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Synthesis of Nucleotides from Low Oxidation State Phosphorus Starting Materials

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Philosophy

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Abbreviations

A	Adenine
AdCOCl	1-Adamantanecarboxylic acid
AIDS	Acquired immune deficiency syndrome
app	Apparent
ASO	Antisense Oligonucleotide
bp	base pairs
br	Broad
C	Cytosine
CMV	cytomegalovirus
d	Doublet
DCC	Dicyclohexylcarbodiimide
DMTr	Dimethoxytrityl
DNA	Deoxyribonucleic acid
dquin	doublet of quintet
ds	Double stranded
dt	Doublet of triplets
dt	Doublet of triplets
FDA	Food and Drug Administration
G	Guanine
Lev	LevulinyI
MeCN	Acetonitrile
miRNA	microRNA
MMTr	Monomethoxytrityl

mRNA	messenger RNA
NMR	Nuclear magnetic resonance
PivCl	Pivaloyl chloride
PS	Phosphorothioate
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	RNA interference
siRNA	small interfering RNA
SSOs	Splice switching Oligonucleotides
T	Thymine
TBDPS	<i>Tert</i> -Butyldiphenylsilyl
TBS	<i>Tert</i> -butyldimethylsilyl
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TOF	Time of flight
TPS	Triisopropylbenzenesulfonyl
Tr	Trityl

Abstract

In this thesis we present our work towards the synthesis of oligonucleotides from a H-Phosphinate source instead of the current synthetic methods which use PCl_3 derived compounds.

Chapter 1 introduces oligonucleotides, their use in the pharmaceutical industry and the previous synthetic routes used to obtain these.

Chapter 2 documents our synthetic effort for the synthesis of H-phosphinates in part 1, and the synthesis of H-phosphonates in part 2. We use both previously established esterification and coupling reagents, as well as metal catalysis for these transformations. We find that H-phosphinic acid is too acidic for use with nucleosides and develop conditions for the synthesis of H-phosphinates and H-phosphonates from phosphinic acid salts and phosphonic acid salts. We then go on to discuss our work towards the synthesis of dinucleotides from H-phosphonates, containing the H-phosphonate, phosphonate and phosphorothioate linker. We then detail our attempts to develop this reaction using solid supported reagents with the view to performing this reaction "in-flow".

Chapter 1

Introduction to Oligonucleotides;

Applications and Synthesis

1.1 An Introduction to Oligonucleotides and Their Therapeutic Applications

The majority of currently approved drugs are small molecules that target proteins such as enzymes and receptors, representing a small proportions of the total number of cellular proteins.¹ Therapeutic oligonucleotides, normally 12-30 nucleotides in length, offer a different way of treating disease, by targeting pre-mRNA and mRNA which are involved in the pathological pathway of a disease.²

They were first proposed for use as therapeutics in 1978 by Zamencik and Stephenson, and over the last 40 years there has been intense research and development leading to numerous clinical trials and as of January 2017 six approvals.^{3,4}

The principle of oligonucleotide therapy is the utilization of Watson-Crick base pairing rules, to induce sequence-specific binding of an oligonucleotide to pre-mRNA and mRNA.⁵ Oligonucleotides initially showed promise to researchers as a way of treating targets and diseases not accessible through small molecule drugs (as mRNAs encode for all cellular proteins), and the opportunity for the rapid and rational design of drugs against any genetic target, however it has taken over 40 years for these therapies to reach clinical maturity.^{1,6}

1.1.1 Mechanisms of Action

There are three broad mechanisms of actions that oligonucleotides are known to work by; RNA interference (RNAi), antisense oligonucleotides (ASOs) and steric-blocking oligonucleotides. The first two of these affect gene expression by inducing enzymatic

degradations of the targeted mRNA.¹ Steric-blocking oligonucleotides act by blocking targeted RNA without inducing its degradation, the result of which can depend on the sequence elements in the target, but there have been two major clinical uses so far; splice switching and microRNA (miRNA) inhibition.^{1,6}

RNA Interference

RNAi became prominent in 2001 through the development of small interfering RNAs (siRNAs), 19-22 bp RNA duplexes that enable gene silencing by incorporating their guide strands into the RNA-induced silencing complex (RISC), which then binds to the target mRNA, and then there is cleavage between the 10th and 11th nucleotide (relative to the 5' end), leading to mRNA degradation.^{1,7}

Antisense Oligonucleotides

ASOs are 15-20 bp single stranded (unlike siRNAs) nucleotides with a sequence that is antisense and complementary to the target mRNA resulting in hybridisation, leading to specific gene inhibition by various mechanisms, the most common of which being RNase H mediated cleavage of RNA.⁷

Steric-blocking

Steric blocking oligonucleotides do not recruit RNase H, and have currently have two major applications;

Splice switching Oligonucleotides (SSOs)– Oligonucleotides that modulate pre-mRNA splicing by blocking RNA sequences that are essential for splicing and prevent the interaction of splicing factors, which can result in the repair of defective RNA, restoration of essential proteins or generation of novel proteins.¹

microRNAs (miRNA)- miRNAs are small non-coding RNAs which have a crucial role in regulating gene expression, and can be targeted with complementary sequence resulting in duplex formations which can lead to direct inhibition and in some cases inhibition of miRNA maturation.^{6,8}

1.1.2 Existing Drugs

The first reported use of a synthetic oligonucleotide as an effective inhibitor of protein expression was in 1978, when Zamecnik synthesised a tridecamer oligodeoxynucleotide with a complementary sequence to the Rous sarcoma virus.⁹ Since then there has been numerous clinical trials of various oligonucleotides, with different chemical modifications being used to improve the poor pharmacokinetic and pharmacodynamic properties that unmodified oligonucleotides show, and as of January 2017 there have been 6 approved for clinical use.^{4,10}

Fomivirsen

The first FDA approved oligonucleotide was fomivirsen in 1998, as 21-mer phosphorothioate (PS) with a complementary sequence to the UL123 gene within cytomegalovirus (CMV), and was used for the treatment of CMV retinitis in patients who were immunocompromised due to AIDS.^{4,11,12} This has been synthesised in Reese *et al.* using H-phosphonate nucleotides in a block approach.¹³ Fomivirsen has since been discontinued as there has been a sharp decrease in the number of CMV cases, due to the development of high-activity anti-retroviral therapy.⁴

Pegaptanib

Pegaptanib is a RNA aptamer, targeting vascular endothelial growth factor, approved in late 2014 for the treatment of neovascular age-related macular degeneration.¹⁴ Consisting of 27 nucleobases; with all purine ribose sugars being 2'-O-methylated and all pyrimidine ribose sugars bearing a 2'-F modification, a 3'-3' deoxythymidine cap and 5' PEGylation modification as well in order to improve therapeutic activity and half-life in vivo.^{4,15}

Mipomersen

A second generation ASO, mipomersen has been approved for use in the USA since 2013 for the treatment of homozygous familial hypercholesterolemia.¹⁶ A 20-mer which has PS backbone and has 2' methoxyethyl substituted nucleotides at the 5' and 3' ends to increase the potency, nuclease resistance and distribution to peripheral tissues, mipomersen targets apolipoprotein B-100 mRNA and cleaves this via RNase H activity.¹⁵

Eteplirsen

A 30-mer phosphorodiamidate morpholino-based oligomer was approved by the FDA in 2016.⁶ Eteplirsen is a SSO used to treat Duchenne muscular dystrophy, and binds specifically to a portion of exon 51 of dystrophin and induces exon skipping.⁴

Nusinersen

Nusinersen was developed to treat spinal muscular atrophy and was approved for use by the FDA in late 2016.⁴ A 18-mer oligonucleotide

with a PS backbone, 2' methoxyethyl substitutions and methyl-modified at the 5 position on cytidine bases, it redirects the splicing of the SMN2 gene.^{4,6}

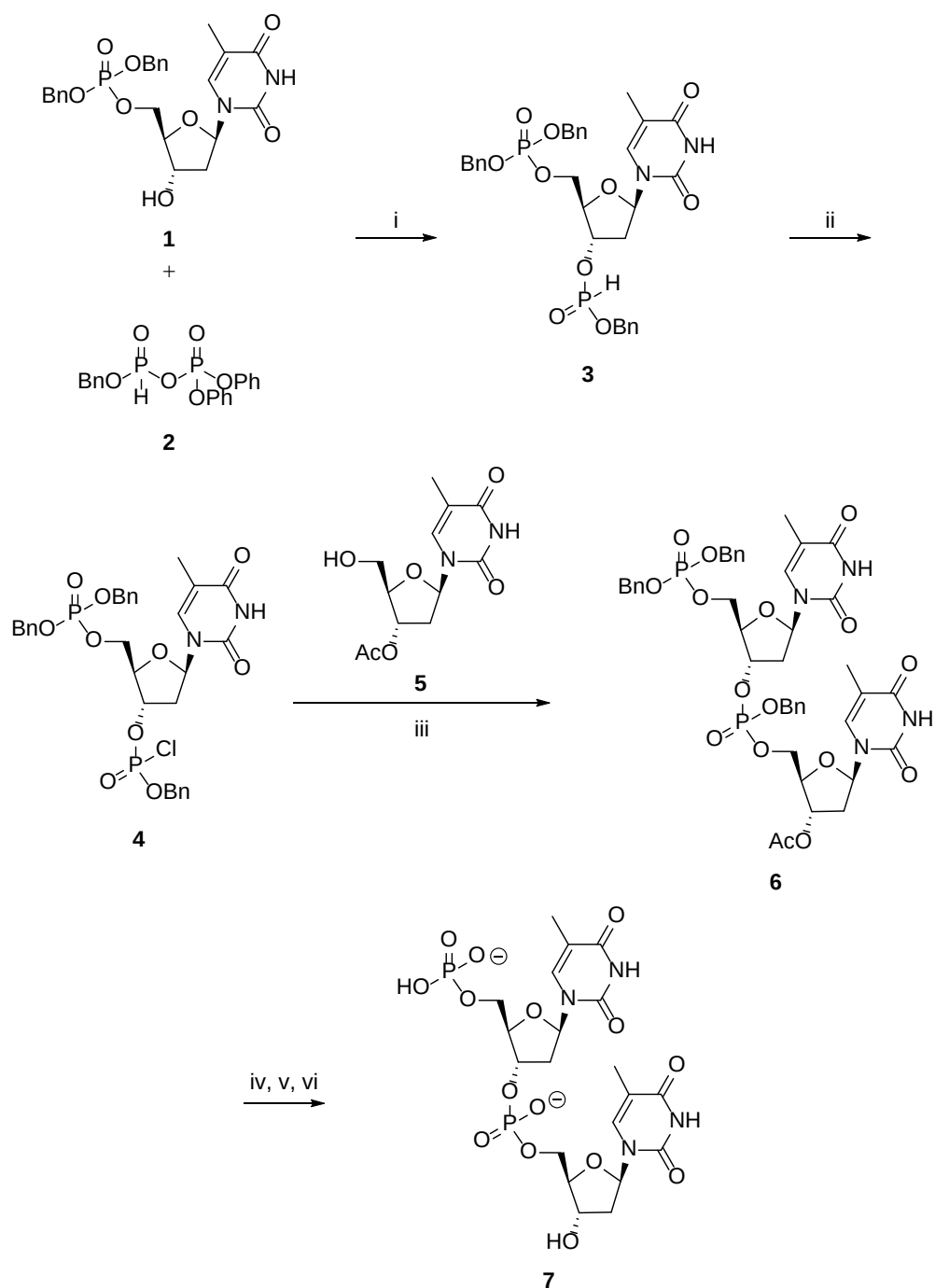
Defibrotide

Defibrotide is different to other FDA approved therapies above, in that it is a natural product, which is isolated and purifies from the intestinal mucosa of pigs rather than being constructed on a DNA synthesizers. As such it consists of a mixture of single and double stranded phosphodiester oligonucleotides. At the present time it is not possible to resolve the structure of the individual stands, and has a complex mechanism of actions which is controversial and not completely understood.⁴

1.2 Synthesis of Oligonucleotides

Except for defibrotide, the above drugs are synthesized chemically for mass production. The first chemical synthesis of a 3'-5' deoxyribonucleotide was published in 1955, by Todd *et al.* using thymine as the base in both nucleosides (Scheme 1).¹⁷

Since this publication, research has been carried out on the development of different synthetic methods of making oligonucleotides, addressing the difficulties of the selective protection and deprotection of various reactive moieties present in these molecules.¹⁸

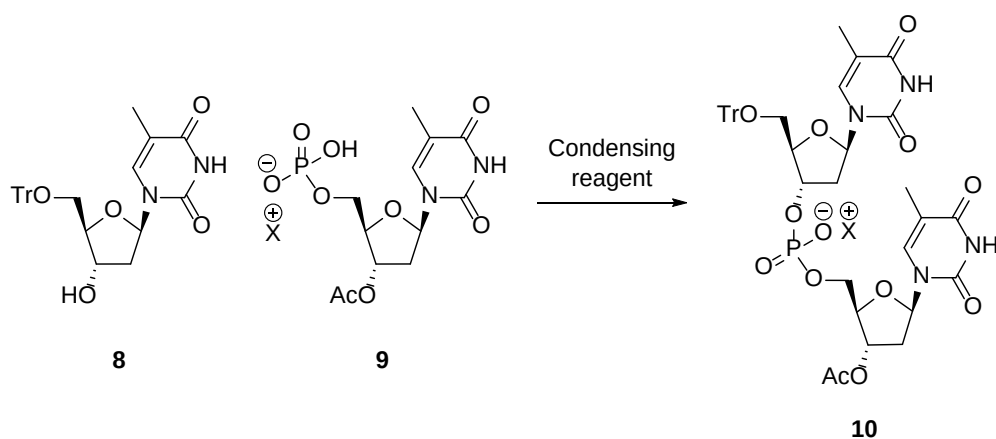


Reagents and Conditions: (i) 2,6-Lutidine, MeCN; (ii) NCS, MeCN, Benzene;
 (iii) 2,6-Lutidine, MeCN; (iv) H₂SO₄, EtOH, H₂O; (v) Ba(OH)₂, H₂O;
 (vi) H₂, PdO, Pd/C

Scheme 1: First reported synthesis of a dinucleotide with correct 3'-5' orientation.¹⁷

1.2.1 Phosphodiester approach

The first gene was chemically synthesized by Khorana *et al.* in 1979 using what is termed as the “phosphodiester approach”. The key step in the synthesis of this 207 bp DNA was a condensation reaction which made >90% of the internucleotide bonds present. In the phosphodiester approach a nucleoside with a free 3'OH group (**8**) was reacted with a 5'-phosphomonoester (**9**) which has been activated directly with another suitable reagent (Scheme 2).¹⁹



Scheme 2: Synthesis of a dinucleotide using the phosphodiester approach.¹⁹

In the development of this method a variety reagents for the activation of the 5'phosphomonester were tried; including *N*-ethyl-5-phenylisoxazolium fluoroborate (**11**), ethoxyacetylene (**12**), dicyclohexylcarbodiimide (DCC) (**13**) and a variety of substituted arylsulfonyl chlorides (**14a-c**) (Figure 1).^{19,20}

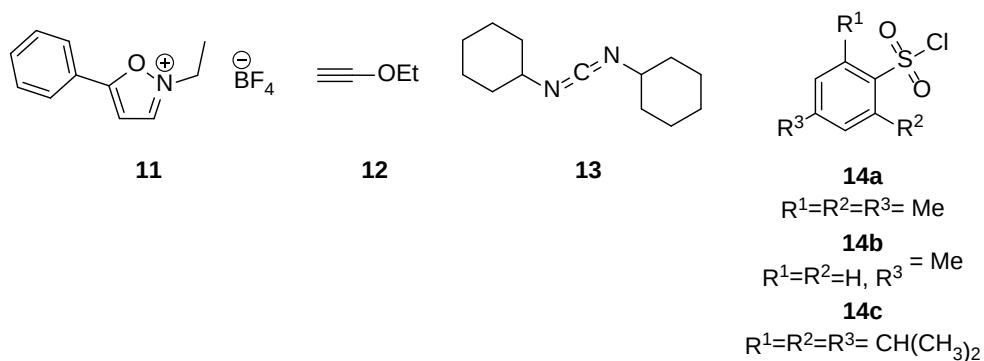


Figure 1: Different reagents used for the condensation of **8** with **9**.

During this investigation it was found that DCC and arylsulfonyl chlorides were the better coupling reagents, giving the highest yields (90-95%) with stoichiometric quantities of the nucleotide and nucleoside starting materials, and also crucially did not require the protection of the internucleotide phosphodiester functionality.²¹

While no protection of the phosphodiester was required, it soon became apparent to Khorana *et al.* that when using deoxynucleosides besides thymidine, the amino group present in the base would require protection. This would be needed to avoid reaction with the activated 5'phosphate group to form the phosphoroamidate, and also to increase the solubility of these compounds.²¹

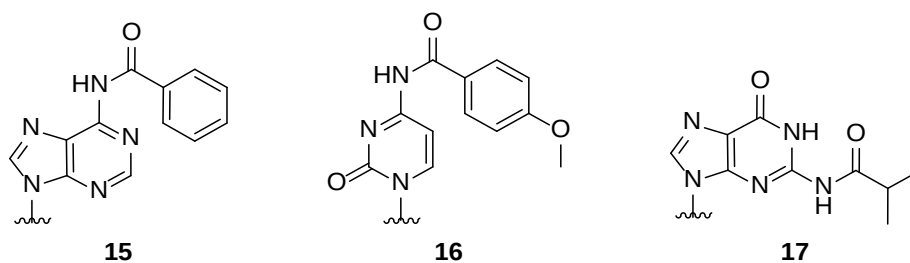


Figure 2: Protecting group strategy used for adenine **15**, cytosine **16** and guanine **17** by Khorana.¹⁹

While the 5'OH functionality in nucleoside **8** (Scheme 2) was protected using a trityl (Tr) group, it was found that the amine protecting groups (Figure 2) were not stable to long exposure to acetic acid, required for the removal of the Tr group, therefore a moiety that was more labile to acid was required to protect the 5'OH. The introduction of a *p*-methoxy group to one of the aryl rings (MMTr) increased the lability to acid in the region of about 10-fold, and the addition of another *p*-methoxy group (DMTr) increased this even further.²¹

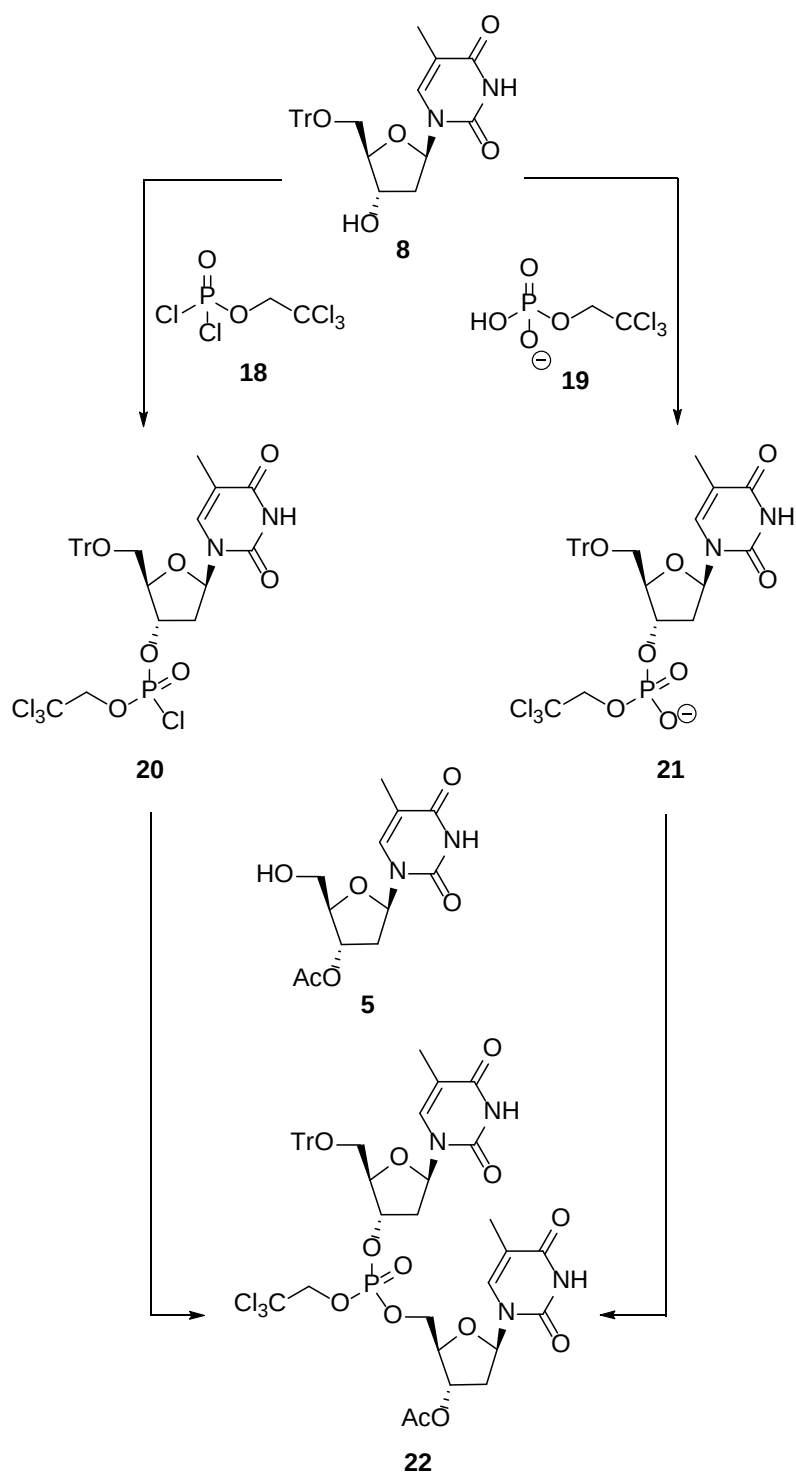
The phosphodiester method has been applied in the two approaches towards oligonucleotide synthesis; the stepwise approach in which mononucleotides are added individually to a growing chain, and the block approach wherein many “blocks” of oligonucleotides are made, and then reacted together.²² This approach did have its drawbacks, as the charged internucleotide linkages proved problematic, causing side reactions and therefore lower yields.²³

1.2.2 Phosphotriester approach

The problems caused by the charged internucleotide linkages lead to researchers reinvestigating the phosphotriester approach first used by Todd *et al.* (Scheme 1), however it was clear that the benzyl group used would be susceptible to nucleophilic attack, so a different protecting group was required.^{17,23}

During the 1960s numerous laboratories used different protecting groups, and starting materials to synthesis first diesters and longer sequences; Eckstein and Rick developed a method of synthesising

diesters using 2,2,2-trichloroethyl group on either a monoalkyl phosphate (**19**) or a monoalkyl phosphorodichloridate (**18**) (Scheme 3) to synthesize a protected dinucleotide (**22**) which could be deprotected using zinc dust in acetic acid, or be further reacted to make a tetramer first.^{24,25}

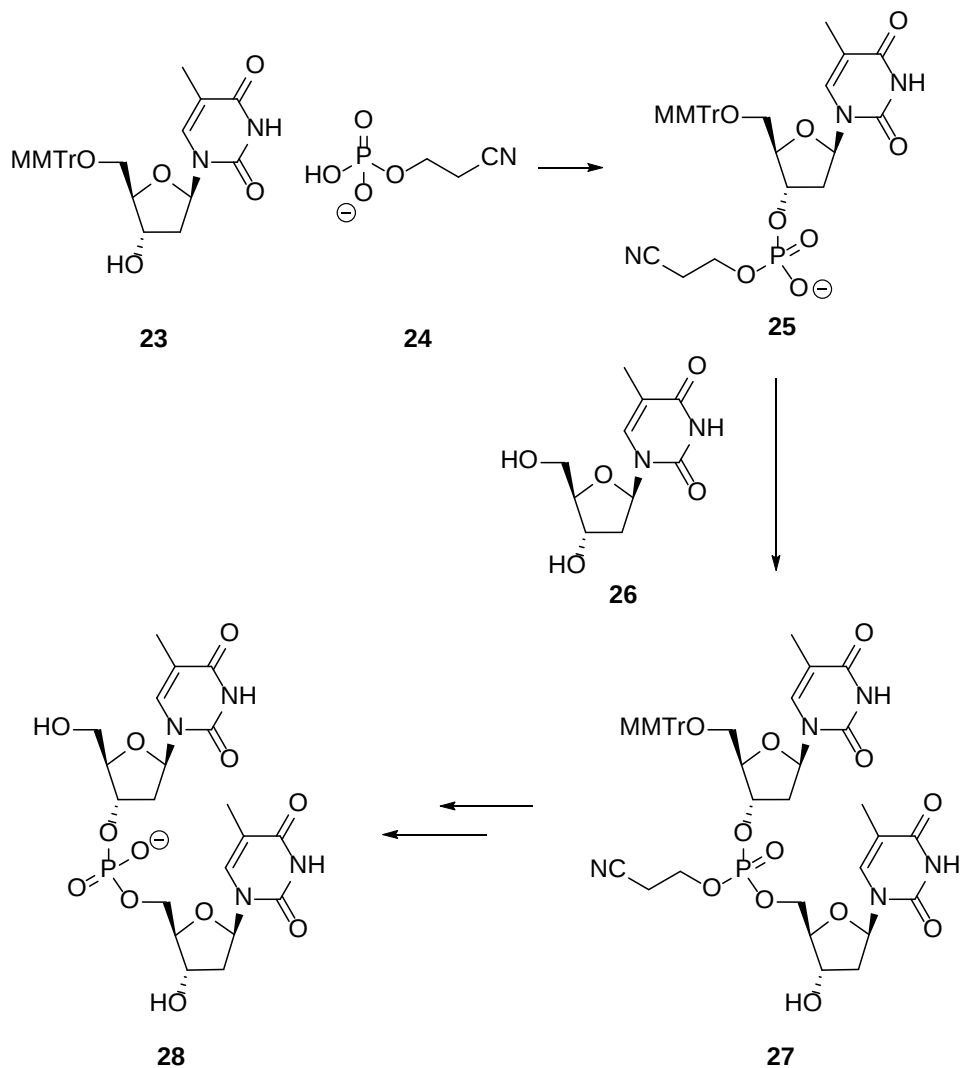


Scheme 3: Eckstein and Rick's method of synthesising a dinucleotide using a 2,2,2-trichloroethyl protecting group.

