, *J* 1.7, C_2-CH-3′), 63.00 (C_1-CH-5′), 62.97 (C_1-CH-5′), 41.45 (C_2-CH-2′), 41.39 (C_2-CH-2′), 41.42 (d, *J* 4.2, C_1-CH-2′), 41.41 (d, *J* 4.2, C_1-CH-2′), 25.99 ($SiC(CH_3)_3$), 25.79 ($SiC(CH_3)_3$), 25.76 ($SiC(CH_3)_3$), 18.37 (2 × $SiC(CH_3)_3$), 18.01 (2 × $SiC(CH_3)_3$), -4.52 ($Si(CH_3)_2$), -4.54 ($Si(CH_3)_2$), -4.69 ($Si(CH_3)_2$), -4.71 ($Si(CH_3)_2$), -5.34 ($Si(CH_3)_2$), -5.57 ($Si(CH_3)_2$), -5.43 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 14.49, 14.43; *m/z* (ESI^+) 1059.3666 (M+H. $C_{51}H_{65}ClN_6O_{11}PSi_2$ requires 1059.3671), 1081.3502 (M+Na. $C_{51}H_{64}ClN_6NaO_{11}PSi_2$ requires 1081.3490).

5.4.4 Synthesis of G*C dinucleotide **130**

(2*R*,3*S*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **130**



Chemical Formula: $C_{49}H_{66}ClN_8O_{11}PSi_2$
Exact Mass: 1064.3816

A mixture of phosphonate **126** (300 mg, 475 µmol), alcohol **104** (237 mg, 520 µmol) and *N*-methylimidazole (245 µL, 3.09 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (360 mg, 1.19 mmol) were added. The reaction mixture was stirred at RT for 28 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **130** (350 mg, 69%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2951, 2928, 2856, 1681, 1606, 1555, 1401, 1317, 1251, 833, 778; δH (500MHz, $CDCl_3$): 12.09 (0.5H, s, N-H), 12.05 (0.5H, s, N-H), 9.88 (0.5H, s, N-H), 9.53 (0.5H, s, N-H), 8.96 (1H, br s, N-H), 8.03-7.67 (4H, m, C-H-6 + G-H-8 + 2 × ArH), 7.71-7.56 (2H, m, ArH + C-H-5),

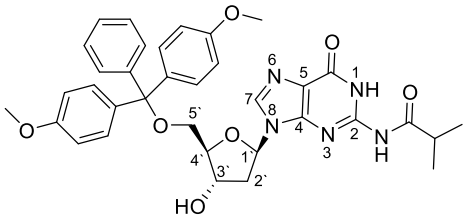
7.53-7.39 (4H, m, ArH), 7.29-7.24 (1H, s, ArH), 7.20-7.15 (1H, m, ArH), 7.01-6.85 (1H, m, C-H-5′), 6.32-6.14 (2.4H, m, G-H-1′+ C-H-1′ + 0.4 × C-H-6′), 6.10 (0.6H, dd, *J* 7.5, 6.0, C-H-1′), 5.72-5.66 (0.5H, m, G-H-3′), 5.59-5.53 (0.5H, m, G-H-3′), 4.57-4.51 (0.5H, m, C-H-4′), 4.50-4.45 (0.5H, m, C-H-4′), 4.33 (0.5H, dt, *J* 6.2, 4.3, C-H-3′), 4.30 (0.5H, q, *J* 2.9, G-H-4′), 4.20 (0.5H, q, *J* 2.8, G-H-4′), 4.14 (0.5H, q, *J* 5.8, C-H-3′), 3.84-3.63 (2H, m, G-$H_{A,B}$-5′), 2.84-2.54 (4H, m, G-$H_{A,B}$-2′ + C-H_A-2′ + $^{iBu}CH(CH_3)_2$), 2.36-2.29 (0.5H, m, C-H_A-2′), 2.25-2.18 (0.5H, m, C-H_B-2), 1.26-1.21 (6H, m, $^{iBu}CH(CH_3)_2$), 0.90-0.79 (18H, $SiC(CH_3)_3$), 0.08-(-)0.06 (12H, $Si(CH_3)_2$); δc (121 MHz, $CDCl_3$): 179.1 (^{iBu}CO), 178.7 (^{iBu}CO), 166.7 (2 × ^{Bz}CO), 162.8 (2 × C-C-4), 155.9 (G-C-6), 155.8 (G-C-6), 150.3 (d, *J* 6.0, C-CH-5′), 154.4 (d, *J* 5.0, C-CH-5), 155.2 (2 × C-C-2), 148.0 (2 × G-C-2), 146.9 (G-C-4), 147.8 (G-C-4), 146.2 (d, *J* 6.8, ArC), 146.1 (d, *J* 6.8, ArC), 144.6 (CH-6-C), 143.9 (CH-6-C), 137.3 (CH-8-G), 137.1 (CH-8-G), 135.5 (4 × ArCH), 132.8 (ArCH), 132.9 (2 × ^{Bz}ArC), 131.0 (ArCH), 130.9 (ArCH), 129.2 (4 × ArCH), 128.3 (ArCH), 128.2 (ArCH), 127. 7 (2 × ArCH), 125.8 (d, *J* 6.0, ArC), 125.7 (d, *J* 5.9, ArC), 122.5 (d, *J* 2.4, ArCH), 122.3 (d, *J* 2.5, ArCH), 121.6 (G-C-5), 121.4 (G-C-5), 118.5 (d, *J* 191, G-CH-6′), 116.8 (d, *J* 190, G-CH-6′), 96.74 (2 × C-C-5), 88.16 (d, *J* 26.1, G-CH-4′), 96.9 (2 × C-CH-5), 89.1 (G-CH-1′), 87.9 (G-CH-1′), 87.1 (d, *J* 22.9, C-CH-4′), 86.1 (d, *J* 22.3, C-CH-4′), 85.7 (d, *J* 6.5, G-CH-4′), 85.6 (d, *J* 6.0, G-CH-4′), 83.5 (C-CH-1′), 83.4 (C-CH-1′), 79.05 (d, *J* 7.1, G-CH-3′), 78.08 (d, *J* 6.1, G-CH-3′), 74.8 (C-CH-3′), 74.4 (C-CH-3′), 62.8 (2 × G-CH-5′), 41.5 (C-CH-2′), 41.4 (C-CH-2′), 40.0 (d, *J* 1.7, G-CH-2′), 39.6 (d, *J* 2.6, G-CH-2′), 36.5 ($^{iBu}CH(CH_3)_2$), 36.4 ($^{iBu}CH(CH_3)_2$), 25.9 ($SiC(CH_3)_3$), 25.8 ($SiC(CH_3)_3$), 25.875 ($SiC(CH_3)_3$), 19.26 ($^{iBu}CH(CH_3)_2$), 19.1 ($^{iBu}CH(CH_3)_2$), 19.08 ($^{iBu}CH(CH_3)_2$), 18.98 ($^{iBu}CH(CH_3)_2$), 18.43 (2 × $SiC(CH_3)_3$), 18.05 (2 × $SiC(CH_3)_3$), -4.55 ($Si(CH_3)_2$), -4.71 ($Si(CH_3)_2$), -4.76 ($Si(CH_3)_2$), -5.35 ($Si(CH_3)_2$), -5.48 ($Si(CH_3)_2$), -5.51 ($Si(CH_3)_2$), δ_P (162 MHz, $CDCl_3$): 15.02; 13.80 *m/z* (ESI^+)

1065.3903 (M+H. $C_{49}H_{67}ClN_8O_{11}PSi_2$ requires 1065.3888), 1087.3707 (M+Na. $C_{49}H_{66}ClN_8NaO_{11}PSi_2$ requires 1087.3708).

5.5 Synthesis of phosphonic acid (G) **135**

N-(9-((2*R*,4*S*,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

241

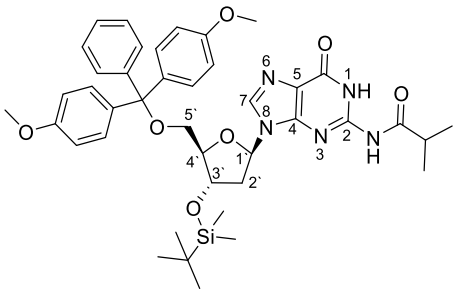


Chemical Formula: $C_{35}H_{37}N_5O_7$
Exact Mass: 639.2693

2-N-^{i}Bu-deoxyguanosine **111** (3.25 g, 9.60 mmol) was dissolved in pyridine (10 mL), and the water was removed by azeotropic distillation with pyridine (3 × 10 mL). The residue was dissolved in pyridine (12 mL), Dimethoxytrityl chloride (3.30 g, 9.80 mmol) was added in portions at 0 °C and the reaction mixture was stirred this temperature for 10 min then allowed to warm up to room temperature. The resultant mixture was stirred at RT for 3 d. when the reaction is complete, pyridine was removed by reduced pressure and the residue was dissolved in ethyl acetate (200 mL) and washed with water (100 mL) the separated aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over anhydrous sodium sulphate. The volatiles were removed by reduced pressure and the residue was purified by column chromatography using petrol: ethyl acetate (4:1 → 1:1) to afford the desired product **241** as a white foam (5.50 g, 89%). FTIR (ATR) ν_{max}/cm^{-1} 3430, 3216, 2953, 2934, 2835, 1668, 1604, 1558, 1508, 1483, 1419, 1249, 1097, 826, 785; δH (400MHz, $CDCl_3$): 12.1 (1H, s, N-H), 8.73 (1H, s, N-H), 7.80 (1H, s, H-8), 7.47-7.40 (2H, m, ArH), 7.33-7.28 (4H, m, ArH), 7.24-7.15 (3H, m, ArH), 6.80-6.72 (4H, m, ArH), 6.21 (1H, dd, *J* 7.5, 5.8, H-1'), 4.75-

4.66 (1H, m, H-3′), 4.13 (1H, q, *J* 3.9, H-4′), 3.75 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.36 (1H, dd, *J* 10.4, 3.9, H_A-5′), 3.27 (1H, dd, *J* 10.4, 4.5, H_B-5′), 2.86-2.75 (1H, m, H_A-2′), 2.44 (1H, ddd, *J* 13.2, 5.8, 3.3 H_B-2′), 2.3 (1H, h, *J* 6.9 $^{iBu}CH(CH_3)_2$), 1.10 (3H, d, *J* 6.8, $^{iBu}CH(CH_3)_2$), 1.00 (3H, d, *J* 6.8, $^{iBu}CH(CH_3)_2$); δc (101 MHz, $CDCl_3$): 178.9 (^{iBu}CO), 158.8 (ArC), 155.63 (C-6), 148.2 (C-2), 147.5 (C-4), 144.8 (ArC), 137.8 (CH-8), 136.0 (ArC), 135.8 (ArC), 130.1 (4 × ArH), 129.3 (2 × ArH), 128.2 (2 × ArH), 128.1 (2 × ArH), 127.2 (ArH), 127.0 (ArH), 122.0 (C-5), 113.35 (4 × ArH), 86.5 (CH-4′ + CAr_3), 84.5 (CH-1′), 72.4 (CH-3′), 64.0 (CH-5′), 55.4 (2 × OCH_3), 40.3 (CH-2′), 36.3 ($^{iBu}CH(CH_3)_2$), 18.94 ($^{iBu}CH(CH_3)_2$), 18.89 ($^{iBu}CH(CH_3)_2$); *m/z* (ESI$^+$) 640.2790 (M+H. $C_{35}H_{38}N_5O_7$ requires 640.2766), 662.2599 (M+Na. $C_{35}H_{37}N_5NaO_7$ requires 662.2585).

N-(9-((2*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide **131**



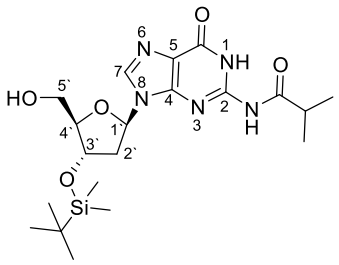
Chemical Formula: $C_{41}H_{51}N_5O_7Si$
Exact Mass: 753.3558

Imidazole (2.00 g, 23.5 mmol) was added in one portion to a stirring solution of alcohol **241** (5.00 g, 7.82 mmol) in DMF (13 mL) at 0 °C and the resulting solution was stirred at this temperature for 5 min. TBDMSCl (1.77 g, 11.7 mmol) was added in one portion and the reaction mixture was allowed to warm up to RT and stirred for 25 h at this temperature. DMF was removed *via* azeotrope with toluene (5 × 100 mL) to afford a sticky oily residue. Diethyl

ether (150 mL) and water (100 mL) were added and the separated aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo* to afford the desired product **131** (5.38 g, 91%) as a white foam.

FTIR (ATR) ν_{max}/cm^{-1} 3149, 2951, 2929, 2856, 1674, 1605, 1556, 1508, 1463, 1401, 1248, 1031, 944, 829, 779, 751; δH (400MHz, $CDCl_3$): 11.97 (1H, br s, N-H), 8.06 (1H, br s, N-H), 7.80 (1H, s, H-8), 7.49-7.44 (2H, m, ArH), 7.34 (4H, d, *J* 8.8, ArH), 7.29-7.15 (3H, m, ArH), 6.83-6.66 (4H, m, ArH), 6.18 (1H, dd, *J* 8.1, 5.5, H-1'), 4.54 (1H, dt, *J* 5.6, 2.7 H-3'), 4.04 (1H, q, *J* 3.5, H-4'), 3.77 (6H, d, $(OCH_3)_2$), 3.36 (1H, dd, *J* 10.5, 3.5, H_A-5'), 3.14 (1H, dd, *J* 10.5, 4.0, H_B-5'), 2.76 (1H, ddd, *J* 12.8, 8.1, 5.7 H_A-2'), 2.30 (1H, ddd, *J* 12.8, 5.6, 2.7, H_B-2'), 2.08 (1H, h, *J* 6.8, C*H*$(CH_3)_2$), 1.06 (3H, d, *J* 6.8, CH(C*H*$_3)_2$), 0.96 (3H, d, *J* 6.8, CH(C*H*$_3)_2$), 0.84 (9H, s, SiC$(CH_3)_3$), 0.02 (3H, s, Si$(CH_3)_2$), -0.02 (3H, s, Si$(CH_3)_2$); δc (101 MHz, $CDCl_3$): 178.3 (CO), 158.8 (C-6), 155.63 (2 × ArC), 148.2 (C-2), 147.3 (C-4), 144.5 (ArC), 137.6 (CH-8), 136.0 (ArC), 135.8 (ArC), 130.1 (4 × ArH), 128.2 (2 × ArH), 128.0 (2 × ArH), 127.2 (2 × ArH), 122.3 (C-5), 113.35 (4 × ArH), 87.2 (CH-4'), 86.4 (CAr_3), 84.5 (CH-1'), 72.6 (CH-3'), 63.4 (CH-5'), 55.4 (2 × OCH_3), 40.8 (CH-2'), 36.4 (*C*H$(CH_3)_2$), 25.9 (SiC(*C*H$_3)_3$), 18.9 (CH(*C*H$_3)_2$), 18.09 (Si*C*$(CH_3)_3$), -4.60 (Si(*C*H$_3)_2$), -4.70 (Si(*C*H$_3)_2$); *m/z* (ESI$^+$) 754.3619 (M+H. $C_{41}H_{52}N_5O_7Si$ requires 754.3631), 776.3424 (M+Na. $C_{41}H_{51}N_5NaO_7Si$ requires 776.3450), 822.3964 (M + $C_3H_5N_2$. $C_{44}H_{56}N_7O_7Si$ requires 822.4005).

N-(9-((2*R*,4*S*,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide **132**

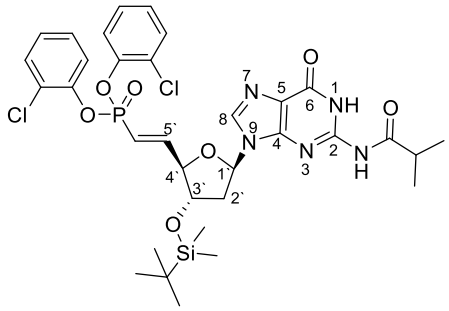


Chemical Formula: $C_{20}H_{33}N_5O_5Si$
Exact Mass: 451.2251

Compound **131** (3.00 g, 3.98 mmol) was dissolved in 3% (v/v) dichloroacetic acid in dichloromethane (55 mL) and stirred for 15 min at room temperature. The mixture was neutralized by saturated solution of sodium bicarbonate and stirred for an additional 10 min, brine (50 mL), dichloromethane (50 mL) were added and after separation the organic layer, the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using gradient elution; petrol : ethyl acetate (1:4) → ethyl acetate : methanol (9:1) to afford the desired product **132** (1.62, 90%). FTIR (ATR) ν_{max}/cm^{-1} 3157, 2953, 2929, 2855, 1673, 1603, 1555, 1470, 1400, 1250, 1091, 831, 777; δH (400MHz, $CDCl_3$): 12.09 (1H, br s, N-H), 8.92 (1H, br s, N-H), 7.84 (1H, s, H-8), 6.17 (1H, dd, *J* 8.5, 5.8, H-1′), 5.12 (1H, br s, OH), 4.59 (1H, dt, *J* 5.7, 2.1 H-3′), 4.05 (1H, q, *J* 2.1, H-4′), 3.94 (1H, dd, *J* 12.4, 2.4, H_A-5′), 3.72 (1H, dd, *J* 12.4, 2.1, H_B-5′), 2.82-2.64 (2H, m, H_A-2′, C*H*$(CH_3)_2$), 2.25 (1H, ddd, *J* 13.1, 5.8, 2.2, H_B-2′), 1.24 (6H, dd, *J* 8.9, 6.9, CH(C*H*$_3)_2$), 0.90 (9H, s, SiC$(CH_3)_3$), 0.09 (6H, s, Si$(CH_3)_2$); δc (101 MHz, $CDCl_3$): 179.0 (CO), 155.4 (C-6), 147.7 (C-2), 147.3 (C-4), 138.6 (CH-8), 122.5 (C-5), 89.3 (CH-4′), 86.4 (CH-1′), 73.4 (CH-3′), 62.9 (CH-5′), 41.5 (CH-2′), 36.5 (*C*H$(CH_3)_2$), 25.9 (SiC(*C*H$_3)_3$), 19.07 (CH(*C*H$_3)_2$), 19.04 (CH(*C*H$_3)_2$), 18.11 (Si*C*$(CH_3)_3$), -4.58 (Si(*C*H$_3)_2$), -5.65

(Si(CH_3)$_2$); *m/z* (ESI$^+$) 452.2323 (M+H. $C_{20}H_{34}N_5O_5Si$ requires 452.2324), 474.2145 (M+Na. $C_{20}H_{33}N_5NaO_5Si$ requires 474.2143).

bis(2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate

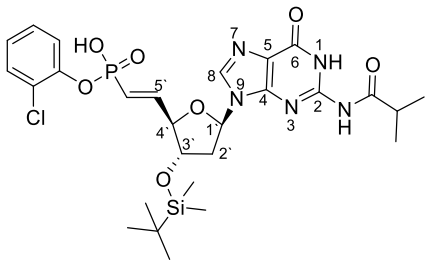


Chemical Formula: $C_{33}H_{40}Cl_2N_5O_7PSi$
Exact Mass: 747.1812

To a stirred solution of the alcohol **132** (1.10 g, 2.44 mmol) in dichloromethane (15 mL), Dess-martin periodinane (1.03 g, 2.44 mmol) was added in one portion and the reaction mixture was stirred at room temperature for 4 h. After which time, a solution of saturated; sodium bicarbonate (50 mL), sodium thiosulphate (50 mL) and brine (50 mL) were added and the mixture was stirred for 1 h and diluted with dichloromethane (80 mL). The mixture was separated, the aqueous layer was extracted with dichloromethane (4 × 40 ml) and the combined organic layers were dried over anhydrous $MgSO_4$, the volatiles were evaporated to afford the desired aldehyde **133** as white foam (Quantitative). Yield **24** (1.93 g, 2.22 mmol) was added in one portion to a stirring solution of freshly prepared aldehyde **133** (1.00 g, 2.22 mmol) in dichloromethane (10 mL) and the resulting solution was stirred at RT for 20 h. The volatiles were removed *in vacuo* and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1 → 7:3) to afford the desired product **134** (1.25, 83% over two steps) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2953, 2929, 2855, 1678, 1602, 1556, 1475, 1391, 1249, 1103, 832, 771; δH

(500MHz, $CDCl_3$): 12.28 (1H, br s, N-H-1), 10.24 (1H, br s, N-H), 8.24 (1H, ddd, *J* 26.2, 17.6, 7.8, H-5′), 7.66 (1H, s, H-8), 7.49-7.45 (1H, m, ArH), 7.42-7.35 (2H, m, ArH), 7.3-7.24 (1H, m, ArH), 7.23-7.17 (2H, m, ArH), 7.16-7.11 (2H, m, ArH), 6.30 (1H, ddd, *J* 23.8, 17.6, 1.2, H-6′), 6.24 (1H, dd, *J* 10.0, 5.4, H-1′), 4.66 (1H, dd, *J* 7.8, 3.3, H-4′), 4.23 (1H, d, *J* 3.7, H-3′), 2.93 (1H, ddd, *J* 13.2, 10.0, 3.7, H_A-2′), 2.64 (1H, h, *J* 6.8 C*H*$(CH_3)_2$), 2.14 (1H, dd, *J* 13.2, 5.4, H_B-2′), 1.11 (3H, d, *J* 6.9, CH(C*H*$_3)_2$), 1.01 (3H, d, *J* 6.8, CH(C*H*$_3)_2$), 0.93 (9H, s, SiC$(CH_3)_3$), 0.13 (3H, d, Si$(CH_3)_2$), 0.12 (3H, d, Si$(CH_3)_2$); δc (121 MHz, $CDCl_3$): 180.4 (CO), 156.6 (d, *J* 6.4, CH-5′), 155.7 (C-6), 147.9 (C-2), 147.6 (C-4), 146.1 (d, *J* 8.0, ArC), 146.0 (d, *J* 7.8, ArC), 139.4 (CH-8), 131.00 (2 × ArH), 128.1 (ArH), 127.9 (ArH), 126.9 (2 × ArH), 126.3 (d, *J* 5.5, ArC), 126.2 (d, *J* 6.0, ArC), 123.6 (C-5), 122.8 (d, *J* 2.8, ArH), 122.3 (d, *J* 2.9, ArH), 115.0 (d, *J* 189, CH-6′), 88.3 (d, *J* 27, CH-4′), 88.0 (CH-1′), 77.8 (CH-3′), 38.3 (CH-2′), 35.4 (*C*H$(CH_3)_2$), 25.88 (SiC(*C*H$_3)_3$), 19.58 (CH(*C*H$_3)_2$), 18.54 (CH(*C*H$_3)_2$), 18.22 (Si*C*$(CH_3)_3$), -4.60 (Si(*C*H$_3)_2$); δ_P (162 MHz, $CDCl_3$): 13.55; *m*/*z* (ESI^+) 748.1871 (M+H. $C_{33}H_{41}Cl_2N_5O_7PSi$ requires 748.1884), 770.1669 (M+Na. $C_{33}H_{40}Cl_2N_5NaO_7PSi$ requires 770.1704).

2-chlorophenyl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **135**

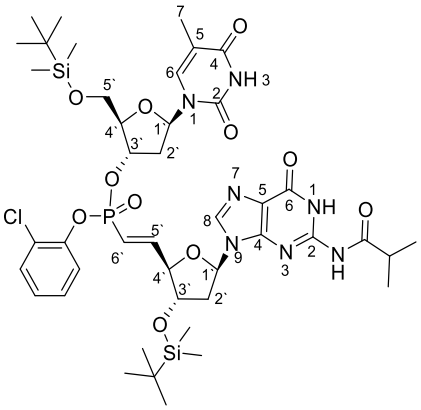


Chemical Formula: $C_{27}H_{37}ClN_5O_7PSi$
Exact Mass: 637.1888

Vinylphosphonate **134** (1.25 g, 1.67 mmol), 2-nitrobenzaldoxime (332 mg, 2.00 mmol), tetramethylguanidine (252 µL, 2.00 mmol), triethylamine (1.5 mL, 10.8 mmol) and dioxane (50 mL). The reaction mixture was stirred at RT for 16 h, then dry loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **135** (800 mg, 75%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} 3128, 2953, 2929, 2855, 1675, 1604, 1561, 1476, 1389, 1221, 1159, 831, 800, 761; δH (400 MHz, CD_3OD): 8.04 (1H, s, H-8), 8.43 (1H, d, *J* 8.0, ArH), 7.32 (1H, d, *J* 7.0, ArH), 7.1 (1H, t, *J* 7.0, ArH), 7.06-6.91 (2H, m, ArH + H-5'), 6.34 (1H, dd, *J* 9.0, 5.5, H-1'), 6.17 (1H, t, *J* 17.6, H-6'), 4.50 (1H, br d, *J* 5.5, H-3'), 4.30 (1H, br d, *J* 3.5, H-4'), 2.93-2.78 (2H, m, H_A-2' + $^{iBu}CH(CH_3)_2$), 2.24 (1H, dd, *J* 12.7, 5.5, H_B-2'), 1.20 (3H, d, *J* 6.9,$^{iBu}CH(CH_3)_2$), 1.15 (3H, d, *J* 6.8,$^{iBu}CH(CH_3)_2$), 0.93 (9H, s, $SiC(CH_3)_3$), 0.11 (6H, s, $Si(CH_3)_2$); δc (121 MHz, CD_3OD): 182.5 (^{iBu}CO), 157.6 (C-6), 150.2 (C-4 + ArC), 149.4 (C-2), 145.7 (d, *J* 4.5, CH-5'), 141.0 (CH-8), 131.1 (ArH), 128.4 (ArH), 126.9 (d, *J* 5.6, ArC), 125.5 (d, *J* 180, CH-6'), 126.3 (ArH), 123.7 (d, *J* 2.5, ArH), 122.3 (C-5), 89.8 (d, *J* 22.4, CH-4'), 87.2 (CH-1'), 78.1 (CH-3'), 39.5 (CH-2'), 36.5 ($^{iBu}CH(CH_3)_2$), 26.3 ($SiC(CH_3)_3$), 19.9 ($^{iBu}CH(CH_3)_2$), 19.1 ($^{iBu}CH(CH_3)_2$), 18.9 ($SiC(CH_3)_3$), -4.60 ($Si(CH_3)_2$), -4.8 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 9.4; *m/z* (ESI^+) 638.1951 (M+H. $C_{27}H_{38}ClN_5O_7PSi$ requires 638.1961).

5.5.1 Synthesis of T*G dinucleotide **137**

(2*R*,3*S*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **137**



Chemical Formula: $C_{43}H_{63}ClN_7O_{11}PSi_2$
Exact Mass: 975.3550

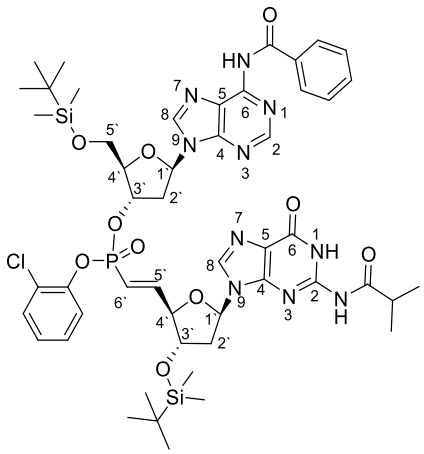
A mixture of phosphonate **135** (200 mg, 0.313 mmol), alcohol **70** (117.5 mg, 0.33 mmol) and *N*-methylimidazole (161 µL, 2.03 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (236 mg, 0.78 mmol) were added. The reaction mixture was stirred at RT for 19 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **137** (290 mg, 95%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 3222, 3064, 2952, 2929, 2857, 1756, 1683, 1603, 1556, 1472, 1393, 1250, 831, 776; δH (500MHz, $CDCl_3$): 12.30 (0.4H, s, G-N-H), 12.15 (0.6H, s, G-N-H), 10.32 (0.4H, s, G-N-H), 9.88 (0.6H, s, G-N-H), 8.69 (0.6H, br s, T-N-H), 8.67 (0.4H, br s,

T-N-H), 8.13 (0.4H, ddd, *J* 25.2, 17.7, 7.1, G-H-5′), 7.97 (0.6H, ddd, *J* 25.6, 17.4, 8.6, G-H-5′), 7.68 (0.6H, s, G-H-8), 7.66 (0.4H, s, G-H-8), 7.50 (0.6H, q, *J* 1.4, T-H-6), 7.46 (0.4, dd, *J* 8.1, 1.0, ArH), 7.41 (0.4H, q, *J* 1.4, T-H-6), 7.35 (0.6, dd, *J* 8.1, 1.0, ArH), 7.33-7.30 (0.4H, m, ArH), 7.29-7.24 (0.4H, m, ArH), 7.23-7.17 (0.4H, m, ArH), 7.10-7.14 (0.6H, m, ArH), 6.99-6.94 (0.6H, m, ArH), 6.86 (0.6H, td, *J* 7.8, 1.6, ArH), 6.49 (0.6H, dd, *J* 9.5, 5.0, T-H-1′), 6.51 (0.4H, dd, *J* 9.3, 5.1, T-H-1′), 6.29-6.08 (2H, m, G-H-1′ + G-H-6′), 5.49 (0.6H, dd, *J* 8.3, 5.3, T-H-3′), 5.17 (0.4H, dd, *J* 8.2, 5.2, T-H-3′), 4.68-4.61 (1H, m, G-H-4′), 4.46 (0.6H, app q, *J* 1.7, T-H-4′), 4.43 (0.6H, d, *J* 3.7, G-H-3′), 4.29 (0.4H, q, *J* 1.8, T-H-4′), 4.11 (0.4H, d, *J* 3.8, G-H-3′), 3.95 (0.6H, dd, *J* 11.5, 1.8, G-H_A-5′), 3.88 (0.6H, dd, *J* 11.5, 2.0 T-H_B-5′), 3.87 (0.4H, dd, *J* 11.5, 2.0, T-H_A-5′), 3.76 (0.4H, dd, *J* 11.5, 2.0, T-H_B-5′), 3.08-2.96 (0.6H, ddd, *J* 13.5, 9.9, 3.9, G-H_A-2′), 2.88-2.76 (0.8H, m, 0.4 × G-H_A-2′ + 0.4 × C*H*$(CH_3)_2$), 2.68 (0.6H, dd, 13.8, 5.0, T-H_A-2′), 2.60 (0.6H, h, *J* 6.8, C*H*$(CH_3)_2$), 2.53 (0.4H, dd, T-H_A-2′), 2.28-2.19 (1H, m, G- H_B-2′ + T-H_B-2′), 2.17-2.17 (1H, m, G-H_B-2′ + T-H_B-2′), 1.94 (1.60H, d *J*, 1.4, T-H-7), 1.90 (1.4H, d, *J* 1.4, T-H-7), 1.18 (2.4H, 2 × d, *J* 6.8, CH(C*H*$_3)_2$), 1.14 (1.8H, d, *J* 7.0, CH(C*H*$_3)_2$), 1.01 (1.8H, d, *J* 6.7, CH(C*H*$_3)_2$), 0.95 (9H, SiC$(CH_3)_3$), 0.92 (0.4H, SiC$(CH_3)_3$), 0.88 (0.4H, SiC$(CH_3)_3$), 0.17 (1.5H, Si$(CH_3)_2$), 0.15 (3H, Si$(CH_3)_2$), 0.14 (1.5H, Si$(CH_3)_2$)), 0.12 (1.5H, Si$(CH_3)_2$), 0.10 (1.5H, Si$(CH_3)_2$), 0.80 (1.5H, Si$(CH_3)_2$), 0.60 (1.5H, Si$(CH_3)_2$); *δ*c (121 MHz, $CDCl_3$): 180.16 (^{iBu}CO), 179.86 (^{iBu}CO), 163.52 (2 × T-C-4), 156.7 (d, *J* 6.2, G-CH-5′), 155.7 (G-C-6), 155.6 (G-C-6), 154.4 (d, *J* 6.5, G-CH-5′), 150.43 (T-C-2), 150.3 (T-C-2), 147.8 (G-C-2), 147.6 (G-C-2), 147.5 (2 × G-C-4), 146.1 (d, *J* 6.8, ArC), 146.00 (d, *J* 7.0, ArC), 139.6 (G-CH-8), 139.3 (G-CH-8), 134.8 (2 × T-CH-6), 131.1 (ArCH), 130.8 (ArCH), 128.2 (ArCH), 127.9 (ArCH), 127.0 (ArCH), 126.9 (ArCH), 126.1 (d, *J* 4.8, ArC), 126.0 (d, *J* 5.8, ArC), 123.7 (G-C-5), 123.6 (G-C-5), 122.25 (d, *J* 2.5, ArCH), 121.79 (d, *J* 2.7, ArCH), 116.81 (d, *J* 189.8, G-CH-6′), 114.8 (d, *J* 187.5, G-

CH-6′), 111.6 (T-C-5), 111.5 (T-C-5), 88.3 (d, *J* 26.2, G-CH-4′), 88.2 (d, *J* 26.2, G-CH-4′), 87.94 (G-CH-1′), 87.89 (G-CH-1′), 86.1 (d, *J* 4.0, T-CH-4′), 86.8 (d, *J* 4.2, T-CH-4′), 84.7 (T-CH-1′), 84.6 (T-CH-1′), 79.05 (d, *J* 7.0, T-CH-3′), 78.08 (d, *J* 6.1, T-CH-3′), 77.98 (G-CH-3′), 76.76 (G-CH-3′), 63.4 (T-CH-5′), 63.3 (T-CH-5′), 39.8 (d, *J* 4.8, C-CH-2′), 39.13 (d, *J* 4.5, T-H-2′), 38.5 (2 × G-CH-2′), 35.5 (^{iBu}*C*H(CH$_3$)$_2$), 36.2 (^{iBu}*C*H(CH$_3$)$_2$), 26.00 (SiC(*C*H$_3$)$_3$), 25.96 (SiC(*C*H$_3$)$_3$), 25.86 (SiC(*C*H$_3$)$_3$), 20.10 (^{iBu}CH(*C*H$_3$)$_2$), 19.43 (^{iBu}CH(*C*H$_3$)$_2$), 19.04 (^{iBu}CH(*C*H$_3$)$_2$), 18.39 (2 × Si*C*(CH$_3$)$_3$), 18.21 (Si*C*(CH$_3$)$_3$), 18.16 (Si*C*(CH$_3$)$_3$), 17.99 (^{iBu}CH(*C*H$_3$)$_2$), 12.65 (C-7, T), -4.51 (Si(CH$_3$)$_2$), -4.55 (Si(CH$_3$)$_2$), -4.66 (2 × Si(CH$_3$)$_2$), -5.24 (Si(CH$_3$)$_2$), -5.28 (Si(CH$_3$)$_2$), -5.40 (Si(CH$_3$)$_2$), -5.47 (Si(CH$_3$)$_2$); δ_P (162 MHz, CDCl$_3$): 17.7, 16.3; *m/z* (ESI$^+$) 976.3585 (M+H. $C_{43}H_{64}ClN_7O_{11}PSi_2$ requires 976.3623), 998.3411 (M+Na. $C_{43}H_{63}ClN_7NaO_{11}PSi_2$ requires 998.3442).

5.5.2 Synthesis of A*G dinucleotide **136**

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **136**



Chemical Formula: $C_{50}H_{66}ClN_{10}O_{10}PSi_2$
Exact Mass: 1088.3928

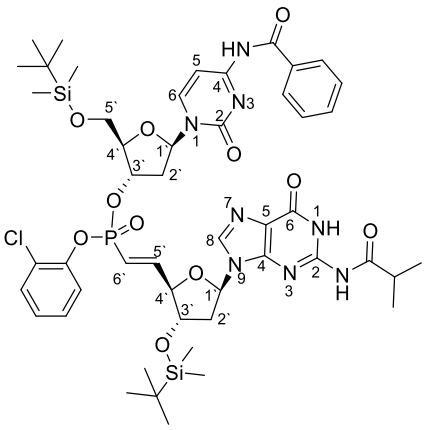
A mixture of phosphonate **135** (300 mg, 638 µmol), alcohol **71** (243 mg, 470 µmol) and *N*-methylimidazole (242 µL, 3.05 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (356 mg, 1.17 mmol) were added. The reaction mixture was stirred at RT for 20 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were removed *via* reduced pressure and the residue was purified by column chromatography (5%→10% methanol in dichloromethane) to afford the desired product **136** (320 mg, 63%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2951, 2928, 2856, 1686, 1604, 1554, 1477, 1391, 1247, 833, 777; δH (400MHz, $CDCl_3$): 12.30 (0.4H, s, G-N-H), 12.16 (0.6H, s, G-N-H), 10.33 (0.4H, s, ^{iBu}N-H), 9.92 (0.6H, s, ^{iBu}N-H), 9.07 (0.6H, br s, A-N-H), 9.08 (0.4H, br s, A-N-H), 8.80 (0.6H, s, A-H-2), 8.87 (0.4H, s, A-H-2), 8.35

(0.6H, s,A- H-8), 8.27 (0.4H, s, A-H-8), 8.13 (0.4H, ddd, *J* 25.7, 17.6, 7.2, G-H-5′), 8.08-7.94 (2.6H, m, 2 × ArH, + 0.6 × G-H-5′), 7.68 (0.6H, s, G-H-8), 7.65 (0.4H, s, G-H-8), 7.63-7.56 (1H, m, ArH), 7.55-7.45 (2.4H, m, ArH), 7.38-7.34 (1, m, ArH), 7.31-7.26 (0.4H, m, ArH), 7.23-7.18 (0.4H, m, ArH), 7.10-7.04 (0.6H, m, ArH), 7.03-6.99 (0.6H, m, ArH), 6.92-6.86 (0.6H, app td, *J* 7.8, 1.6, ArH), 6.69 (0.6H, dd, *J* 8.8, 5.6, A-H-1′), 6.57 (0.4H, dd, *J* 8.3, 5.7, A-H-1′), 6.33-6.11 (2H, m, G-H-1′, G-H-6′), 5.66 (0.6H, dd, *J* 8.3, 5.3, A-H-3′), 5.47-5.40 (0.4H, m, A-H-3′), 4.68-4.61 (1H, m, G-H-4′), 4.56 (0.6H, app q, *J* 2.3, A-H-4′), 4.45 (0.6H, d, *J* 3.7, G-H-3′), 4.39 (0.4H, td, *J* 3.3, 1.7, A-H-4′), 4.10 (0.4H, d, *J* 3.7, G-H-3′), 3.98 (0.6H, dd, *J* 11.4, 2.8, A-H_A-5′), 3.94-3.88 (1H, m, A-H_B-5′), 3.80 (0.4H, dd, *J* 11.3, 3.0, A-H_A-5′), 3.06 (0.6H, ddd, *J* 13.5, 9.9, 4.0, G-H_A-2′), 2.99-2.75 (2.8H, m, 0.4 × G-H_A-2′ + 2 × A-H-2′ + 0.4 × $^{iBu}CH(CH_3)_2$), 2.64 (0.6H, h, *J* 6.8, $^{iBu}CH(CH_3)_2$), 2.22 (0.6H, dd, 13.2, 5.5, G-H_B-2′), 2.53 (0.4H, dd, *J* 13.2, 5.5, G-H_B-2′), 1.18 (2.0H, d, *J* 6.5, $^{iBu}CH(CH_3)_2$), 1.15 (2.2H, d, *J* 7.1, $^{iBu}CH(CH_3)_2$), 1.02 (1.8H, d, *J* 6.8, $^{iBu}CH(CH_3)_2$), 0.95 (5H, $SiC(CH_3)_3$), 0.922 (3.7H, $SiC(CH_3)_3$), 0.916 (5.3H, $SiC(CH_3)_3$), 0.85 (4H, $SiC(CH_3)_3$), 0.18 (1.7H, $Si(CH_3)_2$), 0.16 (1.7H, $Si(CH_3)_2$), 0.12 (3H, $Si(CH_3)_2$)), 0.11 (1.7H, $Si(CH_3)_2$), 0.10 (1.3H, $Si(CH_3)_2$), 0.08 (1.3H, $Si(CH_3)_2$), 0.04 (1.3H, $Si(CH_3)_2$); *δ*c (101 MHz, $CDCl_3$): 180.18 (^{iBu}CO), 179.90 (^{iBu}CO), 164.72 (^{Bz}CO), 164.69 (^{Bz}CO), 156.6 (d, *J* 6.3, G-CH-5′), 155.7 (G-C-6), 155.6 (G-C-6), 154.3 (d, *J* 6.4, G-CH-5′), 152.86 (A-CH-2), 152.80 (A-CH-2), 151.75 (A-C-4), 151.61 (A-C-4), 149.75 (A-C-6), 149.71 (A-C-6), 147.88 (G-C-2), 147.61 (G-C-2), 147.5 (2 × G-C-4), 146.1 (d, *J* 6.7, ArC), 145.98 (d, *J* 6.9, ArC), 141.3 (A-CH-8), 141.0 (A-CH-8), 139.6 (G-CH-8), 139.3 (G-CH-8), 133.69 (ArC), 133.65 (ArC), 132.97 (ArCH), 132.92 (ArCH), 131.06 (ArCH), 130.79 (ArCH), 128.99 (ArCH), 128.89 (ArCH), 128.19 (ArCH), 127.98 (2 × ArCH), 127.90 (ArCH), 126.96 (ArCH), 126.83 (ArCH), 126.1 (d, *J* 4.8, ArC), 126.0 (d, *J* 5.7, ArC), 126.0 (d, *J* 5.8, ArC), 123.7 (G-C-5), 123.6 (G-C-5), 123.47 (A-C-5),

123.37 (A-C-5), 122.3 (d, *J* 2.7, ArCH), 121.8 (d, *J* 2.8, ArCH), 116.7 (d, *J* 190.2, G-CH-6′), 113.9 (d, *J* 187.4, G-CH-6′), 88.28 (d, *J* 26.4, G-CH-4′), 88.0 (d, *J* 26.3, G-CH-4′), 87.96 (G-CH-1′), 87.88 (G-CH-1′), 86.64 (d, *J* 4.2, A-CH-4′), 86.24 (d, *J* 4.9, A-CH-4′), 84.49 (A-CH-1′), 84.31 (A-CH-1′), 78.8 (d, *J* 6.9, A-CH-3′), 78.2 (d, *J* 6.4, A-CH-3′), 77.97 (G-CH-3′), 76.95 (G-CH-3′), 63. 4(A-CH-5′), 63.0 (A-CH-5′), 40.58 (d, *J* 4.6, A-CH_2-2′), 39.78 (d, *J* 3.8, A-CH_2-2′), 38.52 (2 × G-CH_2-2′), 35.5 (^{iBu}*C*H$(CH_3)_2$), 36.2 (^{iBu}*C*H$(CH_3)_2$), 26.00 (3 × SiC(*C*$H_3)_3$), 25.97 (3 × SiC(*C*$H_3)_3$), 25.86 (6 × SiC(*C*$H_3)_3$), 20.04 (^{iBu}CH(*C*$H_3)_2$), 19.40 (^{iBu}CH(*C*$H_3)_2$), 19.03 (^{iBu}CH(*C*$H_3)_2$), 18.44 (Si$(CH_3)_3$), 18.41 (Si*C*$(CH_3)_3$), 18.19 (^{iBu}CH(*C*$H_3)_2$), 18.16 (^{iBu}CH(*C*$H_3)_2$), 18.04 (^{iBu}CH(*C*$H_3)_2$), -4.54 (Si$(CH_3)_2$), -4.55 (Si$(CH_3)_2$), -4.68 (2 × Si$(CH_3)_2$), -5.22 (Si$(CH_3)_2$), -5.29 (Si$(CH_3)_2$), -5.44 (Si$(CH_3)_2$), -5.47 (Si$(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 17.53, 16.19; *m/z* (ESI$^+$) 1089.3950 (M+H. $C_{50}H_{67}ClN_{10}O_{10}PSi_2$ requires 1089.4001), 1111.3779 (M+Na. $C_{50}H_{66}ClNNa_{10}O_{10}PSi_2$ requires 1111.3820).

5.5.3 Synthesis of C*G dinucleotide **138**

(2*R*,3*S*,5*R*)-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **138**



Chemical Formula: $C_{49}H_{66}ClN_8O_{11}PSi_2$
Exact Mass: 1064.3816

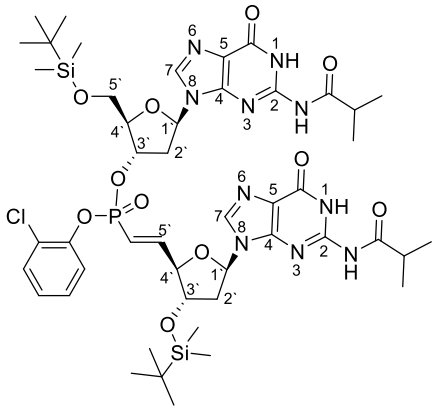
A mixture of phosphonate **135** (300 mg, 0.47 mmol), alcohol **103** (218 mg, 0.49 mmol) and *N*-methylimidazole (241 µL, 3.05 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (356 mg, 1.17 mmol) were added. The reaction mixture was stirred at RT for 23 h, into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na2SO4. The volatiles were removed via reduced pressure and the residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **138** (350 mg, 64%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} 2953, 2928, 2853, 1687, 1603, 1554, 1478, 1392, 1248, 833, 779; δH (400MHz, $CDCl_3$): 12.31 (0.4H, s, G-N-H), 12.15 (0.6H, s, G-N-H), 10.33 (0.4H, s, ^{iBu}N-H), 9.95 (0.6H, s, ^{iBu}N-H), 8.82 (1H, br s, ^{Bz}N-H), 8.28 (0.6H, d, *J* 7.5, C-H-6), 8.23 (0.4H, d, *J* 7.5, C-H-6), 8.13 (0.4H,

ddd, *J* 26, 17.9, 7.23, G-H-5′), 8.00 (0.6H, ddd, *J* 25.6, 17.5, 8.6, G-H-5′), 7.92-7.85 (2H, m, ArH), 7.68 (0.6H, s, G-H-8), 7.66 (0.4H, s, G-H-8), 7.63-7.57 (1H, m, ArH), 7.55-7.45 (3H, m, ArH + C-H-5), 7.45-7.42 (0.4H, m, ArH), 7.37-7.33 (0.4H, m, ArH), 7.33-7.30 (0.4H, m, ArH), 7.29-7.23 (0.6H, m, ArH), 7.22-7.16 (0.4H, m, ArH), 7.09-7.03 (0.6H, m, ArH), 7.00-6.96 (0.6H, m, ArH), 6.92-6.85 (0.6H, m. ArH), 6.51 (0.6H, dd, *J* 8.6, 5.3, C-H-1′), 6.51 (0.4H, dd, *J* 7.7, 5.6, C-H-1′), 6.28-6.04 (2H, m, G-H-1′ + G-H-6′), 5.56-5.48 (0.6H, m, C-H-3′), 5.20-5.13 (0.4H, m, C-H-3′), 4.67-4.61 (1H, m, G-H-4′), 4.5 (0.6H, app q, *J* 1.7, C-H-4′), 4.43 (0.6H, d, *J* 3.7, G-H-3′), 4.37 (0.4H, q, *J* 2.2, C-H-4′), 4.09 (0.4H, d, *J* 3.8, G-H-3′), 3.99 (0.6H, dd, *J* 11.5, 2.0, C-H_A-5′), 3.94-3.85 (1H, m, C-H_B-5′), 3.78 (0.4H, dd, *J* 11.5, 2.2, C-H_A-5′), 3.08-2.96 (1H, m, G-H_A-2′ + C-H_A-2′), 2.92-2.73 (1.4H, m, C-H_A-2′ + G-H_A-2′ + 0.4 × C*H*$(CH_3)_2$), 2.61 (0.6H, h, *J* 6.9, C*H*$(CH_3)_2$), 2.27-2.09 (2H, m, G-H_B-2′ + C-H_B-2′), 1.18 (2.4H, d, *J* 6.8, CH(C*H*$_3)_2$), 1.13 (1.8H, d, *J* 7.0, CH(C*H*$_3)_2$), 1.10 (1.8H, d, *J* 6.7, CH(C*H*$_3)_2$), 0.96-0.85 (18H, SiC$(CH_3)_3$), 0.20-0.02 (12H, Si$(CH_3)_2$); *δ*c (101 MHz, $CDCl_3$): 180.13 (^{iBu}CO), 179.84 (^{iBu}CO), 166.5 (2 × ^{Bz}CO), 162.4 (2 × C-C-4), 156.8 (d, *J* 6.1, G-CH-5′), 155.7 (G-C-6), 155.6 (G-C-6), 154.8 (2 × C-C-2), 154.4 (d, *J* 6.6, G-CH-5′), 147. 83(G-C-2), 147.57 (G-C-4), 147.54 (G-C-2), 147.51 (G-C-4), 146.0 (d, *J* 6.8, ArC), 145.96 (d, *J* 6.8, ArC), 144.27 (2 × C-CH-6), 139.56 (G-CH-8), 139.28 (G-CH-8), 133.41 (ArCH), 133.36 (ArCH), 132.99 (2 × ArC), 131.0 (ArCH), 130.73 (ArCH), 129.19 (2 × ArCH), 129.16 (2 × ArCH), 128.18 (ArCH), 127.91 (ArCH), 127.64 (4 × ArCH), 126.89 (ArCH), 126.79 (ArCH), 126.04 (d, *J* 4.7, ArC), 125.95 (d, *J* 5.8, ArC), 123.59 (G-C-5), 123.54 (G-C-5), 122.25 (d, *J* 2.7, ArCH), 121.79 (d, *J* 2.8, ArCH), 116.63 (d, *J* 189.8, G-CH-6′), 114.64 (d, *J* 187.2, G-CH-6′), 96.74 (2 × C-CH-5), 88.16 (d, *J* 26.1, G-CH-4′), 88.10 (d, *J* 26.2, G-CH-4′), 87.85 (G-CH-1′), 87.83 (G-CH-1′), 87.21 (C-CH-1′), 87.13 (C-CH-1′), 86.91 (d, *J* 3.6, C-CH-4′), 86.56 (d, *J* 4.7, C-CH-4′), 79.05 (d, *J* 7.1, C-CH-3′), 78.08

(d, *J* 6.1, C-CH-3′), 77.96 (G-CH-3′), 76.83 (G-CH-3′), 63.32 (C-CH-5′), 63.94 (C-CH-5′), 41.38 (d, *J* 5.2, C-CH-2′), 41.13 (d, *J* 4.2, C-CH-2′), 38.53 (2 × G-CH-2′), 35.53 (^{iBu}*C*H(CH$_3$)$_2$), 36.16 (^{iBu}*C*H(CH$_3$)$_2$), 25.92 (SiC(*C*H$_3$)$_3$), 25.89 (SiC(*C*H$_3$)$_3$), 25.83 (SiC(*C*H$_3$)$_3$), 20.00 (^{iBu}CH(*C*H$_3$)$_2$), 19.35 (^{iBu}CH(*C*H$_3$)$_2$), 19.04 (^{iBu}CH(*C*H$_3$)$_2$), 18.31 (2 × Si*C*(CH$_3$)$_3$), 18.16 (Si*C*(CH$_3$)$_3$), 18.13 (Si*C*(CH$_3$)$_3$), 18.03 (^{iBu}CH(*C*H$_3$)$_2$), -4.54 (Si(CH$_3$)$_2$), -4.59 (Si(CH$_3$)$_2$), -4.69 (Si(CH$_3$)$_2$), -5.34 (Si(CH$_3$)$_2$), -5.39 (Si(CH$_3$)$_2$), -5.54 (Si(CH$_3$)$_2$), -5.57 (Si(CH$_3$)$_2$); δ_P (162 MHz, CDCl$_3$): 17.40; 17.23 *m/z* (ESI$^+$) 1065.3910 (M+H. $C_{49}H_{67}ClN_8O_{11}PSi_2$ requires 1065.3888), 1087.3686 (M+Na. $C_{49}H_{66}ClN_8NaO_{11}PSi_2$ requires 1087.3708).

5.5.4 Synthesis of G*G dinucleotide **139**

(2*R*,3*S*,5*R*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(6-oxo-2-propionamido-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **139**



Chemical Formula: $C_{47}H_{68}ClN_{10}O_{11}PSi_2$
Exact Mass: 1070.4034

A mixture of phosphonate **135** (300 mg, 0.51 mmol), alcohol **104** (230 mg, 0.51 mmol) and *N*-methylimidazole (240 µL, 3.05 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1.5 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (356 mg, 1.17 mmol) were added. The reaction mixture was stirred at

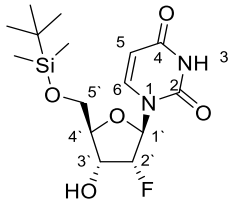
RT for 19 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na2SO4. The volatiles were removed via reduced pressure and the residue was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **139** (350 mg, 70%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) $\nu_{max/}cm^{-1}$ 2951, 2929, 2856, 1680, 1603, 1555, 1475, 1360, 1249, 832, 778; δH (400MHz, $CDCl_3$): 12.24 (0.5H, s, G-N-H), 12.19 (0.5H, s, G-N-H), 12.15 (0.5H, s, G-N-H), 12.07 (0.5H, s, G-N-H), 10.36 (0.5H, br s, ^{iBu}N-H), 9.98 (0.5H, br s, ^{iBu}N-H), 9.81 (0.5H, br s, ^{iBu}N-H), 9.45 (0.5H, br s, ^{iBu}N-H), 7.93 (0.5H, s, G_1-H-8), 7.88 (0.5H, s, G_1-H-8), 7.88 (0.5H, s, G_2-H-8), 7.78 (0.5H, s, G_2-H-8), 7.84-7.58 (1H, m, H-5′), 7.45 (0.5H, dd, *J* 7.9, 1.0, ArH), 7.36-7.30 (1H, m, ArH), 7.28-7.23 (0.5H, m, ArH), 7.20-7.15 (0.5H, m, ArH), 7.08-6.98 (1H, m, ArH), 6.93-6.87 (0.5H, m, ArH), 6.36-6.06 (3H, m, G_1-H-1′ + G_2-H-1′ + H-6′), 5.57-5.49 (0.5H, m, G_1-H-3′), 5.41-5.35 (0.5H, m, G_1-H-3′), 4.64-4.15 (1H, m, G_2-H-4′), 4.47-4.41 (1H, m, G_1-H-4′ + G_2-H-3′), 438-4.34 (0.5H, m, G_2-H-3′), 4.28 (0.5H, q, *J* 2.5, G_1-H-4′), 3.85 (1H, d, *J* 2.5, G_1-H_A-5′), 3.79-3.72 (1H, m, G_1-H_B-5′), 3.08-2.96 (1H, m, G_2-H_A-2′), 2.94-2.65 (3H, m, G_1-H_A-2′ + G_1-H_B-2′ + C*H*$(CH_3)_2$), 2.64-2.54 (1H, m, C*H*$(CH_3)_2$)), 2.3-2.20 (1H, m, G_2-H_B-2′), 1.25-1.99 (12H, m, CH(C*H*$_3)_2$), 0.93 (4.5H, s, SiC$(CH_3)_3$), 0.90 (4.5H, s, SiC$(CH_3)_3$), 0.87 (4.5H, s, SiC$(CH_3)_3$), 0.85 (4.5H, s, SiC$(CH_3)_3$), 0.14 (3H, d, Si$(CH_3)_2$), 0.10 (3H, d, Si$(CH_3)_2$), 0.06 (3H, d, Si$(CH_3)_2$), 0.04 (3H, d, Si$(CH_3)_2$); δc (101 MHz, $CDCl_3$): 179.94 (^{iBu}CO), 179.79 (^{iBu}CO), 179.77 (^{iBu}CO), 179.09 (^{iBu}CO), 155.88 (C-6), 155.71 (C-6), 155.65 (C-6), 155.59 (C-6), 154.02 (d, *J* 6.6, G_2-CH-5′), 153.98 (d, *J* 6.4, G_2-CH-5′), 148.41 (C-2), 148.28 (C-2), 148.07 (2 × C-2), 147.97 (C-4), 147.77 (C-4), 147.59 (C-4), 147.46 (C-4), 146.11 (d, *J* 6.9, ArC), 146.01 (d, *J* 6.9, ArC), 139.65 (G_2-CH-8), 139.51 (G_2-CH-8), 136.39 (G_1-CH-8),

136.12 (G_1-CH-8), 131.03 (ArCH), 130.75 (ArCH), 128.20 (ArCH), 127.86 (ArCH), 126.83 (ArCH), 126.69 (ArCH), 125.98 (d, *J* 5.0, ArC), 125.88 (d, *J* 5.8, ArC), 123.14 (C-5), 122.97 (C-5), 122.05 (d, *J* 2.6, ArCH), 121.89 (d, *J* 2.6, ArCH), 121.36 (C-5), 121.24 (C-5), 117.01 (d, *J* 190, CH-6′, G_2), 116.34 (d, *J* 190, G_2-CH-6′), 87.97 (d, *J* 24, G_2-CH-4′), 87.82 (d, *J* 24, G_2-CH-4′), 87.48 (G_2-CH-1′), 87.16 (G_2-CH-1′), 86.58 (d, *J* 5.0, G_1-CH-4′), 86.10 (d, *J* 5.0, CH-4′,G_1), 84.07 (G_1-CH-1′), 83.51 (G_1-CH-1′), 78.62 (d, *J* 6.7, G_1-CH-3′), 78.14 (d, *J* 6.7, G_1-CH-3′), 76.76 (G_2-CH-3′), 76.56 (G_2-CH-3′), 63.40 (G_1-CH-5′), 63.21 (G_1-CH-5′), 40.67 (d, *J* 3.8, G_1-CH-2′), 40.55 (d, *J* 3.8, G_1-CH-2′), 38.45 (G_2-CH-2′), 38.42 (G_2-CH-2′), 36.38 (CH$(CH_3)_2$), 36.09 (CH$(CH_3)_2$), 35.90 (CH$(CH_3)_2$), 35.42 (CH$(CH_3)_2$), 26.00 (SiC($CH_3)_3$), 25.83 (SiC($CH_3)_3$), 19.85 (CH($CH_3)_2$), 19.61 (CH($CH_3)_2$), 19.23 (CH($CH_3)_2$), 19.21 (CH($CH_3)_2$), 19.14 (CH($CH_3)_2$), 19.12 (CH($CH_3)_2$), 19.59 (CH($CH_3)_2$), 18.43 (SiC($CH_3)_3$), 18.41 (SiC($CH_3)_3$), 18.14 (CH($CH_3)_2$), (2 × SiC($CH_3)_3$), -4.56 (Si($CH_3)_2$),-4.59 (Si($CH_3)_2$), -4.66 (Si($CH_3)_2$), -4.72 (Si($CH_3)_2$), -5.30 (2 × Si($CH_3)_2$), -5.51 (2 × Si($CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 17.16; 15.79; *m/z* (ESI$^+$) 1071.4102 (M+H. $C_{47}H_{69}ClN_{10}O_{11}PSi_2$ requires 1071.4106), 1093.3913 (M+Na. $C_{47}H_{68}ClN_{10}NaO_{11}PSi_2$ requires 1093.3926).

5.6 Synthesis 2'substituted dinucleotides

5.6.1 Synthesis of ^{F}U*A dinucleotide **154**

1-((2*R*,3*R*,4*R*,5R)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **149**

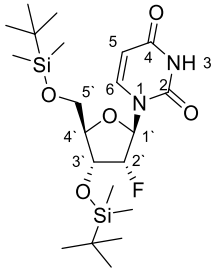


Chemical Formula: $C_{15}H_{25}FN_2O_5Si$
Exact Mass: 360.1517

2'-F-Uridine **148** (2.00 g, 8.123 mmol) was dissolved in pyridine (10 mL) and dried by azeotropic distillation with pyridine (3 × 10 mL), the residue was then dissolved in anhydrous DMF (13 mL). Imidazole (1.22 g, 17.9 mmol) was added at 0 °C, and stirred at this temperature for 5 min. *tert*-butyldimethylsilyl chloride (1.35 g, 8.93 mmol) was added at this temperature and the reaction was allowed to warm up to RT and stirred for 24 h. The solvent was then evaporated by azeotropic distillation with toluene (3 × 50 mL) and the sticky oily residues was dissolved in ethyl acetate (100 mL) and washed with water (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were evaporated, and the crude product was purified by column chromatography using gradient elution; petrol: ethyl acetate (1:1) → ethyl acetate) to afford the desired product **150** as white solid (2.52 g, 87%). And 3',5'-bis(OTBDMS) nucleoside **151** as a by-product (87 mg, 6%), FTIR (ATR) ν_{max}/cm^{-1}: 2953, 2929, 2857, 1673, 1461, 1255, 1111, 1064, 827; δ_H (400 MHz, $CDCl_3$): 9.14 (1H, br. s, NH), 7.94 (1H, d, *J* 8.2, H-6), 6.11 (1H, dd, *J* 15.2, 2.1, H-1'), 5.69 (1H, d, *J* 8.2, H-5), 4.96 (1H, ddd, *J* 53, 4.3, 2.1, H-2'), 4.36 (1H, ddd, *J* 18.4, 7.2, 4.3, H-3'), 4.15-4.13 (2H, m, H-4' + H_A-5'), 3.87 (1H, dd, *J* 12.0, 2.0, H_B-5'), 2.65 (1H, br s, OH), 0.92 (9H, $SiC(CH_3)_3$), 0.12 (6H, $Si(CH_3)_2$); δ_C (101

MHz, $CDCl_3$): 163.3 (C-4), 150.15 (C-2), 139.92 (CH-6), 102.55 (CH-5), 94.10 (d, *J* 188, CH-2′), 87.31 (d, *J* 33.6, CH-1′), 83.76 (CH-4′), 68.61 (d, *J* 16.8, CH-3′), 61.31 (CH-5′), 26.03 (3 × SiC(*C*H_3)$_3$), 18.56 (SiC(CH_3)$_3$), -5.34 (Si(*C*H_3)$_2$), -5.43 (Si(*C*H_3)$_2$); δF (376 MHz, $CDCl_3$): 22.04; *m/z* (ESI$^+$) 361.1589 (M+H. $C_{15}H_{26}FN_2O_5Si$ requires 361.1590), 383.1408 (M+H. $C_{15}H_{25}FN_2NaO_5Si$ requires 383.1409).

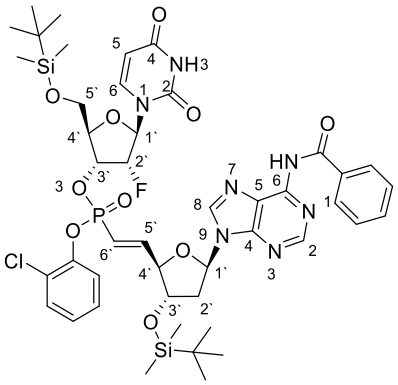
1-((2*R*,3*R*,4*R*,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **150**



Chemical Formula: $C_{21}H_{39}FN_2O_5Si_2$
Exact Mass: 474.2382

FTIR (ATR) ν_{max}/cm^{-1}: 2952, 2927, 2856, 1706, 1695, 1454, 1252, 1120, 1074, 811; δ_H (400 MHz, $CDCl_3$): 8.84 (1H, br. s, NH), 7.92 (1H, d, *J* 8.2, H-6), 6.06 (1H, dd, *J* 15.3, 2.0, H-1′), 5.69 (1H, d, *J* 8.2, H-5), 4.77 (1H, ddd, *J* 52, 4.2, 2.0, H-2′), 4.28 (1H, ddd, *J* 19.0, 7.1, 4.1, H-3′), 4.11-4.03 (2H, m, H-4′ + H_A-5′), 3.87 (1H, dd, *J* 11.7, 1.6, H_B-5′), 0.93 (9H, SiC(CH_3)$_3$), 0.90 (9H, SiC(CH_3)$_3$), 0.12 (6H, Si(CH_3)$_2$), 0.11 (3H, Si(CH_3)$_2$), 0.10 (3H, Si(CH_3)$_2$); δ_C (101 MHz, $CDCl_3$): 163.1 (C-4), 150.0 (C-2), 139.9 (CH-6), 102.5 (CH-5), 94.10 (d, *J* 193.4, CH-2′), 87.9 (d, *J* 33.9, CH-1′), 83.9 (CH-4′), 68.7 (d, *J* 16.3, CH-3′), 60.85 (CH-5′), 26.0 (3 × SiC(*C*H_3)$_3$), 25.7 (3 × SiC(*C*H_3)$_3$), 18.54 (SiC(CH_3)$_3$), 18.21 (SiC(CH_3)$_3$), -4.50 (Si(*C*H_3)$_2$), -4.90 (Si(*C*H_3)$_2$); -5.35 (Si(*C*H_3)$_2$), -5.39 (Si(*C*H_3)$_2$), δF (376 MHz, $CDCl_3$): -202.45; *m/z* (ESI$^+$) 475.2460 (M+H. $C_{21}H_{40}FN_2O_5Si_2$ requires 475.2454), 497.2269 (M+Na. $C_{21}H_{39}FN_2NaO_5Si_2$ requires 497.2274).