This protecting group was then used by other groups in both oligodeoxyribonucleotide and oligoribonucleotide synthesis, however

it was found that the final deprotection step lead to unsatisfactory yields.²³

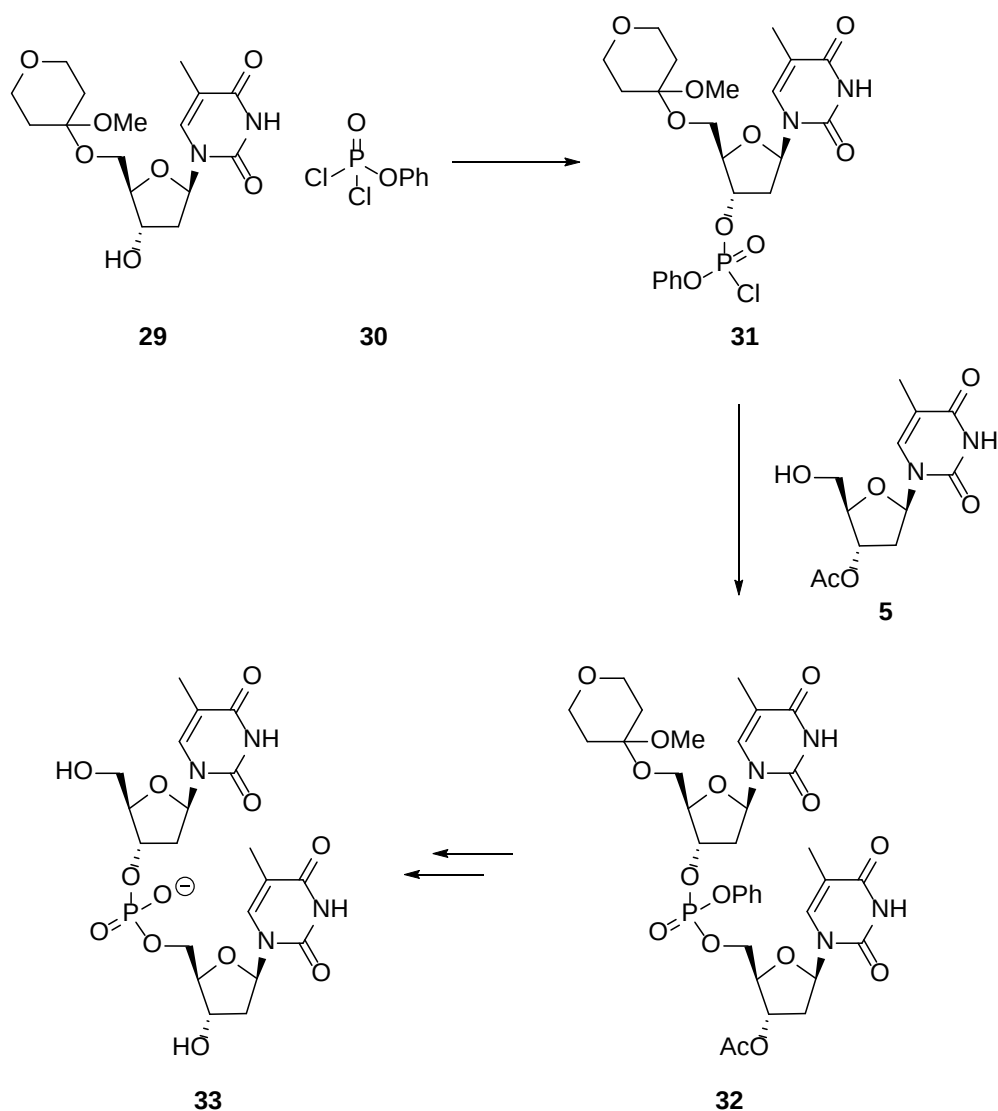
At the same time Letsinger *et al.* used the cyanoethyl protecting group, first using polymer supported reagents and then in solution phase (Scheme 4).²⁶



Scheme 4: Synthesis of TpT (F) by Letsinger *et al.* using cyanoethyl protecting group.²⁶

This protecting group could be removed through brief treatment with ammonium hydroxide, and although is still used today in solid-phase synthesis, was found to be too labile for use in solution phase.²³

In 1968 Reese and Saffhill used a phenyl protecting group in the synthesis of TpT (**32**) (Scheme 5).²⁷



Scheme 5: Synthesis of a dinucleotide using the phenyl protecting group.²⁷

While initially this protecting group showed great promise, the removal of the group using alkaline hydrolysis can lead to the desired product (**33**), but also can result in removal of either the nucleosides, giving two further products (Figure 3).²³

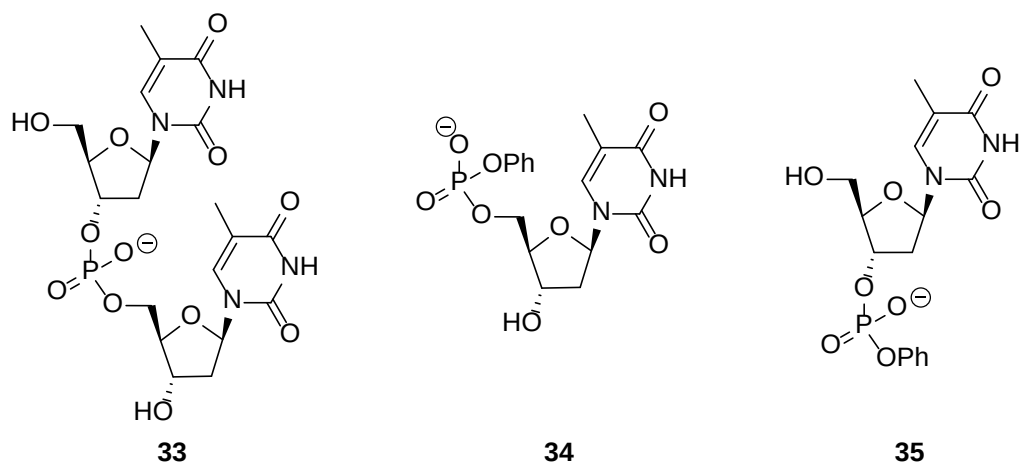
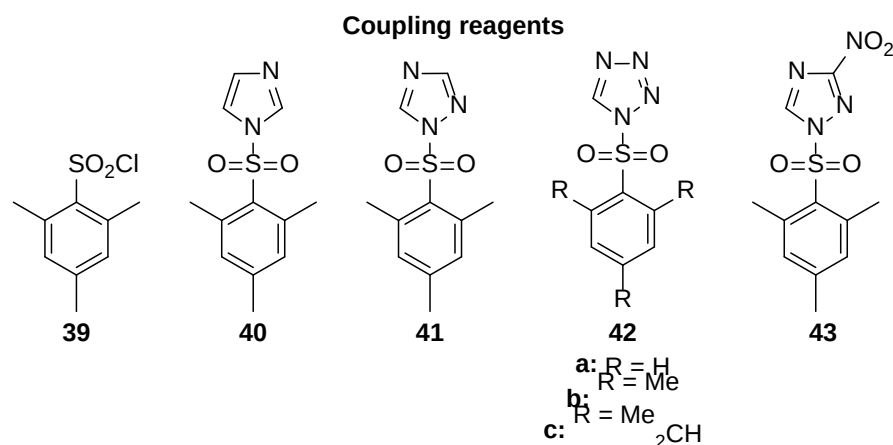
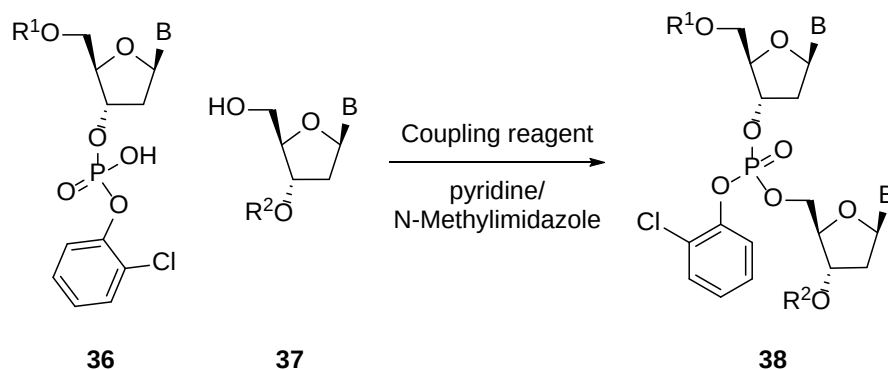


Figure 3: Three possible products from alkaline hydrolysis when using the phenyl protecting group.²³

It was found that this problem could be overcome by increasing the unblocking rate by having electron withdrawing groups present on the ring, and changing the reagents used to deprotect these groups, a variety of modifications were tried, and it was found that 2-chlorophenyl was the best protecting group, and 2-nitrobenzaldehyde and pyridine-2-carboxaldehyde being the best unblocking reagents.²³

With the optimal protecting group being found for the internucleotide linkage in this approach, research was then carried out on the formation of the protected dinucleotide.



Scheme 6: Various coupling reagents used to form the 3',5'-phosphodiester bond.

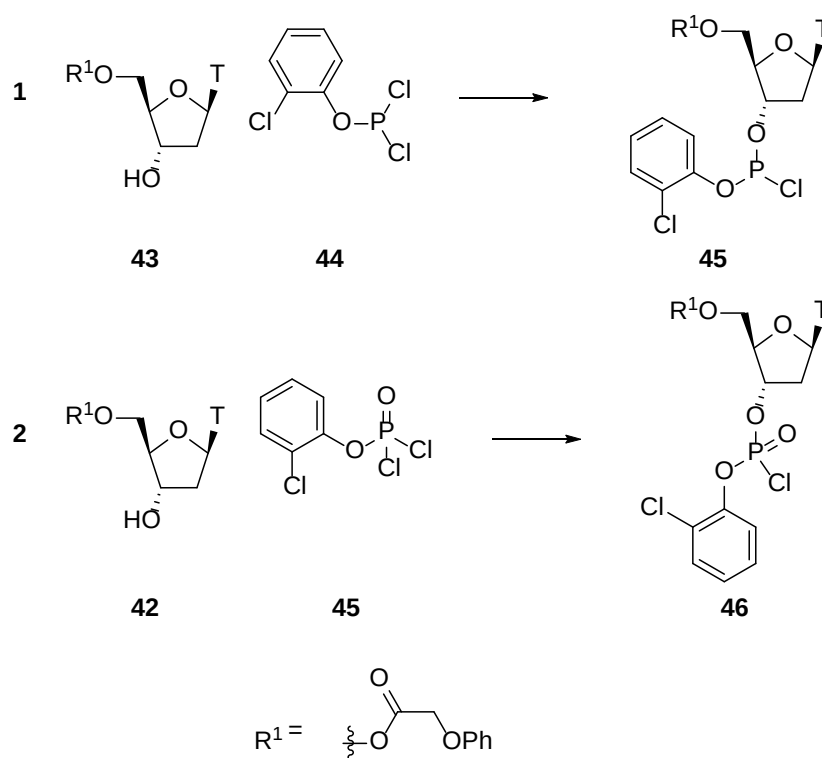
Originally arenesulfonyl chlorides such as **39** were used, but then Berlin *et al.* used arenesulfonyl derivatives of imidazole, such as **40**, successfully. Although lower coupling rates were observed, no darkening of the reaction was found.²⁸ This breakthrough led to arenesulfonyl derivatives of 1,2,4-1*H*-triazole and then 1*H*-tetrazole being tried (Compounds **41**, **42a-c**), and were found to be more proficient, giving higher yields and faster reactions.²³

The 1*H*-tetrazole derivatives were found to be unstable however, so a different reagent was developed. In the late 1970s an alternative was found; 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-1*H*-triazole

(MSNT), **43**. This was found to give quick, clean coupling reactions similar to **42a-c**, however was relatively stable, and in the 1980s was the coupling reagent of choice.^{23,29,30}

1.2.3 Phosphite triester and Phosphoramidate approach

In 1976, Letsinger and Lunsford reported a novel coupling procedure which used intermediate phosphite links and it was coined the phosphite triester approach. The basis of this methodology arose from observations that phosphorochloridites (e.g. **44**), P(III) compounds, reacted very quickly at the 3'-OH of nucleosides in pyridine at low temperatures, much quicker than the corresponding P(V) compounds such as **46**.³¹



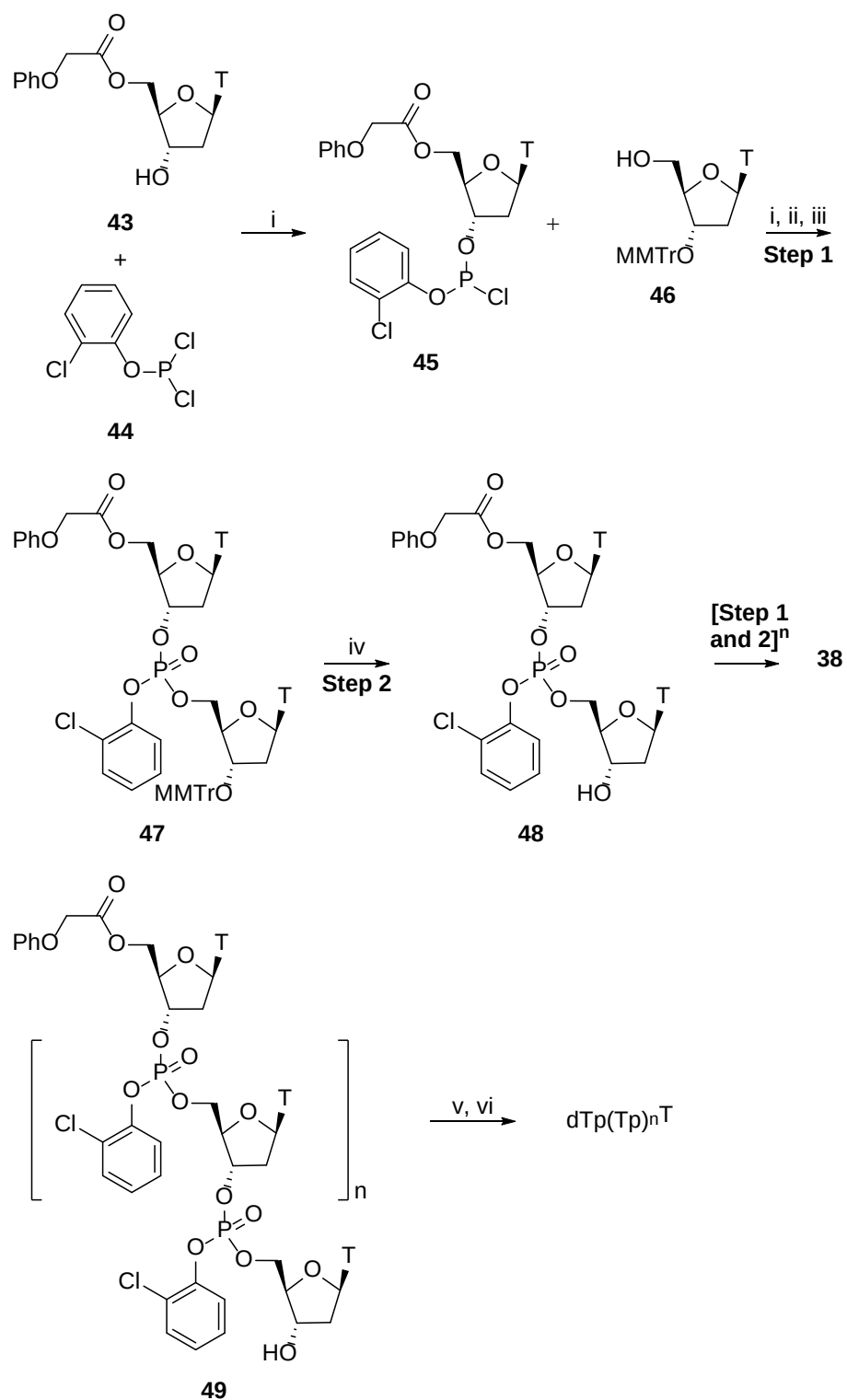
Scheme 7: Reaction 1 proceeds quicker and at lower temperatures than reaction 2.³¹

Further investigation into this reaction produced two more findings;

1) dinucleoside monophosphite triesters can be easily oxidised into their analogous phosphates through the addition of iodine and water.

2) the condensation reaction was quicker when performed in tetrahydrofuran (THF) with small amounts of pyridine or 2,6-lutidine than in pyridine exclusively.

The group investigated the stability of different protecting groups in these conditions, and selected phenoxyacetyl to protect the 5' hydroxyl, MMtr to protect the 3' hydroxyl and eventually settled on 2,2,2- trichloroethyl as the protecting group for the phosphoryl function. Using this method (Scheme 8) the group successfully carried out the stepwise synthesis of dTpTpTpT in 69-71% yield.³¹

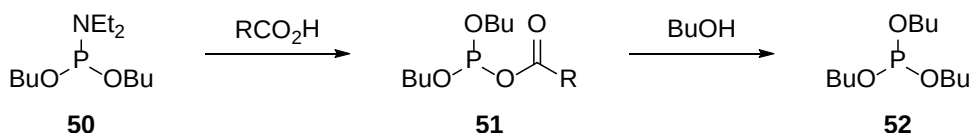


(i) 2,6-lutidine, THF, -78 °C;
Reagents and conditions:
 (ii) 2,6-lutidine, I₂, THF, H₂O; (iii) OH⁻ or NH₄OH;
 (iv) CH₃CO₂H; (v) Sodium naphthalene, HMPA; (vi) H₂O

Scheme 8: Phosphite triester approach developed by Letsinger and

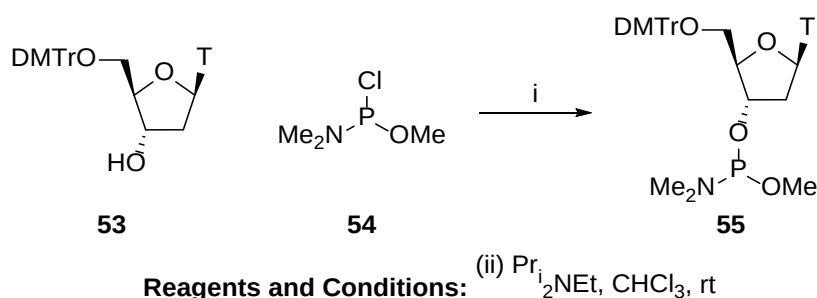
Lunford.³¹

Another way of utilising the P(III) reactivity is through the phosphoramidate approach, which was led by Caruthers and co-workers in the 1980s.³² This development was only possible following the work carried out by Evdakov *et al.* in the 1970s that explored the reactivity of dibutyl *N,N*-diethylphosphoramidate, **50**, (Scheme 9).³³



Scheme 9: Reactivity of dibutyl *N,N*-diethylphosphoramidate, **39**, with acetic acid ($\text{R} = \text{CH}_3$) or fluoroacetic acid ($\text{R} = \text{CH}_2\text{F}$).³³

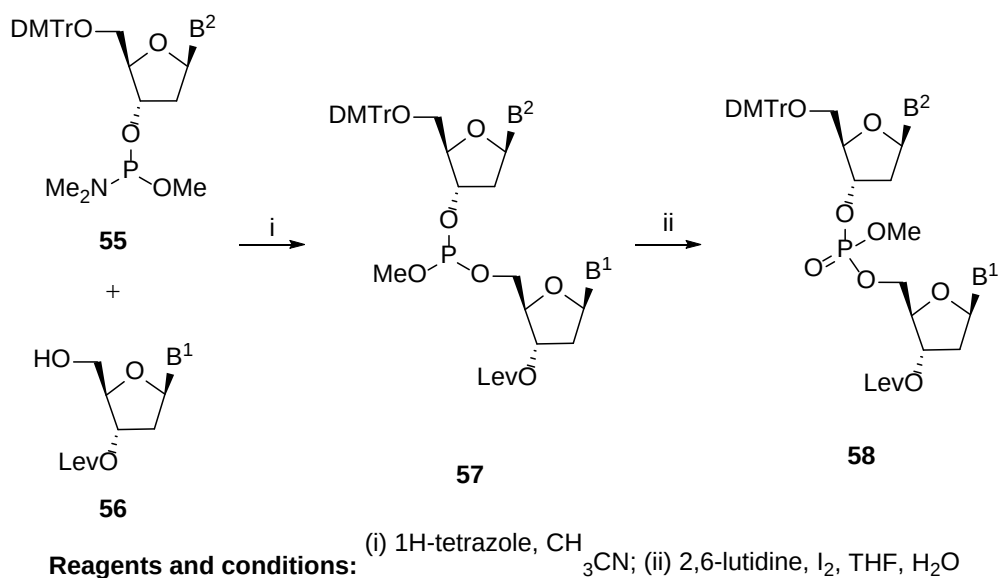
Evdakov *et al.* reported that **50** reacted with fluoroacetic or acetic acid at 30 °C to make the intermediate **51**, which they then reacted with butanol to synthesise tributyl phosphite **52**. This encouraged Caruthers *et al.* to try to react 5'-*O*-DMTr-thymidine, **53**, with the monofunctional phosphitylating agent, **54**. This gave the corresponding protected nucleoside phosphoramidite, **55**. They also attempted this with all the other bases in their 5'-*O*-DMTr-*N*-acyl forms, and isolated these in $\geq 90\%$ yields.³²



Scheme 10: Synthesis of a nucleoside phosphoroamidite.³²

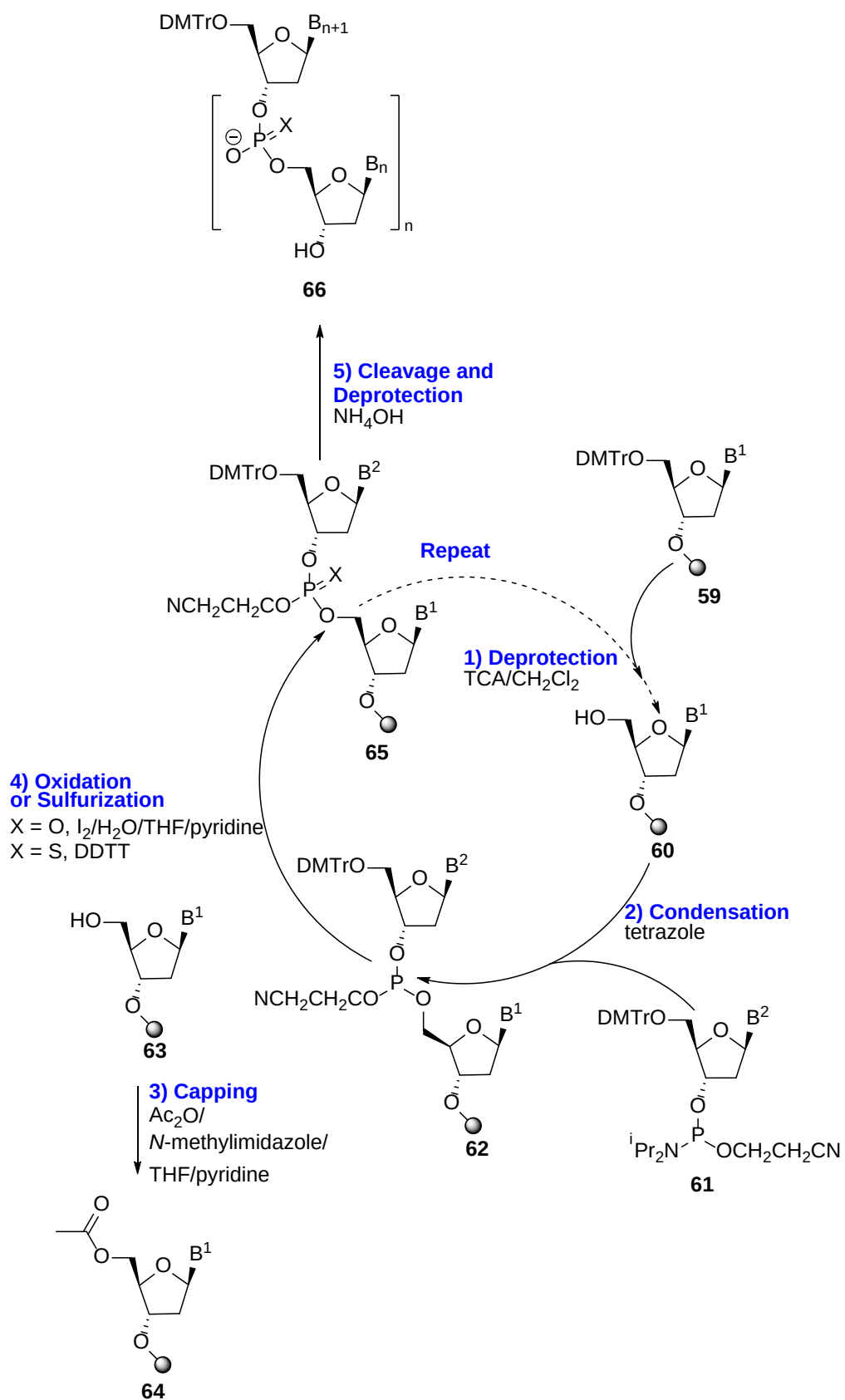
These nucleoside phosphoramidates can be further reacted with nucleosides such as **56** in the presence of the acid catalyst 1*H*-

tetrazole to form protected dinucleosides phosphites (**57**), in a fast, high yielding reaction, and then can be transformed into the corresponding phosphates in the presence of iodine and water, such as in the phosphite triester approach.³²



Scheme 11: Synthesis of dinucleotides using phosphoramidates.²³

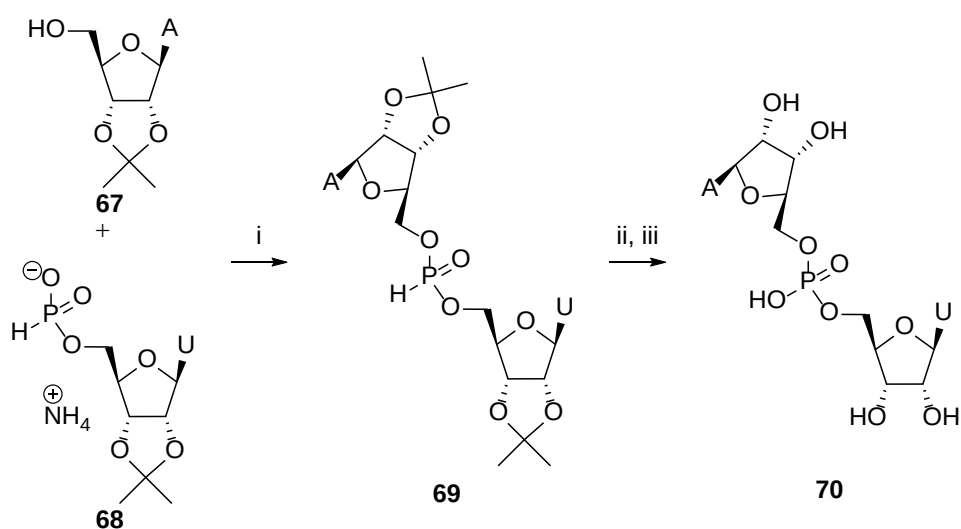
This approach has been utilised in solid phase synthesis, with slightly different protecting groups (Scheme 12) and is used daily in many labs around the world using an automated synthesizer.



Scheme 12: Steps in solid-phase synthesis of oligonucleotides.

1.2.4 H-Phosphonate approach

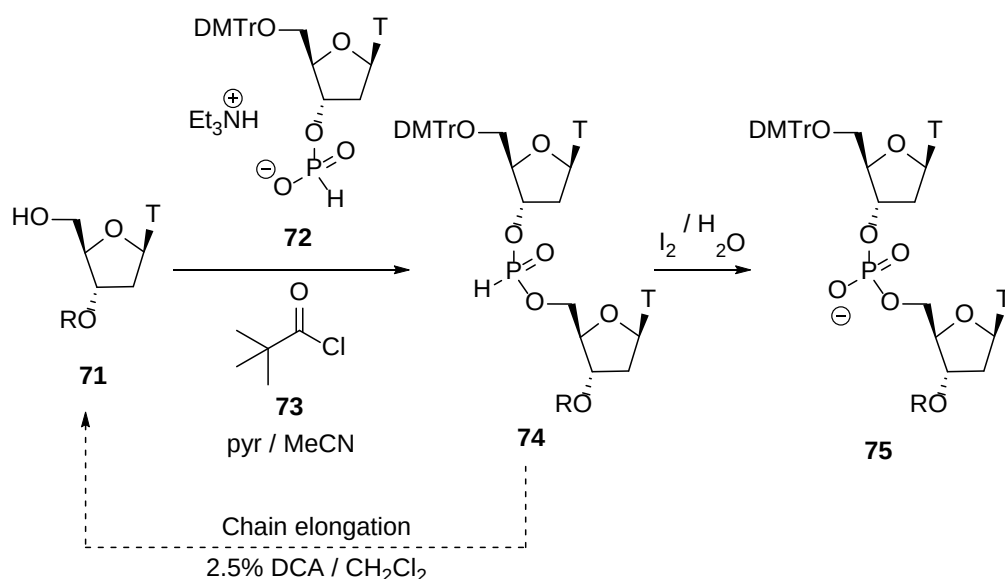
H-Phosphonates were first used as starting materials for dinucleotide synthesis by Todd *et al.* in the 1950's, to make the unnatural 5'-5' dinucleoside, using diphenyl phosphorochloridate to generate a mixed anhydride which then react with nucleoside **67** to give the H-Phosphonate **69**, subsequent oxidation and acetal deprotection affords the phosphate **70** (Scheme 13).^{23,34,35}



Reagents and conditions: (i) $(\text{PhO})_2\text{POCl}$, 2,6-lutidine, MeCN; (ii) (a) NCS, 2,6-lutidine, MeCN, (b) NaHSO_3 , H_2O ; (iii) HCl, H_2O

Scheme 13: Synthesis of a 5'-5' dinucleoside from the H-phosphonate **Y**.

This reaction remained unexplored until the 1980s when various groups began publishing research on the synthesis of oligonucleotides using H-phosphonates; Froehler *et al.*^{36,37} first synthesised eicosathymidylic (T_{20}) and tetracontathymidylic (T_{40}) acid using a solid supported nucleoside and pivoyl chloride as the acylating agent (Scheme 14). Later Froehler *et al.* then used the same method to synthesise DNA strands of up to 107 bases.^{36,37}



Scheme 14: Method developed by Froehler *et al.* for the synthesis of oligonucleotides using H-Phosphonates.³⁶

The H-Phosphonate approach allows for the synthesis of diesters in 2 steps; a coupling then oxidation, rather than the 3 steps required when using phosphoramidites due to the need for a protecting group on the P-atom that has to be removed after oxidation.³⁸

H-Phosphonates (such as **72** Scheme 14) are more stable to spontaneous oxidation, and acidic conditions than phosphoramidates (**61** Scheme 12), yet are more reactive than P(V) compounds (**9** Scheme 2) allowing for comparable reaction rates found in the phosphoramidate approach.³⁸

These favourable qualities are found in H-Phosphonates due to the fact that these compounds exist as an equilibrium mixture of two tautomeric forms, both in the +3 oxidation state; a tetra-coordinated phosphonate form ($\lambda^5\sigma^4$) **76** which is relatively stable and resistant to spontaneous oxidation, and a tri-coordinated phosphite form ($\lambda^3\sigma^3$)

77 which will react with electrophiles due to the lone pair of electrons present.³⁹

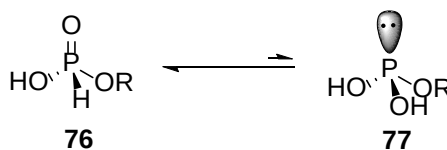


Figure 4: Tautomeric forms of H-Phosphonates.

Because the equilibrium is shifted almost completely towards the phosphonate form, it is this that determines the overall reactivity of these compounds. This means that they can be stored more easily, and that the H-Phosphonate intermediates have a linkage that is stable to the acidic conditions needed for the 5'-DMTr group. This allows for oxidation of the whole chain at the end of the cycle.⁴⁰ This is key, as it allows for this step to be modified, facilitating the synthesis of phosphoramidates (**78**), phosphorothioates (**79**) and phosphoroselonomates (**80**).^{41–43}

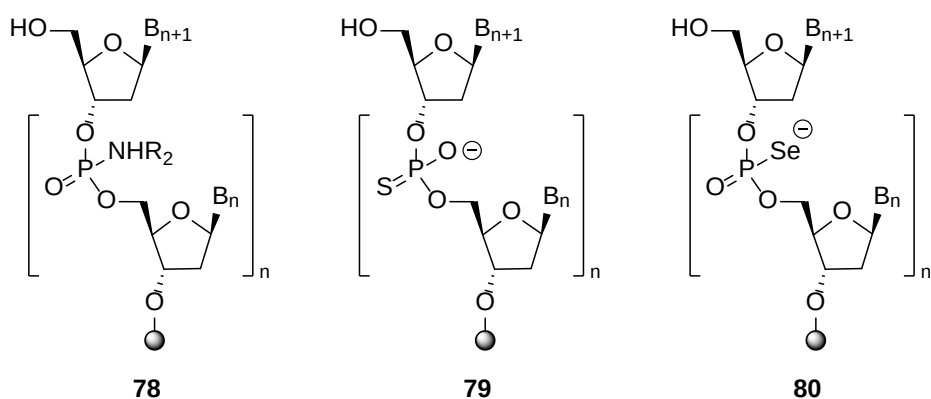
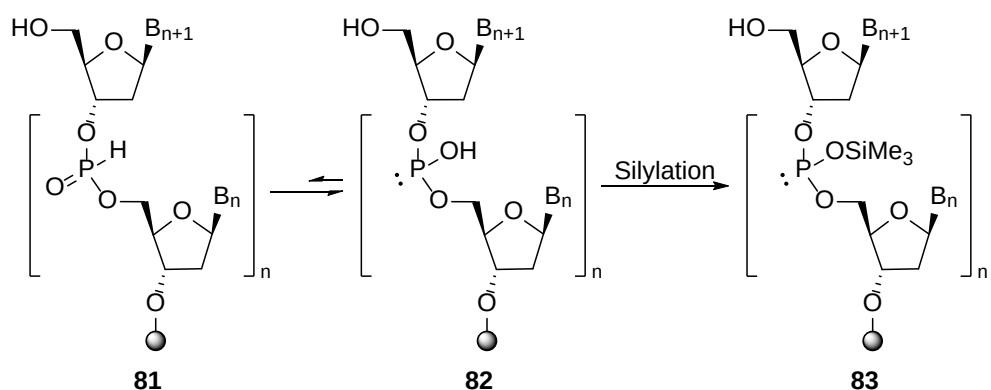


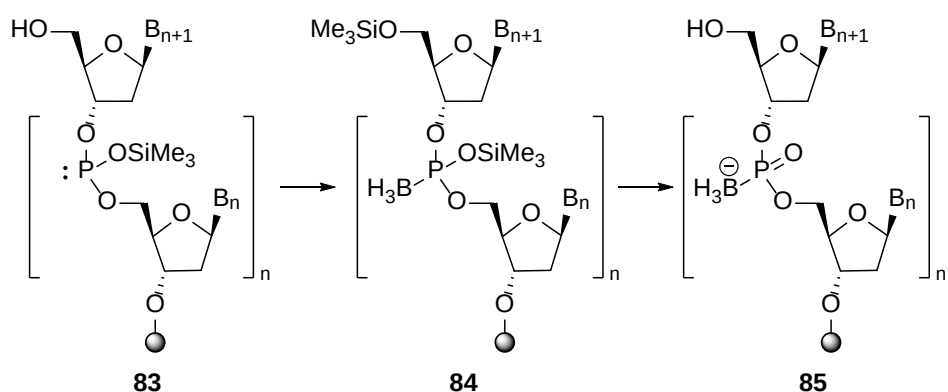
Figure 5: Phosphoramidate, Phosphorothioate and Phosphoroselomate.

We can also utilise the equilibria present to synthesise various other synthetic analogues through the trapping of the phosphite form using silylation, which can be performed at the end of the synthetic cycle.



Scheme 15: P(=O) P-OH equilibria and subsequent silylation of the phosphite form.⁴⁰

The lone pair of electrons on the phosphorus atom (**83** Scheme 15) can then form stable complexes with Lewis acids, which then enables oxidation or attack by electrophiles, facilitating the synthesis of other modified oligonucleotides, such as boranephosphonate oligomers.^{40,44}



Scheme 16: Synthesis of boranephosphonate oligomer.⁴⁴

1.2.5 Issues with current synthetic methods

The previous sections outline current approaches used for oligonucleotide synthesis, and all these methods use phosphorus containing compounds which are all derived from PCl₃, which is toxic, corrosive and is controlled under the Chemical Weapons Convention.

Currently the majority of commercially available organophosphorus compounds are synthesized from PCl_3 (Figure 6). This pathway involves the initial reduction of phosphate rock to elemental phosphorus, then direct exothermic chlorination with chlorine; itself dangerous and energetically demanding to prepare, to make three phosphorus-chlorine bonds. These all then have to be broken in order to make the final oligonucleotide.^{45,46}

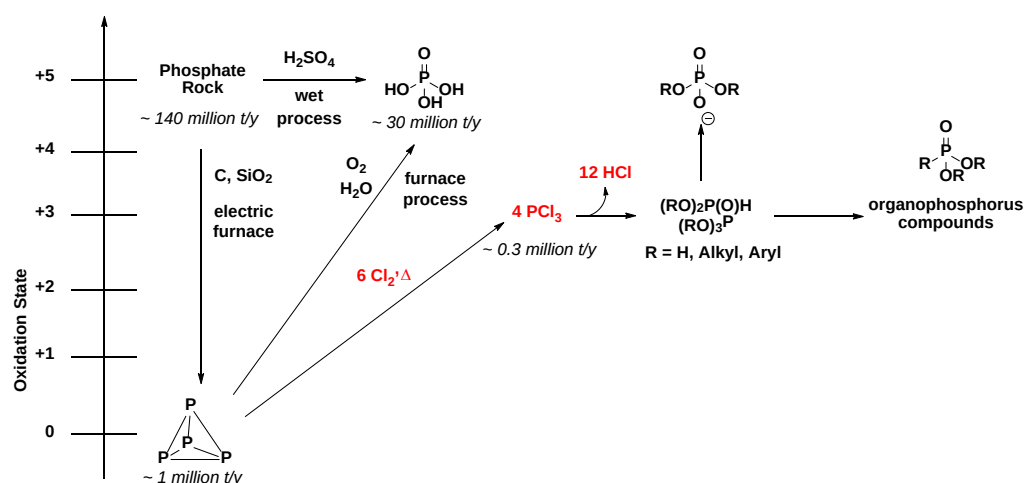


Figure 6: Current industrial pathway to organophosphorus compounds including oligonucleotides.⁴⁶

Ideally we would be able to synthesise oligonucleotides from phosphorous acid (H_3PO_4), removing the need for any reduction and oxidation steps, however $\text{P}(\text{V})$ compounds are less efficient in synthetic transformations and react significantly more slowly than $\text{P}(\text{III})$ compounds.³⁸

There has been research carried out on the “ P_4 -activation” pathway, where instead of making PCl_3 , P_4 is used to make the organophosphorus compounds directly. This method has its own

drawbacks as not all the phosphorous atoms are used, P_4 is toxic and pyrophoric, as well as being insoluble in standard organic solvents.⁴⁷

H-Phosphinates ($H_2P(O)(OR)$) provide an alternative phosphorus source.

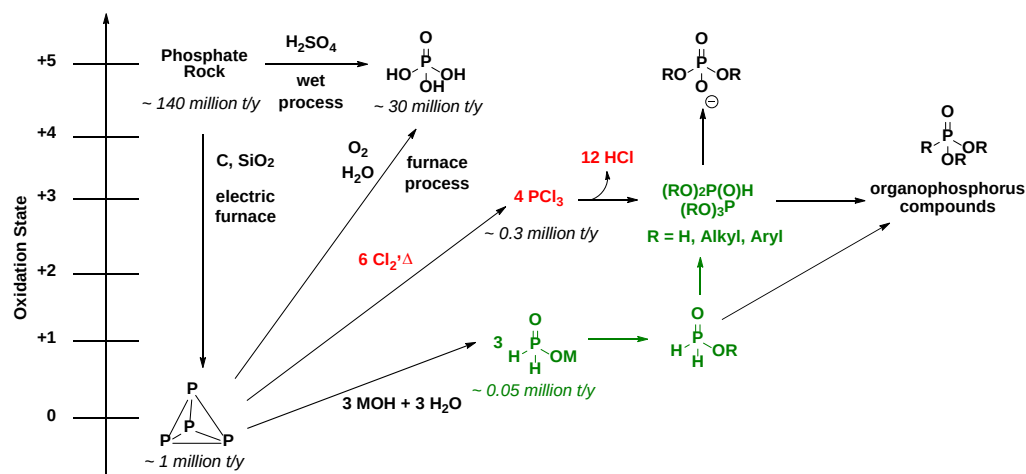
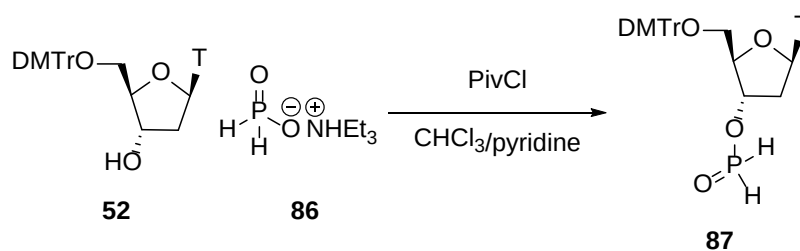


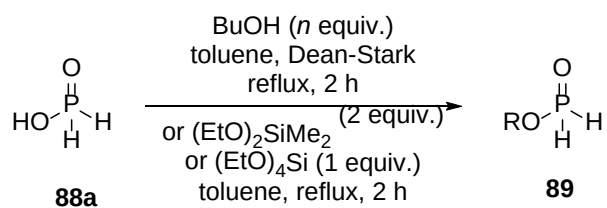
Figure 7: Pathway to organophosphorus compounds from PCl_3 and H-Phosphinates.⁴⁶

Previously Stawinski *et al.* have synthesised the phosphinate intermediate **Z**, using phosphinic acid salts, (Scheme 17).^{48,49}



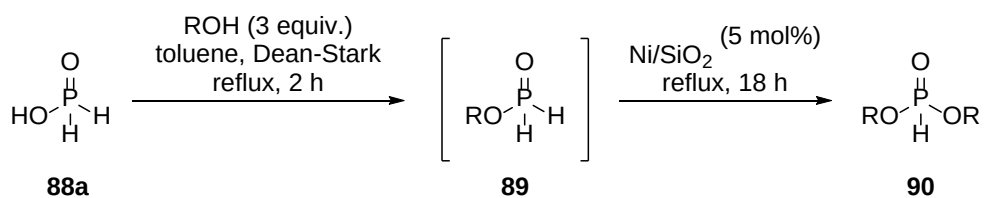
Scheme 17: Synthesis of phosphinate intermediate from phosphinic acid salts.⁴⁸

These types of compounds have also been made by Montchamp *et al.* in a variety of ways, from phosphinic acid (Scheme 18).⁴⁷



Scheme 18: Synthesis of phosphinate intermediate from phosphinic acid.⁴⁷

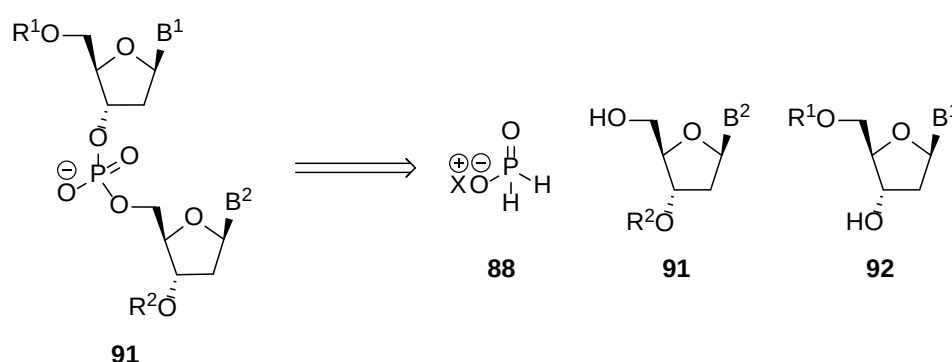
Montchamp's group have then used intermediate **89** to make symmetrical H-phosphonate diester using a nickel catalyst (Scheme 19).⁴⁷



Scheme 19: Conditions used for the synthesis of H-phosphonate diesters.⁴⁷

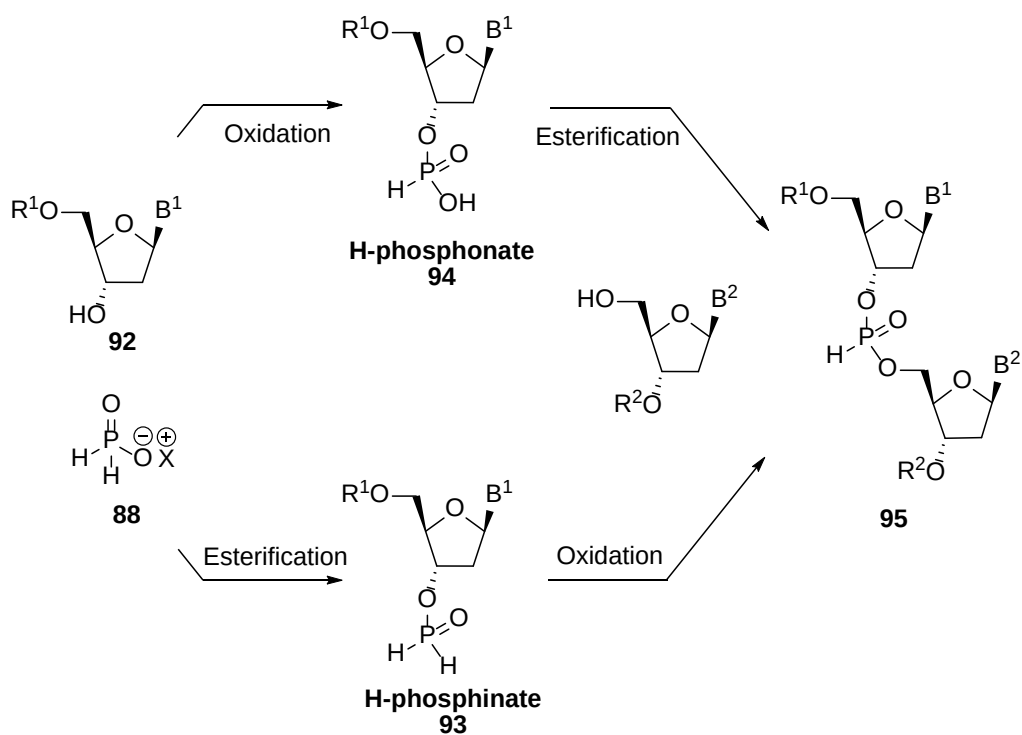
Aims and Objectives

Due to the issues with the current synthesis and the potential of phosphonic acid as a precursor to di-nucleotides as discussed previously, we wished to develop a sustainable synthesis of dinucleotides which avoids the use of PCl_3 . We envisaged that this could be achieved by the reaction of a suitably protected nucleoside with phosphinic acid (Scheme 20).



Scheme 20: Retrosynthetic approach to dinucleosides from phosphinic acid and/or its salts.

This overall conversion would involve an esterification and oxidation, which could be done in either order, forming either a H-phosphinate (**93**) or H-phosphonate (**94**) first (Scheme 21).



Scheme 21: Proposed synthesis of H-phosphonate dinucleotide via H-phosphonate or H-phosphinates.

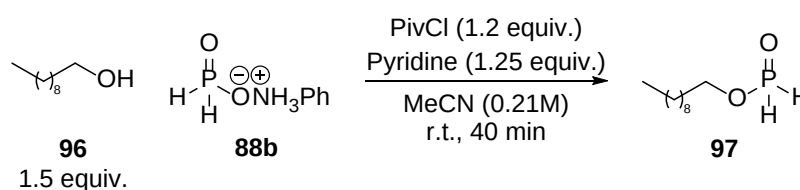
Chapter 2

Results and Discussion

2.1 Synthesis of H-Phosphinates

2.1.1 Esterifications

We initiated our studies by first trying to repeat the reaction carried out by Stawinski *et al.* but using decanol (**96**) as the starting alcohol instead of 5-ODMTr-Thymidine as previous unpublished work within the Denton group had synthesised this H-phosphinate (**97**) and coupled it under metal catalysed conditions.



Scheme 22: Attempted synthesis of the H-phosphinate **97**.

However, this failed to yield the desired H-phosphinate **97**. After 40 min the ^{31}P NMR spectrum showed two peaks; 14.0 ppm and 6.4 ppm. We believe that the peak at 14.0 is **97**, and 6.4 is an anhydride, such as **98**.

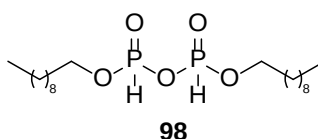


Figure 8: Suspected structure of compound at 6.41 ppm.

Column chromatography of the mixture was attempted, however the only compound to contain any phosphorus was not clean by ^1H NMR spectroscopy, and the only phosphorus peak detected was at 7.81 ppm. This was found to be **99**, decyl *H*-phosphonate, this was not

unsurprising considering we suspect H-phosphinates to be susceptible to oxidation.

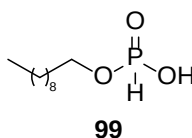


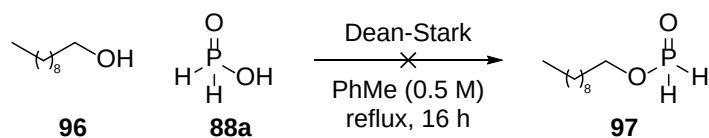
Figure 9: decyl H-phosphonate

This lead us to believe that H-phosphinates were not stable to purification using silica gel chromatography, which was in line with work carried out by others that did not isolate these compounds.^{47,48,50}

We later tried to repeat the experiment shown in *Scheme 22* in order to try and isolate more of compound **99**, however it proved difficult to isolate this compound cleanly with any appreciable yield. We also attempted to leave the reaction mixture stirring open to air once compound **97** was detected by ³¹P NMR spectroscopy in order to oxidise the H-phosphinate into the H-phosphonate, as this would too be a desirable compound (*Scheme 21*), though this failed to give the H-phosphonate cleanly by ³¹P NMR, as did attempts to leave the reaction mixture stirring over silica in air.

2.1.2 Condensation reactions

As the aforementioned conditions were not working in our hands, it was decided to try and use the methodology developed by Montchamp, again first using this with decanol (*Scheme 23*).⁴⁷



Scheme 23: Attempted synthesis of decyl phosphinate **97** using Montchamp's conditions.⁴⁸

This too failed to generate the desired compound. After the 16 hours, there were two sets of peaks present in the ^{31}P NMR spectrum; a doublet (d) at 8.80 ppm with $J = 699$ Hz, corresponding to the acid, **100**, and a doublet of quintets at 8.17 ppm with $J = 693, 10.0$ Hz, corresponding to compound **101** (Figure 10).

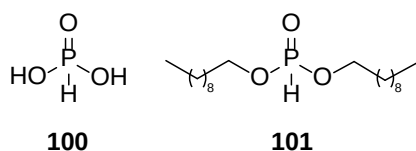


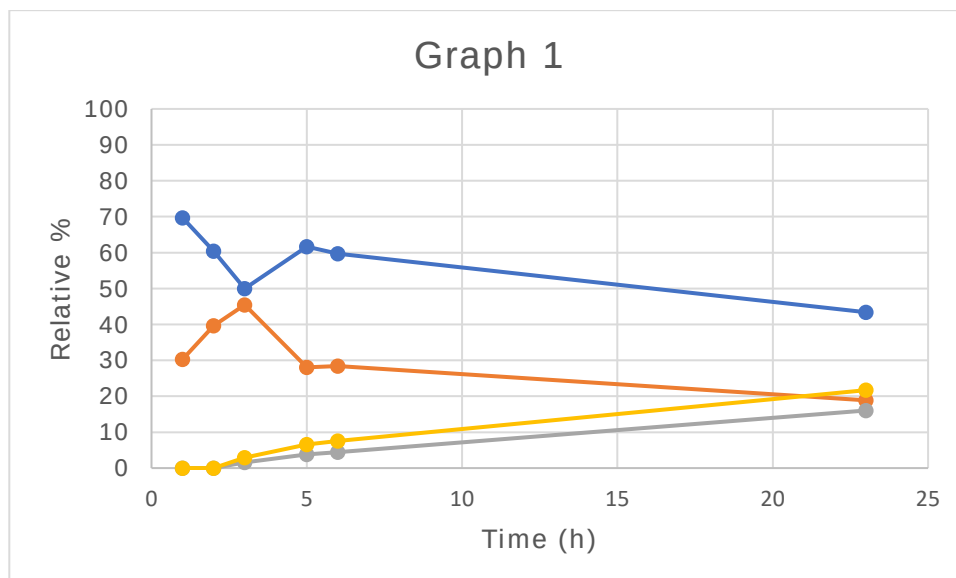
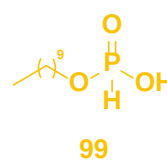
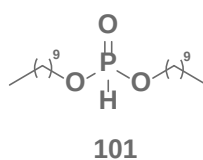
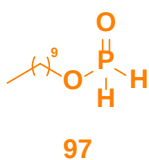
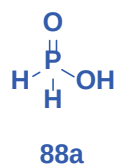
Figure 10: Compounds present in the reaction mixture after 16 hours.