(2*R*,3*R*,4*R*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluorotetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((tert butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **154**



Chemical Formula: $C_{45}H_{58}ClFN_7O_{10}PSi_2$
Exact Mass: 997.3194

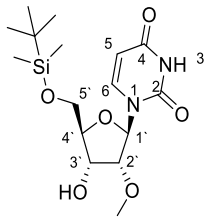
A mixture of phosphonate **69** (300 mg, 460 µmol), alcohol **150** (157 mg, 460 µmol) and *N*-methylimidazole (237 µL, 3.00 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (167 mg, 550 µmol) were added. The reaction mixture was stirred at RT for 21 h, poured into citric acid solution (10%, 50 mL), ethyl acetate (50 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were removed via reduced pressure and the residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate) to afford the desired product **154** (350 mg, 77%, 2:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2951, 2928, 2855, 1695, 1664, 1479, 1251, 1126, 1066, 833; δH (400 MHz, $CDCl_3$): 9.97 (0.65H, bs, NH), 9.90 (0.35H, bs, NH), 9.64 (0.35H, s, A-NH), 9.54 (0.65H, s, A-NH), 8.82 (0.35H, s, A-H-2), 8.77 (0.65H, s, A-H-2), 8.21 (0.35H, s, A-H-8), 8.14 (0.65H, s, A-H-8), 8.09-7.05 (2H, m, ArH), 7.48 (0.65H, d, *J* 8.2, U-H-6), 7.79 (0.35H, d, *J* 8.2, U-H-6), 7.61-7.54 (1, m, ArH), 7.53-7.45 (3H, m, ArH), 7.41-

7.33 (2H, m, ArH), 7.24-7.18 (1H, m, ArH), 7.15-7.01 (2H, m, ArH + A-H-5′), 6.53 (0.35H, dd, *J* 7.3, 6.1, A-H-1′), 6.47 (0.65H, dd, *J* 7.3, 6.1, A-H-1′), 6.28-6.12 (1.35H, m, A-H-6′ + U-H1′), 6.32 (0.65H, dd, *J* 14.9, 2.2, U-H-1′), 5.66 (1H, d, *J* 8.2, U-H-5), 5.20-4.97 (2H, m, U-H-3′ + U-H-2′), 4.64-4.48 (2H, m, A-H-3′ + A-H-4′), 4.31 (0.65H, d, *J* 7.0, U-H-4′), 4.27-4.23 (0.35H, m, U-H-4′), 4.01 (0.65H, dd, *J* 12.0, 1.9, U-H_A-5′), 3.95 (0.35H, dd, *J* 12.0, 2.0, U-H_A-5′), 3.81 (0.65H, dd, *J* 12.1, 1.9, U-H_B-5′), 3.81 (0.35H, dd, *J* 12.0, 1.8, U-H_B-5′), 3.97-2.84 (1H, m, A-H_A-2′), 2.49-2.35 (1H, m, A-H_B-2′), 0.91 (9H, SiC(CH_3)$_3$), 0.88 (6H, SiC(CH_3)$_3$), 0.86 (3H, SiC(CH_3)$_3$), 0.11 (6H, Si(CH_3)$_2$), 0.07 (1.83H, Si(CH_3)$_2$), 0.06 (1.83H, Si(CH_3)$_2$), 0.04 (1.1H, Si(CH_3)$_2$), 0.03 (1.1H, Si(CH_3)$_2$); δ_C (121 MHz, $CDCl_3$): 165.32 (^{Bz}CO), 165.19 (^{Bz}CO), 163.18 (U-C-4), 163.13 (U-C-4), 152.77 (A-CH-2), 152.72 (A-CH-2), 152.07 (A-C-4), 152.72 (A-C-4), 151.51 (d, *J* 6.6, A-CH-5′), 151.27 (d, *J* 6.7, A-CH-5′), 150.27 (U-C-2), 150.21 (U-C-2), 150.15 (A-C-6), 150.10 (A-C-6), 146.1 (d, *J* 6.9, ArC), 145.9 (d, *J* 7.2, ArC), 142.1 (A-CH-8), 141.9 (A-CH-8), 139.5 (U-CH-6), 139.4 (U-CH-6), 133.6 (ArH), 133.6 (ArH), 132.9 (ArH + ArC), 132.8 (ArH + ArC), 130.9 (2 × ArCH), 128.8 (2 × ArCH), 128.7 (2 × ArCH), 128.4 (2 × ArCH), 128.4 (2 × ArCH), 128.3 (2 × ArCH), 128.2 (ArCH), 128.1 (ArCH), 126.5 (2 × ArCH), 126.0 (d, *J* 5.8, ArC), 125.8 (d, *J* 5.8, ArC), 124.12 (A-C-5), 124.04 (A-C-5), 122.4 (d, *J* 2.3, ArCH), 122.2 (d, *J* 2.2, ArCH), 116.6 (d, *J* 192.5, U-CH-6′), 116.4 (d, *J* 191.5, U-CH-6′), 103.0 (U-CH-5), 102.8 (U-CH-5), 91.9 (d, *J* 196.0, 2.0, U-CH-2′), 90.5 (d, *J* 196.3, 2.0, U-CH-2′), 87.32 (d, *J* 33.4, U-CH-1′), 87.0 (d, *J* 22.3, A-CH-4′), 86.9 (d, *J* 33.0, U-CH-1′), 86.7 (d, *J* 22.6, A-CH-4′), 85.22 (A-CH-1′), 85.0 (A-CH-1′), 82.7 (d, *J* 7.0, U-CH-4′), 82.3 (d, *J* 7.0, U-CH-4′), 75.3 (2 × A-CH-3′), 72.0 (dd, *J* 14.9, 5.8, U-CH-3′), 71.2 (dd, *J* 15.2, 6.3, U-CH-3′), 61.2 (U-CH_2-5′), 60.8 (U-CH_2-5′), 39.1 (T-CH_2-2′), 39.0 (T-CH_2-2′), 26.0 (6 × SiC(*C*H_3)$_3$), 25.8 (6 × SiC(*C*H_3)$_3$), 18.4 (2 × Si*C*(CH_3)$_3$), 18.1 (2 × Si*C*(CH_3)$_3$), -4.6 (2 × Si(CH_3)$_2$), -4.7 (2 × Si(CH_3)$_2$), -5.4 (2 ×

$Si(CH_3)_2$), -5.4 (2 × $Si(CH_3)_2$); δP (162 MHz, $CDCl_3$): 16.22, 15.36; δF (376 MHz, $CDCl_3$): -202.32, -202.51; *m/z* (ESI^+) 998.3250 (M+H. $C_{45}H_{59}ClFN_7O_{10}PSi_2$ requires 998.3267), 1020.3044 (M+Na. $C_{45}H_{58}ClFN_7NaO_{10}PSi_2$ requires 1020.3086).

5.6.2 Synthesis of ^{OMe}U*A dinucleotide **155**

1-((2*R*,3*R*,4*R*,5R)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-hydroxy-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **151**

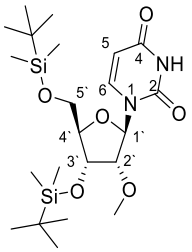


Chemical Formula: $C_{16}H_{28}N_2O_6Si$
Exact Mass: 372.1717

2'-OMe-Uridine **149** (2.00 g, 7.72 mmol) was dissolved in pyridine (10 mL) and dried by azeotropic distillation with pyridine (3 × 10 mL), the residue was then dissolved in anhydrous DMF (12 mL). Imidazole (1.16 g, 16.9 mmol) was added at 0 °C, and stirred at this temperature for 5 min. *Tert*-butyldimethylsilyl chloride (1.28 g, 8.49 mmol) was added at this temperature and the reaction was allowed to warm up to RT and stirred for 17 h. The solvent was then evaporated by azeotropic distillation with toluene (3 × 50 mL) and the sticky oily residues was dissolved in ethyl acetate (100 mL) and washed with water (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were evaporated and the crude product was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate) to afford the desired product **149** as white solid (2.40 g, 83%). And 3',5'-bis(OTBDMS) nucleoside **151** as a by-product (220 mg, 11%), FTIR (ATR) ν_{max}/cm^{-1}: 2952, 2929, 2857, 1672, 1460, 1267, 1118, 826; δ_H (400 MHz, $CDCl_3$): 9.55 (1H, br.

s, NH), 8.07 (1H, d, *J* 8.1, H-6), 5.97 (1H, d, *J* 2.0, H-1′), 5.67 (1H, d, *J* 8.1, H-5), 4.24 (1H, app t, *J* 5.4, H-3′), 4.05 (1H, dd, *J* 11.9, 1.8, H_A-5′), 3.96 (1H, dt, *J* 7.3, 1.8, H-4′), 3.85 (1H, dd, *J* 11.9, 1.8, H_B-5′), 3.73 (1H, dd, *J* 5.4, 2.0, H-2′), 3.61 (3H, s, OCH_3), 2.71 (1H, br d, *J* 5.4, OH), 0.92 (9H, $SiC(CH_3)_3$), 0.12 (6H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 163.7 (C-4), 150.4 (C-2), 140.1 (CH-6), 102.2 (CH-5), 87.0 (CH-1′), 84.5 (CH-4′), 84.28 (CH-2′), 67.8 (CH-3′), 61.3 (CH-5′), 58.8 (OCH_3), 26.0 (3 × $SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), -5.4 ($Si(CH_3)_2$), -5.5 ($Si(CH_3)_2$); *m/z* (ESI^+) 373.1775 (M+H. $C_{16}H_{29}N_2O_6Si$ requires 373.1789), 395.1603 (M+H. $C_{16}H_{28}N_2NaO_6Si$ requires 395.1609).

1-((2*R*,3*R*,4*R*,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **151**

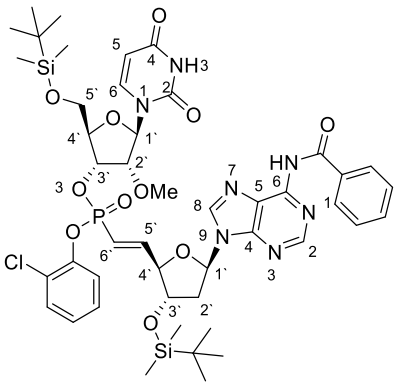


Chemical Formula: $C_{22}H_{42}N_2O_6Si_2$
Exact Mass: 486.2581

FTIR (ATR) ν_{max}/cm^{-1}: 2953, 2928, 2857, 1687, 1459, 1253, 1126, 1096, 833; δ_H (400 MHz, $CDCl_3$): 8.14 (1H, br. s, NH), 8.05 (1H, d, *J* 8.2, H-6), 5.93 (1H, d, *J* 2.0, H-1′), 5.67 (1H, dd, *J* 8.2, 2.0, H-5), 4.24 (1H, dd, *J* 7.10, 4.9, H-3′), 4.07-4.00 (2H, m, H-4′ + H_A-5′), 3.77 (1H, dd, *J* 12.3, 2.2, H_B-5′), 3.60 (1H, dd, *J* 4.9, 2.0, H-2′), 3.54 (3H, s, OCH_3), 0.94 (9H, $SiC(CH_3)_3$), 0.90 (9H, $SiC(CH_3)_3$), 0.12 (3H, $Si(CH_3)_2$), 0.11 (3H, $Si(CH_3)_2$), 0.10 (3H, $Si(CH_3)_2$), 0.08 (3H, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$): 163.0 (C-4), 150.0 (C-2), 140.2 (CH-6), 102.0 (CH-5), 87.6 (CH-1′), 84.1 (CH-2′), 84.5 (CH-4′), 68.7 (CH-3′), 61.0 (CH-5′), 58.4 (OCH_3), 26.1 (3 × $SiC(CH_3)_3$), 25.8 (3 × $SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), 18.3 ($SiC(CH_3)_3$), -4.4 (($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$), -5.3 ($Si(CH_3)_2$),

-5.4 ($Si(CH_3)_2$); *m/z* (ESI^+) 487.2641 (M+H. $C_{22}H_{43}N_2O_6Si_2$ requires 487.2654), 509.2458 (M+H. $C_{22}H_{42}N_2NaO_6Si_2$ requires 509.2474).

(2*R*,3*R*,4*R*,5R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-methoxytetrahydrofuran-3-yl (2-chlorophenyl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **155**



Chemical Formula: $C_{46}H_{61}ClN_7O_{11}PSi_2$
Exact Mass: 1009.3394

A mixture of phosphonate **69** (300 mg, 460 µmol), alcohol **151** (171 mg, 460 µmol) and *N*-methylimidazole (237 µL, 3.00 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (1 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (167 mg, 550 µmol) were added. The reaction mixture was stirred at RT for 22 h, poured into citric acid solution (10%, 50 mL), ethyl acetate (50 mL) and stirred for 30 min. The separated aqueous layers were extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were removed *via* reduced pressure and the residue was purified by column chromatography using gradient elution; ethyl acetate: petrol 1:1→ ethyl acetate) to afford the desired product **155** (285 mg, 62%, 2:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1}: 2953, 2929, 2856, 1690, 1477, 1252, 1122, 1071, 331; δH (500 MHz, $CDCl_3$): 9.93 (0.65H, bs, NH), 9.61 (0.65H, bs, NH), 9.49 (0.7H, s, A-NH), 8.82 (0.35H, s, A-H-2), 8.76

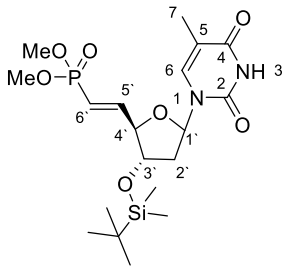
(0.65H, s, A-H-2), 8.21 (0.35H, s, A-H-8), 8.11 (0.65H, s, A-H-8), 8.08-8.01 (2H, m, ArH), 7.9 (0.65H, d, *J* 8.2, U-H-6), 7.8 (0.35H, d, *J* 8.2, U-H-6), 7.61-7.54 (1, m, ArH), 7.53-7.45 (3H, m, ArH), 7.41-7.33 (1.65H, m, ArH), 7.24-7.18 (1H, m, ArH), 7.15-6.98 (2.3H, m, ArH + A-H-5′), 6.52 (0.35H, t, 6.5, A-H-1′), 6.47 (0.65H, t, *J* 6.5, A-H-1′), 6.25-6.13 (1H, m, A-H-6′), 6.11 (0.35H, d, *J* 5.3, U-H-1′), 6.02 (0.65H, d, *J* 4.3, U-H-1′), 5.66 (1H, d, *J* 8.2, U-H-5), 5.11-5.02 (1H, m, U-H-3′), 4.61-4.50 (2H, m, A-H-3′ + A-H-4′), 4.34-4.31 (0.65H, m, U-H-4′), 3.77 (0.35H, dt, *J* 3.8, 1.9, U-H-4′), 3.96-3.92 (1H, m, *J* U-H_A-5′), 3.90-3.86 (1H, m, U-H-5′ + U-H-2′), 3.80 (0.65H, dd, *J* 12.0, 1.7, U-H-5′), 3.70 (0.35H, dd, *J* 12.0, 1.7, U-H-5′), 3.43 (1H, s, 2′-OMe), 3.36 (2H, s, 2′-OMe), 3.97-2.86 (1H, m, A-H_A-2′), 2.48-2.37 (1H, m, A-H_B-2′), 0.91 (9H, SiC(CH_3)$_3$), 0.88 (6H, SiC(CH_3)$_3$), 0.88 (3H, SiC(CH_3)$_3$), 0.11 (6H, Si(CH_3)$_2$), 0.07 (6H, m, Si(CH_3)$_2$); δ_C (126 MHz, $CDCl_3$): 165.3 (^{Bz}CO), 165.1 (^{Bz}CO), 163.3 (U-C-4), 163.2 (U-C-4), 152.8 (A-CH-2), 152.7 (A-CH-2), 152.0 (A-C-4), 151.8 (A-C-4), 150.9 (d, *J* 6.7, A-CH-5′), 150.5 (U-C-2), 150.4 (U-C-2), 150.23 (d, *J* 6.7, A-CH-5′), 150.1 (2 × A-C-6), 146.3 (d, *J* 6.9, ArC), 146.2 (d, *J* 7.2, ArC), 142.1 (A-CH-8), 141.9 (A-CH-8), 139.7 (U-CH-6), 133.7 (ArH), 133.6 (ArH), 132.9 (ArH + ArC), 132.8 (ArH + ArC), 130.9 (ArCH), 130.8 (ArCH), 128.84 (2 × ArCH), 128.7 7(2 × ArCH), 128.3 (2 × ArCH), 128.1 (2 × ArCH), 126.3 (2 × ArCH), 126.0 (d, *J* 5.8, ArC), 125.8 (d, *J* 5.8, ArC), 124.1 (A-C-5), 124.0 (A-C-5), 122.3 (2 × combined, ArCH), 117.6 (d, *J* 194, U-CH-6′), 116.9 (d, *J* 191, U-CH-6′), 102.9 (U-CH-5), 102.6 (U-CH-5), 87.13 (d, *J* 23, A-CH-4′), 86.7 (d, *J* 22.3, A-CH-4′), 86.6 (U-CH-1′), 86.3 (U-CH-1′), 85.2 (A-CH-1′), 85.0 (A-CH-1′), 83.7 (d, *J* 6.3, U-CH-4′), 83.4 (d, *J* 5.7, U-CH-4′), 82.9 (d, *J* 2.2, U-CH_2-2′), 82.8 (d, *J* 3.0, U-CH_2-2′), 75.4 (A-CH-3′), 75.2 (A-CH-3′), 73.5 (d, *J* 6.3, U-CH-3′), 72.6 (d, *J* 6.5, U-CH-3′), 62.2 (U-CH_2-5′), 61.7 (U-CH_2-5′), 58.8 (2′-OCH_3), 58.6 (2′-OCH_3), 39.3 (A-CH_2-2′), 39.0 (A-CH_2-2′), 26.0 (6 × SiC(*C*H_3)$_3$), 25.8 (6 × SiC(*C*H_3)$_3$), 18.4 (2 × Si*C*(CH_3)$_3$), 18.1 (2 × Si*C*(CH_3)$_3$), -4.6 (2 ×

$Si(CH_3)_2$), -4.7 (2 × $Si(CH_3)_2$), -5.48 (2 × $Si(CH_3)_2$), -5.4 (2 × $Si(CH_3)_2$); δP (162 MHz, $CDCl_3$): 15.6, 15.3; *m/z* (ESI^+) 1010.3479 (M+H. $C_{46}H_{62}ClN_7O_{11}PSi_2$ requires 1010.3466), 1032.3258 (M+Na. $C_{46}H_{61}ClN_7NaO_{11}PSi_2$ requires 1032.3286).

5.7 Synthesis of terminal 5'-(*E*)-vinylphosphonate

5.7.1 Synthesis of T-monomers phosphoramidite **177**

Synthesis of dimethyl ((*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4 dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **181**

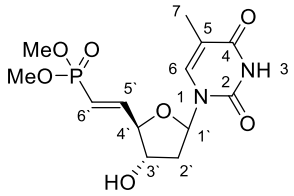


Chemical Formula: $C_{19}H_{33}N_2O_7PSi$
Exact Mass: 460.1795

To a suspension of NaH (146 mg, 6.10 mmol) in dry THF (5 mL) at 0 °C was added a solution of tetramethyl methylenebisphosphonate **180** (1.25 mL, 1.57 mmol) in 10 mL of dry THF. The reaction mixture was stirred at 0 °C for 10 min. The resulting suspension was then added dropwise to a solution of aldehyde **23** (1.20 g, 3.39 mmol) in 10 mL of dry THF at 0 °C. The reaction mixture was stirred for 3 h and allowed to slowly warm up to RT. It was then quenched with saturated NH_4Cl (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. The residue was purified by silica gel column chromatography using gradient elution; ethyl acetate → ethyl acetate : methanol (9:1) to afford the desired the product **181** as a colourless oil (1.30 g, 75%). FTIR (ATR) ν_{max}/cm^{-1}: 2953, 2929, 2853, 1695, 1677, 1651, 1471, 1277, 1250, 1111, 1026, 832;

δ_H (400 MHz, $CDCl_3$): 8.86 (1H, br. s, NH), 7.08 (1H, q, *J* 1.3, H-6), 6.86 (1H, ddd, *J* 22, 17, 5.0, H-5′), 6.32 (1H, t, *J* 6.7 H-1′), 5.98 (1H, ddd, *J* 19, 17, 1.7), 4.40-4.34 (1H, m, H-4′), 4.26 (1H, dt, *J* 6.6, 4.1, H-3′), 3.76 (3H, d, *J* 11.1, $POCH_3$), 3.74 (3H, d, *J* 11.1, $POCH_3$), 2.32-2.23 (1H, m, H_A-2′), 2.17-2.08 (1H, m, H_B-2′), 1.94 (3H, d, *J* 1.3, H-7), 0.89 (9H, $SiC(CH_3)_3$), 0.08 (6H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 163.6 (C-4), 150.3 (C-2), 149.0 (d, *J* 5.9, C-5′), 135.3 (CH-6), 117.8 (d, *J* 189, CH-6′), 111.7 (C-5), 86.2 (d, *J* 22, CH-4′), 85.3 (CH-1′), 75.0 (d, *J* 1.3, CH-3′), 52.8 (d, *J* 12.5, $PO(OCH_3)$), 52.6 (d, *J* 12.5, $PO(OCH_3)$), 39.95 (CH-2′), 25.8 (3 × $SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), 12.8 (CH_3-7), -4.5 ($Si(CH_3)_2$), -4.6 ($Si(CH_3)_2$); δ_P (162 MHz, $CDCl_3$): 19.8; *m/z* (ESI^+) 461.1860 (M+H. $C_{19}H_{34}N_2O_7PSi$ requires 461.1867), 483.1693 (M+Na. $C_{19}H_{33}N_2NaO_7PSi$ requires 483.1687), 478.2127 (M + H_4N. $C_{19}H_{37}N_3O_7Psi$ requires 478.2133).

dimethyl ((*E*)-2-((2*R*,3*S*,5*R*)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **183**

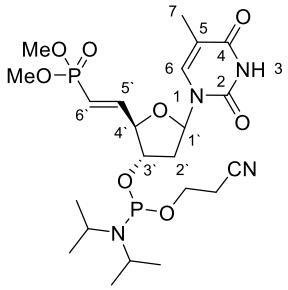


Chemical Formula: $C_{13}H_{19}N_2O_7P$
Exact Mass: 346.0930

Triethylamine trihydrofluoride (3.5 mL, 21.7 mmol) was added in one portion to a stirring solution of vinylphosphonate **181** (1.00 g, 2.17 mmol) in THF (25 mL) and the resulting mixture was stirred at RT for 5 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using; dichloromethane : methanol (9:1) to afford the desired product **183** (900 mg, 100%) as a white powder. FTIR (ATR) ν_{max}/cm^{-1} : 3366, 2955, 2852, 2820, 1680, 1467, 1269 1227, 1019, 830; δ_H (400 MHz, $CDCl_3$): 9.60 (1H, br. s, NH), 7.11 (1H, q, *J* 1.3, H-6), 7.02 (1H, ddd, *J* 22.7, 17, 4.0, H-5′), 6.36 (1H, t, *J*

6.6 H-1′), 6.00 (1H, ddd, *J* 20, 17, 1.8, CH-6′), 4.50-4.45 (1H, m, H-4′), 4.40 (1H, dt, *J* 6.9, 4.5, H-3′), 3.76 (3H, d, *J* 11.1, $POCH_3$), 3.73 (3H, d, *J* 11.1, $POCH_3$), 2.47-2.36 (1H, m, H_A-2′), 2.25-2.16 (1H, m, H_B-2′), 1.92 (3H, d, *J* 1.3 H-7), δ_C (126 MHz, $CDCl_3$): 163.9 (C-4), 150.7 (C-2), 150.0 (d, *J* 6.0, CH-5′), 135.55 (CH-6), 116.3 (d, *J* 190, CH-6′), 111.8 (C-5), 85.8 (d, *J* 22, CH-4′), 85.4 (CH-1′), 74.1 (d, *J* 1.3, CH-3′), 52.9 (d, *J* 12.5, $PO(OCH_3)$), 52.7 (d, *J* 13.1, $PO(OCH_3)$), 39.2 (CH-2′), 12.73 (CH_3-7); δ_P (162 MHz, $CDCl_3$): 20.5; *m/z* (ESI^+) 347.0997 (M+H. $C_{13}H_{20}N_2O_7P$ requires 347.1003), 369.0829 (M+Na. $C_{13}H_{19}N_2NaO_7P$ requires 369.0822).

2-cyanoethyl ((2*R*,3S)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) diisopropylphosphoramidite **177**

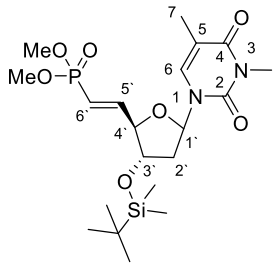


Chemical Formula: $C_{22}H_{36}N_4O_8P_2$
Exact Mass: 546.2008

To a mixture of compound **183** (180 mg, 520 µmol) and phosphordiamidite **52** (148 µL, 490 µmol) in dry dichloromethane (1.5 mL), a solution of (5-Methyl-1*H*-tetrazole 43.7 mg, 520 µmol in dry acetonitrile) (500 µL) was added in one portion and the resultant mixture was stirred at room temperature for 21 h. The solution was diluted with acetonitrile (5mL), quenched with TEA (250 µL) and water (250 µL) and washed with n-hexane (10 mL). After separation the dichloromethane/acetonitrile layer was diluted with ether (50 mL), washed with water (20mL) and brine (10 mL), dried over Na_2SO_4 and the volatiles were removed *in vacuo*. The residue was purified by column chromatography using

3% methanol in dichloromethane to afford the desired product as a colourless oil (1:1 mixture of two diastereoisomers, 180 mg, 63%). FTIR (ATR) ν_{max}/cm^{-1} : 2969, 2934, 2820, 1688, 1409, 1368, 1229, 1030, 974; δ_H (400 MHz, $CDCl_3$): 7.10-7.07 (1H, m, H-6), 7.04-6.81 (1H, m, H-5′), 6.36 (1H, dt, *J* 6.9, 1.7, H-1′), 6.02 (0.5H, ddd, *J* 19.1, 17.1, 1.8, H-6′), 6.00 (0.5H, ddd, *J* 19.2, 17.1, 1.9, H-6′), 4.61-4.54 (0.5H, m, H-4′), 4.54-4.49 (0.5H, m, H-4′), 4.49-4.40 (1H, m, H-3′), 3.93-3.82 (1H, m, OC*H*$_2$CH$_2$CN), 3.77 (1.5H, d, *J* 11.0, POCH$_3$), 3.76 (1.5H, d, *J* 11.0, POCH$_3$), 3.75 (1.5H, d, *J* 11.0, POCH$_3$), 3.74 (1.5H, d, *J* 11.0, POCH$_3$), 3.75-3.66 (1H, m, OC*H*$_2$CH$_2$CN), 3.67-3.56 (2H, m, NC*H*(CH$_3$)$_2$), 2.78-2.62 (2H, m, OCH$_2$C*H*$_2$CN), 2.54-2.41 (1H, m, H$_A$-2′), 2.29-2.19 (1H, m, H$_B$-2′), 1.97-190 (3H, m, H-7), 1.20 (3H, d, *J* 6.8, NCH(C*H*$_3$)$_2$), 1.19 (9H, d, *J* 6.8, NCH(C*H*$_3$)$_2$); δ_C (126 MHz, $CDCl_3$): 163.2 (2 × C-4), 151.2 (C-2), 151.15 (C-2), 148.7 (d, *J* 6.2, CH-5′), 148.5 (d, *J* 6.1, CH-5′), 133.3 (CH-6), 133.2 (CH-6), 117.93 (CN), 117.9 (d, *J* 190, CH-6′), 117.8 (CN), 117.5 (d, *J* 190, CH-6′), 111.9 (2 × C-5), 85.04 (CH-1′), 85.00 (CH-1′), 84.9-84.5 (m, CH-4′), 76.1 (dd, *J* 1.3, 15.9, CH-3′), 75.6 (dd, *J* 1.3, 15.9, CH-3′), 58.3 (d, *J* 6.5, O*C*H$_2$CH$_2$CN), 58.1 (d, *J* 6.7, O*C*H$_2$CH$_2$CN), 52.7 (2 × d, *J* 5.5, PO(OCH$_3$)), 52.8 (d, *J* 5.5, PO(OCH$_3$)), 52.8 (d, *J* 5.5, PO(OCH$_3$)), 43.53 (d, *J* 12.4, N*C*H(CH$_3$)$_2$), 53.5 (d, *J* 12.4, N*C*H(CH$_3$)$_2$), 38.5 (d, *J* 3.5, CH$_2$-2′), 38.4 (d, *J* 4.0, CH$_2$-2′), 24.9-24.6 (m, NCH(*C*H$_3$)$_2$), 20.6 (d, *J* 7.4, OCH$_2$*C*H$_2$CN), 20.5 (d, *J* 7.4, OCH$_2$*C*H$_2$CN), 12.68 (CH$_3$-7), 12.65 (CH$_3$-7); δ_P 162 MHz, $CDCl_3$): 149.3 (2 × P), 20.1, 19.7; *m*/*z* (ESI$^+$) 547.2067 (M+H. $C_{22}H_{37}N_4O_8P_2$ requires 547.2081), 569.1881 (M+Na. $C_{22}H_{36}N_4NaO_8P_2$ requires 569.1901).

dimethyl((*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(3,5-dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **182**

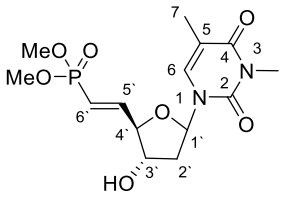


Chemical Formula: $C_{20}H_{35}N_2O_7PSi$
Exact Mass: 474.1951

To a suspension of NaH (233 mg, 10.1 mmol) in dry THF (10 mL) at 0 °C was added a solution of tetramethyl methylenebisphosphonate **180** (1.25 mL, 1.57 mmol) in 10 mL of dry THF. The reaction mixture was stirred at 0 °C for 10 min. The resulting suspension was then added dropwise to a solution of aldehyde **23** (1.20 g, 3.39 mmol) in 30 mL of dry THF at 0 °C. The reaction mixture was allowed to warmup to room temperature and stirred 20 h. It was then quenched with saturated NH_4Cl (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. The residue was purified by silica gel column chromatography using ethyl acetate to afford the desired product **182** (1.50 g, 93%) as a colourless oil. δ_H (400 MHz, $CDCl_3$): 7.08 (1H, q, *J* 1.3, H-6), 6.84 (1H, ddd, *J* 22, 17, 5.0, H-5′), 6.35 (1H, t, *J* 6.7, H-1′), 5.97 (1H, ddd, *J* 19, 17, 1.7, H-6′), 4.40-4.34 (1H, m, H-4′), 4.25 (1H, dt, *J* 6.6, 4.1, H-3′), 3.76 (3H, d, *J* 11.0, $POCH_3$), 3.74 (3H, d, *J* 11.0, $POCH_3$), 3.33 (1H, s, N-CH_3), 2.32-2.25 (1H, m, H_A-2′), 2.14-2.05 (1H, m, H_B-2′), 1.95 (3H, d, *J* 1.3, H-7), 0.88 (9H, $SiC(CH_3)_3$), 0.07 (6H, $Si(CH_3)_2$); δ_C (126 MHz, $CDCl_3$): 163.5 (C-4), 151.1 (C-2), 149.0 (d, *J* 5.9, CH-5′), 133.0 (CH-6), 117.9 (d, *J* 189, CH-6′), 110.7 (C-5), 86.2 (d, *J* 22, CH-4′), 85.9 (CH-1′), 75.0 (d, *J* 1.25, CH-3′), 52.8 (d, *J* 12.5, $PO(OCH_3)$), 52.6 (d, *J* 12.5, $PO(OCH_3)$), 40.0 (CH-2′), 28.0 (N-CH_3), 25.8 (3 × ($SiC(\mathit{C}H_3)_3$), 18.1 ($Si\mathit{C}(CH_3)_3$), 13.5 (CH_3-7), -4.50 ($Si(\mathit{C}H_3)_2$), -4.66

(Si(CH_3)$_2$); δ_P 162 MHz, $CDCl_3$): 19.8; *m/z* (ESI$^+$) 461.1859 (M+H. $C_{19}H_{34}N_2O_7PSi$ requires 461.1867).