In Montchamp's paper, this uncatalysed reaction was observed for only 3 alcohols; 3-butyn-1-ol, 2-chloroethanol and phenol, and they could not fully explain this, only suggesting this must be due to the tautomeric equilibria of $\text{ROP}(\text{O})\text{H}_2$ with its $\text{P}(\text{III})$ form.⁴⁷

This reaction was repeated, and it was found that after 2 hours compound **99** was seen in the ^{31}P NMR spectrum as a doublet of triplets (dt) at 7.62 ppm with $J = 700$ and 8.1 Hz.

2.1.2.1 Investigation into the formation of H-phosphonate diester

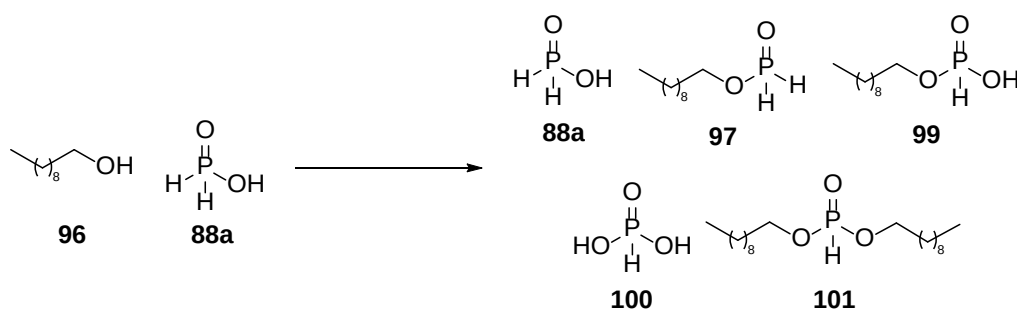
After this discovery, further investigation into this reaction was warranted. The reaction was monitored, and *Graph 1* shows the relative ratios of compounds present over 23 hours.



Graph 1: Relative % as per intensities in ^{31}P NMR spectrum over time

From this we proposed that **97**, the mono ester, was forming first, and then this was getting oxidised into the decyl H-phosphonate **99**, which was in turn reacting with the yet unreacted decanol. It is worth noting that in these experiments the maximum ratio we see of **88a:97** is 1:1, therefore implying that there is always a half equivalent of decanol left to react with **99**.

A variety of conditions were then tried (Table 1) to try and get full conversion of the starting material to decyl phosphinate.



Scheme 24: Overall reaction detailed in Table 1.

Entry	Drying Method	Atmosphere	Temperature	Result
1	Dean-Stark	Argon	Reflux	100 and 101 only (19 h)
2	none	Air	r.t.	No Reaction
3	none	Air	50 °C	No Reaction
4	none	Air	80 °C	No Reaction
5	Dean-Stark	Air	Reflux	Mixture of products
6 ^a	Dean-Stark	Argon	Reflux	100 and 101 only (3 h)
7	Molecular sieves	Argon	Reflux	No Reaction
8 ^b	Dean-Stark	Argon	Reflux	No Reaction
9 ^c	Dean-Stark	Argon	Reflux	100 and 101 only (2 h)

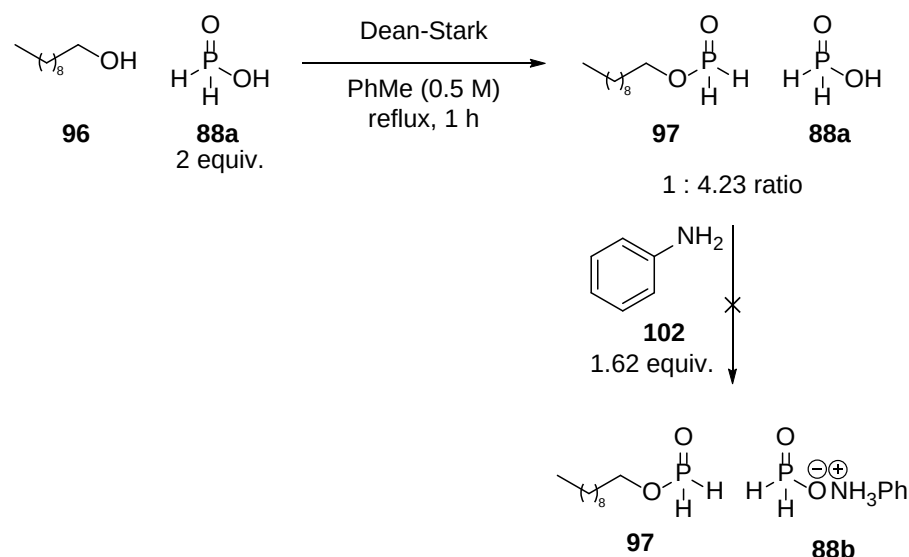
Table 1: Different reaction conditions tried with reagents **96** and **88a**.

Reactions were monitored by ³¹P NMR spectroscopy. Reaction Conditions: decanol (1.0 equiv), H₃PO₂ (1.0 equiv), toluene (0.5 M). a) 2.0 equiv of H₃PO₂ was used b) cyclohexane (0.5 M) instead of toluene was used c) H₃PO₃ (1.0 equiv) was added.

Table 1 entry 1 shows the original conditions, entries 2-5 show that attempts to carry out reaction under air at a variety of temperatures failed, indicating reaction would not proceed at lower temperatures, and when refluxing in air the reaction would not go to completion in a comparable time. Entry 6 shows that when an extra equivalent of

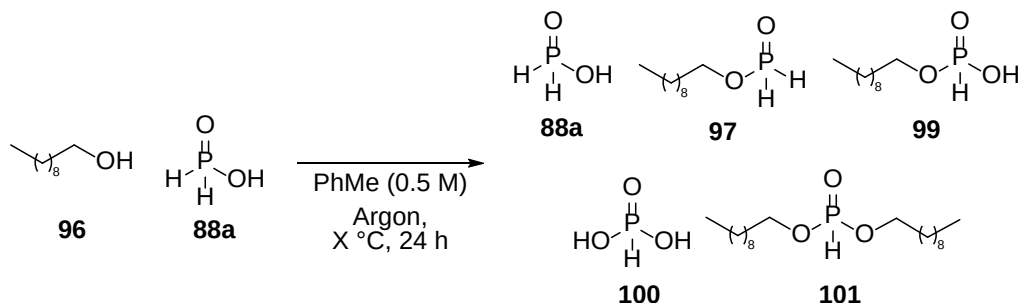
acid was used (compared to entry 1) the reaction proceeded to the diester quicker. From comparing entry 5 to 1 we can infer that air does impede the reaction, but it also insinuates that the oxygen that is incorporated is either coming from the water that is present in the phosphinic acid (it comes as a 50% W/W solution) or as the result of the condensation, not air. It was attempted to remove this water by using molecular sieves instead of a Dean-Stark (Table 1, entry 7), however under these conditions no reaction occurred, it is hypothesized that the acid was destroying the sieves in this reaction. Entry 8 shows our attempts to change the solvent in order to lower the refluxing temperature but yet again this proved futile. We also attempted to use phosphoric acid as well as phosphinic acid, (Table 1, entry 9) and this conversion was comparable to when two equivalents of phosphinic acid was used, indicating that a more acidic environment increased reaction rate.

It was also attempted to try and remove the unreacted phosphinic acid after 1 h with aniline (Scheme 25). 45 % yield of the anilinium salt was produced however the ratio of **97:88a** remained at 1:4, suggesting that compound **97** was being hydrolysed back to the starting acid on addition of aniline.



Scheme 25: Attempts to isolate phosphinate using aniline.

We also investigated the temperature required for the synthesis of the diester, and trialled different temperatures and compared the relative ratios of the products.



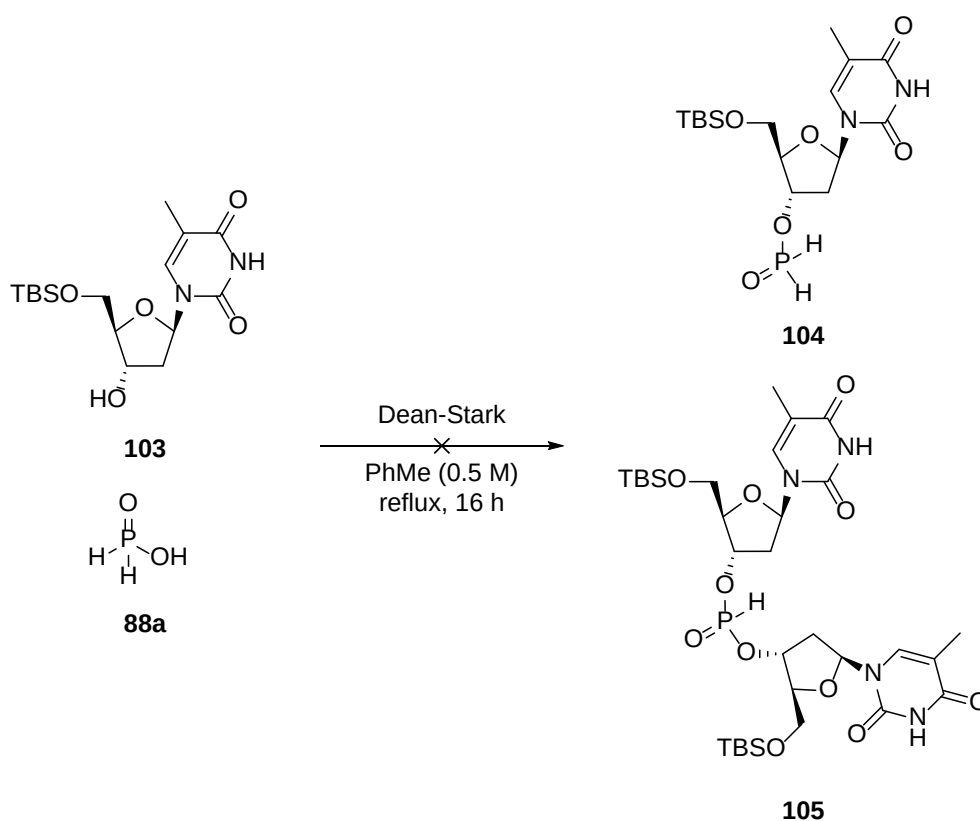
Scheme 26: Reaction trialled at a variety of temperatures.

Entry	Temperature (°C)	Relative %				
		88a	97	99	100	101
1	RT	89	11	0	0	0
2	55	88	12	0	0	0
3	80	81	19	0	0	0
4	100	65	32	3	0	0
5	reflux	11	13	12	43	21

Table 2: Relative Intensities were determined by integration of the corresponding peaks in the ^{31}P NMR spectrum.

From this data we concluded that the formation of the H-phosphinate was very slow at temperatures below 100 °C, and that the diester would only form at reflux. This seems odd, and at present we have no proposed mechanism to explain, nor any calculations to interpret the kinetic restraints of the reaction.

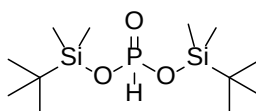
We also attempted the reaction shown in Scheme 27, hoping to get either the H-phosphinate **104** or the H-phosphonate diester **105**.



Scheme 27: Attempted synthesis of nucleoside H-phosphinate **104** or H-phosphonate diester **105**.

However, after 16 hours ^{31}P NMR spectroscopy only showed two sets of peaks; a triplet at 1.18 ppm with a coupling constant of 563 Hz, corresponding to the starting material **88a**, and a doublet at -13.5

ppm with a coupling constant of 692 Hz, which we believe to be compound **106**.



106

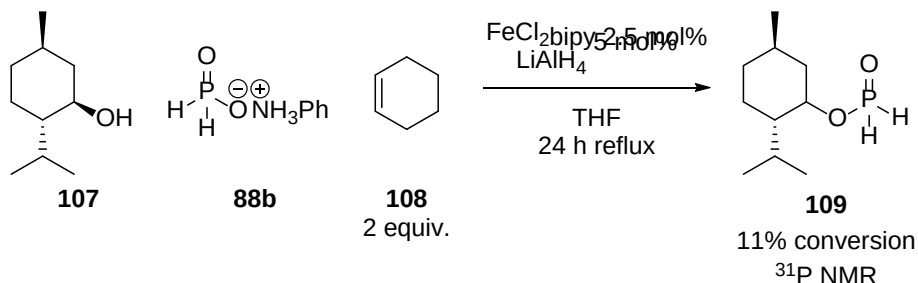
Figure 11: Compound **106**, bis(tert-butyldimethylsilyl) phosphonate.

Also in the flask was a largely insoluble compound, which by TLC matched thymidine, which would be the compound present after the deprotection of **103**.

This indicated that phosphinic acid was too strong an acid to be used in the synthesis of nucleosides.

2.1.3 Anilinium Hypophosphite and Metal Catalysis

During our investigations into the synthesis of H-phosphonates from anilinium hypophosphite (Section 2.2.1.1) we had observed H-Phosphinates as intermediates. With this knowledge in hand, we attempted the reaction shown in Scheme 28, in which pleasingly only the starting material and H-phosphinate **109** were observed.



Scheme 28: Synthesis of H-phosphinate from anilinium hypophosphite with iron catalysis.

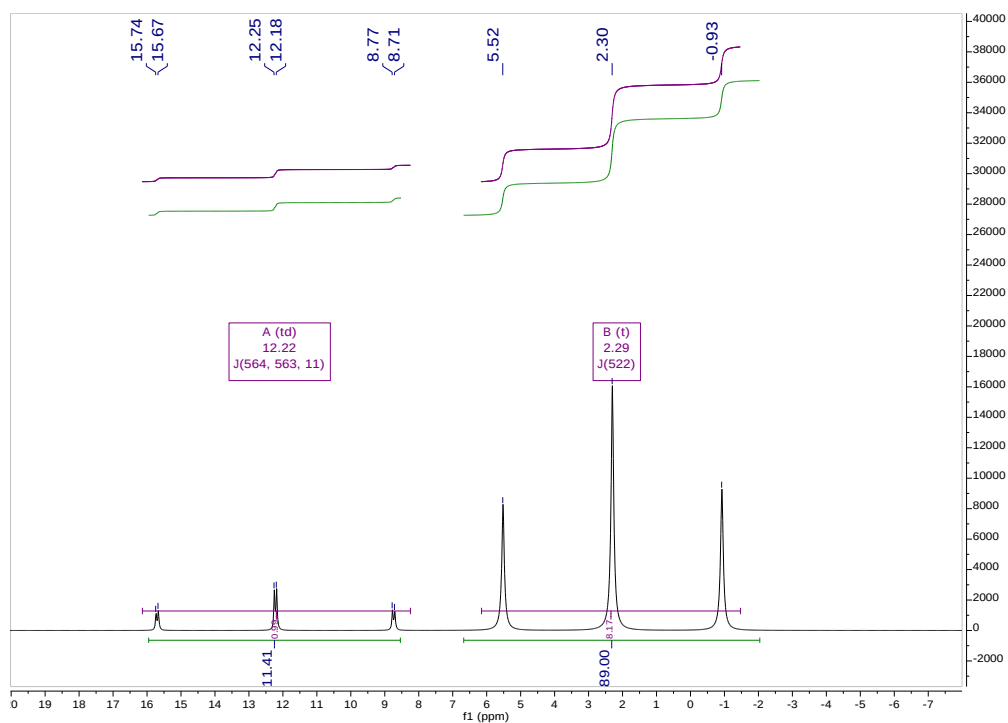
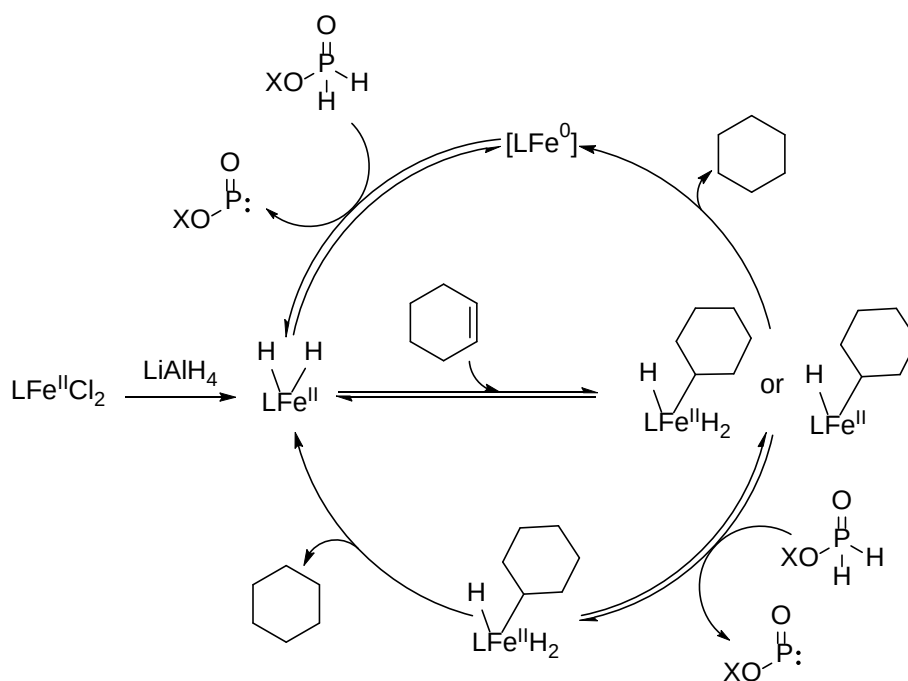


Figure 12: ^{31}P NMR of reaction shown in scheme 28.

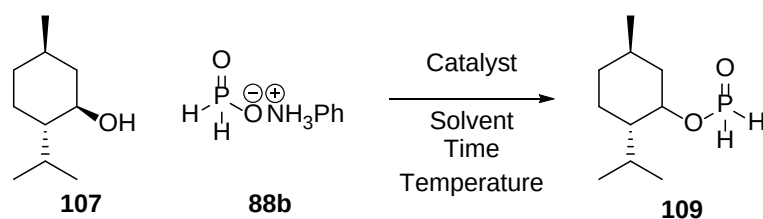
We chose the catalytic system shown above as overall the reaction involves generating hydrogen from anilinium hypophosphite, and iron catalysed hydrogenations have recently drawn much attention due to Fe being relatively abundant, cheap and environmentally benign compared to other transition metals traditionally used for hydrogenation, such as Pd, Rh, Ru, Ir and Os.⁵¹

The original paper citing FeCl_2bipy and LiAlH_4 as the conditions for hydrogenation proposes that these react to generate a bipy-Fe-(H)_2 species which then can react in one of two reaction pathways (Scheme 29).⁵¹



Scheme 30: Our initial expectations for the reaction shown in Scheme 28.

With the result we obtained when using the anilinium salt we propose that this pathway is not occurring and instead we are observing a Lewis acid catalysed esterification, with one of the iron or aluminium species acting as a Lewis acid, which then converts small amounts of anilinium hypophosphite **88b** into acid **88**. To confirm this we carried out this reaction with various known Lewis acids, with and without cyclohexene, Table 3.



Scheme 31: Reactants and conditions used with various catalysts.

Entry	Catalyst	Solvent	Temp	Time (h)	NMR yield of 109 ^[a]
1	AlCl ₃ 5 mol% ^[b]	THF	Reflux	24	0
2	Yb(OTf) ₃ 5 mol% ^[b]	THF	Reflux	24	7
3	FeCl ₂ 5 mol% ^[b]	THF	Reflux	24	0
4	Yb(OTf) ₃ 5 mol%	THF	Reflux	24	5
5	Cu(OTf) ₂ 5 mol%	THF	Reflux	24	5
6	Triflic acid 5 mol%	THF	Reflux	24	2
7	CuI 5 mol%	THF	Reflux	24	5
8	CuBr ₂ 5 mol%	THF	Reflux	24	5
9	Fe(OTf) ₃ 5 mol%	THF	Reflux	24	25
10	Triflic acid 5 mol%	PhMe	Reflux	24	20
11	Fe(OTf) ₃ 5 mol%	PhMe	Reflux	19	49
12	CuI 5 mol%	PhMe	Reflux	2	84
13	-	PhMe	Reflux	2	46

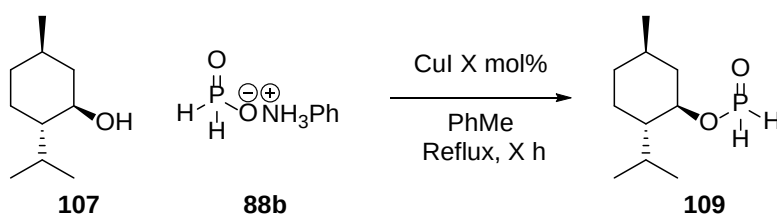
Table 3: Catalyst screen for synthesis of H-phosphinates.

Reaction conditions: **107** (1.6 mmol) was added to salt **88b** (1.6 mmol), and catalyst in Solvent (7.5 ml) under an argon atmosphere and reacted for X hours at X temperature. [a] By ³¹P NMR. [b] 2 equivalents of cyclohexene were added.

We originally used THF as the solvent, as this was the solvent used in the iron catalyzed hydrogenation paper, however we changed due to the low yields observed, we changed to toluene in order to increase the temperature, and repeated with a select number of catalysts in

this solvent. This table shows a significant increase when using copper iodide vs with no catalyst present (entries 12 and 13), indicating that the catalyst is playing an important role in this reaction. We suspect that the copper iodide is better at coordinating with the nitrogen, thus leading to a quicker conversion to the free acid, which then quickly undergoes esterification with menthol.

With copper iodide identified as the best catalyst, we then probed the stoichiometry and molarity of the reaction.



Scheme 32: Reagents using for investigating reaction stoichiometry.

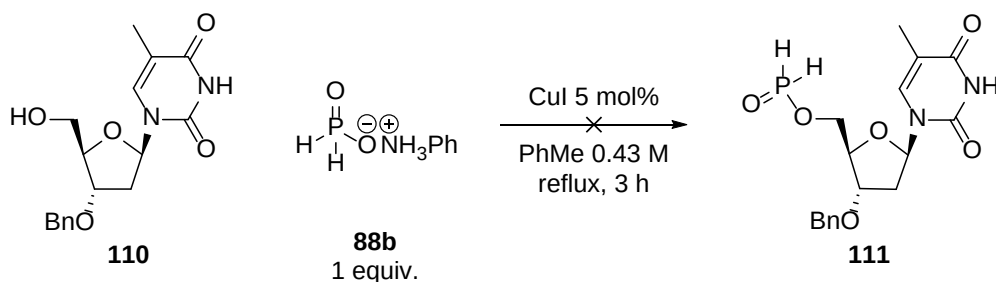
Entry No	Equiv Salt	CuI mol %	Concentration M	Time h	NMR yield of 109 ^[a]
1	1	0	0.21	2	40
2	1	5	0.21	2	84
3	1	5	0.42	3	97
4	1	5	0.42	4	64
5	1	2.5	0.42	3	94
6	1	10	0.42	3	80
7	1	20	0.42	3	90
8	2	5	0.42	3	6 ^[a]
9	2	5	0.42	2	81
10	2	5	0.42	1	57
11	1.5	5	0.42	3	64
12	2	10	0.42	3	41 ^[b]

Table 4: Various equivalents and concentration of reactions trialled.

Reaction conditions: **107** (1.6 mmol) was added to salt **88b** (X equiv.),

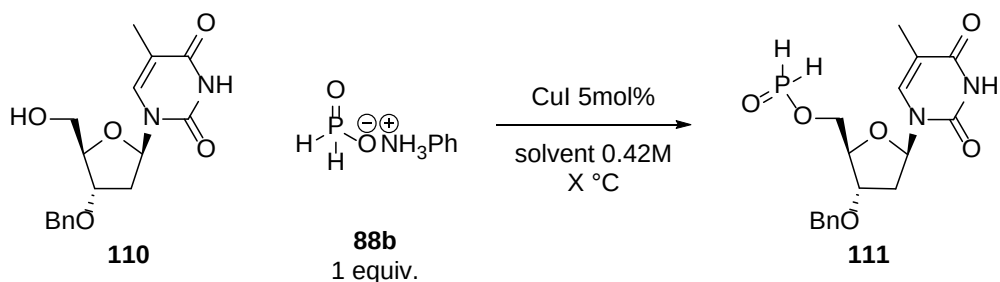
and catalyst in Solvent (X ml) under an argon atmosphere and reacted for X hours at reflux. [a] by ^{31}P NMR [b] averaged over multiple reactions

We found that increasing the concentration of the reaction (entry 3 onwards) led to higher yields but increasing or decreasing the catalyst loading did not improve yields. When we doubled the equivalents of salt used we found that the best results were obtained when the length of reaction was changed to 2 hours, and when longer times were used it led to lower yield and unclear reaction mixtures. Changing the equivalent to 1.5 or increasing the catalyst loading also led to lower yields when sampling at 3 hours. With the optimised conditions in hand, entry 3, we then attempted to replace menthol with 3'OBn Thymidine.



Scheme 33: Attempted synthesis of 3'OBn 5' H-phosphate Thymidine.

When we did attempt this, no reaction was observed, we believe this is due to the insolubility of the starting material (**110**) in toluene. With this in mind we then attempted to replace toluene, using other solvents in which the starting material was soluble (Table 5).

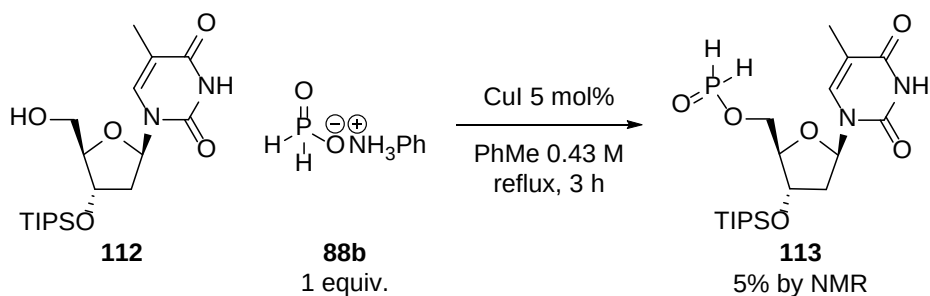


Scheme 34: Reagents for screening of solvents.