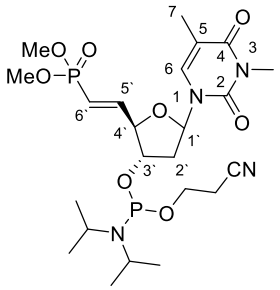
dimethyl ((*E*)-2-((2*R*,3*S*,5*R*)-5-(3,5-dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)vinyl)phosphonate **184**



Chemical Formula: $C_{14}H_{21}N_2O_7P$
Exact Mass: 360.1086

Triethylamine trihydrofluoride (2.4 mL, 14.7 mmol) was added in one portion to a stirring solution of vinylphosphonate **182** (700 mg, 1.47 mmol) in THF (25 mL) and the resulting reaction mixture was stirred at RT for 5 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using; dichloromethane : methanol (9:1) to afford the desired product **184** (500 mg, 94%) as a colourless oil. δ_H (400 MHz, $CDCl_3$): 7.09-6.96 (2H, m, H-6, H-5'), 6.40 (1H, t, *J* 6.5 H-1'), 6.00 (1H, ddd, *J* 20, 17, 2.0, H-6'), 4.48-4.43 (1H, m, H-4'), 4.38-4.33 (1H, m, H-3'), 3.75 (3H, d, *J* 11.0, $POCH_3$), 3.73 (3H, d, *J* 11.0, $POCH_3$), 3.33 (1H, s, N-CH_3), 2.46-2.37 (1H, m, H_A-2'), 2.27-2.18 (1H, m, H_B-2'), 1.95 (3H, d, *J* 1.3, H-7; δ_C (126 MHz, $CDCl_3$): 163.5 (C-4), 151.2 (C-2), 149.0 (d, *J* 6.1, CH-5'), 133.2 (CH-6), 116.9 (d, *J* 190, CH-6'), 110.8 (C-5), 85.8 (CH-1'), 85.5 (d, *J* 21.5, CH-4'), 73.9 (d, *J* 1.25, CH-3'), 52.8 (d, *J* 12.5, PO(OCH_3)), 52.6 (d, *J* 12.5, PO(OCH_3)), 40.0 (CH-2'), 28.0 (N-CH_3), 13.5 (CH_3-7); δ_P 162 MHz, $CDCl_3$): 20.6; *m/z* (ESI$^+$) 361.1168 (M+H. $C_{14}H_{22}N_2O_7P$ requires 361.1159), 383.0981 (M+Na. $C_{14}H_{21}N_2NaO_7P$ requires 383.0979), 378.1431 (M + H_4N. $C_{14}H_{25}N3O_7P$ requires 378.1425).

2-cyanoethyl ((2*R*,3S)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(3,5-dimethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) diisopropylphosphoramidite **185**



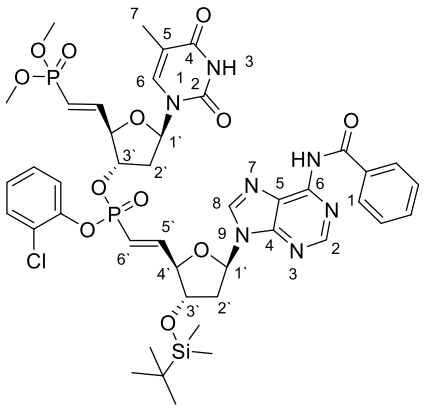
Chemical Formula: $C_{23}H_{38}N_4O_8P_2$
Exact Mass: 560.2165

Vinylphosphonate **184** (380 mg, 1.06 mmol) was added to a round bottom flask which was then flushed with argon. Anhydrous dichloromethane (10 mL) and diisopropyl ethylamine (732 µL, 4.20 mmol) were added. Chloro-cyanoethyl-N,Nisopropylphosphorodiamidite **30** (281 µL, 1.26 mmol) was dissolved in anhydrous dichloromethane (5 mL) and added dropwise *via* syringe. The reaction was stirred at room temperature for 22 h at which time TLC showed complete disappearance of the starting material **184**. The reaction mixture was diluted in dichloromethane (75 mL) and washed with brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography using 2% methanol in dichloromethane eluent to afford the desired product **185** (390 mg, 66%, 1:1 mixture of two diastereoisomers) as a colourless oil. FTIR (ATR) ν_{max}/cm^{-1} : 2967, 2933, 2874, 1704, 1670, 1641, 1469, 1365, 1294, 1240, 1025, 974, 832; δ_H (400 MHz, $CDCl_3$): 7.10-7.06 (1H, m, H-6), 7.04-6.81 (1H, m, H-5'), 6.41 (1H, t, *J* 6.7, H-1'), 6.02 (0.5H, ddd, *J* 19.3, 17.2, 1.8, H-6'), 5.99 (0.5H, ddd, *J* 19.1, 17.1, 1.9, H-6'), 4.61-4.55 (0.5H, m, H-4'), 4.54-4.50 (0.5H, m, H-4'), 4.49-4.39 (1H, m, H-3'), 3.92-3.82 (1H, m, OC*H*$_2$CH$_2$CN),3.80-3.68 (7H, m, OC*H*$_2$CH$_2$CN + 2 × POCH$_3$), 3.67-3.55 (2H, m, NC*H*(CH$_3$)$_2$), 3.34 (3H, m, N-CH$_3$), 2.78-2.61 (2H, m, OCH$_2$C*H*$_2$CN), 2.55-2.42 (1H, m, H$_A$-2'), 2.26-2.15

(1H, m, H_B-2′), 1.98-191 (3H, m, H-7), 1.19 (3H, d, *J* 6.8, NCH(C*H*$_3$)$_2$), 1.18 (9H, d, *J* 6.8, NCH(C*H*$_3$)$_2$); δ_C (126 MHz, $CDCl_3$): 163.5 (2 × C-4), 151.3 (C-2), 151.2 (C-2), 148.8 (d, *J* 6.1, CH-5′), 148.6 (d, *J* 6.1, CH-5′), 133.1 (CH-6), 133.0 (CH-6), 117.91 (d, *J* 190, CH-6′), 117.9 (CN), 117.7 (d, *J* 190, CH-6′), 117.4 (CN), 111.0 (2 × C-5), 85.8 (CH-1′), 85.7 (CH-1′), 85.0-84.4 (m, CH-4′), 76.1 (dd, *J* 15.5, 1.3, CH-3′), 75.6 (dd, *J* 15.5, 1.3, CH-3′), 58.2 (d, *J* 19.6, O*C*H$_2$CH$_2$CN), 58.1 (d, *J* 19.8, O*C*H$_2$CH$_2$CN), 52.8-52.6 (m, PO(OCH$_3$)), 43.5 (d, *J* 12.4, N*C*H(CH$_3$)$_2$), 43.4 (d, *J* 12.4, N*C*H(CH$_3$)$_2$), 38.7 (d, *J* 4.0, CH$_2$-2′), 38.6 (d, *J* 2.5, CH$_2$-2′), 28.1 (N-CH$_3$), 24.85-24.6 (m, NCH(*C*H$_3$)$_2$), 20.6 (d, *J* 7.5, OCH$_2$*C*H$_2$CN), 20.5 (d, *J* 7.3, OCH$_2$*C*H$_2$CN), 13.46 (CH$_3$-7), 13.43 (CH$_3$-7); δ_P 162 MHz, $CDCl_3$): 149.4, 149.3, 20.1, 19.7; *m/z* (ESI$^+$) 561.2232 (M+H. $C_{23}H_{39}N_4O_8P_2$ requires 561.2238), 583.2064 (M+Na. $C_{23}H_{38}N_4NaO_8P_2$ requires 583.2057).

5.8 Synthesis of consecutive 5′-(*E*)-VP dimer *T*A **178**

2-chlorophenyl ((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **186**

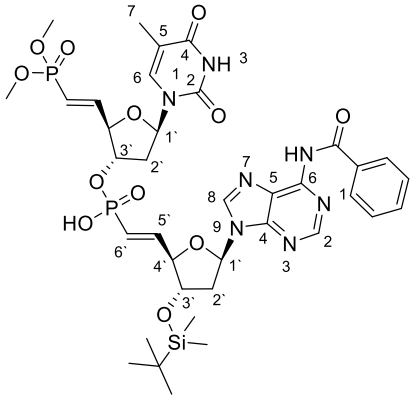


Chemical Formula: $C_{43}H_{52}ClN_7O_{12}P_2Si$
Exact Mass: 983.2607

A mixture of phosphonate **69** (1.33 g, 2.02 mmol), alcohol **183** (700 mg, 2.02 mmol) and *N*-methylimidazole (1.0 mL, 13.3 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (3 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (1.53 g, 5.05 mmol) were added. The reaction mixture was stirred at RT for 21 h, poured into citric acid solution (10%, 100 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers were extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were removed *via* reduced pressure and residue was purified by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate) to afford the desired product **186** (1.50 g, 83%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} :2952, 2928, 2854, 1687, 1607, 1580, 1513, 1476, 1448, 1247, 1225, 1024, 925, 832; δH (400 MHz, CD_3OD): 8.72 (0.7H, s, A-H-2), 8.67 (0.3H, s, A-H-2), 8.53 (0.7H, s, A-H-8), 8.49 (0.3H, s, A-H-8), 8.15-8.01 (2H, m, ArH), 7.68-7.60 (1H, m ArH),

7.58-7.51 (2H, m, ArH), 7.50-7.43 (1H, m, ArH), 7.40-7.35 (2H, m, T-H-6 + ArH), 7.35-7.28 (1H, m, ArH), 7.25-7.05 (2H, m, ArH + A-H-5′), 6.94-6.76 (1H, m, T-H-5′), 6.61-6.52 (1H, m, A-H-1′), 6.298-6.11 (2H, m, T-H-1′ + A-H-6′), 6.10-5.92 (1H, m, T-H-6′), 5.33-5.18 (1H, m, T-H-3′), 4.91-4.80 (1H, m, A-H-3′), 4.68-4.63 (0.3H, m, T-H-4′), 4.62-4.53 (1.7H, m, 0.7 × T-H-4′ + A-H-4′), 3.76-3.60 (6H, m, $PO(CH_3)_2$), 3.19-3.06 (1H, m, A-H_A-2′), 2.68-2.58 (1H, m, T-H_A-2′), 2.57-2.43 (2H, m, A-H_B-2′ + T-H_B-2′), 1.91-1.80 (3H, br.s, H-7), 0.94 (6H, $SiC(CH_3)_3$), 0.16 (9H, $Si(CH_3)_2$); *δ*c (101 MHz, CD_3OD): 168.0 (2 × ^{Bz}CO), 166.2 (2 × T-C-4), 153.8 (d, 6.2, A-H-5′), 153.7 (d, *J* 6.0, A-CH-5′), 153.2 (2 × A-CH-2), 153.0 (A-C-4), 152.9 (A-C-4), 152.0 (2 × T-C-2), 151.3 (2 × A-C-6), 149.7 (d, *J* 5.8, T-CH-5′), 149.6 (d, *J* 5.8, T-CH-5′), 147.24 (d, *J* 6.5, ArC), 147.17 (d, *J* 6.5, ArC), 145.3 (A-CH-8), 145.3 (A-CH-8), 138.74 (T-CH-6), 138.68 (T-CH-6), 134.9 (2 × ^{Bz}ArC), 133.9 (2 × ArCH), 132.0 × ArCH), 129. 8(4 × ArCH), 129.5 (6 × ArCH), 127.9 (2 × ArCH), 126.9 (2 × d, *J* 5.6, ArC), 125.7 (A-5-C), 125.6 (A-5-C), 123.4 (d, *J* 2.7, ArCH), 123.35 (d, *J* 2.7, ArCH), 119.0 (d, *J* 188, T-CH-6′), 118.9 (d, *J* 188, T-CH-6′), 117.4 (d, *J* 190, A-CH-6′), 116.9 (d, *J* 190, A-H-6′), 112.0 (2 × T-C-5), 88.2-88.8 (A-CH-4′ + 2 × T-H-1′), 87.5 (d, *J* 23.0, A-CH-4′), 86.7 (A-H-1′), 86.6 (A-H-1′), 85.5 (2 × dd, *J* 23.4, 5.9, T-CH-4′), 80.5-80.28 (2 × T-CH-3′), 76.8 (d, *J* 1.2, A-CH-3′), 76.6 (d, *J* 1.2, A-CH-3′), 53.6-53.4 (4 × $PO(CH_3)_2$), 39.9 (2 × A-CH_2-2′), 38.1 (d, *J* 3.5, T-CH_2-2′), 37.9 (d, *J* 3.6, T-CH_2-2′), 26.3 ($SiC(CH_3)_3$), 18.9 ($SiC(CH_3)_3$), 12.3 (2 × T-CH_3-7), -4.51 ($Si(CH_3)_2$), -4.58 ($Si(CH_3)_2$); *δ*P (162 MHz, CD_3OD): 20.25, 20.17, 15.94, 15.74; *m/z* (ESI^+) 984.2696 (M+H. $C_{43}H_{53}ClN_7O_{12}P_2Si$ requires 984.2680), 1006.2513 (M+Na. $C_{43}H_{52}ClN_7NaO_{12}P_2Si$ requires 1006.2499).

(2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **187**

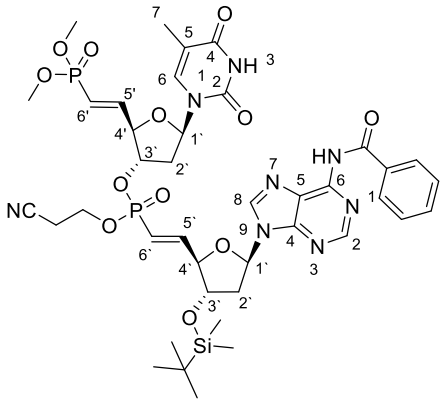


Chemical Formula: $C_{37}H_{49}N_7O_{12}P_2Si$
Exact Mass: 873.2684

To a solution of dinucleotide **186** (4.00 g, 4.06 mmol) in dioxane (80 mL) was added in one portion a solution of tetramethylguanidine (610 µL, 4.87 mmol), triethyl amine (1.7 mL, 12.19 mmol) and 2-nitrobenzaldoxime (808 mg, 4.87 mmol) in dioxane (20 mL) and the resulting mixture was stirred at room temperature for 20 h. The volatiles were evaporated, and the residue was purified by column chromatography using gradient elution; dichloromethane : methanol (9:1 → 4:1) to afford the desired product **187** as yellow-off powder (2.50 g, 67%). FTIR (ATR) ν_{max}/cm^{-1} : 2950, 2929, 2854, 1683, 1604, 1577, 1515, 1454, 1408, 1248, 1217, 1026, 831, 771; δH (400 MHz, CD_3OD): 8.75 (1H, s, A-H-2), 8.63 (1H, s, A-H-8), 8.12-8.06 (2H, m, ArH), 7.69-7.62 (1H, m ArH), 7.60-7.53 (2H, m, ArH), 7.40 (1H, d, *J* 1.3, T-H-6), 6.91 (1H, ddd, *J* 22.2, 17.2, 4.6, T-H-5′), 6.721-6.55 (1H, m, A-H-5′ + A-H-1′), 6.32 (1H, d, *J* 7.0, T-H-1′), 6.08-5.94 (2H, m, A-H-6′ + T-H-6′), 4.77-4.70 (2H, m, T-H-3′ + A-H-1′), 4.63-4.58 (1H, m, T-H-4′), 4.53-4.48 (1H, m, A-H-4′), 3.71 (6H, d, *J* 11.1, $PO(CH_3)_2$), 3.04-2.95 (1H, m, A-H_A-2′), 2.54-2.47 (1H, m, A-H_B-2′), 2.40-2.33 (T-H_A-2′ +T-H_A-2′), 1.87 (3H, d, *J* 1.2, H-7), 0.97 (9H, $SiC(CH_3)_3$), 0.19 (6H, $Si(CH_3)_2$); δc (101 MHz, CD_3OD); 168.1 (^{Bz}CO), 166.3 (2 × T-C-4), 153.3 (A-

CH-2), 153.1 (A-C-4), 152.2 (T-C-2), 151.2 (d, *J* 5.5, T-H-5′), 151.17 (A-C-6), 144.6 (A-CH-8), 149.7 (d, *J* 4.7, A-CH-5′), 138.04 (T-CH-6), 135.0 (ArC), 133.87 (ArCH), 129.8 (2 × ArCH), 129.4 (2 × ArCH), 126.7 (d, *J* 178, T-CH-6′), 125.3 (A-C-5), 117.5 (d, *J* 189, A-CH-6′), 112.0 (T-C-5), 89.1 (d, *J* 21, A-CH-4′), 88.0 (T-H-1′), 87.9 (T-H-1′), 87.5 (d, *J* 22.8, A-CH-4′), 87.0 (T-H-1′), 86.2 (dd, *J* 22.5, 5.3, T-CH-4′), 77.5 (dd, *J* 5.0, 1.5, T-CH-3′), 77.1 (A-CH-3′), 53.5 (d, *J* 5.9, PO(*C*H$_3$)$_2$), 53.4 (d, *J* 5.9, PO(*C*H$_3$)$_2$), 40.46 (A-CH$_2$-2′), 38.3 (d, *J* 3.3, T-CH$_2$-2′), 26.3 (SiC(*C*H$_3$)$_3$), 18.9 (Si*C*(CH$_3$)$_3$), 12.4 (T-CH$_3$-7), -4.50 (Si(CH$_3$)$_2$), -4.55 (Si(CH$_3$)$_2$); δP (162 MHz, CD$_3$OD): 20.85, 11.49; *m/z* (ESI$^+$) 874.2743 (M+H. $C_{37}H_{50}N_7O_{12}P_2Si$ requires 874.2756), 896.2546 (M+Na. $C_{37}H_{49}N_7NaO_{12}P_2Si$ requires 896.2576).

2-cyanoethyl ((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **188**



Chemical Formula: $C_{40}H_{52}N_8O_{12}P_2Si$
Exact Mass: 926.2949

Method A (monomers coupling)

A mixture of phosphonate **194** (260 mg, 430 µmol), alcohol **183** (150 mg, 430 µmol) and *N*-methylimidazole (221 µL, 2.79 mmol) was dried by azeotropic removal of water with pyridine (3 × 3 mL). The residue was dissolved in pyridine (1 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (130

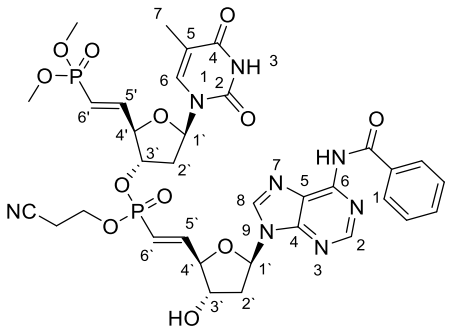
mg, 430 µmol) were added. The reaction mixture was stirred at RT for 24 h, pyridine was removed *via* reduced pressure. The residue was dry-loaded and purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **188** (350 mg, 87%, 1:1 mixture of diastereoisomers) as a white foam.

Method B (cyanoethyl alcohol)

A mixture of phosphonate **69** (2.5 g, 2.86 mmol), 3-hydroxypropionitrile**190** (976 µL, 14.3 mmol) and *N*-methylimidazole (1.47 mL, 18.6 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (8 mL). Molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (2.16 g, 7.15 mmol) were added. The reaction mixture was stirred at RT for 18 h, most of pyridine was volatiles was removed *via* reduced pressure. The residue was dry-loaded and purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **188** (2.00 g, 75%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) $\nu_{max/}cm^{-1}$: 2954, 2930, 2854, 1683, 1608, 1579, 1454, 1245, 1024, 832; δH (400 MHz, CD_3OD): 8.75 (0.5H, s, A-H-2), 8.74 (0.5H, s, A-H-2), 8.55 (1H, s, A-H-8), 8.11-8.05 (2H, m, ArH), 7.68-7.61 (1H, m ArH), 7.60-7.53 (2H, m, ArH), 7.40-7.36 (1H, m, T-H-6), 7.07 (1H, ddd, *J* 22.5, 17.1, 4.6, A-H-5′), 6.96-6.80 (1H, m, T-H-5′), 6.62-6.56 (1H, m, A-H-1′), 6.28-6.19 (1H, m, T-H-1′), 6.15-5.96 (2H, m, A-H-6′ + T-H-6′), 5.15-5.05 (1H, m, T-H-3′), 4.98-4.90 (1H, m, A-H-3′), 4.70-4.56 (2H, m, A-H-4′ + T-H-4), 4.28-4.20 (2H, m, C*H*$_2$CH$_2$CN), 3.77-3.69 (6H, m, 2 × OCH_3), 3.19-3.12 (1H, m, A-H_A-2′), 2.93-3.85 (2H, m, CH_2C*H*$_2$CN), 2.66-2.45 (3H, m, A-H_B-2′ + T-H_A-2′ + T-H_B-2′), 1.87 (1.5H, d, *J* 1.2, H-7), 1.86 (1.5H, d, *J* 1.2, H-7); δc (101 MHz, CD_3OD): 168.1 (2 × ^{Bz}CO), 166.2 (2 × T-C-4), 153.2 (2 × A-CH-2), 153.1 (2 × A-C-4), 152.9 (d, *J* 6.1, A-H-5′), 152.7 (d, *J* 6.0, A-CH-5′), 152.1 (2 × T-C-2), 151.3

(2 × A-C-6), 149.8 (d, *J* 5.8, T-CH-5′), 149.7 (d, *J* 5.8, T-CH-5′), 145.1 (2 × A-CH-8), 138.6 (2 × T-CH-6), 134.9 (2 × ArC), 133.9 (2 × ArCH), 129.8 (4 × ArCH), 129.5 (4 × ArCH), 125.8 (2 × A-C-5), 118.9 (d, *J* 191.3, T-CH-6′), 118.86 (d, *J* 190, T-CH-6′), 118.75 (CN), 118.66 (CN), 117.5 (d, *J* 190, A-H-6′), 117.4 (d, *J* 190, A-H-6′), 112.0 (2 × T-C-5), 88.1 (d, *J* 23.4, A-CH-4′), 80.0 (d, *J* 22.7, A-CH-4′), 87.7 (2 × T-H-1′), 86.6 (2 × A-H-1′), 85.5 (dd, *J* 5.1, 2.0, T-CH-4′), 87.2 (dd, *J* 6.4, 3.5, T-CH-4′), 79.7-79.4 (2 × T-CH-3′), 76.7 (d, *J* 1.7, A-CH-3′), 76.6 (d, *J* 9.5, A-CH-3′), 62.7 (d, *J* 9.5, *C*$H_2$$CH_2$CN), 62.6 (d, *J* 5.1, *C*$H_2$$CH_2$CN), 53.5 (d, *J* 5.9, 3 × OCH_3), 53.5 (d, *J* 5.9, OCH_3), 40.0 (2 × A-CH_2-2′), 40.0 (d, *J* 3.4, T-CH_2-2′), 37.9 (d, *J* 3.5, T-CH_2-2′), 26.3 (6 × SiC(*C*H_3)$_3$), 20.4 (d, *J* 4.1, CH_2*C*H_2CN), 20.3 (d, *J* 4.1, CH_2*C*H_2CN), 18.8 (2 × Si*C*$(CH_3)_3$), 12.3 (2 × T-CH_3-7), -4.5 (4 × Si$(CH_3)_2$); *δ*P (162 MHz, CD_3OD): 20.36, 20.30, 18.96, 18.95; *m/z* (ESI^+) 927.3027 (M+H. $C_{40}H_{53}N_8O_{12}P_2Si$ requires 927.3022), 949.2861 (M+Na. $C_{40}H_{52}N_8NaO_{12}P_2Si$ requires 949.2841).

2-cyanoethyl ((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)vinyl)phosphonate **189**

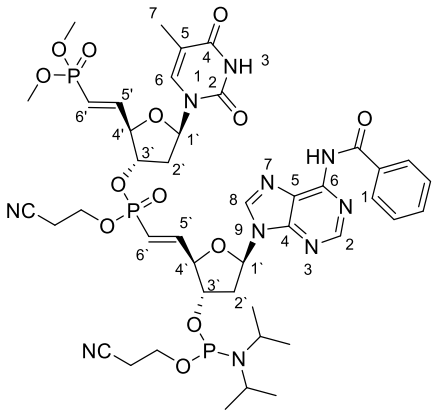


Chemical Formula: $C_{34}H_{38}N_8O_{12}P_2$
Exact Mass: 812.2084

Triethylamine trihydrofluoride (457 µL, 2.80 mmol) was added in one portion to a stirring solution of dinucleotide **188** (520 mg, 560 µmol) in THF (6 mL) and the resulting mixture was stirred at RT for 5 h. The mixture was dry-loaded

onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **189** (400 mg, 88%, 3:2 mixture of diastereoisomers) as a white powder. FTIR (ATR) $\nu_{max/}cm^{-1}$:3306, 2953, 1683, 1608, 1578, 1455, 1240, 1023, 938, 833; δH (400 MHz, CD_3OD): 8.7 5(0.4H, s, A-H-2), 8.74 (0.5H, s, A-H-2), 8.55 (0.5H, s, A-H-8), 8.54 (0.5H, s, A-H-8), 8.12-8.04 (2H, m, ArH), 7.69-7.61 (1H, m ArH), 7.59-7.52 (2H, m, ArH), 7.39 (0.5H, q, *J* 1.2, T-H-6), 7.37 (0.5H, q, *J* 1.2, T-H-6), 7.12 (1H, ddd, *J* 23.4, 17.2, 4.3, A-H-5′), 6.97-6.81 (1H, m, T-H-5′), 6.66-6.58 (1H, m, A-H-1′), 6.26-6.18 (1H, m, T-H-1′), 6.16-5.93 (2H, A-H-6′ + T-H-6′), 5.15-5.03 (1H, m, T-H-3′), 4.80-4.71 (1H, m, A-H-3′), 4.70-4.58 (2H, m, A-H-4′ + T-H-4), 4.30-4.17 (2H, m, C*H*$_2$CH$_2$CN), 3.77-3.68 (6H, m, 2 × OCH_3), 3.18-3.08 (1H, m, A-H_A-2′), 2.94-2.85 (2H, m, CH_2C*H*$_2$CN), 2.15-2.04 (3H, m, A-H_B-2′ + T-H_A-2′ + T-H_B-2′), 1.87 (1.5H, d, *J* 1.2, H-7), 1.85 (1.5H, d, *J* 1.2, H-7); δc (101 MHz, CD_3OD): 168.1 (2 × ^{Bz}CO), 166.2 (2 × T-C-4), 153.5 (d, 6.0, A-H-5′), 153.3 (d, *J* 6.0, A-CH-5′ + 2 × A-CH-2 + 2 × A-C-4), 152.1 (2 × T-C-2), 151.3 (2 × A-C-6), 149.9 (d, *J* 5.8, T-CH-5′), 149.8 (d, *J* 5.8, T-CH-5′), 144.86 (2 × A-CH-8), 138.75 (T-CH-6), 138.7 (T-CH-6), 135.0 (2 × ArC), 133.9 (2 × ArCH), 129.7 (4 × ArCH), 129.5 (4 × ArCH), 125.7 (2 × A-C-5), 118.8 (d, *J* 188, T-CH-6′), 118.86 (d, *J* 188, T-CH-6′), 118.76 (CN), 118.69 (CN), 116.9 (d, *J* 189, A-H-6′), 116.9 (d, *J* 189, A-H-6′), 112.0 (2 × T-C-5), 88.0 (d, *J* 22.5, A-CH-4′), 87.9 (d, *J* 22.7, A-CH-4′), 87.8 (2 × T-H-1′), 86.7 (A-H-1′), 86.6 (A-H-1′), 85.5 (dd, *J* 8.1, 6.2, T-CH-4′), 87.2 (dd, *J* 8.2, 6.1, T-CH-4′), 79.6 (2 × d, *J* 5.6, T-CH-3′), 75.4 (2 × A-CH-3′), 62.7 (d, *J* 5.5, *C*H_2CH$_2$CN), 62.6 (d, *J* 5.4, *C*H_2CH$_2$CN), 53.5 (d, *J* 5.9, 4 × OCH_3), 39.3 (2 × A-CH_2-2′), 38.0 (d, *J* 3.3, T-CH_2-2′), 37.9 (d, *J* 3.9, T-CH_2-2′), 20.3 (d, *J* 6.7, CH_2*C*H_2CN), 20.3 (d, *J* 6.7, CH_2*C*H_2CN), 12.3 (2 × T-CH_3-7); δP (162 MHz, CD_3OD): 20.41, 20.36, 19.2 (2 × P); *m/z* (ESI^+) 813.2135 (M+H. $C_{34}H_{39}N_8O_{12}P_2$ requires 813.2157), 835.1935 (M+Na. $C_{34}H_{38}N_8NaO_{12}P_2$ requires 835.1977).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((*E*)-2-((2 cyanoethoxy)(((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5 methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3 yl)oxy)phosphoryl)vinyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite **178**



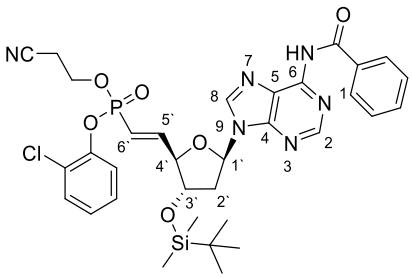
Chemical Formula: $C_{43}H_{55}N_{10}O_{13}P_3$
Exact Mass: 1012.3163

Compound **189** (130 mg, 160 µmol) was dissolved in dry DMF (100 µL) and applied for azeotropic distillation to remove the water with toluene (3 × 1 mL), the sticky residue was dissolved in dry dichloromethane (200 µL). A mixture of 5-methyl-1-H-tetrazole (27 mg, 320 µmol), phosphoramidite **52** (100 µL, 320 µmol) solution in dichloromethane (100 µL) was added and the resultant mixture was stirred at RT for 1 h. The reaction mixture was diluted in ethyl acetate (10 mL), washed with water (10 mL) and brine (10 mL). The separated aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4. The volatiles were removed, and the residue was purified by column chromatography using gradient elution; 1% → 3% methanol in dichloromethane) afford the desired product **178** (100 mg, 62%, 1:1:1:1 mixture of diastereoisomers) as a white foam. *δ*H (400 MHz, $CDCl_3$): 9.77 (0.6H, s, N-H), 9.53 (0.4H, s, N-H), 8.81 (0.6H, s, A-H-2), 8.79 (0.4H, s, A-H-2), 8.27 (0.2H, s, A-H-8), 8.26 (0.3H, s, A-H-8), 8.24 (0.2H, s, A-H-8), 8.22 (0.3H, s, A-H-8), 8.09-8.00 (2H, m, ArH), 7.61-7.53 (1H, m ArH),

7.52-7.43 (2H, m, ArH), 7.22-7.00 (2H, m, H-5′ + T-H-6), 6.95-6.77 (1H, m, H-5′), 6.58-6.49 (1H, m, A-H-1′), 6.40-6.33 (0.5H, m, T-H-1′), 6.32-6.24 (0.5H, m, T-H-1′), 6.09-5.84 (2H, A-H-6′ + T-H-6′), 5.04-4.95 (1H, m, T-H-3′), 4.94-4.74 (2H, m, A-H-3′ + A-H-4′), 4.64-4.59 (1H, m, T-H-4), 4.29-4.10 (2H, m, C*H*$_2$CH$_2$CN), 3.97-3.87 (1H, m, C*H*$_2$CH$_2$CN), 3.80-3.60 (9H, m, C*H*$_2$CH$_2$CN + PO(OCH$_3$)$_2$ + 2 × NHC*H*(CH$_3$)$_2$), 3.22-3.07 (1H, m, T-H$_A$-2′), 2.78-2.48 (6H, m, 2 × CH$_2$C*H*$_2$CN + T-H$_B$-2′ + A-H$_A$-2′), 2.37-2.25 (1H, m, A-H$_B$-2′), 1.89 (3H, H-7), 1.26-1.19 (12H, m, NHCH(C*H*$_3$)$_2$); *δ*c (101 MHz, CD$_3$OD): 165.4 (2 × ^{Bz}CO), 165.3 (2 × ^{Bz}CO), 163.8 (2 × T-C-4), 163.3 (2 × T-C-4), 152.6 (2 × A-CH-2), 152.6 (2 × A-CH-2), 151.1 (2 × A-C-4), 151.1 (2 × A-C-4), 151.2-150.4 (4 × A-C-6 + 4 × CH-5′), 150.3 (2 × T-C-2), 150.1 (2 × T-C-2), 147.4-147.1 (4 × CH-5′), 142.6-142.2 (4 × A-CH-8), 135.38 (T-CH-6), 135.33 (T-CH-6), 135.27 (2 × T-CH-6), 133.5 (4 × ArC), 133.9 (4 × ArCH), 128.8 (4 × ArCH), 128.7 (4 × ArCH), 128.5 (4 × ArCH), 128.3 (4 × ArCH), 124.4 (2 × A-C-5), 124.2 (2 × A-C-5), 120.1-115.1 (m, 4 × T-CH-6′ + 4 × A-CH-6′ + 8 × CN), 112.1 (2 × T-C-5), 112.0 (2 × T-C-5), 86.1-84.8 (m, 4 × T-CH-1′ + 4 × A-CH-1′ + 4 × A-CH-4′), 84.2-83.5 (m, 4 × T-CH-4′), 77.7 (m, 4 × T-CH-3′), 76.5-75.8 (4 × A-CH-3′), 61.0 (2 × d, *J* 4.7, *C*H$_2$CH$_2$CN), 60.7 (2 × d, *J* 4.7, *C*H$_2$CH$_2$CN), 58.4-57.9 (m, 4 × *C*H$_2$CH$_2$CN), 52.9-52.6 (m, PO(OCH$_3$)$_2$), 43.6 (8 × d, *J* 12.3, NH*C*H(CH$_3$)$_2$), 43.5 (2 × d, *J* 12.3, NH*C*H(CH$_3$)$_2$), 38.0-37.4 (m, 4 × A-CH$_2$-2′ + 4 × T-CH$_2$-2′), 24.9-24.6 (8 × NHCH(*C*H$_3$)$_2$), 20.6 (4 × d, *J* 7.2, CH$_2$*C*H$_2$CN), 20.1 (2 × d, *J* 6.5, CH$_2$*C*H$_2$CN), 20.0 (2 × d, *J* 6.6, CH$_2$*C*H$_2$CN), 12.6 (2 × T-CH$_3$-7), 12.5 (2 × T-CH$_3$-7); *δ*P (162 MHz, CDCl$_3$): 149.5, 149.4, 148.9, 148.8, 19.5, 19.4, 19.3, 19.22, 19.21, 19.20, 19.18, 19.00; *m/z* (ESI$^+$) 1013.3228 (M+H. $C_{43}H_{56}N_{10}O_{13}P_3$ requires 1013.3236), 1035.3039 (M+Na. $C_{43}H_{55}N_{10}NaO_{13}P_3$ requires 1035.3055).