Entry	Solvent	Temp (° C)	Time h	NMR Yield 111
1	MeCN	Reflux	3	0
2	MeCN ^[b]	120	3	0
3	Anisole	120	3	0
4	Sulfolane	120	3	0
5	PhMe/Anisole 2:1	120	3	0
6	PhMe/MeCN 1:1	120	2	<1

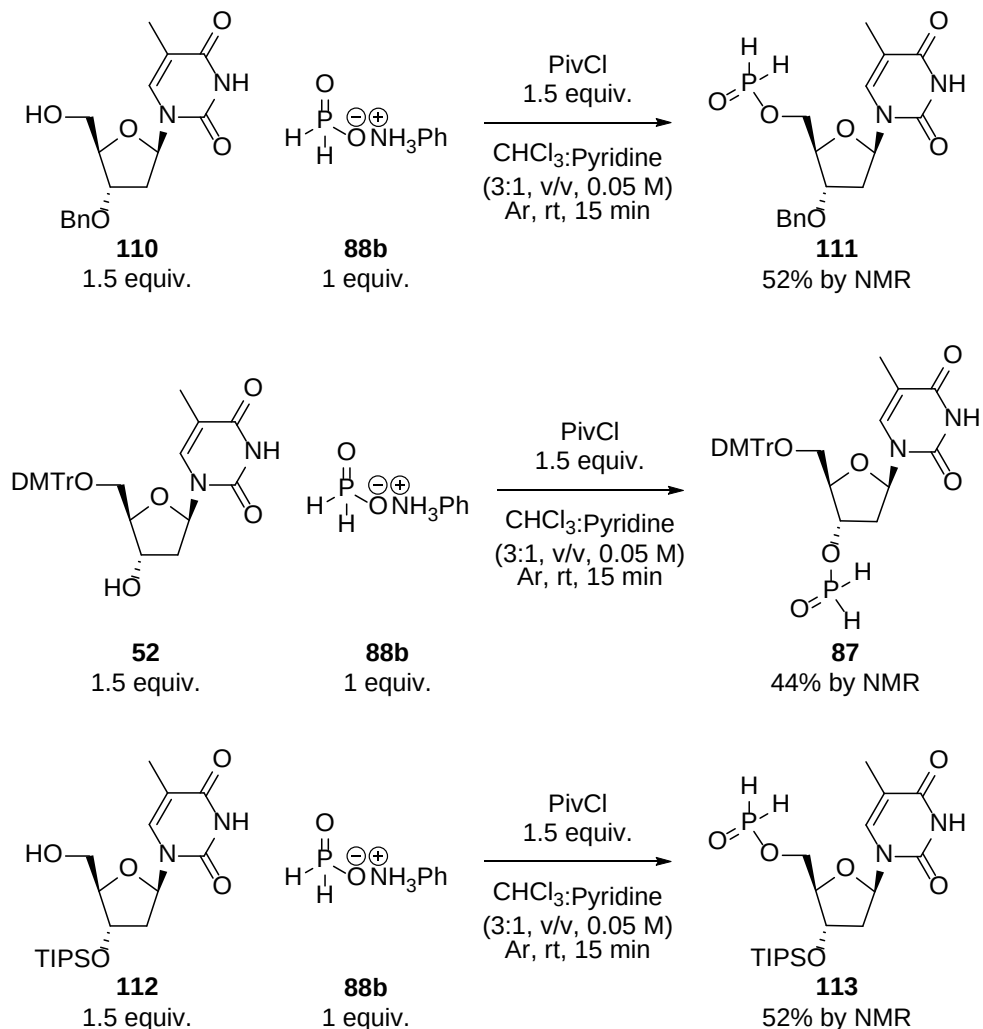
Table 5: Various solvents used. Reaction conditions: **110** (1.6 mmol) was added to salt **88b** (1 equiv.), and CuI (5 mol%) in Solvent (3.8 mL) under an argon atmosphere and reacted for X hours at XX °C. [a] by ³¹P NMR [b] sealed tube.

Changing the solvent did not lead to any increase in reactivity, so instead we attempted to change the protecting group on the thymidine to improve solubility in toluene.



Scheme 53: Synthesis of 3'OTIPS 5'H-phosphinate thymidine using copper iodide.

Although this reaction did generate the desired product, it was in a low amount and at this point it seemed prudent to compare this to the literature method.



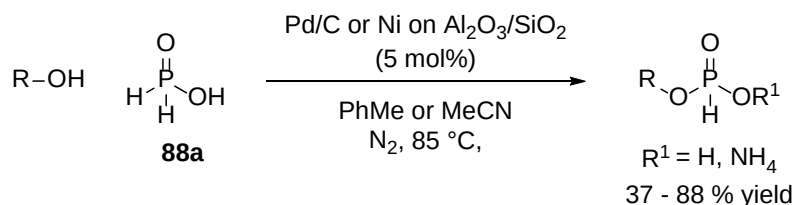
Scheme 54: Synthesis of H-phosphinates using pivaloyl chloride.

The literature method of condensation with pivaloyl chloride has a higher conversion (by ^{31}P NMR) than the method we have investigated and is also compatible with a larger range of protecting groups. Because of this our future work will be to develop this reaction, with a view to using this in-flow.

2.2 Synthesis of H-phosphonates

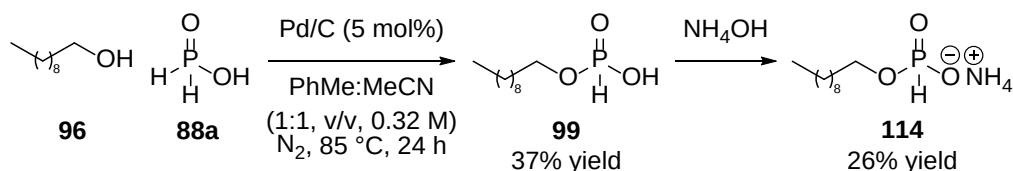
2.2.1 Metal Catalysis

Montchamp *et al.* have previously successfully synthesized a variety of H-phosphonates from phosphinic acid using transition metal catalysis in a transfer dehydrogenation process (Scheme 55).⁵²



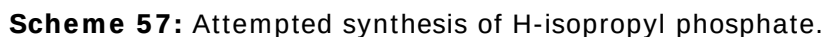
Scheme 55: Synthesis of H-phosphonates using transfer hydrogenation.

We first attempted to use these conditions with decanol, to mirror our work when attempting to synthesise the H-phosphinates, and we synthesised both the *H*-decanyl phosphonate (**99**) and its ammonium salt (**114**).



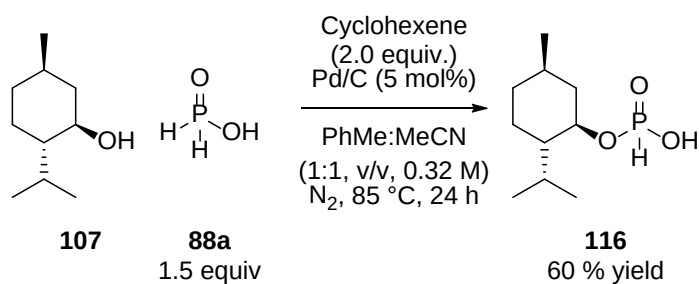
Scheme 56: Synthesis of H-decanyl phosphate and its ammonium salt.

This low yield we believe is due to the susceptibility of these compounds to be hydrolysed. We also attempted to make H-isopropyl phosphate, **74**, and even though the product was observed in the phosphorus NMR spectrum (dd, 6.79 ppm, $J = 699$ and 9 Hz), we were unable to isolate this compound, probably due to its volatility and the compound being hydrolysed on work up.

[illegible]

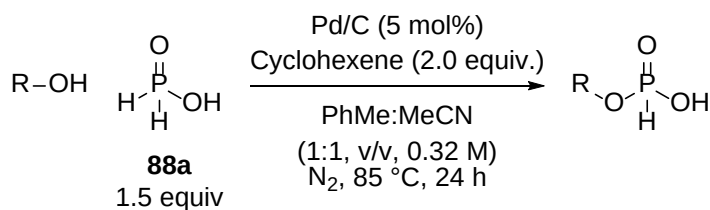
Scheme 58: Synthesis of H-menthyl phosphonate.

The reported yield for this reaction is 88%, however in our hands the reaction only yielded 18%. We suspected that this lower yield may be due to the generated H₂ preventing turnover of the catalyst, so we then attempted to add in 2 equivalents of cyclohexene in order to sequester H₂, which allowed us to isolate **116** in a 60% yield.



Scheme 59: H-menthyl phosphonate synthesis with cyclohexene.

This was a marked improvement on our previous result, so we then used these conditions on a selection of alcohols (Table 6).



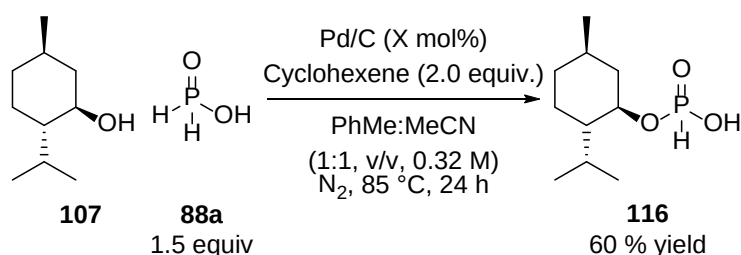
Scheme 60: H-phosphonate synthesis with cyclohexene

Entry	Compound No	Alcohol	Isolated yield (Conversion) %
1	116	Menthol	60 (86)
2	99	Decanol	50 (76)
3	117	Cyclohexanol	40 (79)
4	118	Phenol	18 (72)

Table 6: Different alcohols used in the synthesis of H-phosphonates.

Conversion measured using ^{31}P NMR.

Although the conversion by ^{31}P NMR for the alcohols used were high and reasonable, the isolated yields for the H-phosphonates were low. This is likely due to the susceptibility of the H-phosphonates to hydrolysis, leading to difficulty in isolation. However, with menthol giving the highest yield we then proceeded to trial different amounts of Pd/C (Table 7).

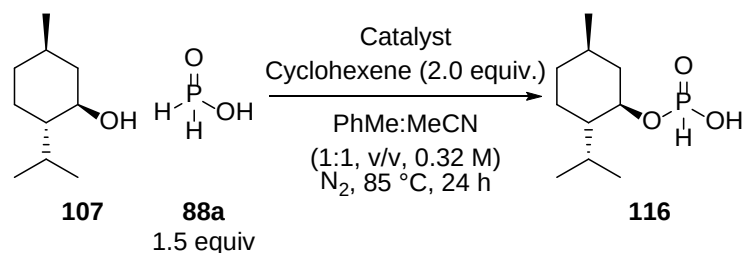


Scheme 61: Reaction conditions used with different amounts of Pd/C.

Entry	mol% Pd/C	isolated yield (Conversion) %
1	5	60 (86)
2	0	0 (0)
3	10	35 (81)
4	2.5	61 (84)
5	1	0 (42)

Table 7: Different amounts of Pd/C. Conversion measured using ^{31}P NMR.

Entry 2 shows that there is no background reaction, and entry 3 shows an increase in catalyst loading does not correlate to an increased yield. When 2.5 mol% Pd/C was used there was a negligible increase in yield, however further lowering (entry 5) did not lead to any improvement. As 2.5 mol% loading lead to a comparable yield, we decided to use this as our catalyst loading amount. We then tried different catalysts to see if there could be any more improvement made.



Scheme 62: Reactants and conditions used with various catalysts.

Entry	Catalyst	isolated yield (Conversion) %
1	No Catalyst	0 (0)
2	2.5 mol% Pd/C	61 (84)
3	2.5 mol% RhCl ₃	56 (56)
4	2.5 mol% RhCl(PPh ₃) ₃	61 (66)
5	2.5 mol% Pd(OH) ₂ /C	41 (59)
6	2.5 mol% Pd(dba) ₂	50 (63)
7	2.5 mol% Pd(PPh ₃) ₄	20 (41)
8	2.5 mol% Rh on Alumina	26 (44)
9	2.5 mol% CuCl ₂	0 (0)
10	2.5 mol% [Ru(COD)Cl ₂] _n	46 (52)
11	2.5 mol% RuCl ₃	0 (0)
12	5 mol% RhCl(PPh ₃) ₃	64 (87)
13	2.5 mol% PtO ₂ ·H ₂ O	0 (41)
14	2.5 mol% Rh/C	58 (80)
15	2.5 mol% FeCl ₂ Bipy	0 (0)
16	2.5 mol% FeCl ₂ Bipy 12.5 mol% LiAlH ₄ ^(a)	0 (9)
17	2.5 mol% FeCl ₂ Bipy 12.5 mol% LiAlH ₄ ^{(a) (b)}	0 (15)
18	12.5 mol% LiAlH ₄ ^(c)	0 (0)
19	5 mol% FeCl ₂ Bipy 5 mol% LiAlH ₄ ^(a)	0 (0)
20	2.5 mol% FeCl ₂ Bipy 12.5 mol% LiAlH ₄ ^{(a) (c)}	0 (0)
21	2.5 mol% Fe(BF ₄) ₂ ·6H ₂ O 2.5 mol% tetrachos ^{(a) (c)}	0 (0)
22	2.5 mol% Fe(OTf) ₃ 1.5 eq NaBH ₄ ^{(a) (c)}	0 (0)
23	2.5 mol% FeCl ₃ 2 eq LiAlH ₄ ^{(a) (c)}	0 (0)
24	2.5 mol% FeCl ₃ 7.5 mol% EtMgCl ^{(a) (c)}	0 (0)

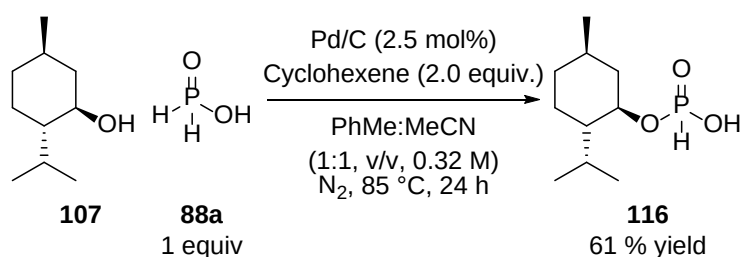
Table 8: Different catalysts and the isolated yields. Conversion measured

using ³¹P NMR (a) THF as solvent; (b) 48 hours (c) no cyclohexene and

propargyl acetate 2 eq used as terminal alkyne

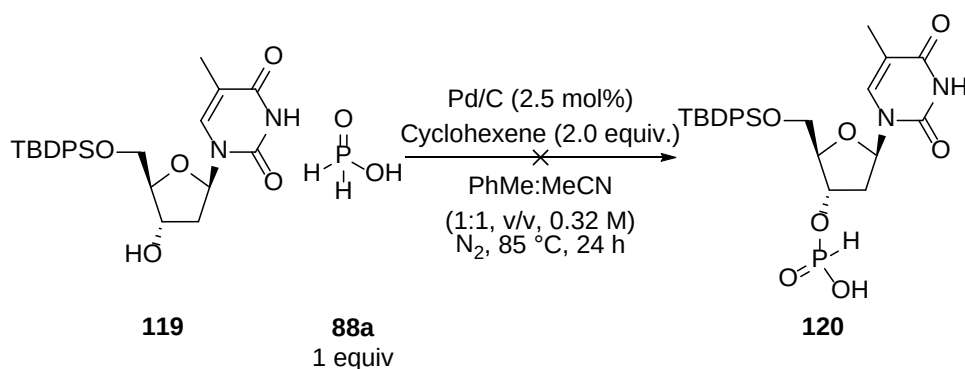
We did explore a limited range of catalysts, but found that the rhodium based catalysts (entries 3, 4, 12 and 14) looked promising, but unless the yield could be substantially increased, the lower cost and ease of use of Pd/C makes this the preferred catalyst moving forward.

We also found when using 2.5 mol% Pd/C we could drop the equivalents of acid to 1 with no decrease in yield.



Scheme 63: Optimised conditions for H-menthyl phosphonate.

We then attempted to apply these optimised conditions for the synthesis of thymidine H-phosphonate (**120**).



Scheme 64: Attempted synthesis of thymidine H-phosphonate.

Unfortunately, none of the desired product was observed in the ³¹P NMR (Figure 13).

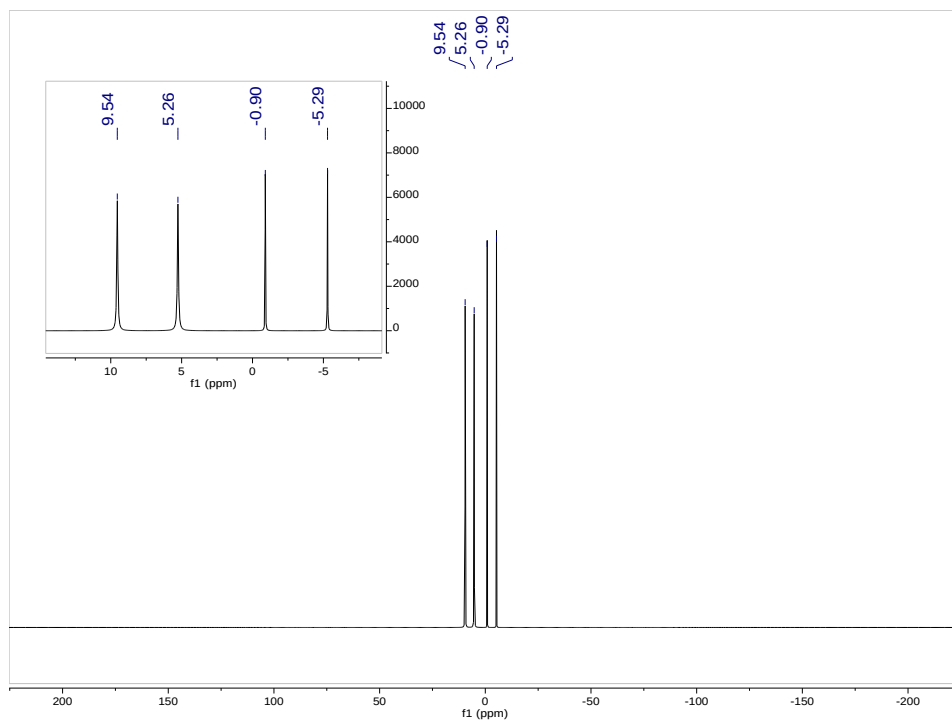


Figure 13: ^{31}P NMR of the reaction shown in Scheme 64.

The NMR showed two products, compound **100** resulting from oxidation of the starting phosphinic acid and compound **121** resulting from silyl deprotection.

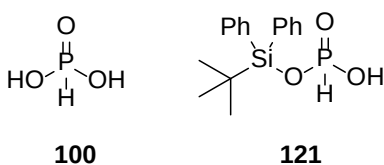


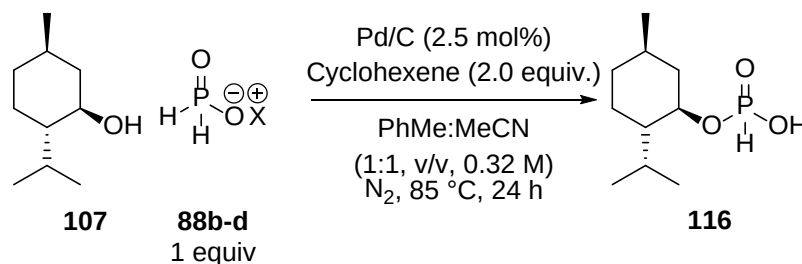
Figure 14: Compounds detected by ^{31}P NMR from Scheme 64.

This deprotection is occurring due to the acidity and temperature of the reaction, we were hoping that the phosphinic acid transfer hydrogenation reaction would out-compete the deprotection reaction, however this was not found. As it is well known that under acidic conditions the glycosidic bonds on the purine bases (adenine and guanosine) are cleaved, resulting in depurination, we decided to

investigate whether a phosphinic acid salt could be used in this reaction instead.

2.2.1.1 Phosphinic acid salts

We began by trying three different phosphinic acid salts with our optimised conditions for use with H-phosphinic acid.

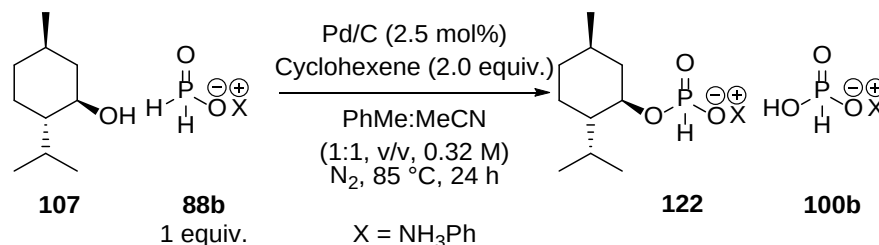


Scheme 65: Reactants and conditions used with various salts

Entry	X (Compound No)	Result
1	N ⁺ H ₃ Ph (88b)	By ³¹ P NMR spectroscopy two compounds: 122 δ 11.1 (td, J 560, 10.0 Hz) 100b δ 3.20 (dd, J 652, 9.00 Hz)
2	Na ⁺ (88a)	No reaction
3	N ⁺ H ₄ (88d)	No reaction

Table 9: Different salts used and the results

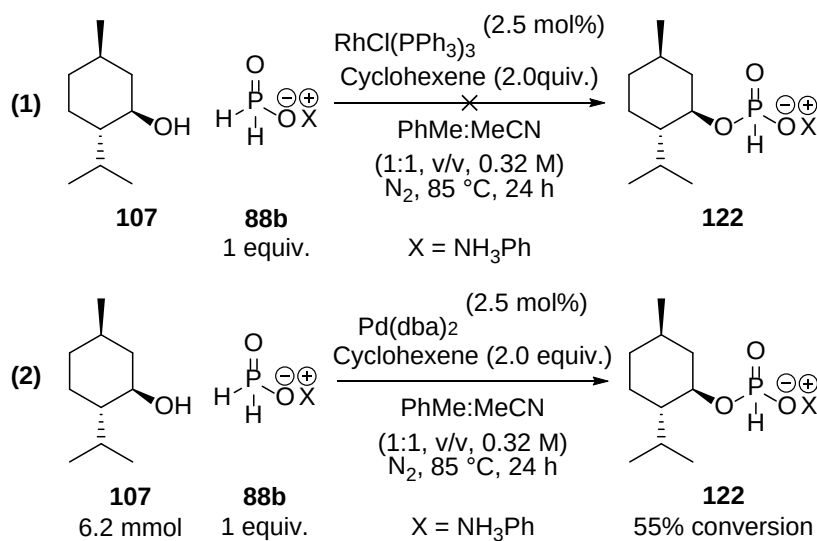
By separate synthesis we were able to confirm that the compounds **122** and **100b** are the compounds shown in Scheme 66.



Scheme 66: Reaction with anilinium hypophosphite.

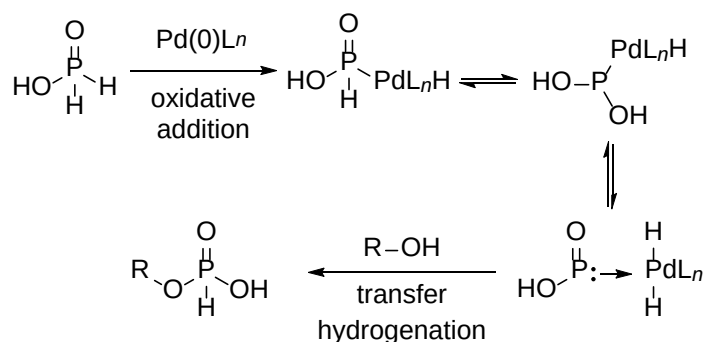
This reaction proved capricious on repetition and scale up with insoluble material forming in these conditions. We then chose to trial

Pd(dba)_2 (Scheme 67, reaction 2), as this was another palladium based catalyst that had worked reasonably well previously (Table 8, entry 6), and pleasingly we found the reaction was scalable to 1 g. We did also try to use Wilkinsons catalyst (Scheme 67 reaction 1), as previously this had worked with the free acid (Table 8, entry 4), however the product was not observed in the NMR spectrum of the crude reaction.



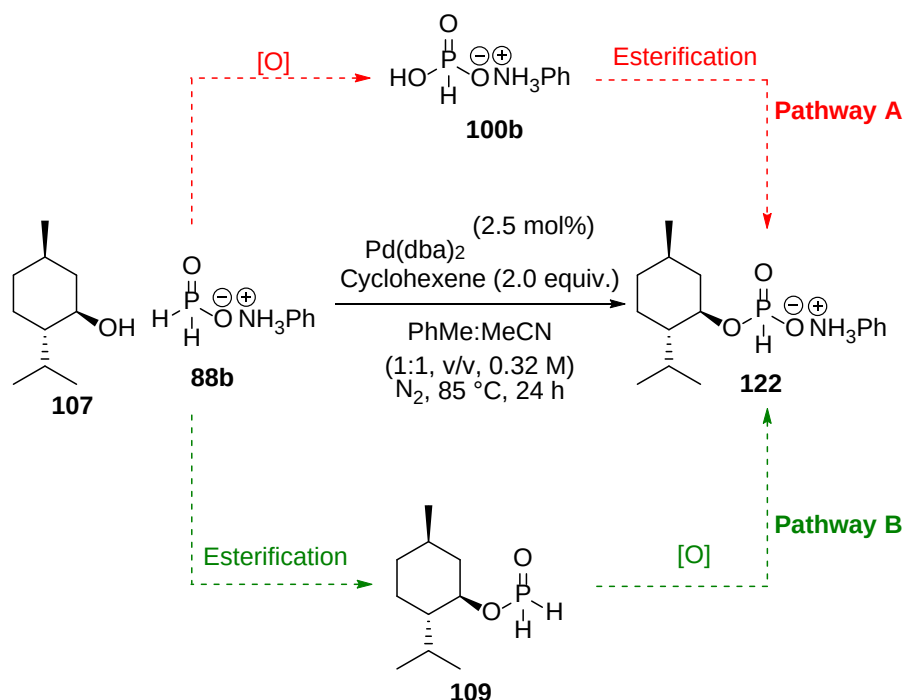
Scheme 67: Scalable reaction conditions.

Montchamp's proposed reaction mechanism for this transformation when using the free acid is shown in scheme 68.⁵² However the group were unsuccessful when attempting perform this reaction using H_3PO_2 salts, whereas we did find success when using the anilinium hypophosphite, which then made us question the mechanism.