5.9 Synthesis of consecutive VP trimer *T*A*A **179**

Synthesis of 2-chlorophenyl (2-cyanoethyl) ((*E*)-2-((2*R*,3*S*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)vinyl)phosphonate **195**

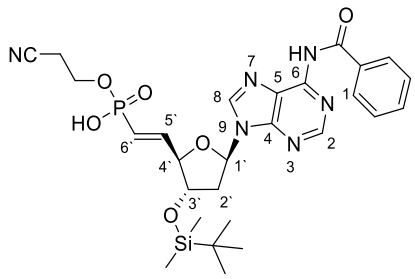


Chemical Formula: $C_{33}H_{38}ClN_6O_6PSi$
Exact Mass: 708.2048

A mixture of phosphonate **69** (1.28 g, 1.95 mmol), 3-hydroxypropionitrile**190** (665 µL, 9.75 mmol) and *N*-methylimidazole (1.54 mL, 19.5 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (4 mL). Molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (1.47 g, 4.88 mmol) were added. The reaction mixture was stirred at RT for 22 h, poured into citric acid solution (20%, 50 mL), ethyl acetate (100 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4 and the volatiles were removed *via* reduced pressure. The residue was purified by column chromatography using ethyl acetate to afford the desired product **196** (900 mg, 65%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2953, 2928, 2856, 1692, 1608, 1580, 1511, 1477, 1452, 1250, 1223, 1034, 924, 833; δH (400MHz, $CDCl_3$): 9.08 (1H, br s, N-H), 8.78 (0.4H, s, H-2), 8.74 (0.6H, s, H-2), 8.16 (0.4H, s, H-8), 8.10 (0.6H, s, H-8), 8.02 (2H, d, *J* 7.8, ArH), 7.63-7.56 (1H, m, ArH), 7.54-7.49 (2H, m, ArH), 7.42-732 (2H, m, ArH), 7.25-7.28 (1H, m, ArH), 7.16-7.00 (2H, m, ArH + H-5’), 6.52 (0.4H, dd, *J* 7.3, 6.0, H-1’), 6.48 (0.6H, dd, *J* 7.1, 6.0, H-1’), 6.25-6.10 (1H, m, H-6’), 4.64-4.52 (2H, m, H-3’

+ H-4′), 4.45-4.27 (2H, m, OC*H*$_2$CH$_2$CN), 2.99-2.87 (1H, m, H$_A$-2′), 2.80-2.71 (2H, m, OCH$_2$C*H*$_2$CN), 2.48-2.39 (1H, m, H$_B$-2′), 0.92 (9H, s, SiC(CH$_3$)$_3$), 0.12 (6H, Si(CH$_3$)$_2$); *δ*c (101 MHz, CDCl$_3$): 164.71 (2 × ^{Bz}CO), 152.86 (CH-2), 151.85 (C-4), 151.69 (C-4), 151.21 (d, *J* 6.4, CH-5′), 149.83 (2 × C-6), 146.1 (d, *J* 7.0, ArC), 146.0 (d, *J* 7.0, ArC), 141.9 (CH-8), 141.86 (CH-8), 133.68 (ArC), 132.94 (2 × ArH), 130.87 (2 × ArH), 128.99, (4 × ArH), 128.2 (d, *J* 1.5, ArH), 128.1 (d, *J* 1.5, ArH), 127.99 (4 × ArH), 126.4 (ArH), 126.25 (ArH), 125.8 (d, *J* 5.8, 2 × ArC), 123.90 (2 × C-5), 122.3 (d, *J* 2.8, ArH), 122.20 (d, *J* 2.8, ArH), 116.6 (d, *J* 191.8, H-6′), 116.58 (d, *J* 191.5, H-6′), 116.53 (CN), 116.3 (CN), 87.2 (d, *J* 23.5, CH-4′), 86.8 (d, *J* 23.4, CH-4′), 85.05 (CH-1′), 85.04 (CH-1′), 75.27 (d, *J* 2.1, CH-3′), 75.22 (d, *J* 2.1, CH-3′), 61.04 (d, *J* 5.9, OC*H*$_2$CH$_2$CN), 60.92 (d, *J* 5.8, OC*H*$_2$CH$_2$CN), 39.2 (CH-2′): 39.1 (CH-2′), 25.83 (SiC(*C*H$_3$)$_3$), 20.06 (d, *J* 5.6, OCH$_2$*C*H$_2$CN), 19.96 (d, *J* 6.5, OCH$_2$*C*H$_2$CN), 18.09 (Si*C*(CH$_3$)$_3$), -4.59 (Si(CH$_3$)$_2$), -4.63 (Si(CH$_3$)$_2$); *δ*$_P$ (162 MHz, CDCl$_3$): 15.75, 15.65; *m/z* (ESI$^+$) 709.2136 (M+H. $C_{33}H_{39}ClN_6O_6PSi$ requires 709.2121), 731.1940 (M+Na. $C_{33}H_{38}ClN_6NaO_6PSi$ requires 731.1940).

2-cyanoethyl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **194**

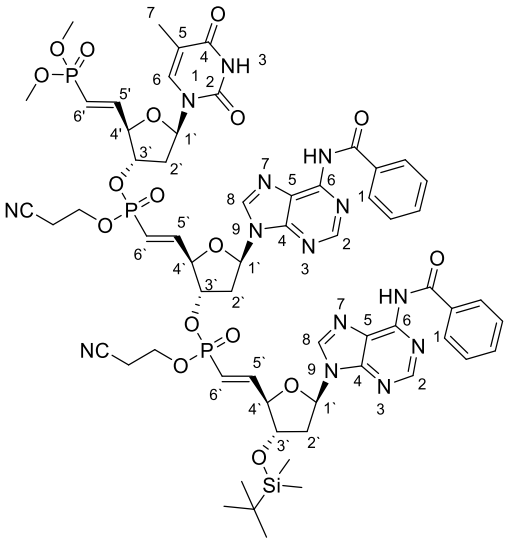


Chemical Formula: $C_{27}H_{35}N_6O_6PSi$
Exact Mass: 598.2125

To a Vinylphosphonate **195** (1.70 g, 2.39 mmol) solution in dioxane (20 mL), a mixture of 2-nitrobenzaldoxime (437 mg, 2.60 mmol), tetramethylguanidine (369 µL, 2.39 mmol) and triethylamine (335 µL, 2.39 mmol) in dioxane (5mL) was added in one portion and the reaction mixture was stirred at RT for 19 h.

The volatiles was removed and the crude product was purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **194** (900 mg, 63%) as a white powder (more polar compound), and phosphonic acid **69** (550 mg, 35%) was separated as a by-product (less polar compound). FTIR (ATR) ν_{max}/cm^{-1} : 2925, 2929, 2856, 1683, 1607, 1577, 1516, 1454, 1251, 1069, 833; δH (400MHz, CD_3OD): 8.75 (1H, s, H-2), 8.62 (1H, s, H-8), 8.13-8.07 (2H, s, ArH), 7.69-7.63 (1H, m, ArH), 7.60-7.53 (2H, m, ArH), 6.71-6.56 (2H, m, H-1′ + H-5′), 6.00 (1H, td, *J* 17.1, 1.5, H-6′), 4.75-4.70 (1H, m, H-3′), 4.53-4.48 (1H, m, H-4′), 3.95 (2H, dt, *J* 7.1, 6.1, OCH_2CH_2CN), 3.01-2.91 (1H, m, H_A-2′), 4.45-4.27 (2H, m, OCH_2CH_2CN), 2.99-2.87 (1H, m, H_A-2′), 2.74 (2H, t, *J* 6.1, OCH_2CH_2CN), 2.54-2.47 (1H, m, H_B-2′), 0.98 (9H, s, $SiC(CH_3)_3$), 0.19 (6H, $Si(CH_3)_2$); δc (101 MHz, CD_3OD): 168.14 (^{Bz}CO), 153.3 (CH-2), 153.2 (C-4), 151.2 (C-6), 144.5 (CH-8), 144.0 (d, *J* 4.6, CH-5′), 135.0 (ArC), 133.9 (ArCH), 129.7 (2 × ArCH), 129.4 (2 × ArCH), 125.9 (d, *J* 177, CH-6′), 125.2 (C-5), 119.4 (CN), 89.2 (d, *J* 21, CH-4′), 85.9 (CH-1′), 77.2 (d, *J* 1.4, CH-3′), 60.5 (d, *J* 4.4, OCH2CH2CN), 40.46 (CH_2-2′), 26.3 ($SiC(CH_3)_3$), 20.5 (d, *J* 7.1, OCH_2CH_2CN), 18.9 ($SiC(CH_3)_3$), -4.5 ($Si(CH_3)_2$), -4.6 ($Si(CH_3)_2$); δ_P (162 MHz, CD_3OD): 11.7; *m*/*z* (ESI^+) 599.2203 (M+H. $C_{27}H_{36}N_6O_6PSi$ requires 599.2198), 621.2006 (M+Na. $C_{27}H_{35}N_6NaO_6PSi$ requires 621.2017).

2-cyanoethyl ((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)vinyl)(2-cyanoethoxy)phosphoryl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **193**

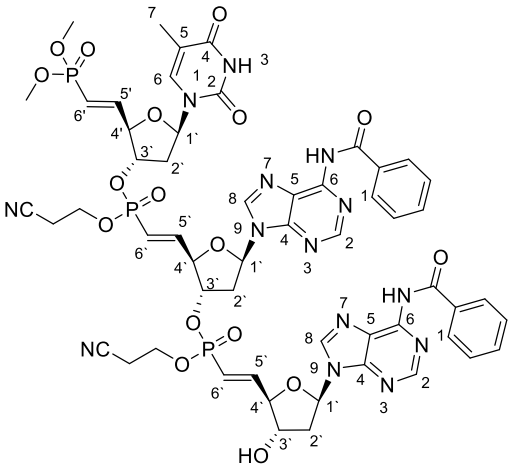


Chemical Formula: $C_{61}H_{71}N_{14}O_{17}P_3Si$
Exact Mass: 1392.4104

A mixture of phosphonate **194** (626 mg, 1.05 mmol), dinucleotide **189** (850 mg, 1.05 mmol) and *N*-methylimidazole (540 µL, 6.83 mmol) was dried by azeotropic removal of water with pyridine (3 × 5 mL). The residue was dissolved in pyridine (2 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (636 mg, 2.10 mmol) were added. The reaction mixture was stirred at RT for 24 h, pyridine was removed *via* reduced pressure. The residue was dry-loaded and purified by column chromatography using gradient elution; 2%→5% methanol in dichloromethane to afford the desired product **193** (950 mg, 68%, 1:1 mixture of diastereoisomers) as a white foam. FTIR (ATR) ν_{max}/cm^{-1} : 2952, 2930, 2854, 1686, 1608, 1608, 1579, 1513, 1454, 1244, 1025, 996, 832; δH (400 MHz, CD_3OD): 8.76-8.66 (2H, A_1-H-2 + A_2-H-2), 8.56-8.49 (2H, A_1-H-8 + A_2-H-8), 8.10-7.98 (4H, m, ArH), 7.66-7.46 (6H, m ArH), 7.36-7.32 (1H, m, T-

H-6), 7.18-7.04 (2H, m, A_1-H-5′ + A_2-H-5′), 6.94-6.79 (1H, m, T-H-5′), 6.65-6.54 (2H, m, A-H-1′), 6.25-6.18 (1H, m, T-H-1′), 6.16-5.96 (3H, A_1-H-6′ + A_2-H-6′ + T-H-6′), 5.53-5.43 (1H, m, A_1-H-3′), 5.12-5.05 (1H, m, T-H-3′), 4.97-4.91 (1H, m, A_2-H-3′), 4.89-4.86 (1H, m, A_1-H-4′), 4.66-4.57 (2H, m, T-H-4′ + A_2-H-4′), 4.36-4.17 (4H, m, 2 × C*H*$_2$CH$_2$CN), 3.74-3.67 (6H, m, 2 × OCH_3), 3.39-3.27 (1H, m, A_1-H_A-2′), 3.19-3.11 (1H, m, A_2-H_A-2′), 2.98-2.84 (4H, m, CH_2C*H*$_2$CN), 2.83-2.71 (1H, m, A_1-H_B-2′), 2.62-2.42 (3H, m, A_2-H_B-2′ + T-H_A-2′ + T-H_B-2′), 1.89-1.78 (3H, m, H-7), 1.04-0.96 (9H, SiC(CH_3)$_3$), 0.23-0.16 (6H, Si(CH_3)$_2$); *δ*c (101 MHz, CD_3OD): 168.0 (8 × ^{Bz}CO), 166.2 (4 × T-C-4), 153.4-152.9 (8 × A-CH-2 + 8 × A-C-4 + 2 × A-CH-5′), 152.7 (2 × d, *J* 5.0, A-CH-5′), 152.0 (4 × T-C-2), 151.3 (8 × A-C-6), 151.08 (d, *J* 6.1, A-CH-5′), 151.1 (d, *J* 6.1, A-CH-5′), 150.9 (2 × d, *J* 6.1, A-CH-5′), 149.8 (d, *J* 5.8, 4 × T-CH-5′), 145.2 (8 × A-CH-8), 138.6-138.46 (4 × T-CH-6), 134.9 (6 × ArC), 134.8 (2 × ArC), 133.9 (16 × ArCH), 129.7 (32 × ArCH), 129.5 (32 × ArCH), 125.8-125.6 (8 × A-C-5), 119.9-119.6 (CH-6′), 119.0-118.8 (82 × CN), 118.4-117.9 (CH-6′), 116.7 (CH-6′), 116.5 (CH-6′), 112.0 (4 × T-C-5), 88.2 (2 × d, *J* 22.7, A_2-CH-4′), 88.0 (2 × d, *J* 22.7, A_2-CH-4′), 87.5 (4 × T-H-1′), 86.8 (2 × A-H-1′), 86.6 (6 × A-H-1′), 86.2 (2 Xd, *J* 23.7, A_1-CH-4′), 86.1 (2 × d, *J* 23.0, A_1-CH-4′), 85.6-85.4 (2 × T-CH-4′), 85.3-85.2 (2 × T-CH-4′), 80.0-79.5 (4 × T-CH-3′ + 4 × A_1-CH-3′), 76.8 (2 × A_2-CH-3′), 76.7 (2 × A_2-CH-3′), 62.9-62.6 (8 × *C*H_2CH$_2$CN), 53.54 (d, *J* 6.3, 4 × OCH_3), 53.5 (d, *J* 6.0, 4 × OCH_3), 39.9 (4 × A_2-CH_2-2′), 37.9-37.5 (4 × A_1-H-2′ + 4 × T-CH_2-2′), 26.30 (2 × SiC(*C*H_3)$_3$), 26.29 (2 × SiC(*C*H_3)$_3$), 20.57-20.22 (8 × CH_2*C*H$_2$CN), 18.9 (2 × Si*C*(CH_3)$_3$), 18.8 (2 × Si*C*(CH_3)$_3$), 12.4 (4 × T-CH_3-7), -4.5 (5 × Si(*C*H_3)$_2$); *δ*P (162 MHz, CD_3OD): 20.35 (2 × P), 20.31 (2 × P), 19.16, 19.15, 19.06 (2 × P), 18.43, 18.37, 18.31, 18.26; *m/z* (ESI$^+$) 1393.4176 (M+H. $C_{61}H_{72}N_{14}O_{17}P_3Si$ requires 1393.4176), 1415.4013 (M+Na. $C_{61}H_{71}N_{14}NaO_{17}P_3Si$ requires 1415.3996).

2-cyanoethyl ((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) ((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((((*E*)-2-((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)vinyl)(2-cyanoethoxy)phosphoryl)oxy)tetrahydrofuran-2-yl)vinyl)phosphonate **196**

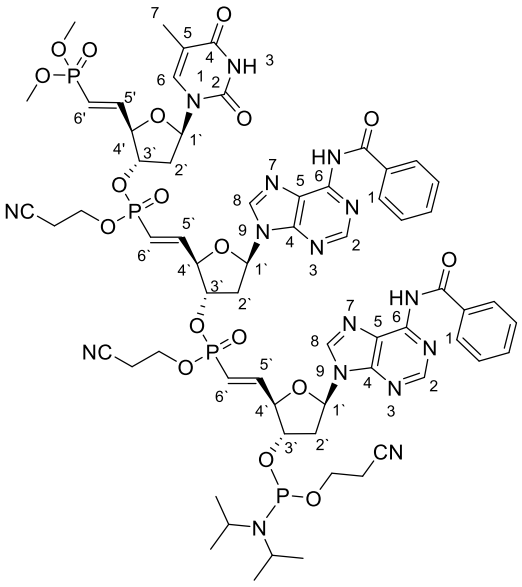


Chemical Formula: $C_{55}H_{57}N_{14}O_{17}P_3$
Exact Mass: 1278.3239

Triethylamine trihydrofluoride (37 µL, 224 µmol) was added in one portion to a stirring solution of trimer **193** (300 mg, 224 µmol) in THF (110 µL) and the resulting reaction mixture was stirred at RT for 6 h. The mixture was dry-loaded onto silica gel and purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **196** (185 mg, 67%) as a white powder, and (70 mg, 23%) of recovered starting material. *δ*H (400 MHz, CD_3OD): 8.76-8.67 (2H, A_1-H-2 + A_2-H-2), 8.57-8.49 (2H, A_1-H-8 + A_2-H-8), 8.16-7.94 (4H, m, ArH), 7.66-7.46 (6H, m ArH), 7.38-7.33 (1H, m, T-H-6), 7.26-7.00 (2H, m, A_1-H-5′ + A_2-H-5′), 6.96-6.78 (1H, m, T-H-5′), 6.65-6.57 (2H, m, A1-H-1′ + A2-H-1′), 6.25-6.18 (1H, m, T-H-1′), 6.14-5.97 (3H, A_1-H-6′ + A_2-H-6′ + T-H-6′), 5.55-5.45 (1H, m, A_1-H-3′), 5.14-5.05 (1H, m, T-H-3′), 4.92-4.87 (1H, m, A_1-H-4′), 4.81-4.75 (1H, m, A_2-H-3′) 4.66-4.55 (2H, m, T-H-4′ + A_2-H-4′), 4.36-4.17 (4H, m, 2 × C*H*$_2$CH$_2$CN), 3.74-3.67 (6H, m, 2 × OCH_3), 3.42-3.30 (1H, m, A_1-H_A-2′), 3.19-3.11 (1H, m, A_1-H_A-2′), 2.98-

2.84 (4H, m, CH_2CH_2CN), 2.83-2.71 (1H, m, H-2′), 2.62-2.42 (1H, m, H-2′), 1.89-1.78 (3H, m, H-7); δc (101 MHz, CD_3OD): 168.0 (8 × ^{Bz}CO), 166.2 (4 × T-C-4), 153.8-152.9 (8 × A-CH-2 + 8 × A-C-4 + 4 × A-CH-5′), 152.1 (4 × T-C-2), 151.4-151.1 (8 × A-C-6 + 2 × A-CH-5′), 151.0 (2 × d, *J* 6.5, A-CH-5′), 149.8 (d, *J* 5.8, 4 × T-CH-5′), 146.3 (4 × A-CH-8), 144.95 (2 × A-CH-8), 144.89 (2 × A-CH-8), 138.6 (2 × T-CH-6), 138.57 (2 × T-CH-6), 134.9 (6 × ArC), 134.8 (2 × ArC), 133.9 (16 × ArCH), 129.8 (32 × ArCH), 129.5 (32 × ArCH), 125.7 (8 × A-C-5), 119.9-119.5 (CH-6′), 118.94 (2 × CN), 118.89 (2 × CN), 118.85 (2 × CN), 118.79 (2 × CN), 118.04-117.7 (CH-6′), 116.0 (CH-6′), 112.0 (4 × T-C-5), 88.1 (2 × d, *J* 22.7, A_2-CH-4′), 88.0 (2 × d, *J* 22.7, A_2-CH-4′), 87.66 (4 × T-H-1′), 86.9 (2 × A-H-1′), 86.6 (6 × A-H-1′), 86.33-85.89 (4 × A_1-CH-4′), 85.62-85.14 (2 × T-CH-4′), 80.02-79.4 (4 × T-CH-3′ + 4 × A_1-CH-3′), 75.4 (4 × A_2-CH-3′), 62.9-62.6 (8 × CH_2CH_2CN), 53.54 (8 × d, *J* 6.3, OCH_3), 39.3 (4 × A_2-CH_2-2′), 37.9-37.6 (4 × A_1-H-2′ + 4 × T-CH_2-2′), 20.53-20.22 (8 × CH_2CH_2CN), 12.36 (4 × T-CH_3-7); δP (162 MHz, CD_3OD): 20.3 (4 × P), 19.3 (2 × P), 19.2 (2 × P), 18.41, 18.37, 18.26 (2 × P); *m*/*z* (ESI^+) 1279.3288 (M+H. $C_{55}H_{58}N_{14}O_{17}P_3$ requires 1279.3312), 1301.3100 (M+Na. $C_{55}H_{57}N_{14}NaO_{17}P_3$ requires 1301.3131).

(2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((*E*)-2-((((2*R*,3*S*,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((*E*)-2-((2-cyanoethoxy)(((2*R*,3*S*,5R)-2-((*E*)-2-(dimethoxyphosphoryl)vinyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)oxy)phosphoryl)vinyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)vinyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite **179**



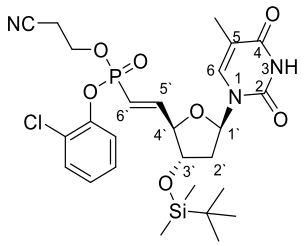
Chemical Formula: $C_{64}H_{74}N_{16}O_{18}P_4$
Exact Mass: 1478.4317

Trimer **196** (140 mg, 110 µmol) was dissolved in dry DMF (100 µL) and applied for azeotropic distillation to remove the water with toluene (3 × 1 mL), the sticky residue was dissolved in dry dichloromethane (200 µL). A mixture of 5-methyl-1-H-tetrazole (18.5 mg, 220 µmol), phosphoramidite **52** (70 µL, 220 µmol) solution in dichloromethane (100 µL) was added and the resultant mixture was stirred at RT for 1 h. The reaction mixture was diluted in ethyl acetate (10 mL), washed with water (10 mL) and brine (10 mL). The separated aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4. The volatiles were removed, and the residue was purified by column chromatography using gradient elution; 3% → 5% methanol in dichloromethane) to afford the desired product **179** (100 mg, 62%) as a white foam in a mixture of eight (1:1:1:1:1:1:1:1) diastereoisomers.

δP (162 MHz, $CDCl_3$): 149.5 (2 × P), 149.43, 149.39, 148.78 (2 × P), 148.70 (2 × P), 19.66, 19.53, 19.22 (12 × P), 19.01 (2 × P), 18.78, 18.49 (2 × P), 18.38, 18.32, 18.29, 18.21, 18.1; *m/z* (ESI^+) 1479.4319 (M+H. $C_{64}H_{75}N_{16}O_{18}P_4$ requires 1479.4390). 1501.4188 (M+Na. $C_{64}H_{74}N_{16}NaO_{18}P4$ requires 1501.4210).

5.10 Synthesis of a consecutive (*E*)-VP trimer with 5'-DMTr

2-chlorophenyl (2-cyanoethyl) ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **229**

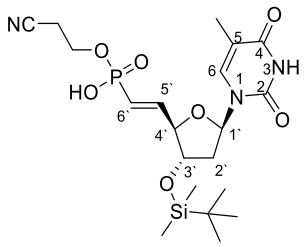


Chemical Formula: $C_{26}H_{35}ClN_3O_7PSi$
Exact Mass: 595.1670

A mixture of phosphonate **26** (2.80 g, 5.16 mmol), 3-hydroxypropionitrile**190** (1.76 mL, 25.8 mmol) and *N*-methylimidazole (4.11 mL, 51.6 mmol) was dried by azeotropic removal of water with pyridine (3 × 10 mL). The residue was dissolved in pyridine (10 mL), molecular sives and 2,4,6-triisopropylbenzenesulfonyl chloride (3.90 g, 12.9 mmol) were added. The reaction mixture was stirred at RT for 19 h, poured into citric acid solution (5%, 50 mL), ethyl acetate (50 mL) and stirred for 30 min. The separated aqueous layers was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were removed *via* reduced pressure and the residue was purified by column chromatography using gradient elution; petrol: ethyl acetate (1:1) → ethyl acetate) to afford the desired product **229** (1.65 g, 54%, 1:1 mixture of diastereoisomers) as a white foam. δH (400MHz, $CDCl_3$): 8.77 (1H, s, N-H), 7.46-7.37 (2H, m, ArH), 7.32-7.21 (1H, m, ArH),

7.18-7.11 (1.5H, m, ArH +0.5 × H-6), 7.10-6.95 (1.5H, m, 0.5 × H-6 + H-5′), 6.31 (1H, dd, *J* 6.4, 12.5, H-1′), 6.27-6.14 (1H, m, *J* 7.1, H-6′), 6.25-6.10 (1H, m, H-6′), 4.50-4.31 (3H, m, H-4′ + OC*H*$_2$CH$_2$CN), 4.28 (1H, dt, *J* 6.6, 4.2, H-3′), 4.22 (1H, dt, *J* 6.6, 4.4, H-3′), 2.81-2.74 (2H, m, OCH$_2$C*H*$_2$CN), 2.31-2.07 (2H, m, H$_{A,B}$-2′), 1.95 (1.5H, d, *J* 1.2, H-7), 1.93 (1.5H, d, *J* 1.2, H-7), 0.88 (9H, SiC(CH$_3$)$_3$), 0.07 (6H, Si(CH$_3$)$_2$); *δ*c (101 MHz, CDCl$_3$): 163.6 (C-4), 163.5 (C-4), 151.1 (d, *J* 6.2, CH-5′), 150.4 (C-2), 150.3 (C-2), 146.1 (2 × d, *J* 7.2, ArC), 135.5 (CH-6), 135.3 (CH-6), 131.0 (ArCH), 130.87 (ArCH), 128.3 (d, *J* 1.0, ArCH), 128.2 (d, *J* 1.0, ArCH), 126.5 (d, *J* 1.0, ArCH), 126.4 (ArCH), 125.8 (d, *J* 5.8, ArC), 125.7 (d, *J* 5.8, ArC), 122.5 (d, *J* 2.8, ArCH), 122.2 (d, *J* 2.8, ArCH), 117.0 (d, *J* 192, H-6′), 116.6 (CN), 116.5 (d, *J* 192, H-6′), 116.4 (CN), 111.8 (2 × C-5), 85.9 (d, *J* 27.7, CH-4′), 85.8 (d, *J* 27.7, CH-4′), 85.3 (CH-1′), 85.2 (CH-1′), 74.9-74.8 (m, CH-3′), 61.2 (d, *J* 5.8, O*C*H$_2$CH$_2$CN), 61.1 (d, *J* 5.8, O*C*H$_2$CH$_2$CN), 39.9 (CH-2′), 39.8 (CH-2′), 25.7 (SiC(*C*H$_3$)$_3$), 20.1 (d, *J* 5.8, OCH$_2$*C*H$_2$CN), 20.0 (d, *J* 5.7, OCH$_2$*C*H$_2$CN), 18.03 (Si*C*(CH$_3$)$_3$), 12.76 (CH$_3$-7), 12.71 (CH$_3$-7), -4.57 (Si(CH$_3$)$_2$), -4.67 (Si(CH$_3$)$_2$); *δ*$_P$ (162 MHz, CDCl$_3$): 15.5, 15.4; *m/z* (ESI$^+$) 596.1722 (M+H. C$_{26}$H$_{36}$ClN$_3$O$_7$PSi requires 596.1743), 618.1562 (M+Na. C$_{26}$H$_{35}$ClN$_3$NaO$_7$PSi requires 618.1563), 613.2006 (M+H$_4$N. C$_{26}$H$_{39}$ClN$_4$O$_7$PSi requires 613.2009).

2-cyanoethyl hydrogen ((*E*)-2-((2*R*,3*S*,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)vinyl)phosphonate **230**



Chemical Formula: $C_{26}H_{35}ClN_3O_7PSi$
Exact Mass: 595.1670

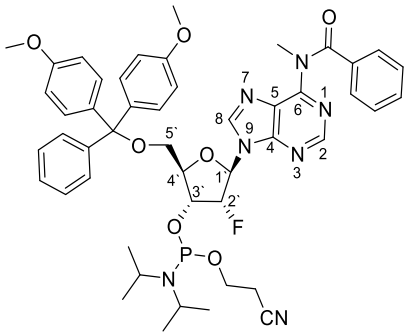
To a Vinylphosphonate **229** (1.65 g, 2.77 mmol) solution in dioxane (20 mL), a mixture of 2-nitrobenzaldoxime (506 mg, 3.05 mmol), tetramethylguanidine (349 µL, 2.77 mmol) and triethylamine (349 µL, 2.77 mmol) in dioxane (5mL) was added in one portion and the reaction mixture was stirred at RT for 22 h. The volitiles was removed and the crude product was purified by column chromatography using gradient elution; dichloromethane: methanol (9:1 → 4:1) to afford the desired product **230** (950 mg, 61%) as a white powder (more polar compound), and phosphonic acid **26** (500 mg, 33%) was separated as a by-product (less polar compound). FTIR (ATR) ν_{max}/cm^{-1} : 2952, 2929, 2855, 1687, 1469, 1209, 1068, 832; δH (400MHz, CD_3OD): 7.49 (1H, q, *J* 1.3, H-6), 6.56 (1H, ddd, *J* 20, 17, 5.7, H-5′), 6.32 (1H, dd, *J* 7.6, 6.1, H-1′), 6.0 (1H, td, *J* 17, 1.5, H-6′), 4.4 (1H, dt, *J* 6.1, 3.3, H-3′), 4.39-4.34 (1H, m, H-4′), 3.99 (1H, dt, J 7.3, 6.2, OC*H*$_2$CH$_2$CN), 2.76 (2H, d, *J* 6.1, OC*H*$_2$CH$_2$CN), 2.31-2.14 (2H, m, H_A-2′ + H_B-2′), 1.90 (3H, d, *J* 1.2, H-7), 0.93 (9H, SiC(CH$_3$)$_3$), 0.14 (3H, Si(CH$_3$)$_2$), 0.13 (3H, Si(CH$_3$)$_2$); δc (101 MHz, $CDCl_3$): 166.3 (C-4), 152.4 (C-2), 143.6 (d, *J* 4.6, CH-5′), 137.7 (CH-6), 126.1 (d, *J* 177, H-6′), 119.4 (CN), 112.1 (C-5), 88.6 (d, *J* 21, CH-4′), 86.3 (CH-1′), 76.9 (CH-3′), 60.5 (d, *J* 4.5, O*C*H$_2$CH$_2$CN), 39.9 (CH-2′), 40.4 (CH$_2$-2′), 26.3 (SiC(*C*H$_3$)$_3$), 20.5 (d, *J* 7.2, OCH$_2$*C*H$_2$CN), 18.8 (Si*C*(CH$_3$)$_3$), 12.4 (CH$_3$-7), -4.57 (Si(CH$_3$)$_2$), -4.61 (Si(CH$_3$)$_2$); δ_P (162 MHz, CD_3OD): 11.7; *m/z* (ESI$^+$) 486.1809 (M+H.

$C_{20}H_{33}N_3O_7PSi$ requires 486.1820), 508.1629 (M+Na. $C_{20}H_{32}N_3NaO_7PSi$ requires 508.1639).

5.11 Alkylation of commercially available phosphoramidite

5.11.1 Methylation of adenine-bearing nucleosides

(2*R*,3*R*,4*R*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-5-(6-(*N*-methylbenzamido)-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite **214**



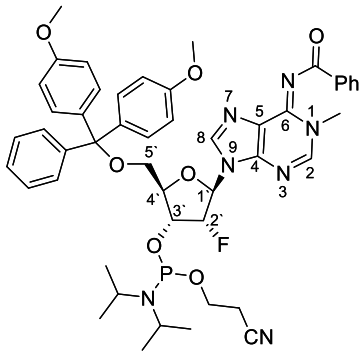
Chemical Formula: $C_{48}H_{53}FN_7O_7P$
Exact Mass: 889.3728

less polar isomer (major)

Phosphoramidite **213** (348 mg, 398 µmol) and methyl iodide (100 µL, 1.60 mmol) were dissolved in dichloromethane (3.5 mL), tetrabutylammonium bromide (130 mg, 398 µmol) and a solution of sodium hydroxide (2 M, 3.5 mL) were added to the solution. After rigorous stirring for 30 min, ether (50 mL) and water (50 mL), were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 50 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column chromatography using gradient elution; pentane: ethyl acetate (1:1) → ethyl acetate) to afford the desired product **214** (240 mg, 67%) as a white foam. And **215** as a minor product (120 mg, 33%). *δ*H (400MHz, $CDCl_3$): 8.52 (0.6H, s, H-2), 8.50 (0.4H, s, H-2), 8.13 (1H, s, H-8), 7.44-7.38 (2H, m, ArH), 7.37-7.32 (2H, m, ArH), 7.29-7.18 (8H, m, ArH), 7.10-7.04 (2H, m, ArH), 6.82-6.73 (4H, m, ArH), 6.27 (0.4H, dd, *J* 16.0, 2.3, H-1'), 6.21 (0.6H, dd, *J* 15.9, 2.3, H-1'), 5.70 (0.4H, ddd, *J* 52.3, 4.5, 2.3),

5.65 (0.6H, ddd, *J* 52.7, 4.5, 2.3), 5.11-4.98 (0.6H, m, H-3′), 4.95-4.83 (0.4H, m, H-3′), 4.36-4.27 (1H, m, H-4′), 3.98-3.81 (1H, m, OC*H*$_2$CH$_2$CN), 3.87 (6H, 2 × OCH$_3$), 3.77 (3H, ^{Bz}N-CH$_3$), 3.67-3.51 (4H, (OC*H*$_2$CH$_2$CN) + 2 (C*H*(CH$_3$)$_2$ + H$_A$-5′), 3.48-3.28 (1H, dd, *J* 11.0, 3.65, H$_B$-5′), 2.59 (0.8H, t, *J* 6.3, OCH$_2$C*H*$_2$CN), 2.40 (1.2H, t, *J* 6.3, OCH$_2$C*H*$_2$CN), 1.21-1.13 (9.4H, m, CH(C*H*$_3$)$_2$), 1.04 (2.6H, d, *J* 6.8, CH(C*H*$_3$)$_2$); *δ*c (101 MHz, CDCl$_3$): 172.3 (^{Bz}CO), 158.67 (4 × ArC), 155.23 (C), 157.19 (C), 152.13 (C), 152.1 (C), 152.05 (CH-2), 152.0 (CH-2), 144.4 (2 × ArC), 142.4 (CH-8), 142.3 (CH-8), 136.0 (2 × ArC), 135.6 (4 × ArC), 130.9 (ArCH), 130.8 (ArCH), 130.2 (8 × ArCH), 128.77 (4 × ArCH), 128.3 (2 × ArCH), 128.2 (2 × ArCH), 128.0 (8X ArCH), 127.0 (2 × ArCH), 126.7 (2 × C), 117.6 (CN), 117.5 (CN), 113.3 (8 × ArCH), 92.36 (dd, *J* 191.6, 2.0, H-2′), 91.78 (dd, *J* 192.4, 3.1, H-2′), 87.6 (d, *J* 33.6, H-1′), 87.6 (d, *J* 33.8, H-1′), 86.6 (CAr$_3$), 86.5 (CAr$_3$), 82.2 (d, *J* 5.7, CH-4′), 82.1 (d, *J* 4.3, CH-4′), 70.15 (m, CH-3′), 62.1 (CH-5′), 61.5 (CH-5′), 58.68 (2 × d, *J* 19.3, OCH$_2$CH$_2$CN), 55.4 (2 × ^{DMT}OCH$_3$), 43.5 (d, *J* 12.4, N-C*H*(CH$_3$)$_2$), 43.4 (d, *J* 12.6, N-C*H*(CH$_3$)$_2$), 36.0 (2 × ^{Bz}N-CH$_3$), 24.7 (4 × CH(*C*H$_3$)$_2$), 20.5 (d, *J* 6.6, OCH$_2$*C*H$_2$CN), 20.3 (d, *J* 7.1, OCH$_2$*C*H$_2$CN); *δ*P (162 MHz, CDCl$_3$): 151.3 (d, $^4J_{P\text{-}F}$ 8.1), 150.6 (d, $^4J_{P\text{-}F}$ 12.3); *δ*F (376 MHz, CDCl$_3$): -201.7 (d, $^4J_{F\text{-}P}$ 8.1), -202.1 (d, $^4J_{F\text{-}P}$ 12.3); *m/z* (ESI$^+$) 890.3841 (M+H. C$_{48}$H$_{54}$FN$_7$O$_7$P requires 890.3801), 912.3647 (M+Na. C$_{48}$H$_{53}$FN$_7$NaO$_7$P requires 912.3620).

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*R*,4*R*,5R)-5-((Z)-6-(benzoylimino)-1-methyl-1,6-dihydro-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl) diisopropylphosphoramidite **215**



Chemical Formula: $C_{48}H_{53}FN_7O_7P$
Exact Mass: 889.3728

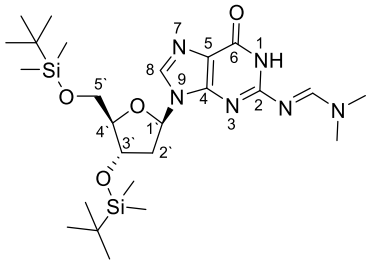
more polar isomer (minor)

*δ*H (400MHz, $CDCl_3$): 8.19-8.14 (2H, ArH), 7.88-7.82 (2H, m, H-2 + H-8), 7.51-7.45 (1H, m, ArH), 7.43-7.35 (4H, m, ArH), 7.30-7.15 (7H, m, ArH), 6.81-6.74 (4H, m, ArH), 6.14 (0.4H, dd, *J* 13.2, 2.8, H-1′), 6.09 (0.6H, dd, *J* 13.7, 2.4, H-1′), 5.5 (0.4H, ddd, *J* 52.4, 4.6, 2.8, H-2′), 5.4 (0.6H, ddd, *J* 52.7, 4.8, 2.4, H-2′), 4.92-4.79 (0.6H, m, H-3′), 4.74-4.62 (0.4H, m, H-3′), 4.33-4.25 (1H, m, H-4′), 3.96-3.77 (1.6H, m, OC*H*$_2$CH$_2$CN), 3.77 (3.4H, s, OCH_3), 3.75 (2.6H, s, OCH_3), 3.70 (3H, CH_3-1), 3.66-3.46 (4.4H, (OC*H*$_2$CH$_2$CN) + 2 (C*H*$(CH_3)_2$ + H_A-5′), 3.31-3.23 (1H, m, H_B-5′), 2.6 (0.8H, t, *J* 6.3, OCH_2CH_2CN), 2.40 (1.2H, td, *J* 6.3, 1.5 OCH_2CH_2CN), 1.21-1.13 (9.6H, m, CH(C*H*$_3)_2$), 1.04 (2.4H, d, *J* 6.7, CH(C*H*$_3)_2$); *δ*C (101 MHz, $CDCl_3$): 172.2 (2 × ^{Bz}CO), 158.64 (4 × ArC), 147.6 (2 × C), 147.3 (CH-2), 147.2 (CH-2), 145.1 (C), 145.0 (C), 144.6 (2 × ArC), 138.8 (CH-8), 138.6 (CH-8), 135.8 (2 × ArC), 135.6 (4 × ArC), 132.1 (2 × ArCH), 130.3 (4 × ArCH), 130.2 (4 × ArCH), 129.86 (4 × ArCH), 128.3 (2 × ArCH), 128.2 (2 × ArCH), 128.16 (4 × ArCH), 127.9 (4 × ArCH), 127.0 (2 × ArCH), 122.9 (2 × C), 117.7 (CN), 117.5 (CN), 113.2 (8 × ArCH), 92.47 (dd, *J*

192.0, 2.0, H-2′), 92.00 (dd, *J* 193.3, 3.0, H-2′), 87.3 (d, *J* 33.3, H-1′), 87.6 (d, *J* 33.8, H-1′), 86.6 (CAr_3), 86.5 (CAr_3), 82.4 (d, *J* 5.3, CH-4′), 82.3 (d, *J* 4.3, CH-4′), 70.25 (m, CH-3′), 62.51 (CH-5′), 61.90 (CH-5′), 58.7 (d, *J* 17.9, OCH_2CH_2CN), 58.65 (d, *J* 18.6, OCH_2CH_2CN), 55.4 ($^{DMT}OCH_3$), 55.3 ($^{DMT}OCH_3$), 43.6 (d, *J* 11.6.4, N-$CH(CH_3)_2$), 43.4 (d, *J* 12.1, N-$CH(CH_3)_2$), 37.0 (2 × ^{Bz}N-CH_3), 24.7 (4 × $CH(CH_3)_2$), 20.4 (d, *J* 6.6, OCH_2CH_2CN), 20.3 (d, *J* 7.1, OCH_2CH_2CN); *δ*P (162 MHz, $CDCl_3$): 151.4 (d, $^4J_{P\text{-}F}$ 8.3), 150.7 (d, $^4J_{P\text{-}F}$ 12.7); *δ*F (376 MHz, $CDCl_3$): -201.6 (d, $^4J_{F\text{-}P}$ 8.3), -202.5 (d, $^4J_{F\text{-}P}$ 12.7); *m/z* (ESI^+) 890.3848 (M+H. $C_{48}H_{54}FN_7O_7P$ requires 890.3801), 912.3660 (M+Na. $C_{48}H_{53}FN_7NaO_7P$ requires 912.3620).

5.11.2 Methylation of guanine-bearing nucleosides

(*E*)-*N*′-(9-((2*R*,4*S*,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)-*N*,*N*-dimethylformimidamide **221**

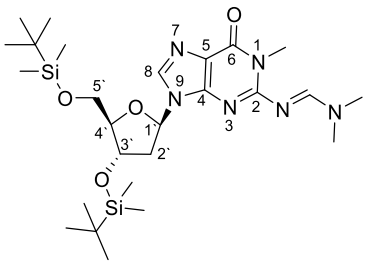


Chemical Formula: $C_{25}H_{46}N_6O_4Si_2$
Exact Mass: 550.3119

2-*N*-*dmf*-2′-deoxyguanosine (1.00 g, 3.12 mmol) was dissolved in pyridine (10 mL) and dried by azeotropic distillation with pyridine (3 × 10 mL), the residue was then dissolved in anhydrous DMF (4 mL). Imidazole (1.06 g, 15.6 mmol) was added at 0 °C, and stirard at this tempreture for 5 min. *Tert*-butyldimethylsilyl chloride (1.17 g, 7.79 mmol) was added at this tempreture and the reaction was allowed to warm up to RT and strried for 2 d. The solvent was then evaporated by azeotropic distillation with toluene (3 × 50 mL) and

the sticky oily residues was dissolved in ethyl acetate (100 mL) and washed with water (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over Na_2SO_4. The volatiles were evaporated and the crude product was purified by column chromatography using 5% methanol in dichloromethane to afford the desired product **221** as white foam (1.35g, 99%), *δ*H (400MHz, $CDCl_3$): 9.2 (1H, br, N-H), 8.61 (1H, s, ^{dmf}C-H), 7.87 (1H, s, H-8), 6.35 (1H, dd, *J* 7.9, 6.6, H-1′), 4.57 (1H, dt, *J* 6.10, 3.5 H-3′), 3.97 (1H, q, *J* 3.5, H-4′), 3.76 (2H, d, *J* 3.5, CH_2-5′), 3.18 (3H, s, $^{dmf}CH_3$), 3.10 (3H, s, $^{dmf}CH_3$), 2.49-2.31 (2H, m, CH_2-2′), (1H, ddd, *J* 13.1, 6.0, 3.6, H_B-2′), 0.92 (9H, s, $SiC(CH_3)_3$), 0.91 (9H, s, $SiC(CH_3)_3$), 0.10 (6H, s, $Si(CH_3)_2$), 0.08 (6H, s, $Si(CH_3)_2$); *δ*c (101 MHz, $CDCl_3$): 158.7 (C), 158.1 (^{dmf}C-H), 156.9 (C), 150.1 (C), 136.1 (CH-8), 120.6 (C), 87.7 (CH-4′), 83.2 (CH-1′), 72.2 (CH-3′), 63.0 (CH-5′), 41.7 (CH-2′), 41.5 ($^{dmf}CH_3$), 35.3 ($^{dmf}CH_3$), 26.1 ($SiC(CH_3)_3$), 25.9 ($SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), 18.2 ($SiC(CH_3)_3$), -4.50 ($Si(CH_3)_2$), -4.60 ($Si(CH_3)_2$), -5.20 ($Si(CH_3)_2$), --5.36 ($Si(CH_3)_2$); *m/z* (ESI^+) 551.3197 (M+H. $C_{25}H_{47}N_6O_4Si_2$ requires 551.3192), 573.3001 (M+Na. $C_{25}H_{46}N_6NaO_4Si_2$ requires 573.3011).

(*E*)-*N*′-(9-((2*R*,4*S*,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-1-methyl-6-oxo-6,9-dihydro-1H-purin-2-yl)-*N*,*N*-dimethylformimidamide **222**

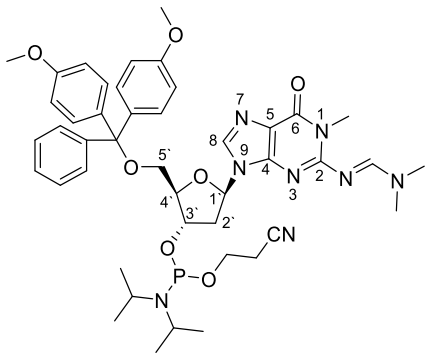


Chemical Formula: $C_{26}H_{48}N_6O_4Si_2$
Exact Mass: 564.3276

Nucleoside **221** (300 mg, 550 µmol) and methyl iodide (136 µL, 2.18 mmol) were dissolved in dichloromethane (6 mL), tetrabutylammonium bromide (177

mg, 550 mmol) and aqueous solution of sodium hydroxide (2M, 6 mL) were added to the solution. After rigorous stirring for 45 min, ethyl acetate (100 mL) and water (100 mL), were added, and the resulting layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 50 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column chromatography using gradient elution; petrol : ethyl acetate (1:1) → ethyl acetate to afford the desired product **222** as a white foam (300 mg, 98%). FTIR (ATR) ν_{max}/cm^{-1} : 2952, 2928, 2856, 1681, 1626, 1536, 1345, 1250, 1110, 1072, 831; δH (400MHz, $CDCl_3$): 8.53 (1H, s, ^{dmf}C-H), 7.84 (1H, s, H-8), 6.35 (1H, dd, *J* 7.3, 6.0, H-1'), 4.57 (1H, dt, *J* 5.7, 3.6 H-3'), 3.97 (1H, q, *J* 3.6, H-4'), 3.76 (2H, d, *J* 3.6, CH_2-5'), 3.65 (1H, s, CH_3-1), 3.19 (3H, s, $^{dmf}CH_3$), 3.13 (3H, s, $^{dmf}CH_3$), 2.45 (1H, ddd, *J* 13.1, 7.3, 5.7, H_A-2'), (1H, ddd, *J* 13.1, 6.0, 3.6, H_B-2'), 0.92 (9H, s, $SiC(CH_3)_3$), 0.91 (9H, s, $SiC(CH_3)_3$), 0.11 (6H, s, $Si(CH_3)_2$), 0.08 (6H, s, $Si(CH_3)_2$); δc (101 MHz, $CDCl_3$): 158.7 (C), 157.5 (^{dmf}C-H), 157.3 (C), 147.8 (C), 135.9 (CH-8), 119.9 (C), 87.9 (CH-4'), 82.91 (CH-1'), 72.3 (CH-3'), 63.1 (CH-5'), 41.6 (CH-2'), 41.3 ($^{dmf}CH_3$), 35.3 ($^{dmf}CH_3$), 30.1 (CH_3-1), 26.12 ($SiC(CH_3)_3$), 25.9 ($SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), 18.2 ($SiC(CH_3)_3$), -4.50 ($Si(CH_3)_2$), -4.60 ($Si(CH_3)_2$), 5.20 ($Si(CH_3)_2$), -5.34 ($Si(CH_3)_2$); *m/z* (ESI^+) 565.3356 (M+H. $C_{26}H_{49}N_6O_4Si_2$ requires 565.3348), 587.3147 (M+Na. $C_{26}H_{48}N_6NaO_4Si_2$ requires 587.3168).

(2*R*,3*S*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(2-(((*E*)-(dimethylamino)methylene)amino)-1-methyl-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite **224**

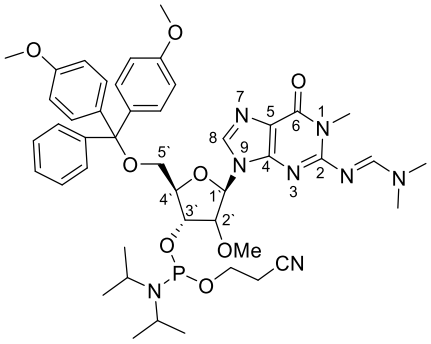


Chemical Formula: $C_{44}H_{55}N_8O_7P$
Exact Mass: 838.3931

Phosphoramidite **223** (328 mg, 398 µmol) and methyl iodide (100 µL, 1.60 mmol) were dissolved in dichloromethane (3.5 mL), tetrabutylammonium bromide (130 mg, 398 µmol) and aqueous solution of sodium hydroxide (2M, 3.5 mL) were added to the solution. After rigorous stirring for 30 min, ether (50 mL) and water (50 mL), were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 50 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column using 5% methanol in dichloromethane to afford the desired product **224** as a white foram (325 mg, 97%). δH (400MHz, $CDCl_3$): 8.52 (1H, s, ^{dmf}C-H), 7.71 (0.4H, s, H-8), 7.70 (0.6H, s, H-8), 7.43-7.37 (2H, m, ^{DMT}ArH), 7.33-7.17 (7H, m, ^{DMT}ArH), 6.83-6.75 (4H, m, ^{DMT}ArH), 6.40-6.33 (1H, m, H-1'), 4.76-4.62 (1H, m, H-3'), 4.31-4.22 (1H, m, H-4'), 3.88-3.52 (4H, m, N-C*H*$(CH_3)_2$ + OC*H*$_2$CH$_2$), 3.77 (6H, 2 × OCH_3), 3.65 (3H, CH_3-1), 3.35-3.25 (2H, m, CH_2-5'), 3.15 (3H, s, $^{dmf}CH_3$), 3.12 (3H, s, $^{dmf}CH_3$), 2.68-2.55 (2.7H, m, H_A-2' + OCH_2CH_2CN), 2.54-2.44 (1.3H, m, 2.7H, m, H_B-2' + OCH_2CH_2CN), 1.23-1.05 (12H, m, $CH(CH_3)_2$; δc (101 MHz, $CDCl_3$): 158.7 (4 × ArC), 158.6 (2 × C), 157.5 (2 × ^{dmf}C-H), 157.3 (C), 157.2 (C), 147.9 (2 ×

C), 144.6 (2 × ArC), 135.79 (CH-8), 135.74 (2 × ArC), 135.69 (2 × ArC), 135.65 (CH-8), 130.2 (8 × ArCH), 128.3 (4 × ArCH), 128.0 (4 × ArCH), 127.1 (2 × ArCH), 120.1 (2 × C), 117.6 (CN), 117.5 (CN), 113.3 (8 × ArCH), 86.6 (2 × CAr_3) 85.6 (d, *J* 5.0, CH-4′), 85.4 (d, *J* 5.2, CH-4′), 83.0 (CH-1′), 74.4 (d, *J* 18.0, CH-3′), 73.8 (d, *J* 18.0, CH-3′), 63.8 (CH-5′), 63.7 (CH-5′), 58.6 (d, *J* 13.9, O*C*$H_2$$CH_2$CN), 58.4 (d, *J* 14.3, O*C*$H_2$$CH_2$CN), 55.4 (4 × O*C*$H_3$), 43.4 (d, *J* 12.5, N-C*H*$(CH_3)_2$), 43.3 (d, *J* 12.2, N-*C*H$(CH_3)_2$), 41.25 ($^{dmf}CH_3$), 40.1 (d, *J* 4.5, CH-2′), 40.0 (d, *J* 4.4, CH-2′), 35.3 ($^{dmf}CH_3$), 30.0 (CH_3-1), 24.7 (8 × CH(*C*H_3)$_2$), 20.5 (d, *J* 7.0, OCH_2*C*H_2CN), 20.4 (d, *J* 7.0, OCH_2*C*H_2CN); δp (162 MHz, $CDCl_3$): 148.8, 148.7 *m/z* (ESI^+) 839.4008 (M+H. $C_{44}H_{56}N_8O_7P$ requires 839.4004), 861.3814 (M+Na. $C_{44}H_{55}N_8NaO_7P$ requires 861.3824).

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*R*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(2-(((*E*)-(dimethylamino)methylene)amino)-1-methyl-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl) diisopropylphosphoramidite **226**

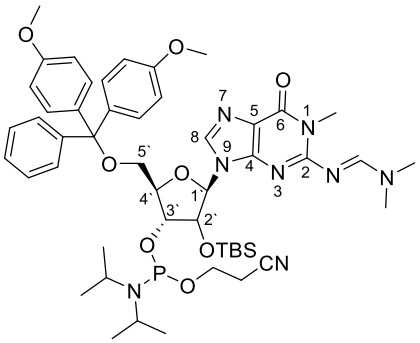


Chemical Formula: $C_{45}H_{57}N_8O_8P$
Exact Mass: 868.4037

Phosphoramidite **225** (128 mg, 150 µmol) and methyl iodide (37 µL, 600 µmol) were dissolved in dichloromethane (1.3 mL), tetrabutylammonium bromide (48 mg, 150 µmol) and aqueous solution of sodium hydroxide (2M, 1.3 mL) were added to the solution. After rigorous stirring for 45 min, ether (25 mL) and water (25 mL), were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 25 mL), the combined organic

extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column using 5% methanol in dichloromethane to afford the desired product **226** as a white foram (98 mg, 76%). *δ*H (400MHz, $CDCl_3$): 8.55 (0.6H, s, ^{dmf}C-H), 8.51 (0.4H, s, ^{dmf}C-H), 7.78 (0.4H, s, H-8), 7.74 (0.6H, s, H-8), 7.43-7.37 (2H, m, ^{DMT}ArH), 7.33-7.17 (7H, m, ^{DMT}ArH), 6.83-6.77 (4H, m, ^{DMT}ArH), 6.10 (0.6H, d, *J* 6.3, H-1′), 6.05 (0.4H, d, *J* 5.7, H-1′), 4.58 (0.4H, ddd, *J* 10.3, 5.1, 4.0, H-3′), 4.51 (0.6H, ddd, *J* 11.3, 4.8, 3.3, H-3′), 4.37-4.25 (1.6H, m, H-4′ + H-2′), 4.23 (0.4H, t, *J* 5.7, H-2′), 3.98-3.82 (1.4H, OC*H*$_2$CH$_2$), 3.78 (6H, 2 × OCH_3), 3.66 (3H, CH_3-1), 3.64-3.52 (2.6H, 0.6 (OC*H*$_2$CH$_2$) + 2 (C*H*$(CH_3)_2$), 3.48-3.28 (5H, m, OMe + CH_2-5′), 3.14 (2H, s, $^{dmf}CH_3$), 3.12 (3H, s, $^{dmf}CH_3$), 3.10 (1H, s, $^{dmf}CH_3$), 2.77-2.57 (1.4H, m, OCH_2C*H*$_2$), 2.37 (0.6H, t, *J* 6.4, OCH_2C*H*$_2$), 1.21-1.15 (8H, m, CH(C*H*$_3)_2$), 1.03 (4H, d, *J* 6.8, CH(C*H*$_3)_2$); *δ*c (101 MHz, $CDCl_3$): 157.7 (4 × ArC), 158.6 (2 × C), 157.7 (^{dmf}C-H), 157.6 (^{dmf}C-H), 157.4 (C), 157.3 (C), 148.4 (C), 148.2 (C), 144.6 (ArC), 144.5 (ArC), 135.7 (2 × ArC), 135.6 (2 × ArC), 135.5 (2 × CH-8), 130.2 (8 × ArCH), 128.2 (8 × ArCH), 127.2 (2 × ArCH), 120.2 (C), 120.1 (C), 117.8 (CN), 117.5 (CN), 113.4 (8 × ArCH), 86.9 (CAr_3), 86.8 (CAr_3), 84.88 (CH-1′), 84.7 (CH-1′), 83.6 (d, *J* 6.0, CH-4′), 83.6 (d, *J* 6.5, CH-4′), 83.5 (d, *J* 3.2, CH-2′), 83.0 (d, *J* 4.5, CH-2′), 71.6 (d, *J* 16.5, CH-3′), 70.9 (d, *J* 19.2, CH-3′), 63.7 (CH-5′), 63.2 (CH-5′), 59.2 (d, *J* 16.9, O*C*H$_2$CH$_2$CN), 59.0 (d, *J* 3.7, OMe), 58.5 (d, *J* 2.8, OMe), 58.2 (d, *J* 19.1, O*C*H$_2$CH$_2$CN) 55.4 (2 × $^{DMT}OCH_3$), 43.51 (d, *J* 12.3, N-C*H*$(CH_3)_2$), 43.3 (d, *J* 12.5, N-C*H*$(CH_3)_2$), 41.3 ($^{dmf}CH_3$), 41.2 ($^{dmf}CH_3$), 35.3 ($^{dmf}CH_3$), 30.0 (2 × CH_3-1), 24.7 (4 × CH(*C*H$_3)_2$), 20.5 (d, *J* 5.8, OCH_2*C*H$_2$CN), 20.3 (d, *J* 7.2, OCH_2*C*H$_2$CN); *δ*p (162 MHz, $CDCl_3$): 150.8; *m/z* (ESI$^+$) 839.4008 (M+H. $C_{44}H_{56}N_8O_7P$ requires 839.4004), 861.3814 (M+Na. $C_{44}H_{55}N_8NaO_7P$ requires 861.3824).