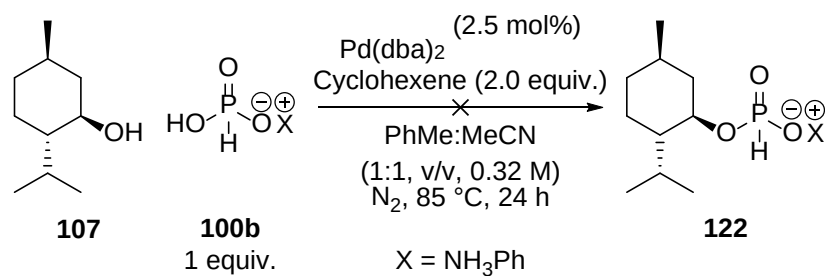
Scheme 68: Proposed reaction mechanism by Montchamp *et. al.*⁵²

We proposed that the reaction could be proceeding through one of two pathways (Scheme 69); pathway A being an oxidation followed by esterification, and pathway B being the reverse, an esterification followed by oxidation.



Scheme 69: Two potential pathways for the synthesis of H-phosphonate.

We did try to mimic pathway A, by separately synthesising the oxidised salt, **100b**, and then using this in place of the anilinium hypophosphite in the reaction, however no reaction took place.



Scheme 70: Attempted synthesis of H-phosphonate from mono-anilinium hyposphosphite.

This also matches with the results obtained when sampling the reaction at earlier time points, e.g. at 3 hours, figure 15. The ³¹P NMR showed the formation of the H-phosphinate **109** as a triplet of doublets at 13.0 ppm, alongside the starting material, the product of oxidation of this salt, and a small amount of the desired product **122**.

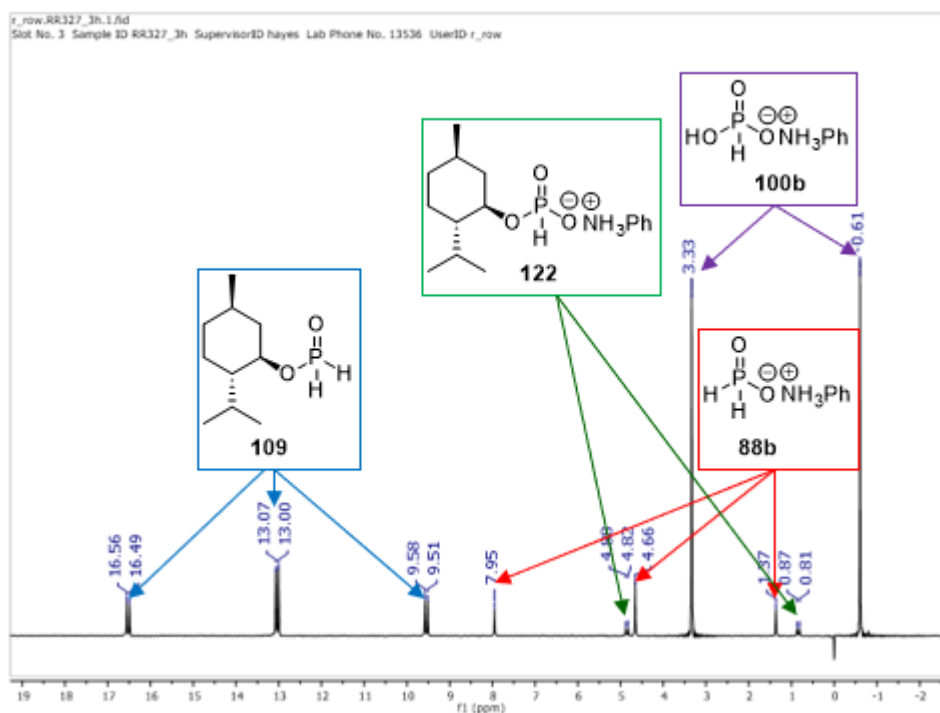
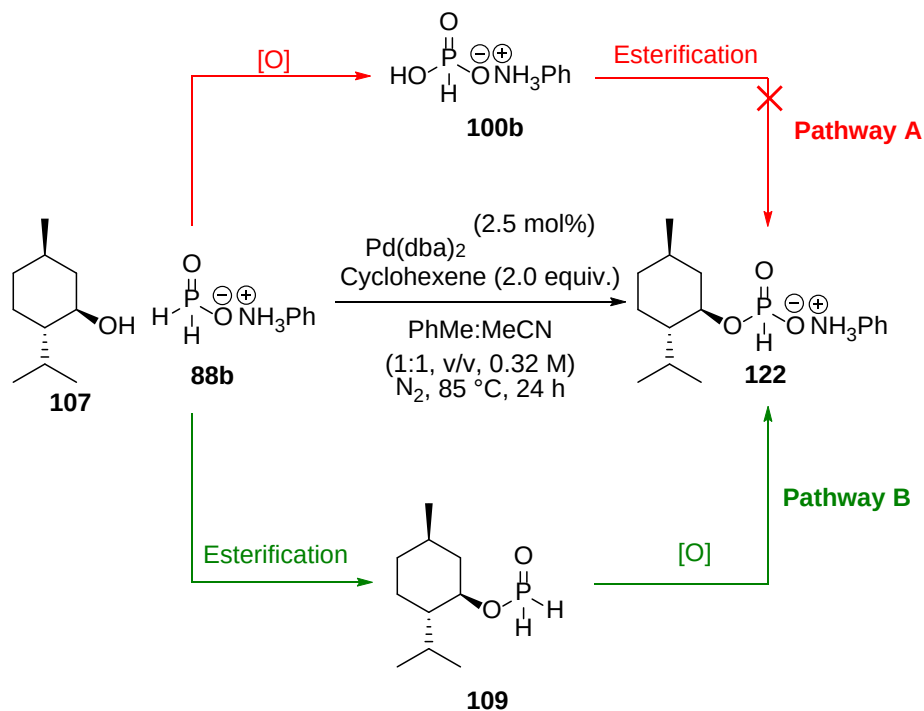


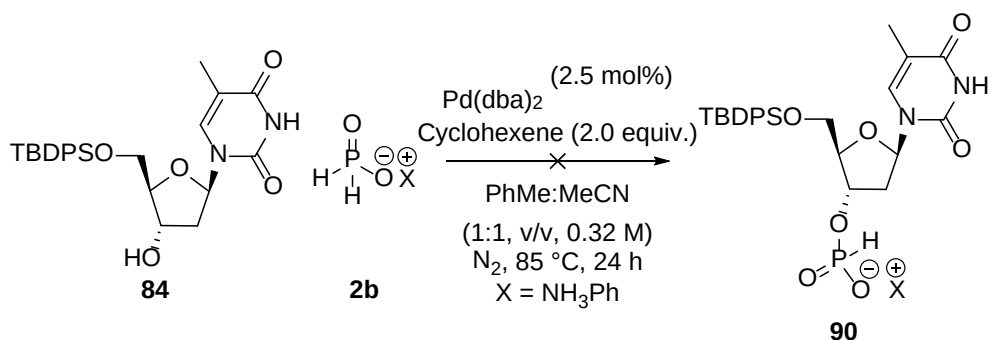
Figure 15: ³¹P NMR of reaction 2 in Scheme 67 at 3 hours.

These results lead us to believe that the menthyl H-phosphonate is forming as a result of pathway B (Scheme 71), however the oxidation of the salt **88b** into **100b** is a competitive reactive pathway, which does not lead to the formation of the product.



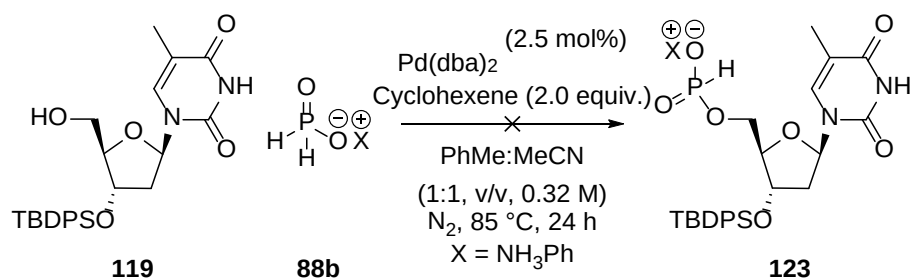
Scheme 71: Pathways for the reaction between menthol and anilinium hypophosphite.

As we had observed the menthyl H-phosphonate salt (Scheme 67) we then attempted to repeat these conditions with 5'OTBDPS Thymidine.



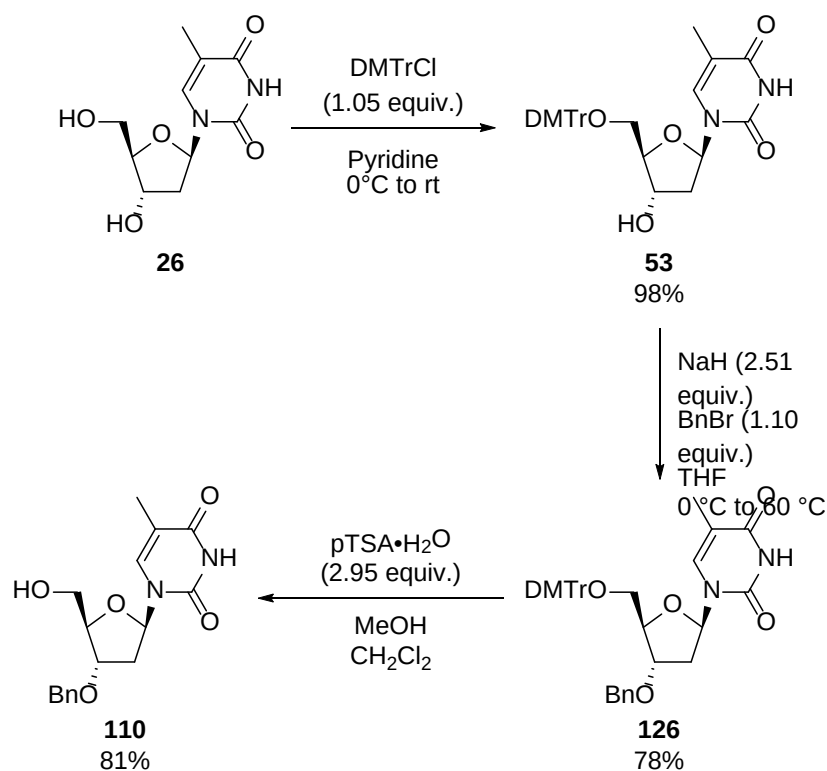
Scheme 72: Attempted reaction between 5'-OTBDPS Thymidine and anilinium hypophosphite.

However, after 24 hours the major products by ³¹P NMR spectroscopy were once again the silyl phosphonate esters. Changing to 3'-OTBDPS, as we thought the primary alcohol may react faster due to less steric hindrance, also generated the silyl phosphonate esters.



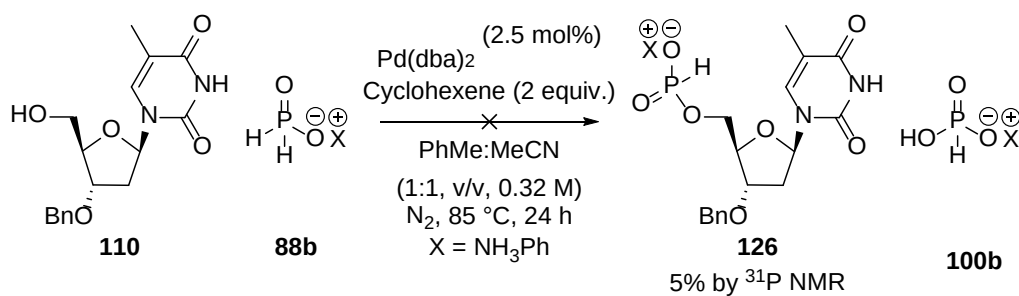
Scheme 73: Reaction with 3'-OTBDPS Thymidine and anilinium hypophosphite.

As we were still observing deprotection of silyl protecting groups when using anilinium hypophosphite, we then synthesized 3'-OBn thymidine from thymidine. Again, this was synthesized over the 5'-OBn thymidine in the hope that the primary 5'-OH would react faster.



Scheme 74: Synthesis of 3'OBn Thymidine.

With 3'OBn Thymidine in hand we then attempted the synthesis of the H-phosphonate **126** using the previously optimised conditions with anilinium hypophosphite.



Scheme 75: Attempted synthesis of H-phosphonate **126**.

Although we did observe the product by ³¹P NMR spectroscopy (as a dt at 4.39 ppm), it was the minority product, with the majority being mono-anilinium phosphite (**100b**) and proved difficult to isolate cleanly.

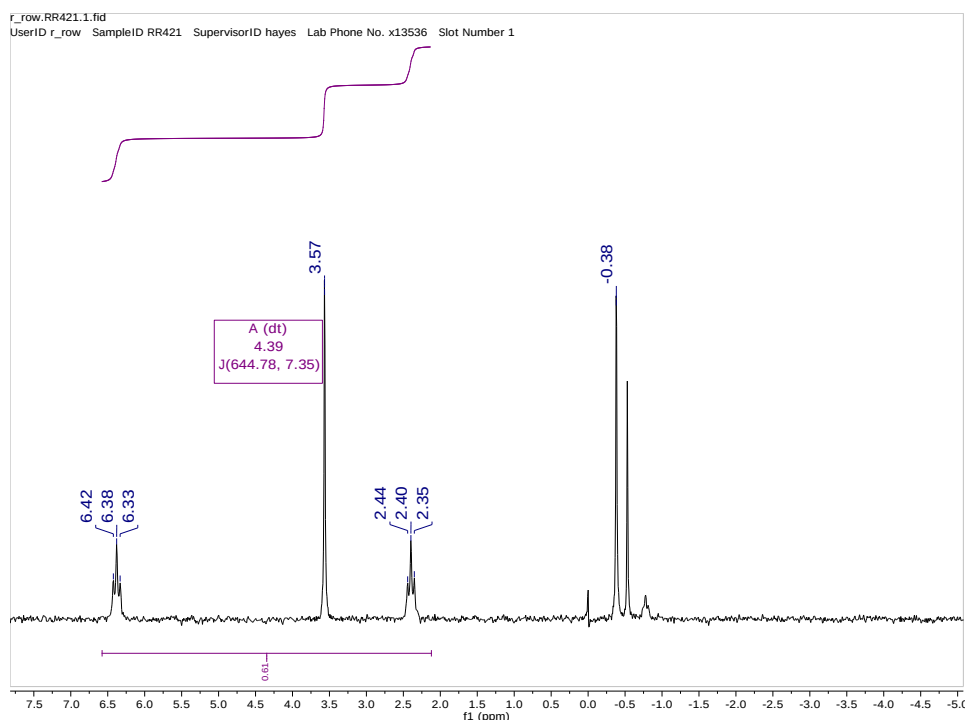


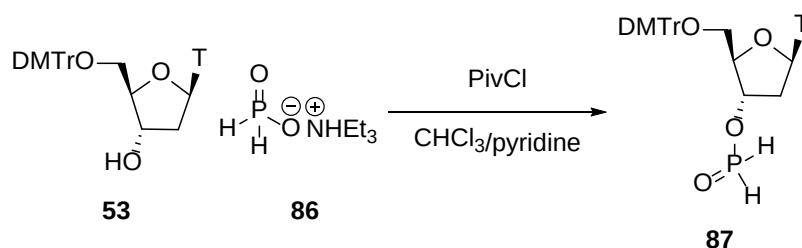
Figure 16: ^{31}P NMR of the crude reaction mixture shown in Scheme 75.

We knew that under these conditions the esterification of mono-anilinium phosphite (**100b**) would not occur, so we began to investigate conditions in which this would happen.

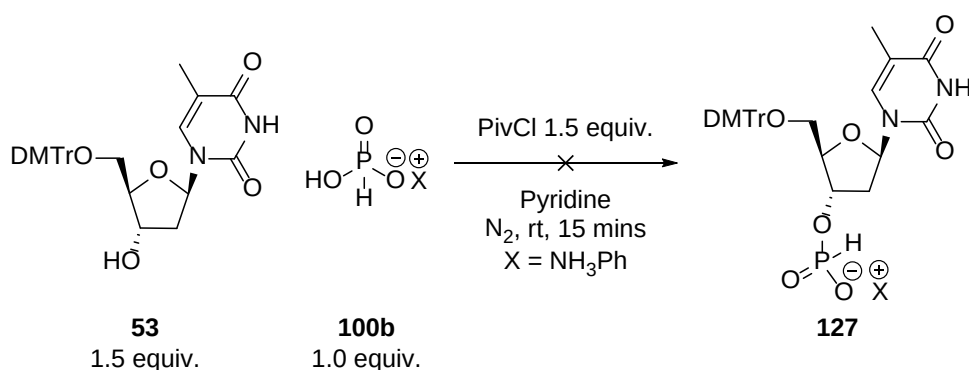
2.2.2 Esterification

As we can oxidise H-phosphinic acid into the mono-anilinium phosphite using palladium catalysis, and Montchamp has previously synthesised phosphonic acid from H-phosphinic acid using a Nickel catalyst, we can still synthesise nucleotides from a more sustainable source when using phosphonic acid and its salts, as we can use H-phosphinic acid as the starting material.⁴⁷

We began by using the literature method for esterification of triethyl ammonium phosphinate with a nucleoside (Scheme 17),^{48,49} but attempting this with mono-anilinium phosphite (**100b**) (Scheme 76).



Scheme 76: Synthesis of phosphinate intermediate from phosphinic acid salts.^{48,49}



Scheme 77: Attempted synthesis of H-Phosphonate **127** using esterification.

It was found that the starting material nucleoside was not consumed in this reaction, and when looking at the ^{31}P NMR of the crude reaction mixture, only one species was observed, the Pyrophosphonate, **128**.

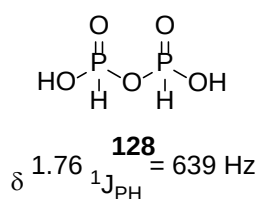
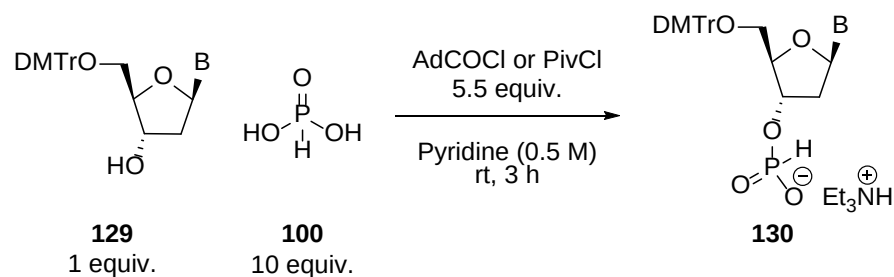


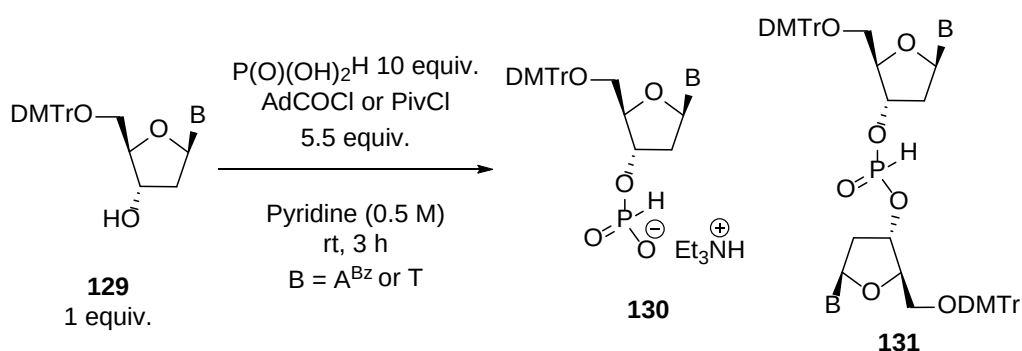
Figure 17: Pyrophosphonate observed as the product of the reaction in Scheme 77.

This species has been used by Stawinski *et al.* to make nucleotide H-phosphonate previously, using the conditions shown in Scheme 78.⁵³



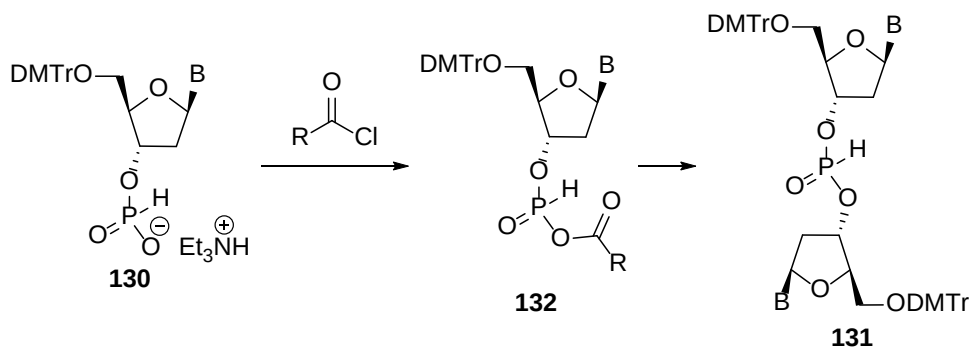
Scheme 78: Stawinski's conditions for the synthesis of nucleotide H-phosphonates.⁵³

When it was attempted to repeat these conditions with both adenosine and thymidine, the desired products were observed, as well as the dinucleotides (Scheme 79).



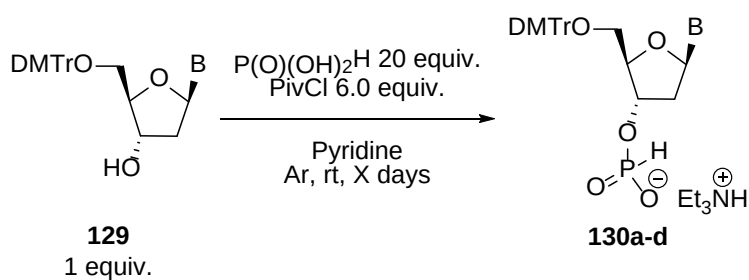
Scheme 79: Synthesis of nucleoside H-phosphonates using Stawinski's previously published conditions.

We believe the unwanted dinucleotides were forming because of the product **130** reacting with any unreacted condensing reagent (AdCOCl or PivCl), and then reacting with the unreacted nucleoside **129**.



Scheme 80: Synthesis of the 3' 3'- dinucleotide **131**.

To avoid this issue, we changed the reaction set up, and first pre-mixed the pivaloyl chloride with the phosphonic acid in a pyridine solution, to form the pyrophosphonate, then added this solution to a solution of the nucleoside in pyridine. A high volume of pyridine was needed, to prevent acidic deprotection of the DMTr group, however, this led to longer reaction times. It was found that the different nucleosides each required different concentrations and different reaction times.

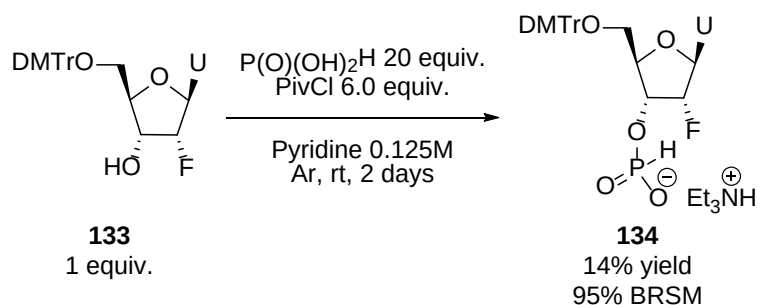


Scheme 81: Synthesis of nucleotide H-phosphonates.

Entry	Compound	Base	Concentration (M)	Time (days)	Yield (%)
1	130a	T	0.14	5	90
2	130b	A ^{Bz}	0.06	3	97
3	130c	C ^{Bz}	0.05	2	98
4	130d	G ^{iBu}	0.13	7	87

Table 10: Best conditions found for each base.

We have also applied these conditions for the synthesis of a 2'F RNA modified nucleoside (Scheme 82).



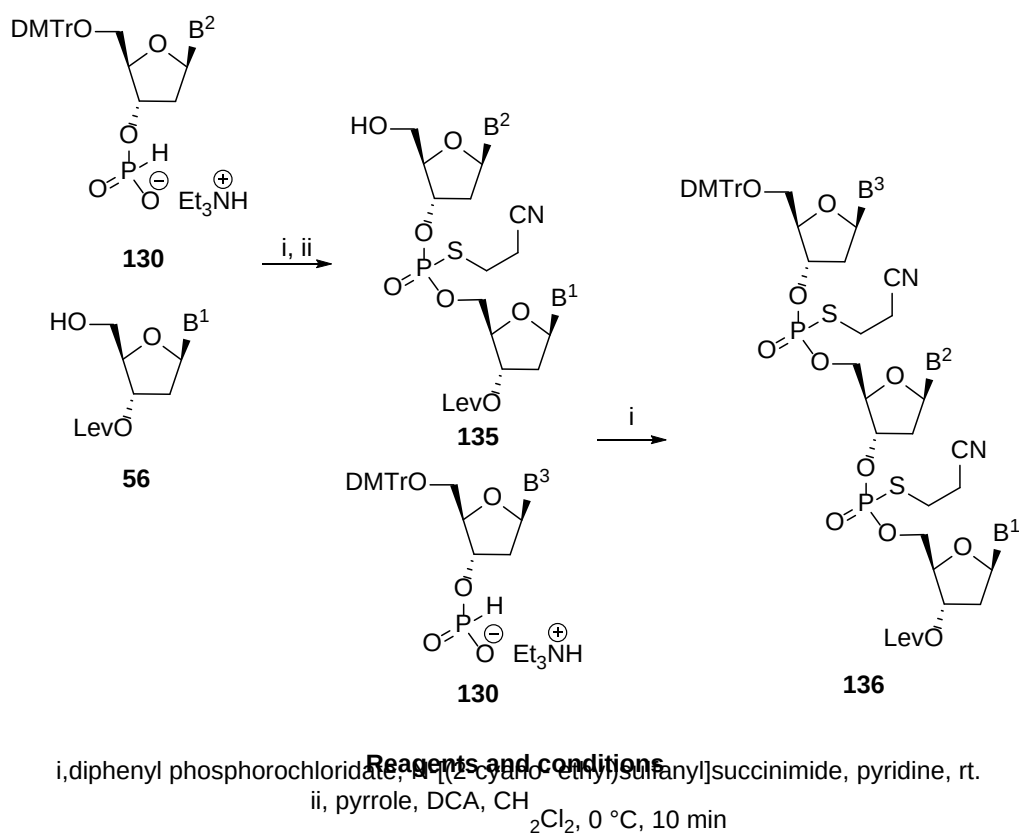
Scheme 82: Synthesis of a 2'F RNA nucleotide

Unfortunately, when using the 2'F Uridine we could not achieve full conversion using the conditions shown in Scheme 82, showing that further optimisation would be required to determine the best conditions for RNA H-Phosphonate synthesis.

With a selection of H-phosphonates synthesised in good yields, our attention then switched to the synthesis of dinucleotides.