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*R*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-5-(2-(((*E*)-(dimethylamino)methylene)amino)-1-methyl-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl) diisopropylphosphoramidite **228**



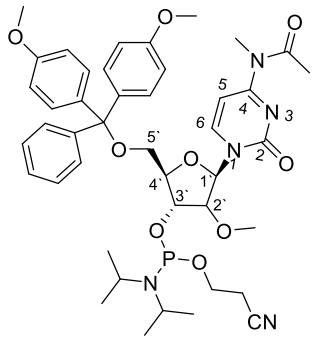
Chemical Formula: $C_{50}H_{69}N_8O_8PSi$
Exact Mass: 968.4745

Phosphoramidite **227** (143 mg, 150 µmol) and methyl iodide (37 µL, 600 µmol) were dissolved in dichloromethane (1.3 mL), tetrabutylammonium bromide (48 mg, 150 µmol) and aqueous solution of sodium hydroxide (2M, 1.3 mL) were added to the solution. After rigorous stirring for 45 min, ether (25 mL) and water (25 mL), were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 25 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column using 5% methanol in dichloromethane to afford the desired product **228** as a white foam (145 mg, quantitative). *δ*H (400MHz, $CDCl_3$): 8.52 (0.4H, s, ^{dmf}C-H), 8.47 (0.6H, s, ^{dmf}C-H), 7.85 (0.6H, s, H-8), 7.82 (0.4H, s, H-8), 7.48-7.40 (2H, m, ^{DMT}ArH), 7.38-7.17 (7H, m, ^{DMT}ArH), 6.85-6.77 (4H, m, ^{DMT}ArH), 6.02 (0.4H, d, *J* 6.4, H-1′), 5.96 (0.6H, d, *J* 6.0, H-1′), 4.71 (0.4H, dd, *J* 6.4, 4.6, H-2′), 4.67 (0.6H, dd, *J* 6.0, 4.8, H-2′), 4.40-4.24 (2H, m, H-3′ + H-4′), 3.99-3.82 (1H, m, OCH_2CH_2), 3.78 (6H, 2 × OCH_3), 3.67 (3H, CH_3-1), 3.65-3.40 (4H, (OCH_2CH_2) + 2 ($CH(CH_3)_2$) + H_A-5′), 3.30 (0.4H, dd, *J* 10.7, 3.9, H_B-5′), 3.26 (0.6H, dd, *J* 10.6, 4.1, H_B-5′), 3.11

(4.4H, $^{dmf}CH_3$), 3.02 (1.6H, s, $^{dmf}CH_3$), 2.74-2.56 (1H, m, OCH_2CH_2CN), 6.40 (1H, q, *J* 6.4, OCH_2CH_2CN), 1.21-1.15 (9H, m, $CH(CH_3)_2$), 1.03-0.93 (3H, $CH(CH_3)_2$), 0.81 (4H, s, $SiC(CH_3)_3$), 0.79 (5H, s, $SiC(CH_3)_3$), 0.00 (3H, s, $Si(CH_3)_2$), 0.12 (1.2H, s, $Si(CH_3)_2$), 0.14 (1.8H, s, $Si(CH_3)_2$); δc (101 MHz, $CDCl_3$): 158.7 (4 × ArC), 157.46 (2 × ^{dmf}C-H), 158.65 (^{dmf}C-H), 157.2 (C), 157.07 (C), 148.5 (C), 148.3 (C), 144.6 (ArC), 144.5 (ArC), 135.9 (2 × ArC), 135.8 (2 × ArC), 135.6 (CH-8), 135.46 (CH-8), 130.2 (8 × ArCH), 128.2 (8 × ArCH), 127.1 (2 × ArCH), 120.2 (C), 120.0 (C), 117.6 (CN), 177.4 (CN), 113.4 (8 × ArCH), 86.9 (CAr_3), 86.7 (CH-1', CAr_3), 86.4 (CH-1'), 83.5 (CH-4'), 83.4 (d, *J* 3.8, CH-4'), 76.6 (d, *J* 2.9, CH-2'), 75.7 (d, *J* 5.5, CH-2'), 73.5 (d, *J* 10.0, CH-3'), 73.0 (d, *J* 15.5, CH-3'), 63.8 (CH-5'), 63.5 (CH-5'), 58.5 (d, *J* 17.5, OCH_2CH_2CN), 57.8 (d, *J* 20.0, OCH_2CH_2CN), 55.4 (2 × OCH_3), 43.4 (d, *J* 12.9, $N\text{-}CH(CH_3)_2$), 43.3 (d, *J* 12.5, $N\text{-}CH(CH_3)_2$), 41.20 ($^{dmf}CH_3$), 41.0 ($^{dmf}CH_3$), 35.3 ($^{dmf}CH_3$), 35.2 ($^{dmf}CH_3$), 30.1 (2 × CH_3-1), 25.8 (6 × $SiC(CH_3)_3$), 24.7 (8 × $CH(CH_3)_2$), 20.4 (d, *J* 6.0, OCH_2CH_2CN), 20.2 (d, *J* 6.8, OCH_2CH_2CN), 18.2 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -4.54 ($Si(CH_3)_2$), -4.57 ($Si(CH_3)_2$), -4.77 ($Si(CH_3)_2$), -4.89 ($Si(CH_3)_2$); δp (162 MHz, $CDCl_3$): 150.67, 149.61; *m/z* (ESI^+) 969.4848 (M+H. $C_{50}H_{70}N_8O_8PSi$ requires 969.4818), 991.4629 (M+Na. $C_{50}H_{69}N_8NaO_8PSi$ requires 991.4637).

5.11.3 Methylation of cytosine-bearing nucleosides

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*R*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-methoxy-5-(4-(*N*-methylacetamido)-2-oxo-3,4-dihydro-1l4,2l5-pyrimidin-1-yl)tetrahydrofuran-3-yl) diisopropylphosphoramidite **217**



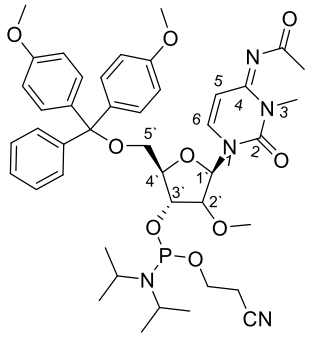
Chemical Formula: $C_{43}H_{54}N_5O_9P$
Exact Mass: 815.3659

more polar isomer (major)

Phosphoramidite **216** (240 mg, 300 µmol) and methyl iodide (72 µL, 1.20 mmol) were dissolved in dichloromethane (2.6 mL), and tetrabutylammonium bromide (100 mg, 300 µmol) and aqueous solution of sodium hydroxide (2M, 2.6 mL) were added to the solution. After rigorous stirring for 30 min, ether (50 mL) and water (50 mL) were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 50 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column chromatography using pentane : ethyl acetate (1:1) afforded **217** (140 mg, 58%, white foam, 1:1 mixture of diastereoisomers) as the major product and **218** (90 mg, 35%) as the minor product. *δ*H (400MHz, $CDCl_3$): 8.35 (0.5H, d, *J* 7.6, H-6), 8.45 (0.5H, d, *J* 7.6, H-6), 7.40-7.21 (9H, m, ArH), 6.89-6.80 (4H, ArH), 6.46 (0.5H, d, *J* 7.6, H-5), 6.38 (0.5H, d, *J* 7.6, H-5), 6.04 (0.5H, s, H-1′), 6.02 (0.5H, s, H-1′), 4.63-4.54 (0.5H, m, H-3′), 4.45-4.37 (0.5H, m, H-3′), 3.89 (1H, H-2′), 4.31-4.23 (1H,

m, H-4′), 3.89 (1H, H-2′), 3.87-3.71 (8H, m, OC*H*$_2$CH$_2$CN, 2 × ^{DMTr}OCH$_3$, CH$_A$-5′), 3.70 (1.5H, CH-2′-OMe), 3.67 (1.5H, CH-2′-OMe), 3.66-3.42 (4H, m, CH$_B$-5′, (OC*H*$_2$CH$_2$CN) + 2 (C*H*(CH$_3$)$_2$), 3.40 (3H, s, N-CH$_3$), 3.39 (3H, s, N-CH$_3$), 2.60 (1H, t, *J* 6.3, OCH$_2$C*H*$_2$CN), 2.41-2.36 (4H, m, OCH$_2$C*H*$_2$CN, ^{Ac}CH$_3$), 1.19-1.11 (9H, m, CH(C*H*$_3$)$_2$), 1.00 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$); *δ*C (101 MHz, CDCl$_3$): 172.8 (2 × ^{Ac}CO), 165.2 (2 × C-4), 158.8 (4 × ArC), 155.1 (2 × C-2), 144.3 (ArC), 144.2 (ArC), 143.2 (CH-6), 143.1 (CH-6), 135.5 (ArC), 135.4 (ArC), 135.26 (2 × ArC), 130.5 (8 × ArCH), 128.7 (4 × ArCH), 128.1 (4 × ArCH), 127.3 (2 × ArCH), 117.7 (CN), 117.5 (CN), 113.4 (8 × ArCH), 99.2 (2 × CH-5), 89.6 (CH-1′), 89.6 (CH-1′), 87.2 (CAr$_3$), 87.1 (CAr$_3$), 83.7 (d, *J* 2.1, CH-2′), 83.0 (d, *J* 2.5, CH-2′), 82.3 (d, *J* 6.3, CH-4′), 82.2 (d, *J* 5.0, CH-4′), 69.3 (d, *J* 15.0, CH-3′), 68.9 (d, *J* 16.3, CH-3′), 60.8 (CH-5′), 60.2 (CH-5′), 58.8 (2 × 2′-OCH$_3$), 58.5 (d, *J* 21, O*C*H$_2$CH$_2$CN), 58.2 (d, *J* 22, O*C*H$_2$CH$_2$CN), 58.4 (2′-OCH$_3$), 55.4 (4 × ^{DMT}OCH$_3$), 43.5 (d, *J* 12.5, N-C*H*(CH$_3$)$_2$), 43.4 (d, *J* 11.5, N-C*H*(CH$_3$)$_2$), 34.2 (2 × ^{Bz}N-CH$_3$), 26.0 (2 × ^{Ac}CH$_3$), 24.7 (8 × CH(*C*H$_3$)$_2$), 20.5 (d, *J* 6.5, OCH$_2$*C*H$_2$CN), 20.3 (d, *J* 7.4, OCH$_2$*C*H$_2$CN); *δ*P (162 MHz, CDCl$_3$): 150.6, 150.2; *m/z* (ESI$^+$) 816.3743 (M+H. C$_{43}$H$_{55}$N$_5$O$_9$P requires 816.3732), 838.3529 (M+Na. C$_{43}$H$_{54}$N$_5$NaO$_9$P requires 838.3551).

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*R*,5R)-5-((Z)-4-(acetylimino)-3-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-methoxytetrahydrofuran-3-yl) diisopropylphosphoramidite **218**



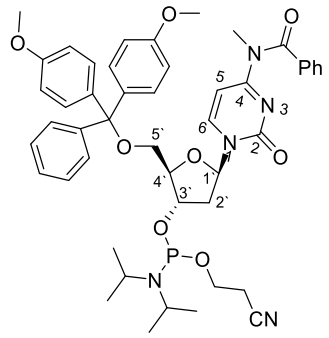
Chemical Formula: $C_{43}H_{54}N_5O_9P$
Exact Mass: 815.3659

Less polar isomer (minor)

*δ*H (400MHz, $CDCl_3$): 7.87 (0.5H, d, *J* 8.2, H-6), 7.77 (0.5H, d, *J* 8.2, H-6), 7.40-7.21 (9H, m, ArH), 6.89-6.81 (4H, ArH), 6.01 (0.5H, d, 2.7, H-1′), 5.96 (0.5H, d, 2.7, H-1′), 5.72 (0.5H, d, *J* 8.2, H-5), 5.68 (0.5H, d, *J* 8.2, H-5), 4.60-4.50 (0.5H, m, H-3′), 4.45-4.38 (0.5H, m, H-3′), 4.26-4.17 (1H, m, H-4′), 3.95-3.74 (8H, m, OC*H*$_2$CH$_2$CN, 2 × $^{DMTr}OCH_3$, CH-2′), 3.67-3.48 (8H, m, CH_2-5′, (OC*H*$_2$CH$_2$CN) + 2 (C*H*(CH_3)$_2$ + 2′-OMe), 3.36 (3H, s, N-CH_3), 2.7 (1H, td, *J* 6.3, 1.9, OCH$_2$C*H*$_2$CN), 2.4 (1H, t, *J* 6.3, OCH$_2$C*H*$_2$CN), 2.18 (3H, s, $^{Ac}CH_3$), 1.21-1.13 (9H, m, CH(C*H*$_3$)$_2$), 1.04 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$); *δ*C (101 MHz, $CDCl_3$): 184.3 (2 × ^{Ac}CO), 158.8 (4 × ArC), 153.3 (2 × C-4), 150.4 (2 × C-2), 144.2 (ArC), 144.1 (ArC), 135.5 (CH-6), 135.5 (CH-6 + ArC), 135.4 (ArC), 135.24 (ArC), 135.15 (ArC), 130.2 (8X ArCH), 128.6 (4 × ArCH), 128.1 (4 × ArCH), 127.3 (2 × ArCH), 117.8 (CN), 117.6 (CN), 113.4 (8 × ArCH), 97.6 (2 × CH-5), 88.3 (2 × CH-1′), 87.3 (CAr_3), 87.1 (CAr_3), 83.8 (d, *J* 2.5, CH-2′), 83.2 (d, *J* 3.4, CH-2′), 82.3 (d, *J* 5.7, CH-4′), 82.2 (d, *J* 4.2, CH-4′), 69.9 (CH-3′), 69.7 (CH-3′), 61.4 (CH-5′), 60.8 (CH-5′), 58.8 (2 × 2′-OCH_3), 58.5 (d, *J* 22, O*C*H$_2$CH$_2$CN), 58.2 (d, *J* 20, O*C*H$_2$CH$_2$CN), 58.4 (2′-OCH_3), 55.4 (4 X$^{DMT}OCH_3$),

43.5 (d, *J* 10.2, N-C*H*$(CH_3)_2$), 43.4 (d, *J* 10.2, N-C*H*$(CH_3)_2$), 29.7 (2 × ^{Bz}N-CH_3), 27.2 (2 × $^{Ac}CH_3$), 24.7 (8X CH(CH_3)$_2$), 20.5 (d, *J* 6.5, OCH_2CH_2CN), 20.4 (d, *J* 7.4, OCH_2CH_2CN); *δ*P (162 MHz, $CDCl_3$): 150.9, 150.3; *m/z* (ESI^+) 816.3740 (M+H. $C_{43}H_{55}N_5O9P$ requires 816.3732), 838.3511 (M+Na. $C_{43}H_{54}N_5NaO_9P$ requires 838.3551).

3-(λ2-azaneylidene)-3λ3-propyl ((2*R*,3*S*,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(4-(*N*-methylbenzamido)-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) diisopropylphosphoramidite **220**



Chemical Formula: $C_{47}H_{54}N_5O_8P$
Exact Mass: 847.3710

Phosphoramidite **219** (332 mg, 227 μmol) and methyl iodide (100 μL, 1.6 mmol) were dissolved in dichloromethane (3.5 mL), tetrabutylammonium bromide (130 mg, 404 μmol) and aqueous solution of sodium hydroxide (2M, 3.5 mL) were added to the solution. After rigorous stirring for 45 min, ether (50 mL) and water (50 mL) were added, and the resulting layers were separated. The aqueous layer was extracted with ether (3 × 50 mL), the combined organic extracts were dried over Na_2SO_4 and the volatiles were removed *in vacuo*. Purification of the residue by column chromatography using 3% methanol in dichloromethane to afford the desired product **220** (260 mg, 77%) as a white foam. *δ*H (400MHz, $CDCl_3$): 8.21-8.11 (2H, m, ArH), 7.71 (0.4H, d, *J* 8.2, H-6), 7.63 (0.6H, d, *J* 8.2, H-6), 7.58-7.52 (1H, m, ArH), 7.49-7.43 (2H, m, ArH), 7.42-7.37 (2H, m, ArH), 7.34-7.20 (7H, m, ArH), 6.89-6.81 (4H, ArH), 6.35

(1H, dd, *J* 13.7, 6.1, H-1′), 6.22 (0.4H, d, *J* 8.2, H-6), 6.20 (0.4H, d, *J* 8.2, H-6), 4.92-4.79 (0.6H, m, H-3′), 4.73-4.59 (0.4H, m, H-3′), 4.21-4.13 (1H, m, H-4′), 3.89-3.74 (7H, m, OC*H*$_2$CH$_2$CN, ^{DMTr}OCH$_3$), 3.71-3.54 (6H, m, (OC*H*$_2$CH$_2$CN) + 2 (C*H*(CH$_3$)$_2$ + ^{Bz}N-CH$_3$), 3.54-3.35 (2H, m, CH$_2$-5′), 2.7 (1.2H, t, *J* 6.3, OCH$_2$C*H*$_2$CN), 2.63-2.57 (1H, m, H$_A$-2′), 2.5 (0.8H, t, *J* 6.4, OCH$_2$C*H*$_2$CN), 2.32-2.24 (1H, m, H$_B$-2′), 1.25-1.16 (8.6H, m, CH(C*H*$_3$)$_2$), 1.04 (3.4H, d, *J* 6.8, CH(C*H*$_3$)$_2$); *δ*C (101 MHz, CDCl$_3$): 177.4 (2 × ^{Bz}CO), 158.8 (4 × ArC), 156.14 (2 × C-4), 150.4 (2 × C-2), 144.2 (2 × ArC), 136.2 (C-6), 135.91 (CH-6 + 2 × ArC), 135.5 (2 × ArC), 135.4 (2 × ArC), 132.4 (2 × ArCH), 130.2 (8 × ArCH), 129.8 (4 × ArCH), 128.3 (8 × ArCH), 128.1 (4 × ArCH), 127.3 (2 × ArCH), 117.6 (CN), 117.5 (CN), 113.4 (8 × ArCH), 98.3 (2 × CH-5), 87.0 (2 × CAr$_3$), 86.0 (2 × CH-1′), 85.6 (d, *J* 4.3, CH-4′), 85.4 (d, *J* 6.0, CH-4′), 72.9 (d, *J* 17.0, CH-3′), 72.3 (d, *J* 17.5, CH-3′), 62.7 (CH-5′), 62.3 (CH-5′), 58.4 (2 × d, *J* 19.1, OCH$_2$CH$_2$CN), 55.4 (4 × ^{DMT}OCH$_3$), 43.4 (d, *J* 12.4, N-C*H*(CH$_3$)$_2$), 43.3 (d, *J* 12.4, N-C*H*(CH$_3$)$_2$), 40.7 (d, *J* 3.1, CH-2′), 40.5 (d, *J* 4.3, CH-2′), 30.1 (2 × ^{Bz}N-CH$_3$), 24.6 (4 × CH(*C*H$_3$)$_2$), 20.5 (d, *J* 7.3, OCH$_2$*C*H$_2$CN), 20.3 (d, *J* 7.1, OCH$_2$*C*H$_2$CN); *δ*P (162 MHz, CDCl$_3$): 149.4, 148.8; *m*/*z* (ESI$^+$) 848.3796 (M+H. C$_{47}$H$_{55}$N5O$_8$P requires 848.3783), 870.3566 (M+Na. C$_{47}$H$_{54}$N$_5$NaO$_8$P requires 870.3602).

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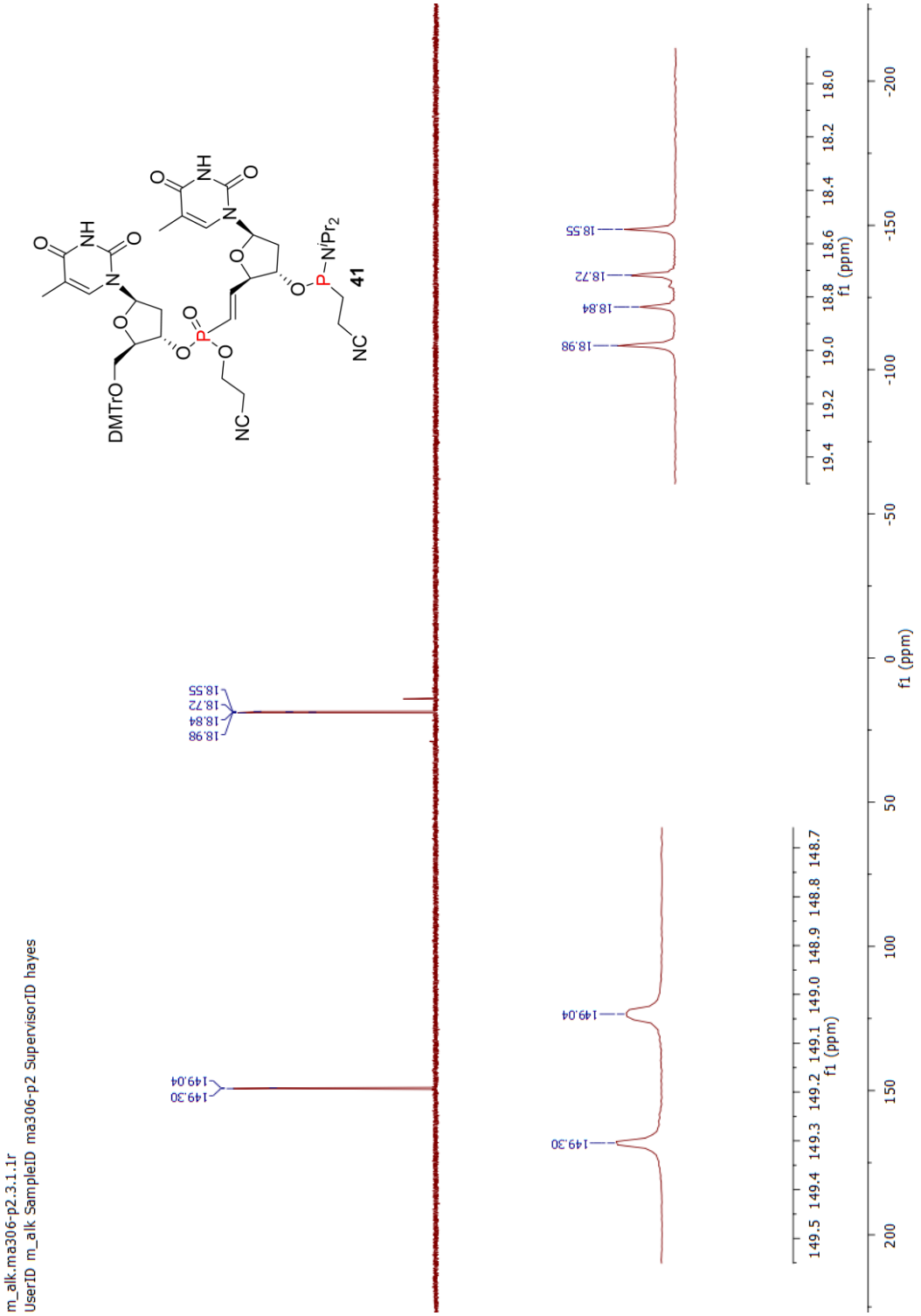
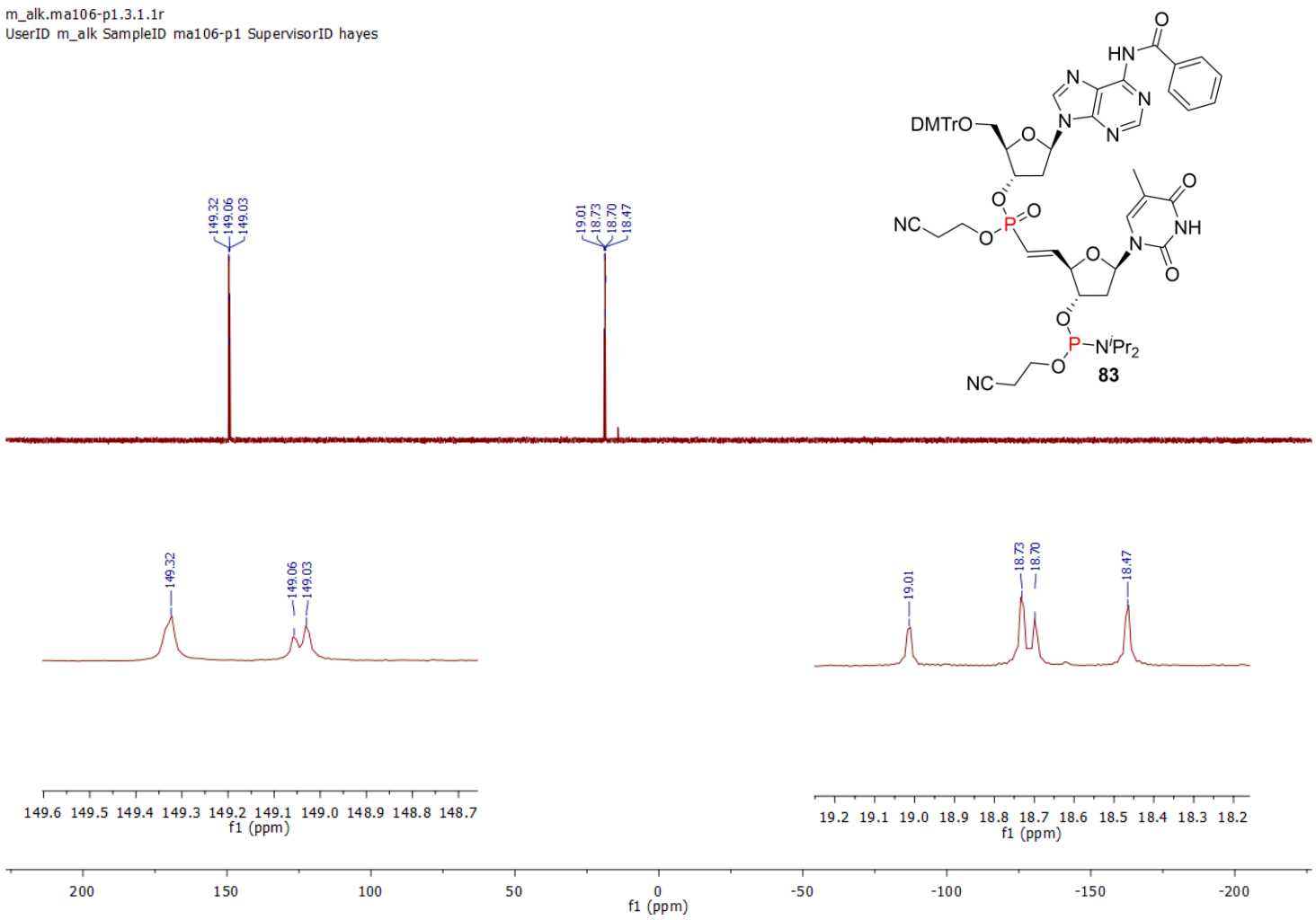
7 Appendix

7.1 ^{31}P NMR data for some of the synthesised phosphoramidite

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149.03
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18.70
18.47
DMTrO
NC
NH
HN
N
O
P
NiPr2
83
149.6 149.5 149.4 149.3 149.2 149.1 149.0 148.9 148.8 148.7
f1 (ppm)
19.2 19.1 19.0 18.9 18.8 18.7 18.6 18.5 18.4 18.3 18.2
f1 (ppm)
200 150 100 50 0 -50 -100 -150 -200
f1 (ppm)

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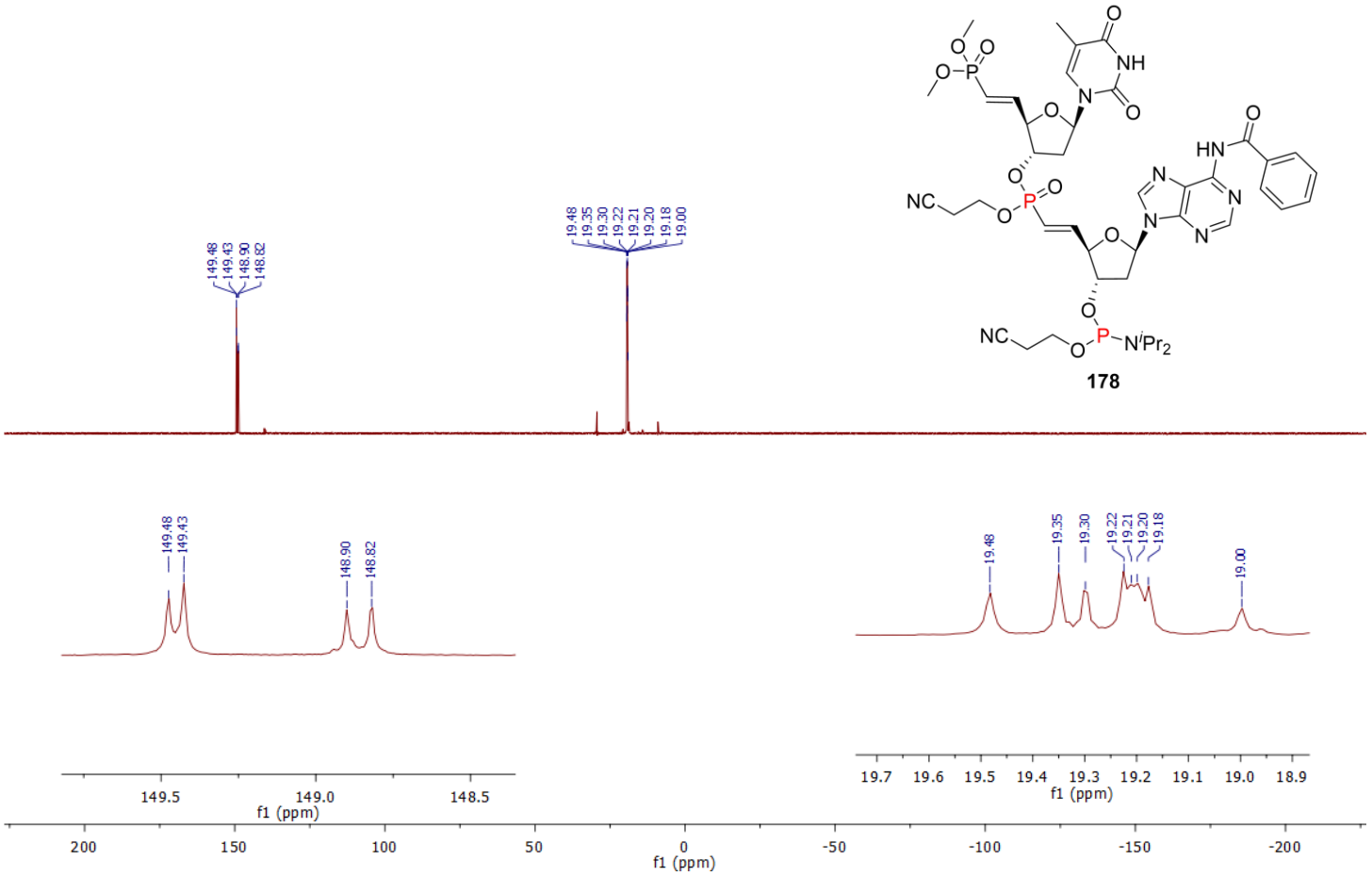

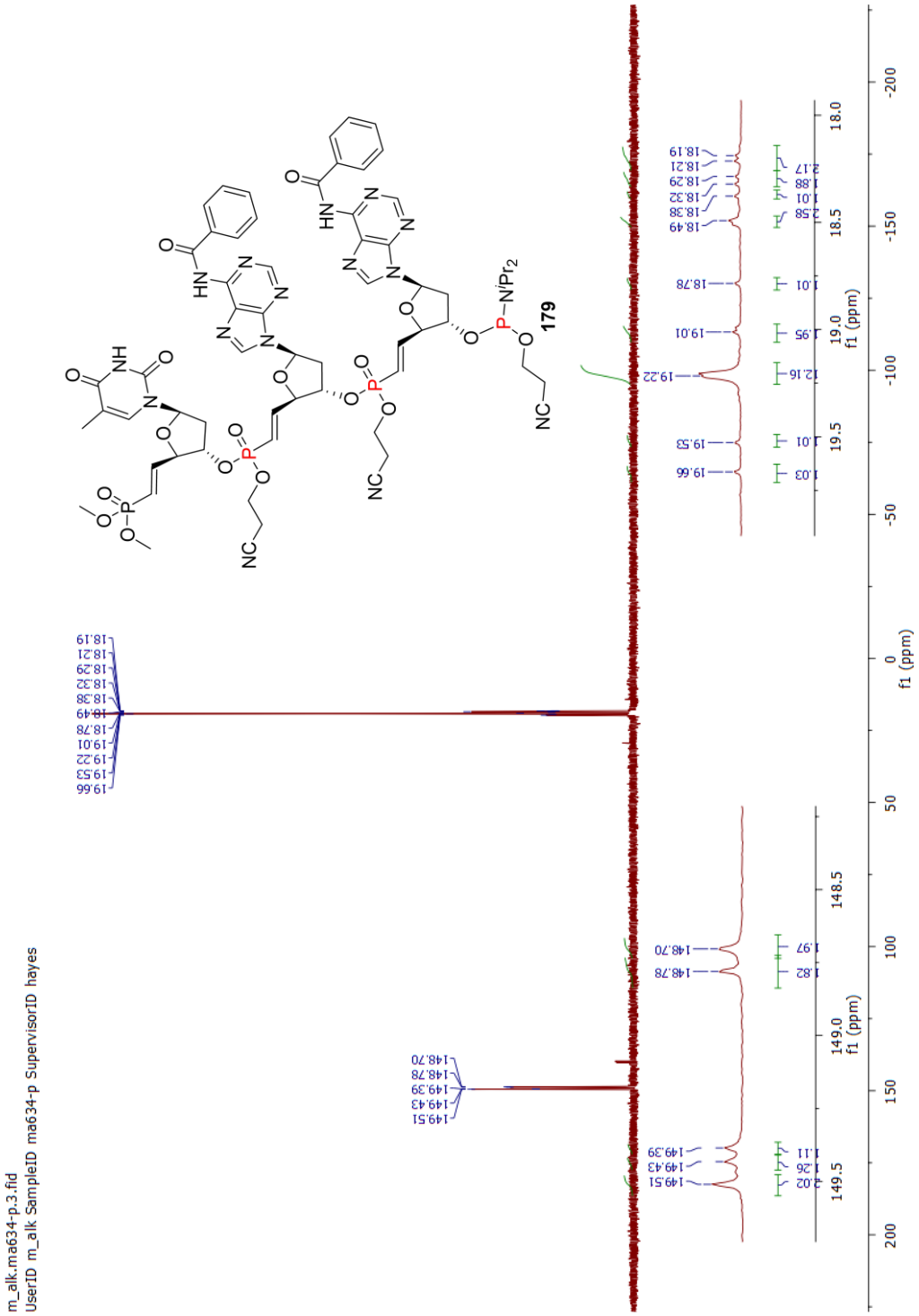
m_alk.ma634-p.3.fid
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179
f1 (ppm)