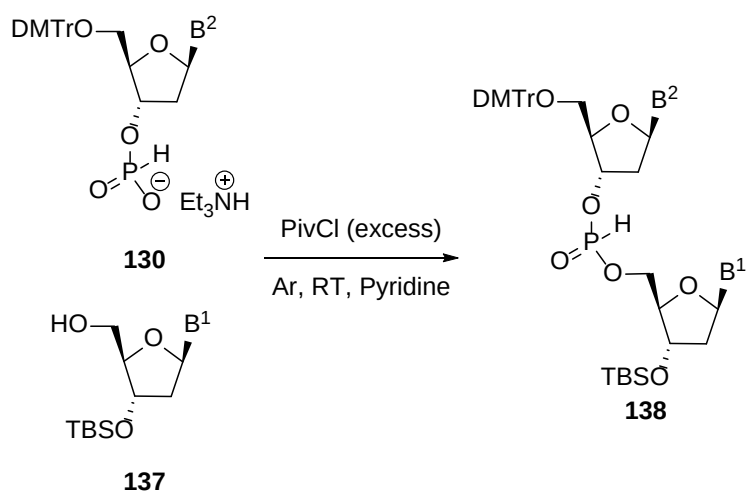
2.3 Synthesis of dinucleotides

Synthesis of di and oligonucleotides from H-phosphonates has been well documented, with Reese *et al.* synthesizing the commercially approved Vitravene in 2002 using a block approach, synthesising four different trimers.¹³



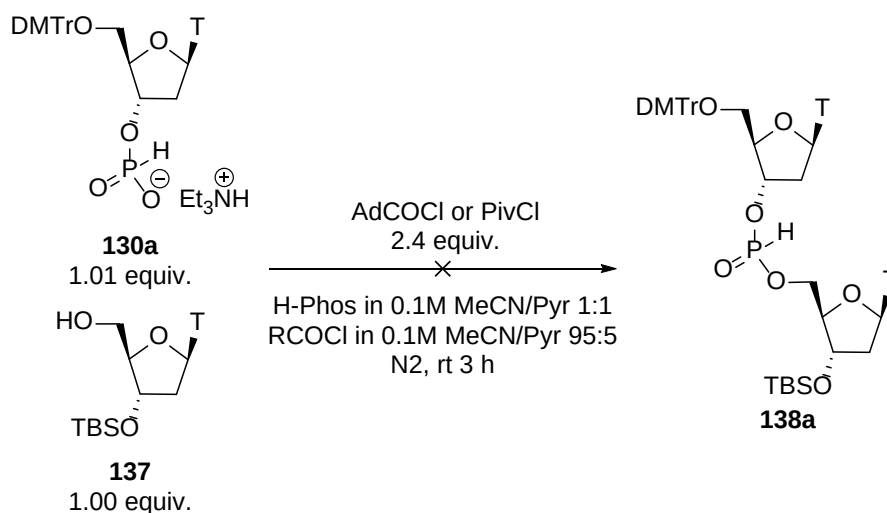
Scheme 83: Reese's synthesis of trimers from H-phosphonates.¹³

Other groups have published the synthesis of the dinucleosides containing the H-phosphonate linker, using 3'-OTBDPS protected nucleosides, and pivaloyl chloride as the condensing agent.^{54,55}



Scheme 84: Synthesis of dinucleotides with the H-phosphonate linker.^{54,55}

As mentioned in the introduction, H-phosphonates have also been used on automated synthesisers, and as such there is a protocol available for this, so we began our studies on the synthesis of dinucleotides using the protocol conditions, but using a 3'TBS protected nucleoside in place of a 3'solid supported nucleoside.⁵⁶



Scheme 85: Attempted synthesis of a dinucleotide using the link protocol.⁵⁶

When monitoring the reaction by HPLCMS and ³¹P NMR of the crude product, the reaction appeared to convert to the desired product cleanly (Figure 18).

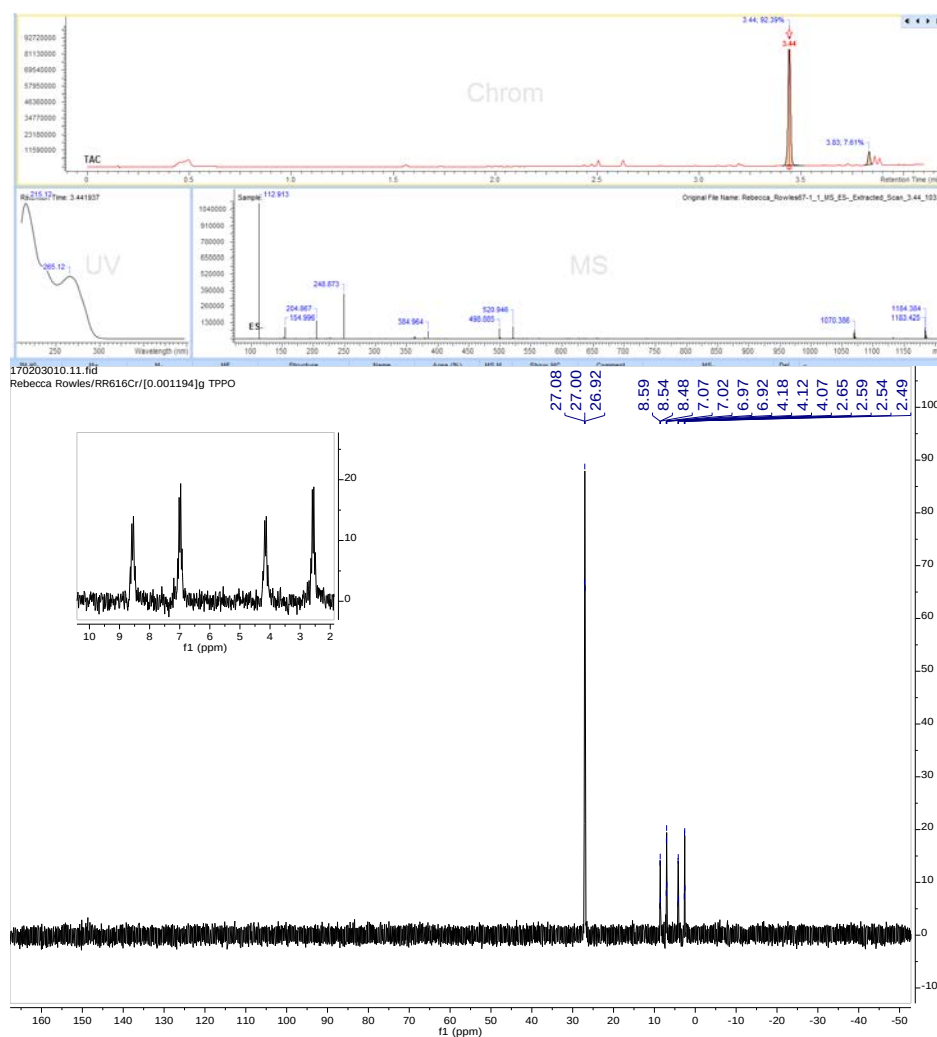


Figure 18: HPLCMS and ^{31}P NMR of the crude reaction mixture.

After purification by column chromatography using silica, with a gradient of 0-10 % MeOH in CH_2Cl_2 with 1% Et_3N , one of the clean products was DMTr Thymidine, **53**, Figure 19, and the HPLCMS and ^{31}P NMR of the fractions containing the desired dinucleotide are shown in Figure 20.

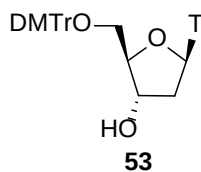


Figure 19: One of the clean products obtained after purification by column chromatography of the reaction shown in Scheme 85.

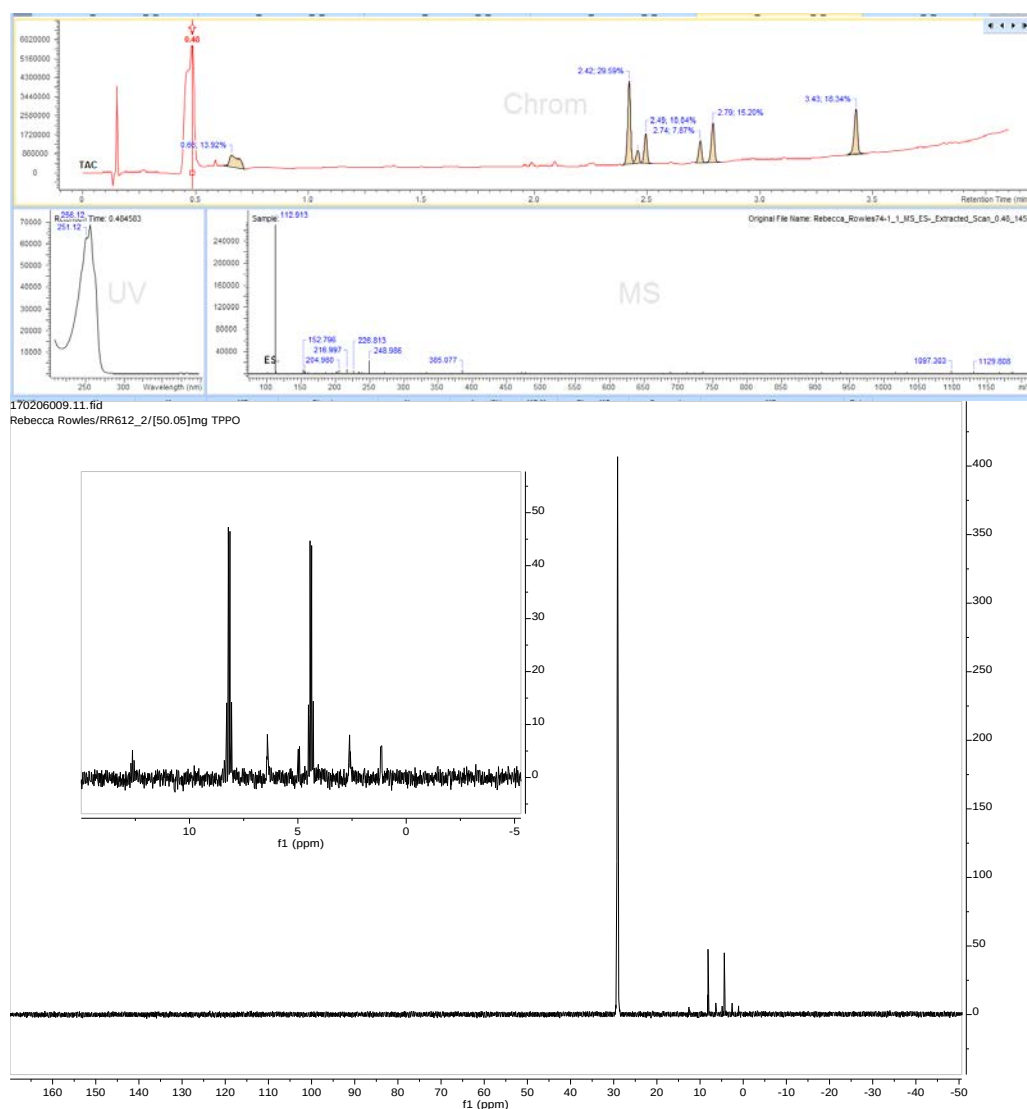
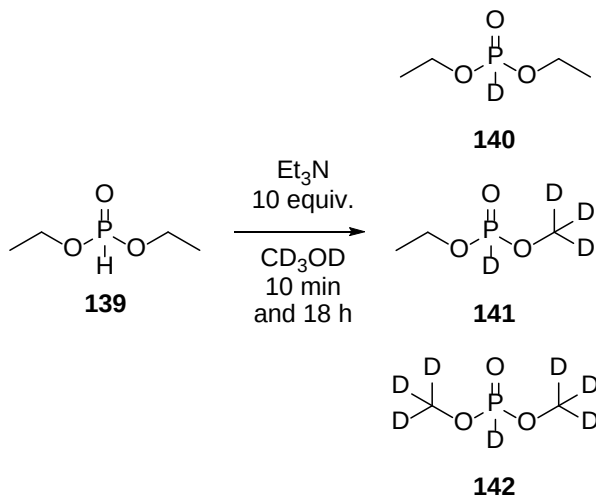


Figure 20: HPLCMS and ³¹P NMR of the fractions containing product after purification by column chromatography.

As the issue appears to happen when the reaction mixture is purified by column chromatography, we investigated what happened to the reaction mixture when MeOD is added, and when MeOD and Et₃N is added (Scheme 86, Figure 21).

transesterification is occurring because of just the methanol and triethylamine, we investigated what happened when diethyl phosphite, **139**, is subjected to MeOD and Et₃N.



Scheme 87: Base enabled transesterification of diethyl phosphite with MeOD.

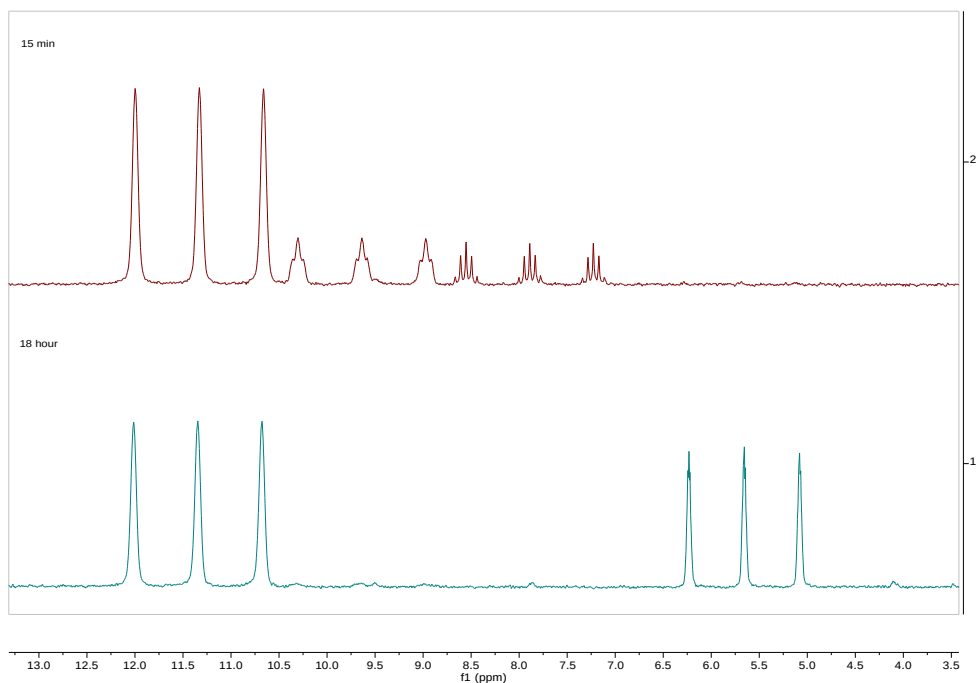
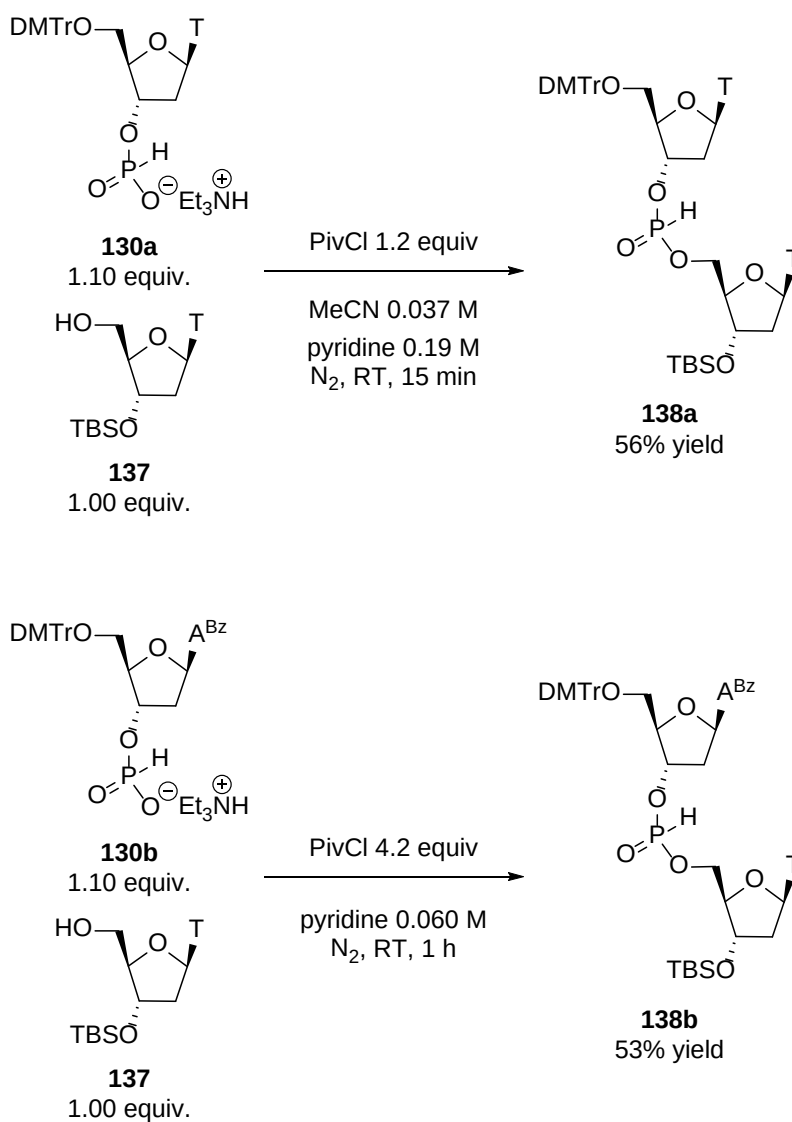


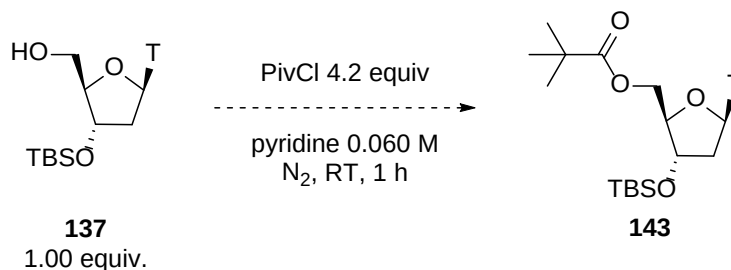
Figure 22: ³¹P NMR of the transesterification of diethyl phosphite with MeOD.

With the results indicating that these dinucleotides would not be suitable for normal phase chromatographic purification on silica gel, when using triethylamine (needed to prevent DMTr deprotection), we then attempted to purify these dinucleotides using reverse phase columns. We used the conditions previously used by Mukhlall and Hersh to make the T-T dimer, and the conditions used by Turks *et al.* to make the A-T dimer (Scheme 88).^{54,55}



Scheme 88: Successful synthesis of dinucleotides purified using reverse phase chromatography.

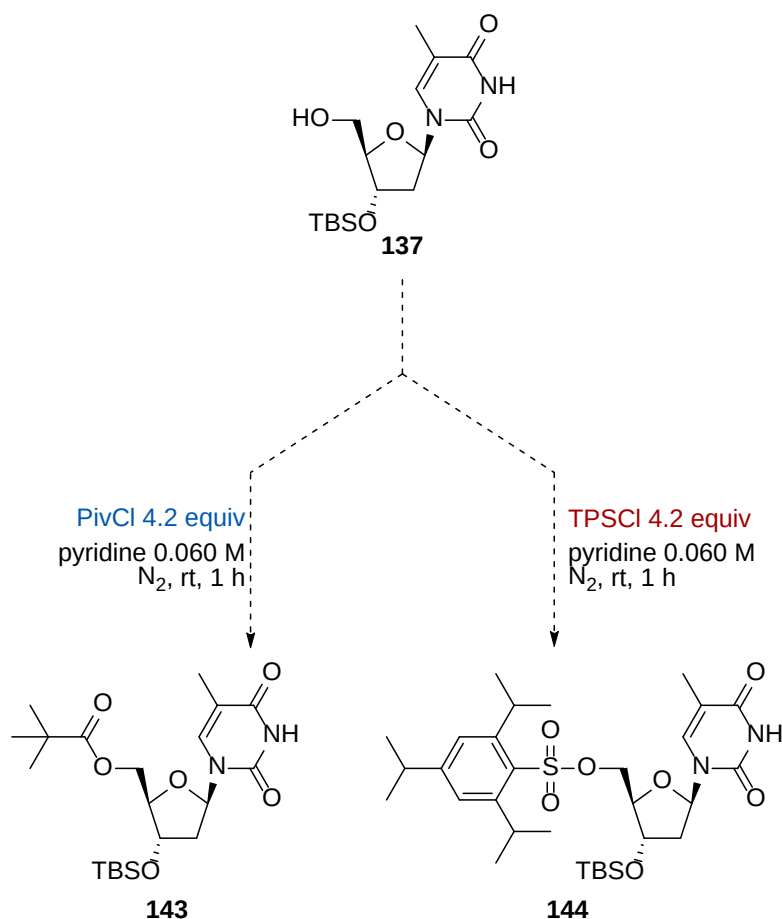
When using reverse phase chromatography the yields were still disappointing, however we were seeing full consumption of the starting material. We believe that the starting alcohol was being consumed in a side reaction – an esterification with the pivaloyl chloride.



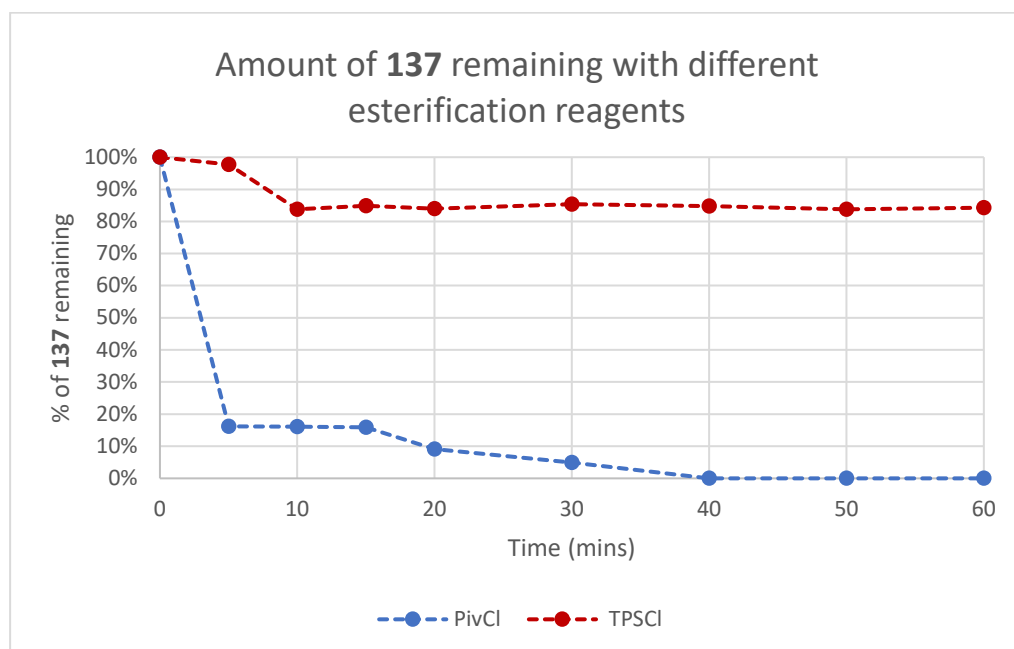
Scheme 89: Suspected esterification of starting alcohol with pivaloyl chloride.

Our group has had success with using 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCI) for the transesterification of phosphotriesters, and we hoped that the esterification of the starting alcohol would be more difficult with this reagent, thus increasing the yield of the desired dinucleotide.

We examined the reaction of the starting alcohol and both esterification reagents, monitoring the amount of alcohol remaining by HPLC. The results can be seen in Graph 2.

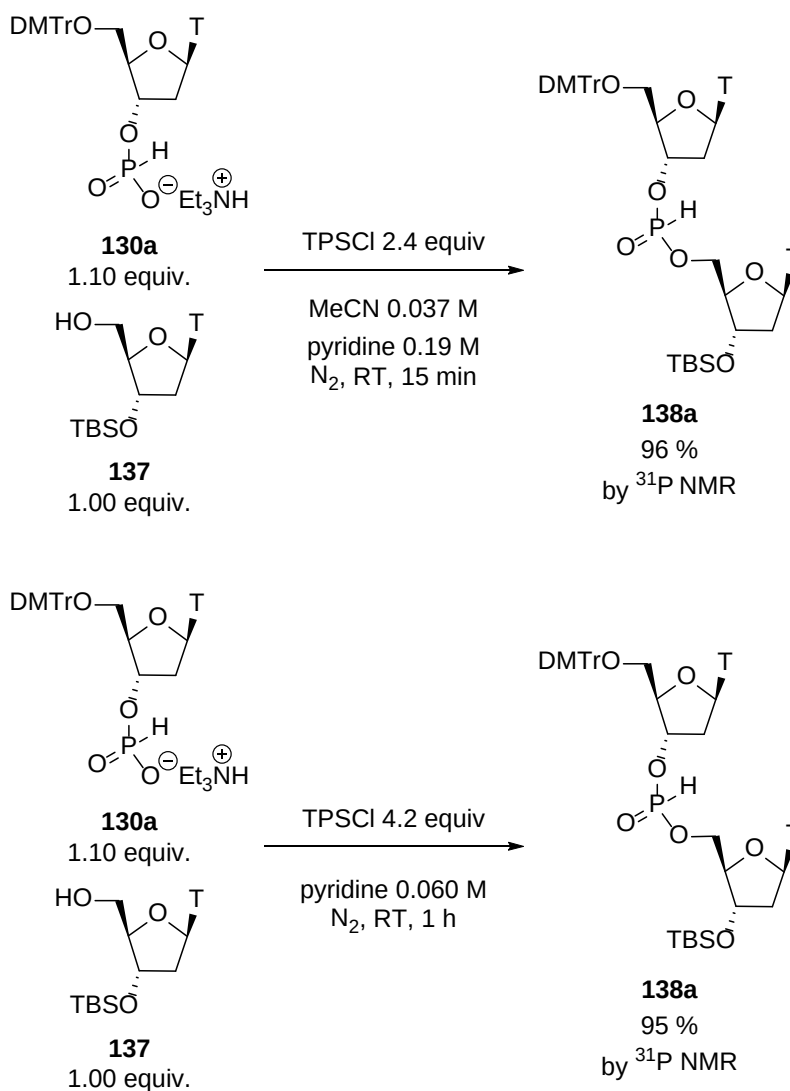


Scheme 90: Reaction monitored using HPLC.