7.2 The HPLC traces and mass spectroscopy data for some synthesized oligonucleotides

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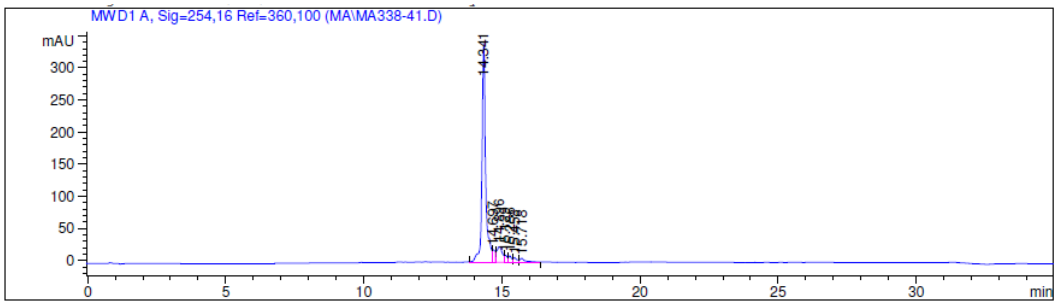
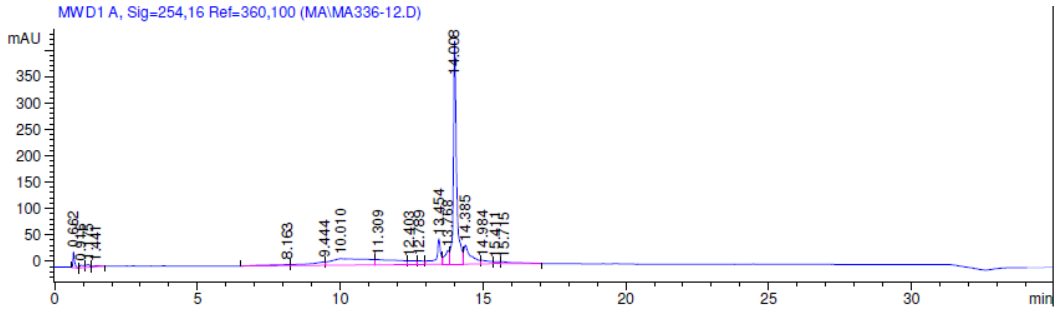
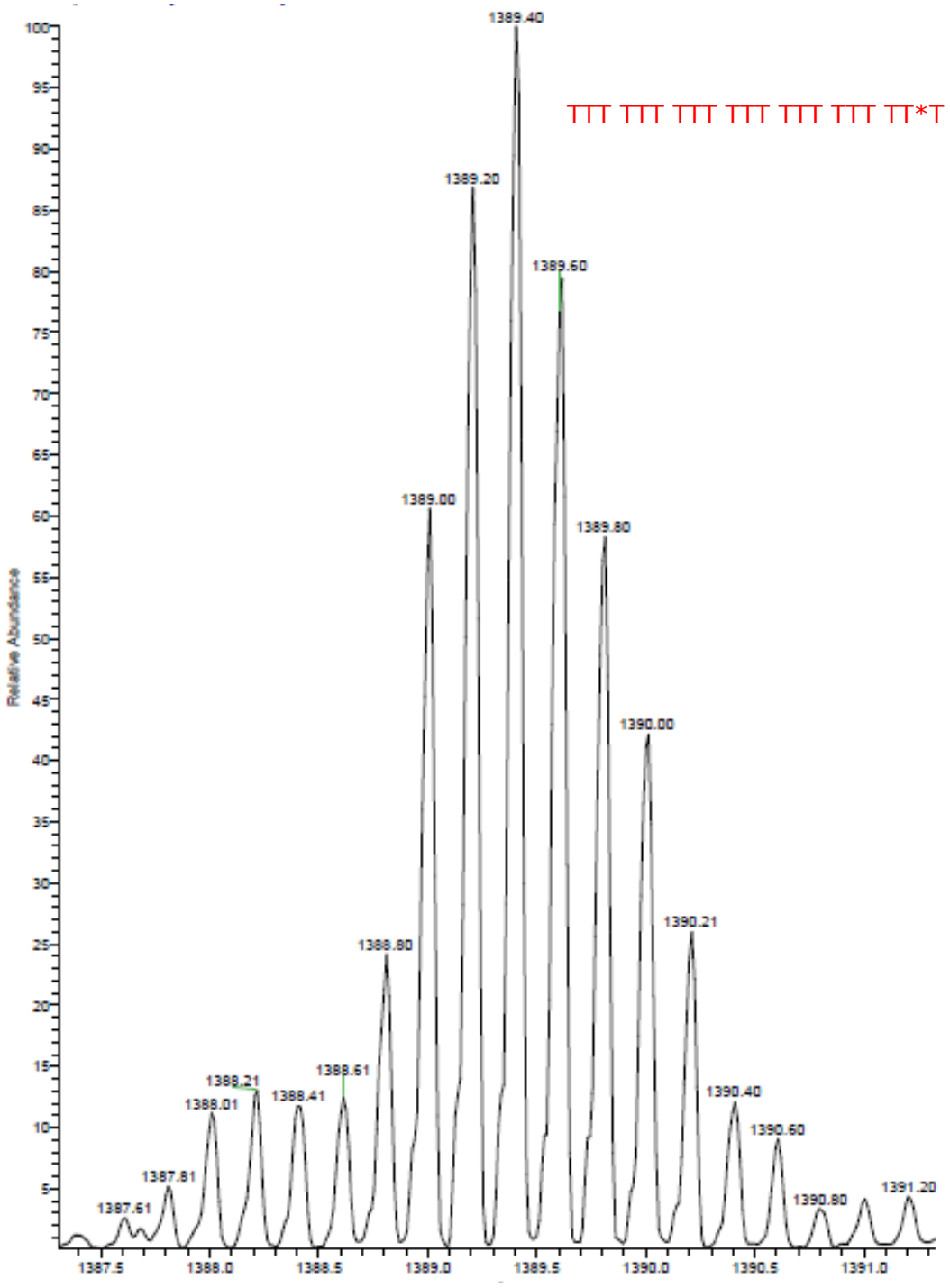
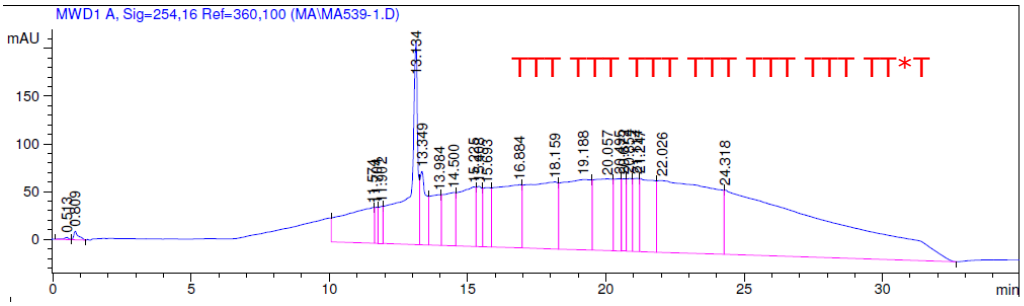


T*AA UGU UUU UGG CAU CsUsUs CsCsAs AsA




Relative Abundance
1389.40
1389.20
1389.60
1389.00
1389.80
1390.00
1390.21
1388.80
1388.21
1388.61
1388.01
1388.41
1390.40
1390.60
1387.81
1387.61
1390.80
1391.20
1387.5
1388.0
1388.5
1389.0
1389.5
1390.0
1390.5
1391.0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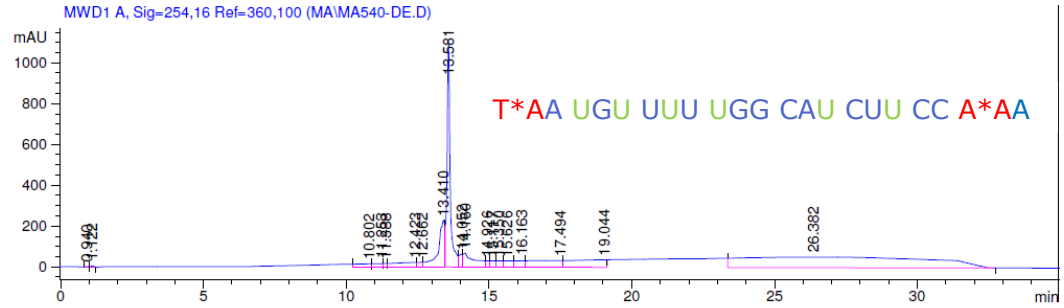
mAU
TTT TTT TTT TTT TTT TTT TT*T
min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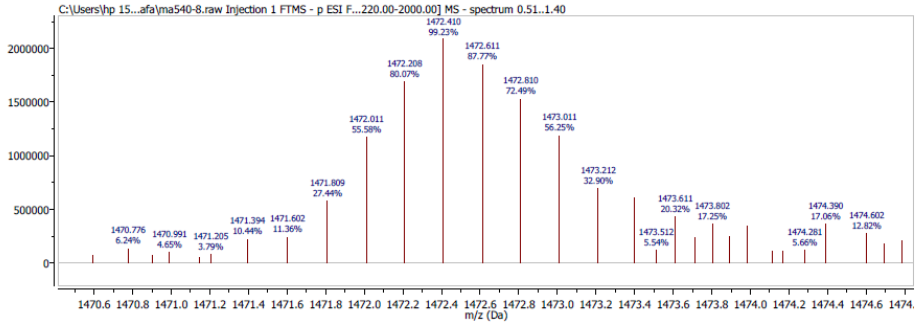
mAU
T*AA UGU UUU UGG CAU CUU CC A*AA
min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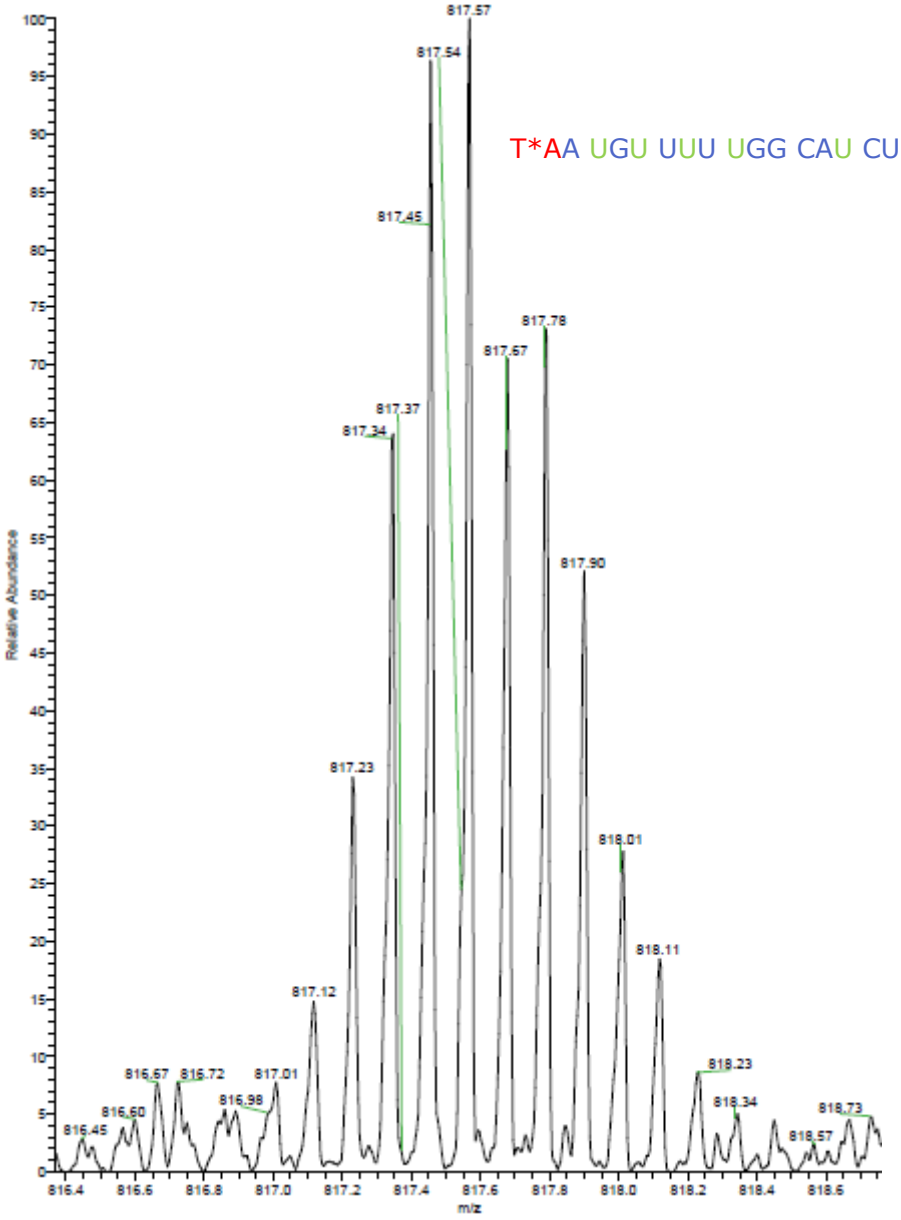
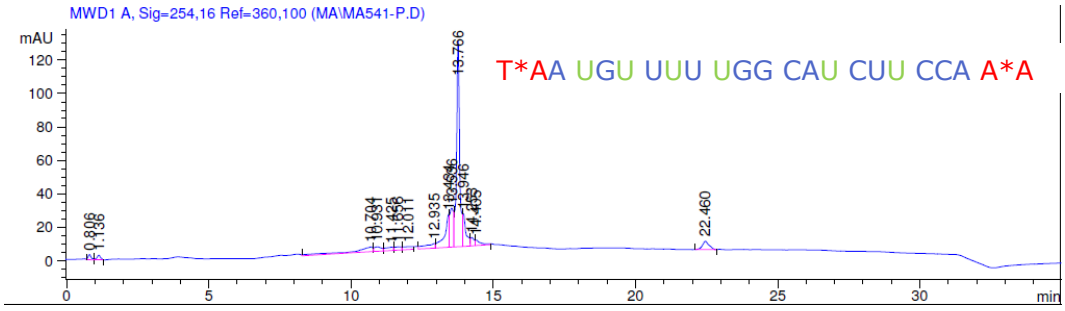
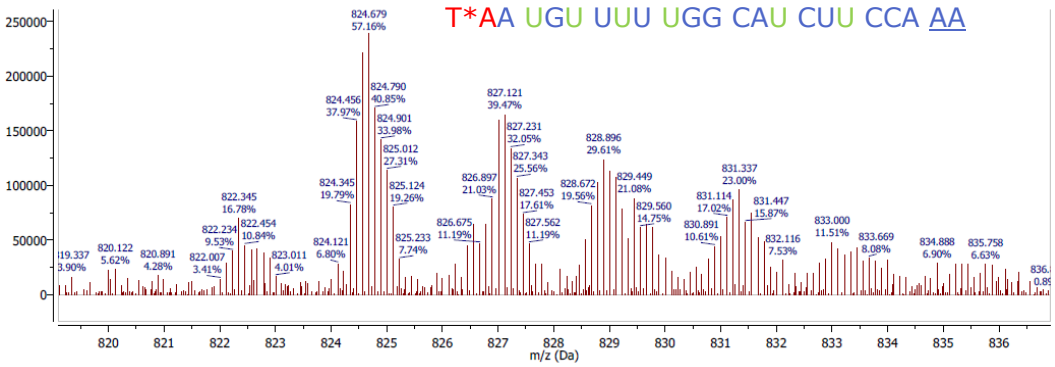
m/z (Da)


T*AA UGU UUU UGG CAU CUU CCA A*A
Relative Abundance
m/z
817.57
817.54
817.45
817.78
817.67
817.37
817.34
817.90
817.23
818.01
818.11
817.12
818.23
818.34
818.73
818.57
816.67
815.72
817.01
816.98
816.60
816.45


MWD1 A, Sig=254,16 Ref=360,100 (MA\MA541-P.D)
T*AA UGU UUU UGG CAU CUU CCA A*A
mAU
min
13.766
22.460
12.935
0.806
1.138


T*AA UGU UUU UGG CAU CUU CCA AA
250000
200000
150000
100000
50000
0
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
m/z (Da)
824.679 57.16%
824.790 40.85%
824.901 33.98%
825.012 27.31%
824.456 37.97%
824.345 19.79%
825.124 19.26%
825.233 7.74%
824.121 6.80%
827.121 39.47%
827.231 32.05%
827.343 25.56%
827.453 17.61%
827.562 11.19%
826.897 21.03%
826.675 11.19%
822.345 16.78%
822.454 10.84%
822.234 9.53%
822.007 3.41%
823.011 4.01%
828.896 29.61%
828.672 19.56%
829.449 21.08%
829.560 14.75%
831.337 23.00%
831.114 17.02%
831.447 15.87%
830.891 10.61%
832.116 7.53%
833.000 11.51%
833.669 8.08%
834.888 6.90%
835.758 6.63%
820.122 5.62%
820.891 4.28%

7.3 Alkylation of commercially available phosporamidites

Recently, Chemical modifications of RNA have been shown to have increased, unprecedented, and species-wide conserved effect on many critical cellular functions, such as Proliferation, survival, differentiation, and mostly through Regulate the stability of RNA.[108–111] *N*-6-methyladenosine (m^6A) is the most prevalent modification of the eukaryotic mRNA including mammals[112,113], plants[112,114,115], Drosophila[116] and yeast[117] as well as viruses[118], which regulate transcript processing and translation. The abundance of m^6A -modified RNAs is linked to the control of cell fate decisions of Both somatic cells and stem cells.[108,119–123] It is necessary for the development and functions of several Tissue, such as, brain, kidney and liver.[124]

m^6A RNA modifications are controlled by methyltransferases (writers), and demethylases or erasers, and apply their role by either directly being recognized by m^6A binding proteins (readers), or indirectly by tuning the structure of the modified RNA to control RNA reader–protein interactions.[125]

Several m^6A -specific readers that affect the metabolism and function of m^6A -RNA in various ways have been identified.[114,126–129] In eukaryotes, the m^6A is catalysed by a 200 kDa complex consisting of the Methyltransferase-Like Protein 3 (METTL3), METTL14 and the connected protein Wilms Tumor 1 Associated Protein (WTAP).[130–133]

Although it is not possible to be excluded that uncharacterized writers play a role in m^6A methylation, mouse embryonic stem cells missing both METTL3 and METTL14 show up to 99% decreasing in m^6A signals.[134,135] These mammalian m^6A sites are located in the consensus RRm^6ACH (R=Guanosine or Adenosine, H=Adenosine, Cytidine or Uridine), which corresponds to the specific binding elements of the METTL14, METTL3 and WTAP.[136] Nevertheless, despite this strong consensus, only a fraction of all possible RRACH sites are found methylated in vivo, which further highlights the dynamic nature of m^6A

modification. Two identified demethylases are contributing to remove the m^{6}A modification, Fat mass and Obesity-associated protein (FTO) and AlkB Homolog 5 (ALKBH5), both of these two demethylases use different mechanisms to revert m^{6}A to A (**Figure 38**).[137,138]

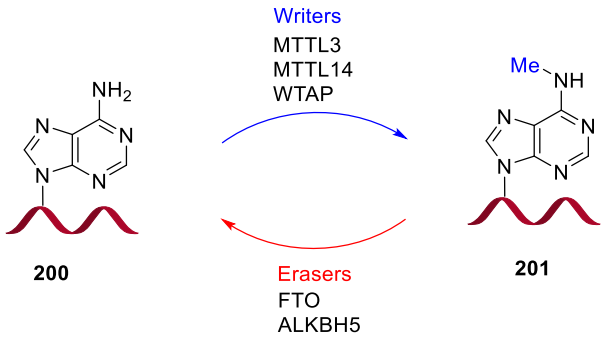
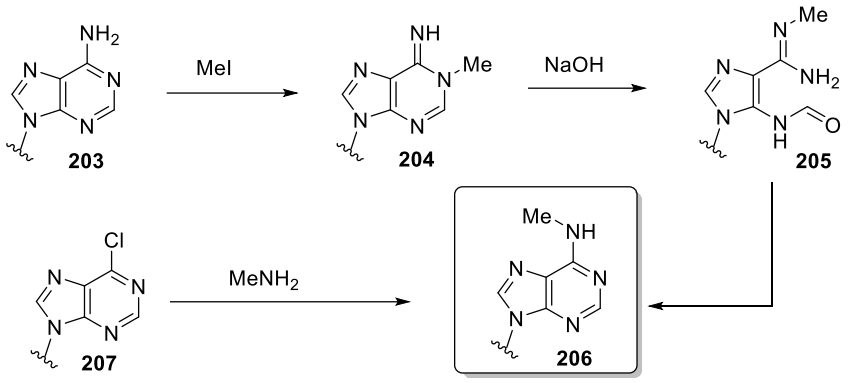


Figure 38. Regulation of m^{6}A RNA modifications. Methylation (CH_3) of adenosine 200 on position N6 to form N6-methylated Adenosine **201** (m^{6}A) is catalysed by writers such as the methyltransferase complex composed of METTL3, METTL14 and WTAP. This process is reversible, and methylation is removed by demethylases or erasers such as FTO and ALKBH5.

7.3.1 Previous synthesis of N^6-methyladenosine

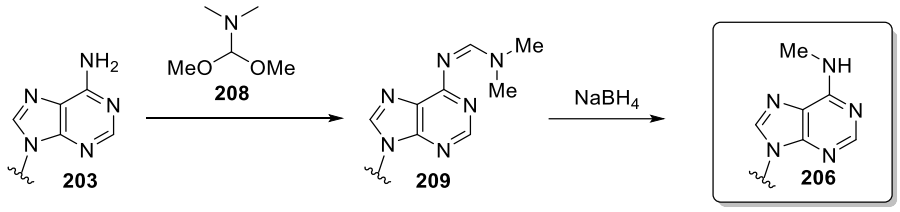
Once it becomes possible to synthesize an oligonucleotide contain m^{6}A modification at a specific location of the oligomer. It should be then possible to study the role of m^{6}A modification *in vitro* and *in vivo*. For this purpose, the phosphoramidite for automated RNA synthesis had to be easily accessible.

Many synthetic strategies have been reported in the literature for the methylation of 6-*N*-position of adenosine. The most commonly used method to access to m^{6}A **206** is Dimroth-rearrangement. This method involves methylation of the N1-position of **203** to **204** followed by a Dimroth-rearrangement *via* intermediate **205** (**Scheme 51**).[139–141] And The other well-known method involves a nucleophilic aromatic substitution of 6-substituted purine such as **207** with methylamine (**Scheme 51**).[142,143]



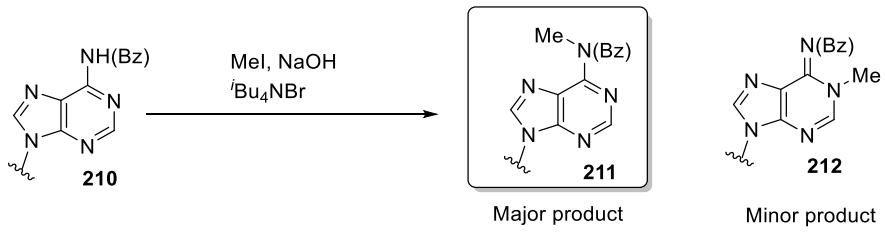
Scheme 51. Methylation of adenine bearing nucleosides **203** and **207**.

Zhang's approach[144] involves the formation of amidines **209** from **203** using *N*,*N*-dimethylformamide dimethylacetal **208** followed by reduction using $NaBH_4$ to give monomethylated adenosines m^6A **206** (**Scheme 48**).



Scheme 48. Zhang's method for methylation of adenine bearing nucleoside **203**.

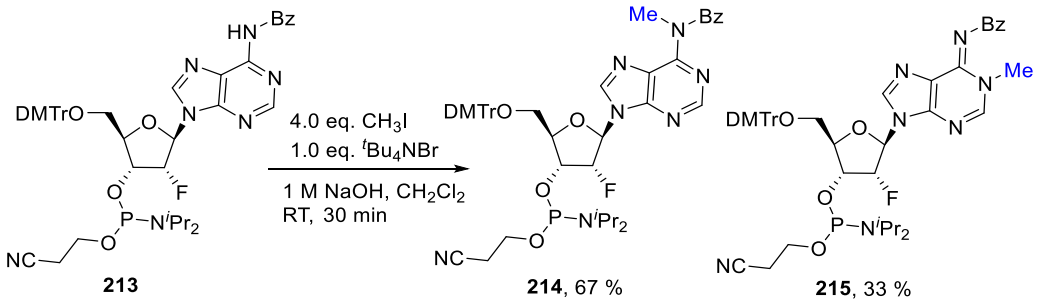
Finally, Aritomo's approach[145] achieves the methylation of 6-*N*-benzoylated adenosine **210** *via* phase transfer catalysis (PTC), which resulted in 6-*N*-methylated compound **211** as the major and *N*1-methylated compound **212** as the minor product in a ratio of 3:2 (**Scheme 52**).



Scheme 52. Aritomo's method for methylation of **210.**

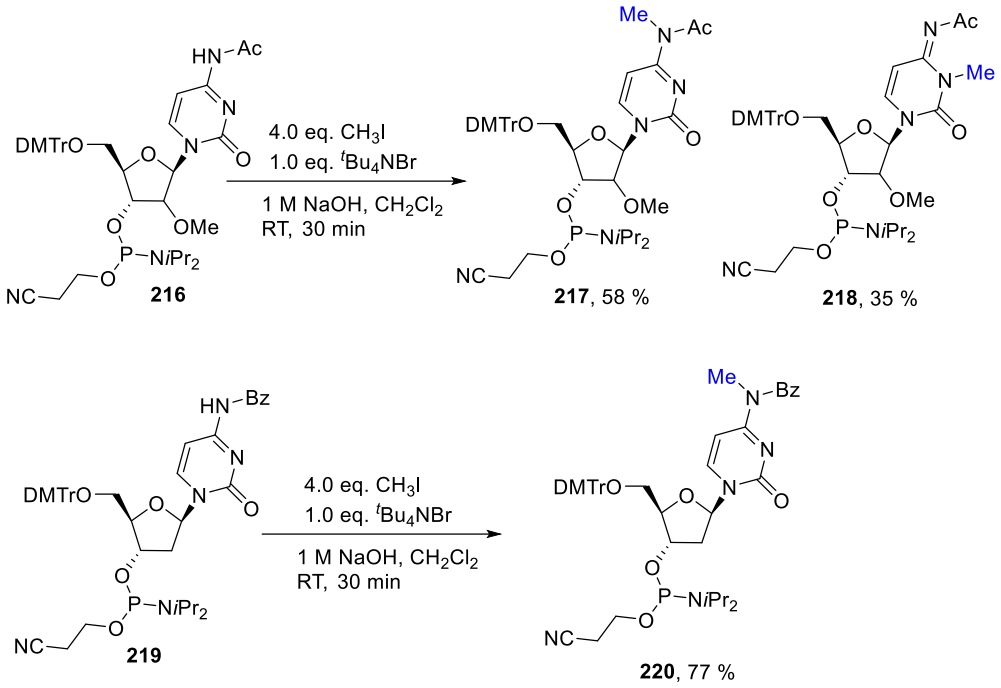
7.3.2 Synthesis of some methylated Phosphoramidites

Due to the high sensitivity of the phosphoramidites towards acids and bases, it was decided to methylation the commercially available phosphoramidite using the Aritomo's condition, which involved the use of phase transfer catalysis. The last condition is relatively mild, and the reaction proceed quickly in one-step to give access to the 6-*N*-methylated adenosine for the solid phase synthesis. Some examples of commercially available phosphoramidites were methylated using the last-mentioned condition. The methylation of N-Benzoyl-2'-F Adenosine phosphoramidite **213** has led to formation of two isomers of methylated phosphoramidites; 6-*N*-methylated **214** as a major product (67%) and 1-*N*-methylated **215** as a minor product (33%). The major and minor products could be easily separated *via* column chromatography, which led to obtain the phosphoramidite in sufficiently clean state and can be incorporated in the solid phase synthesis (**Scheme 53**).



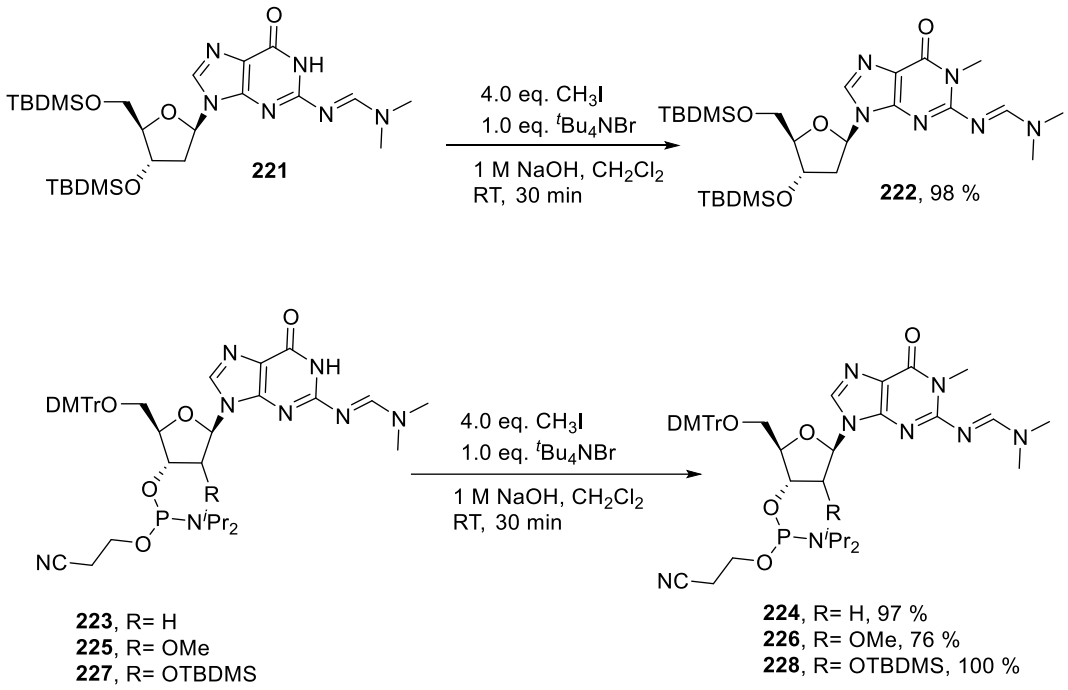
Scheme 53. Methylation of commercially available phosphoramidite **213**.

Another nitrogen base was also methylated, *N*-benzoyldeoxycytidine phosphoramidite **219**, and *N*-acyl-2'-methoxy cytidine phosphoramidite **216**. only one methylated product was separated by the column, which was 4-*N*-methylated 2'-deoxy cytidine phosphoramidite **220** in a good yield, while the RNA phosphoramidte **216** has afforded two products, the major product is 4-*N* methylated phosphoramidite **217**, and 3-*N*-methylated phosphoramidite **218** as a minor product. The separation of the two isomers was possible by column chromatography (**Scheme 54**).



Scheme 54. Methylation of commercially available phosphoramidites **216** and **219.**

In guanine chemistry, we first decided to choose a simpler molecule than the phosphoramidite to examine whether the oxygen at position 6 will be incorporated in the methylation. Therefore, bis (OTBDMS) guanosine **221** was made form the TBDMS protection of 2-*N*-dmf-2′-deoxyguanosine, and the NMR data of this substrate **221** has shown there is no product was obtained which is 1-methylated guanosine **222**. Thus, three different phosphoramidite reagents were selected for methylation, 2′-deoxy guanosine phosphoramidite **223**, 2′-methoxy guanosine phosphoramidite **225,** and 2′-TBDMS protected guanosine phosphoramidite **227**. Only one isomer was separated for each methylation, which is 1-methylted corresponding phosphoramidite **224**, **226**, and **228**, respectively (**Scheme 55**).



Scheme 55. Methylation of guanine-bearing nucleosides.