Graph 2: Amount of 3'TBS Thymidine remaining when pivaloyl chloride (Blue) or TPSCI (Red) added, determined using HPLC.

The results indicated that TPSCI reacts slowly the 3'TBS Thymidine compared to the pivaloyl chloride, so we then attempted to use this as the esterification reagent using both sets of previously used conditions.

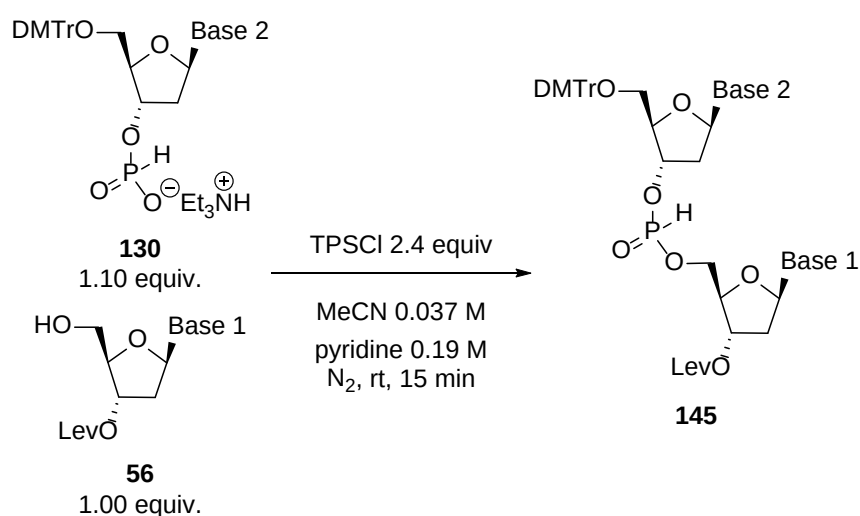


Scheme 91: NMR yields of the TT dinucleotide when using TPSCI instead of PivCl.

The NMR yields of the crude reaction mixture were good, however we still encountered issues when trying to isolate these compounds, so further optimisation of the purification techniques were required. As Reese *et al.* had made the dinucleotides using the 3'Lev protected

nucleoside we also decided to switch to using these when optimising the purification of the dinucleotides.¹³

As we knew the transesterification of the dinucleotides occurred due to the presence of the base triethylamine, we attempted to find ways to avoid the use of the base in the column. We found that if we pre-dried the silica in an oven overnight, we could avoid a significant amount of DMTr deprotection, which occurs when “wet” silica is used. With the use of “dry” silica, we were able to obtain the desired dinucleotides with the H-phosphonate linkage, in moderate yields. The dinucleotides successfully synthesised included a pyrimidine-pyrimidine, purine-purine and purine-pyrimidine, showing that the method could be used of both types of bases

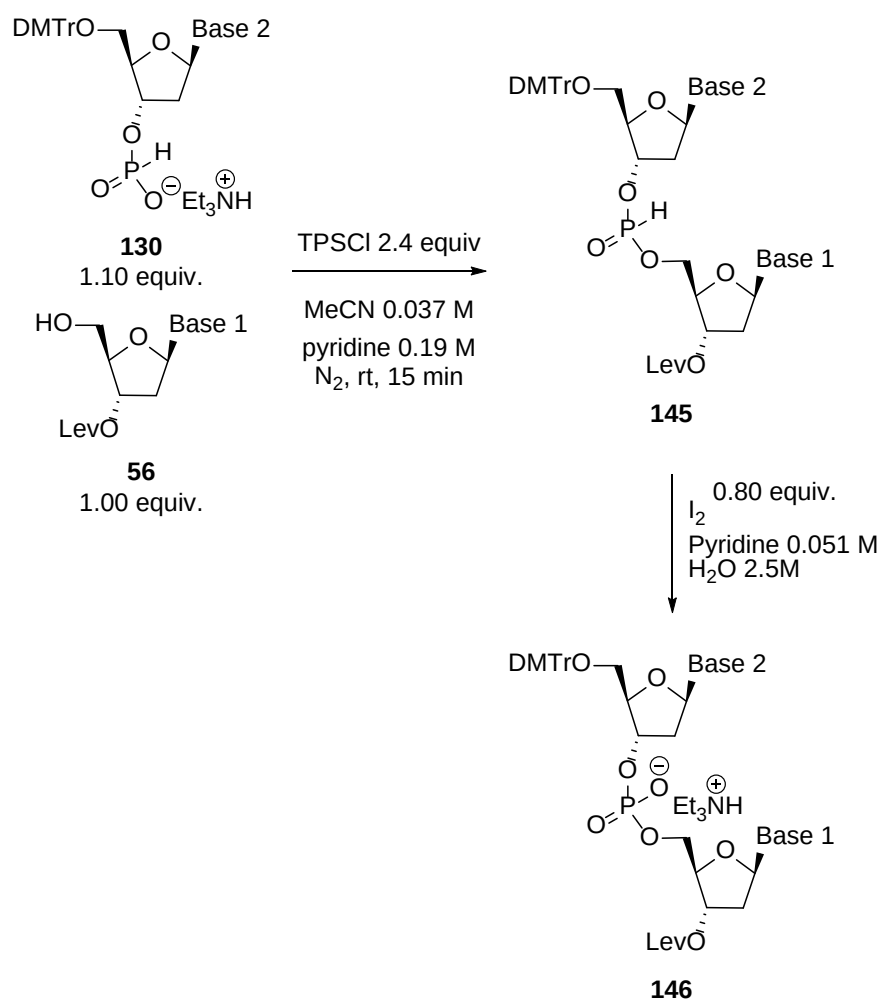


Scheme 92: Synthesis of dinucleotides with the H-phosphonate linkage.

Entry	Compound	Base 1	Base 2	Yield %
1	145a	T	T	73
2	145b	A ^{Bz}	T	72
3	145c	T	A ^{Bz}	59
4	145d	A ^{Bz}	A ^{Bz}	85

Table 11: Yields of the dinucleotides synthesised.

The yields were still disappointing (due to the repeated couplings required for the synthesis a oligonucleotide), albeit better than we had previously obtained for these types of compounds. We believed this was due to the instability of the H-phosphonate linkage, so we then oxidised the crude dinucleotide using iodine and water, to obtain the phosphonate products (Scheme 93, Table 12).



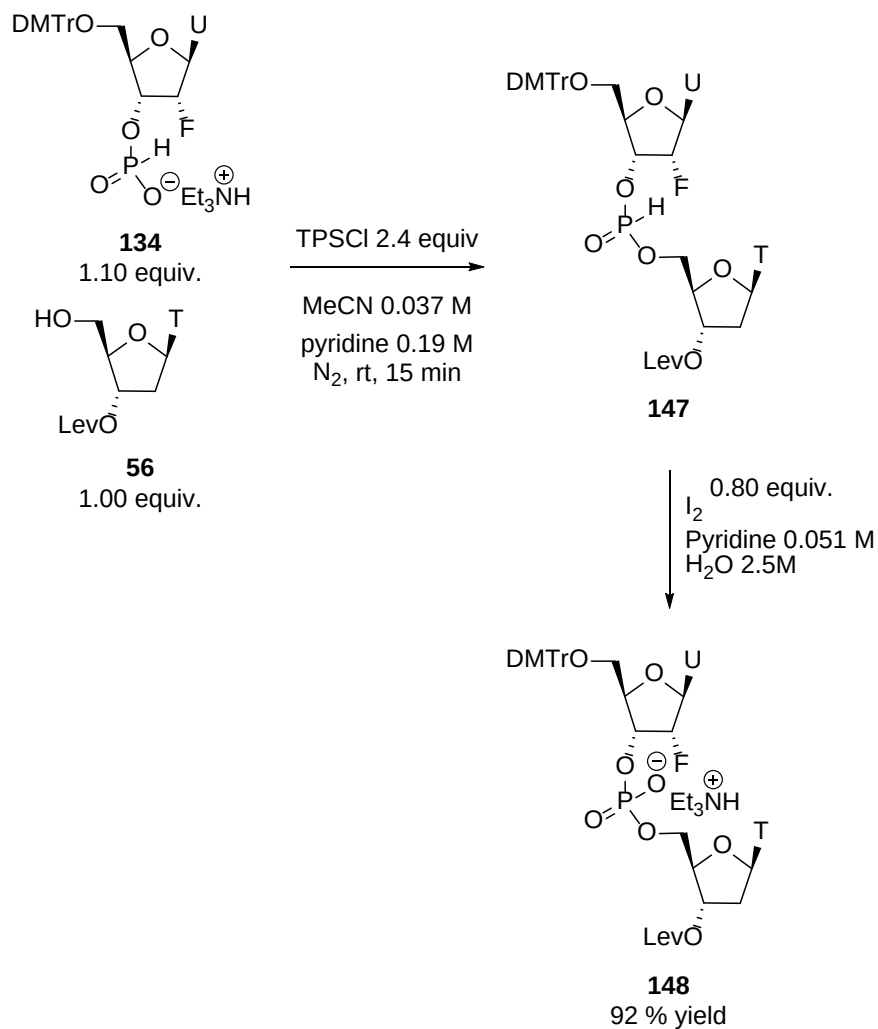
Scheme 93: Synthesis of phosphonate dinucleotides.

Entry	Compound	Base 1	Base 2	Yield %
1	146a	T	T	89
2	146b	T	ABz	92
3	146c	T	CBz	98
4	146d	T	GiBu	94
5	146e	ABz	T	93
6	146f	ABz	ABz	88

Table 12: Yields of phosphonate dinucleotides.

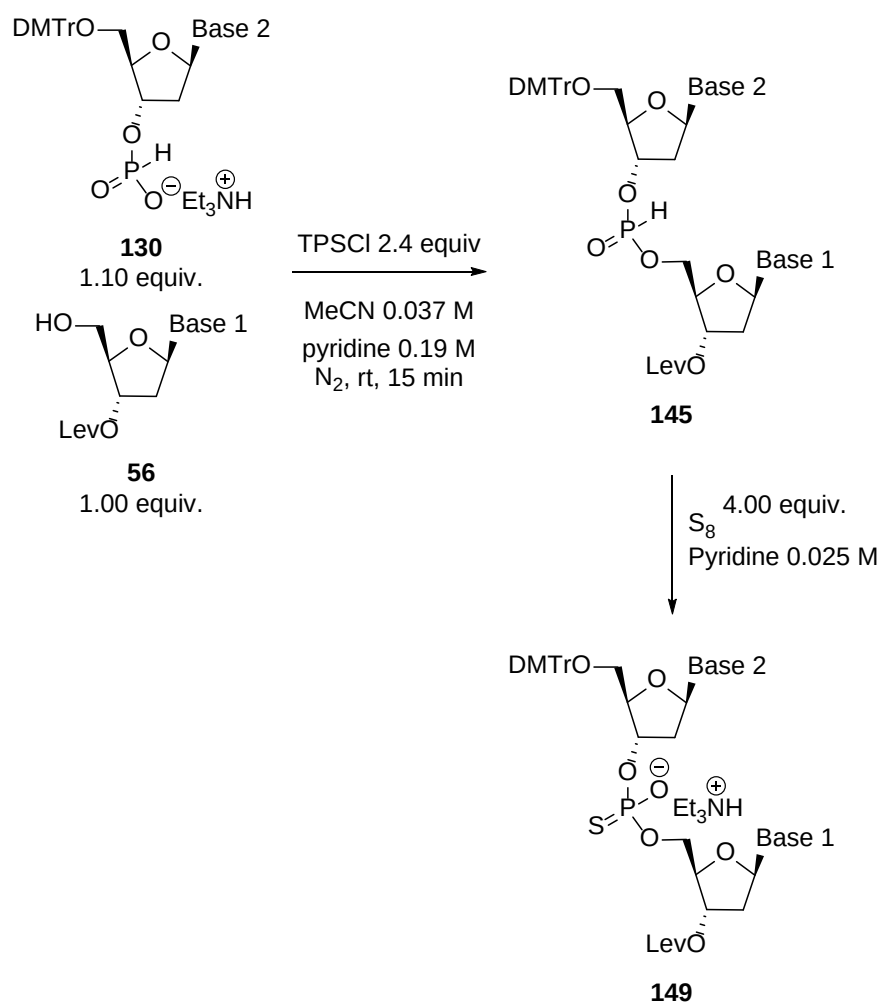
We once again synthesised these dimers using a mixture of pyrimidine and purine bases, showing the compatibility, and found that the yields of the phosphonate dinucleotides were higher than the H-

phosphonate equivalents. This is most probably due to the nature of the H-phosphonates. We also used the 2'F RNA H-phosphonate in this reaction (Scheme 94).



Scheme 94: Synthesis of a dinucleotide using the 2'F H-phosphonate.

As the phosphorothioate is the most common linkage and modification found in oligonucleotides used for clinical purposes, we also synthesised these, again using a mixture of pyrimidines and purines, and pleasingly found that these too could be isolated in high yields.



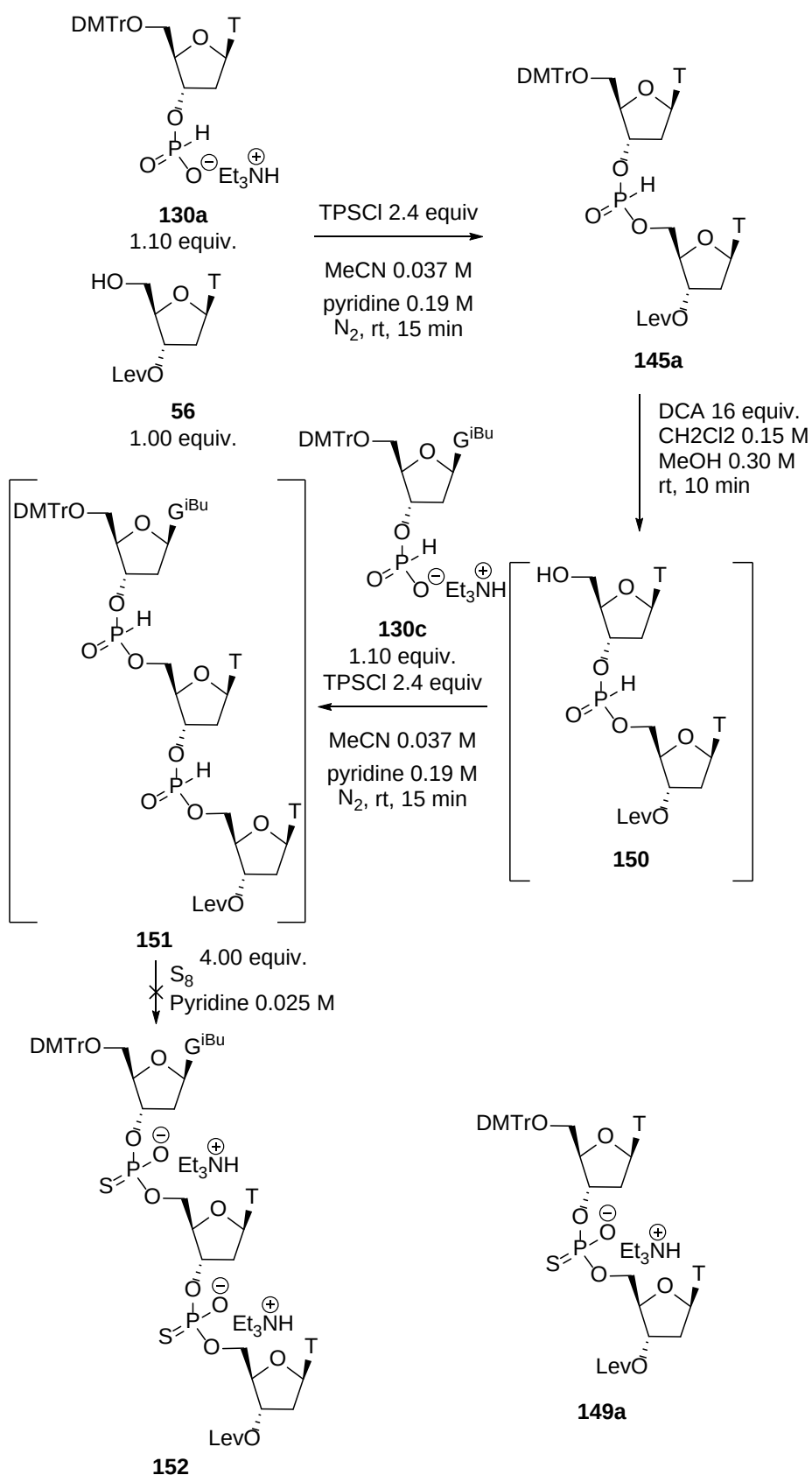
Scheme 95: Synthesis of phosphorothioate dimers.

Entry	Compound	Base 1	Base 2	Yield %
1	149a	T	T	97
2	149b	T	GiBu	98
3	149c	ABz	T	97
4	149d	ABz	GiBu	96

Table 13: Yields of phosphorothioate dimers.

Having now achieved reasonable yields of the dimers, we then attempted to synthesise a trimer. As we knew that the H-phosphonates were difficult to column, we attempted to make the dimer **145a** as previously, remove the DMTr group using standard

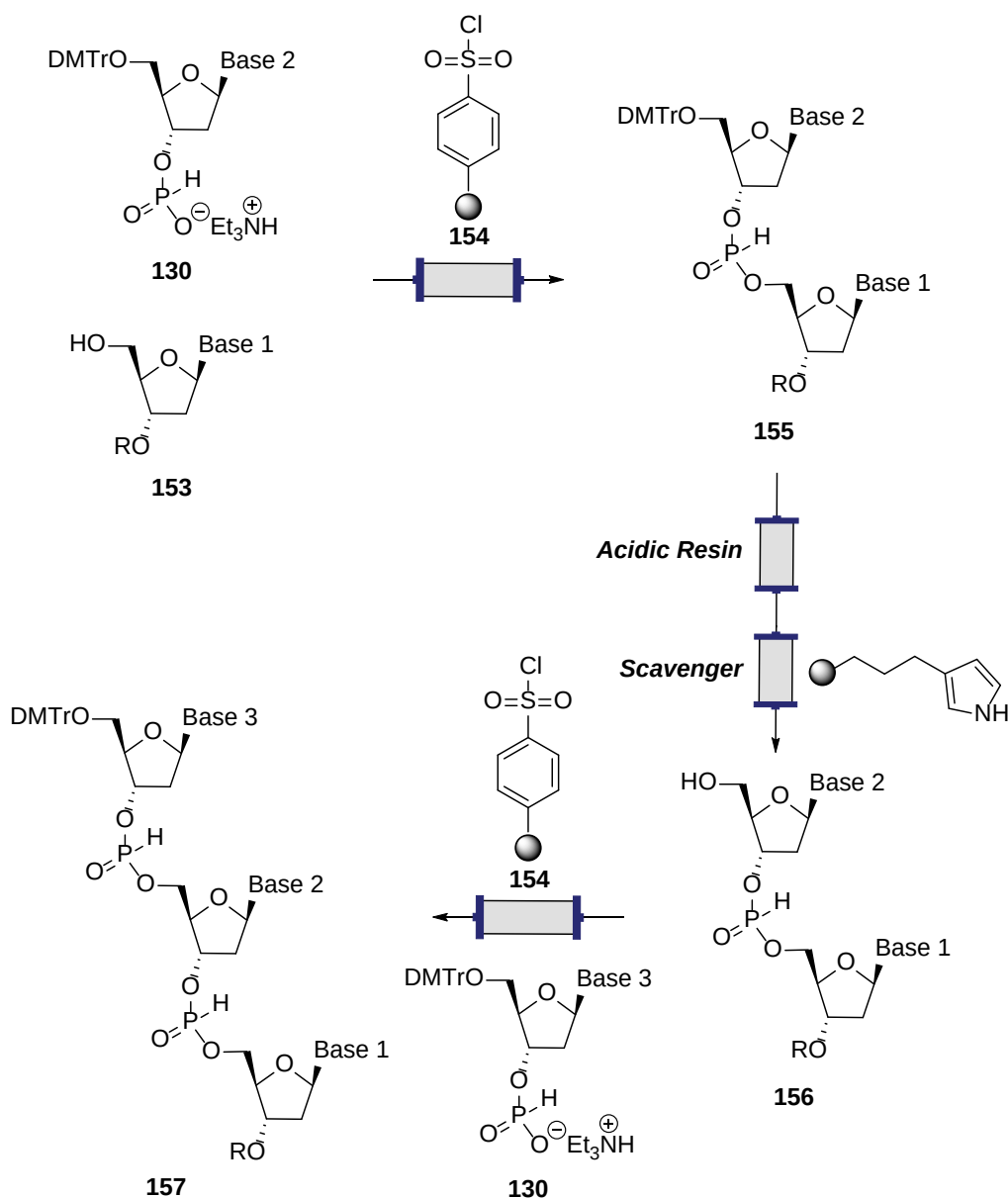
deprotection conditions, and then react this crude alcohol dimer **150** with another H-phosphonate nucleoside **130c**. This should have generated the H-phosphonate trimer **151**, however we could not detect this by mass spectrometry. We did attempt sulfurization of this reaction mixture to generate the phosphorothioate **152**, however no product was isolated cleanly. From the ^{31}P NMR one of the major products was the phosphorothioate dimer **149a**, indicating that the deprotection had not been successful.



Scheme 96: Attempted synthesis of phosphorothioate trimer **152**, and observed product **149a**.

Ideally, we would want to isolate the compound **150**, however our earlier work had indicated that this linkage was unstable to column chromatography. If we were to first oxidise or sulfurize the dimer, we could encounter problems of the phosphonate or phosphorothioate reacting with the TPSCI, lowering yields.

Therefore, in order to make this process applicable to the large scale synthesis of oligonucleotides we would need another way of cleanly generating the H-phosphonate dimer. As previously this has been done by using solid supported nucleosides, we thought it may be possible to do this transformation using a solid supported coupling reagent. Using a solid supported reagent such as polymer supported benzenesulfonyl chloride would be an improvement on current methods as you could potentially re-generate this compound using thionyl chloride.

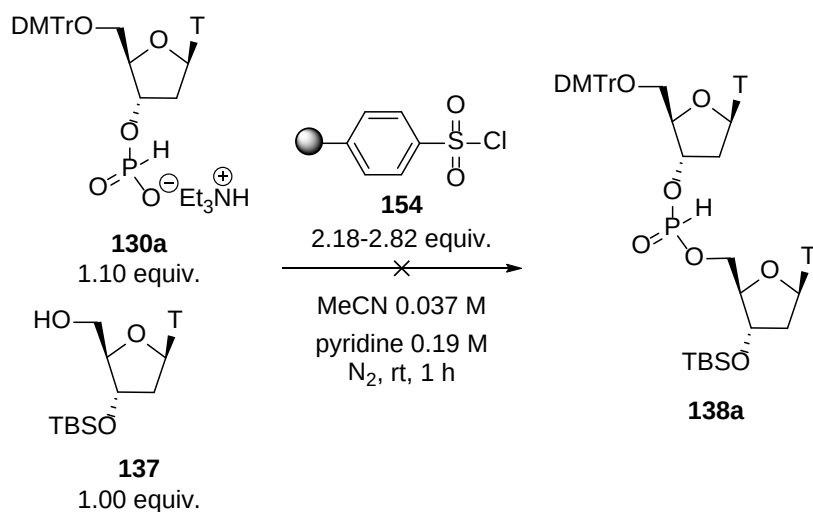


Scheme 97: Proposed synthesis of trimers using solid supported reagents.

This type of transformation has been done previously using solid supported acyl chloride by Morvan *et al.*, however we were unable to source the same resin, and as we had previously shown that the sulfonyl chloride would react more slowly with the free alcohol, we decided to pursue using solid-supported benzenesulfonyl chloride.⁵⁷

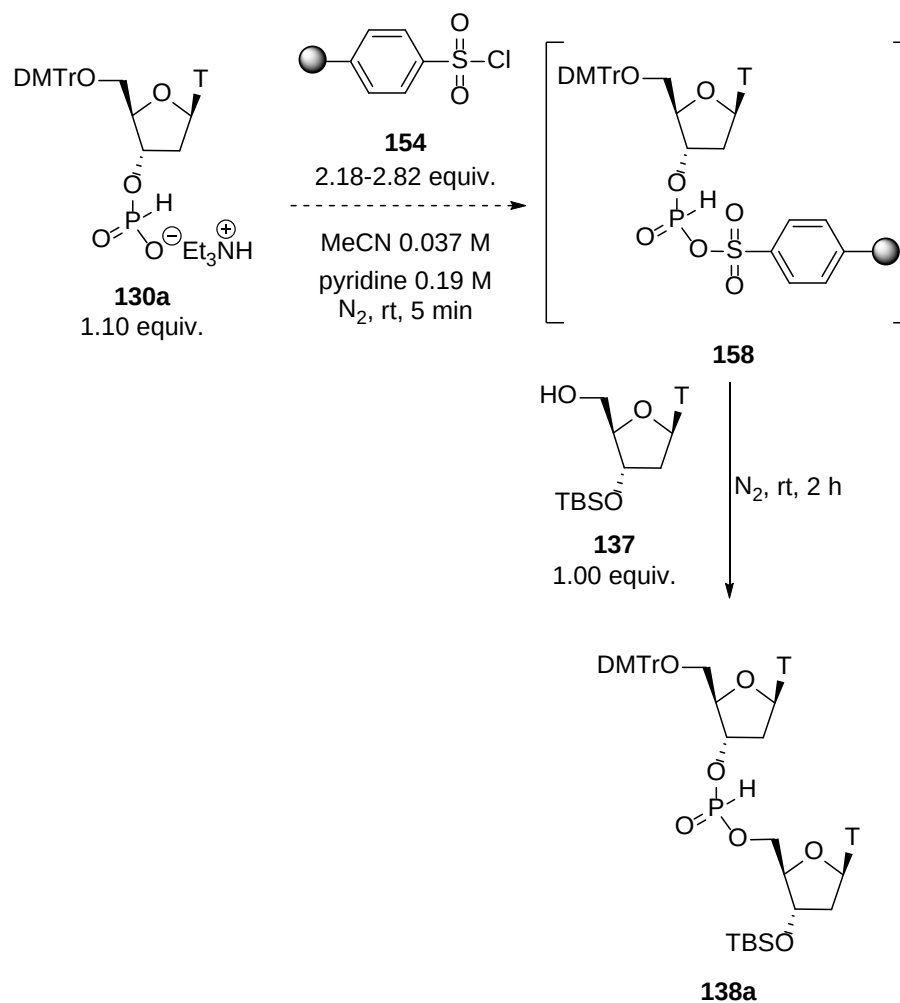
We first tried to replicate the conditions that we had previously had success with when using TPSCl, however no product was observed by

^{31}P NMR, and we saw full consumption of the alcohol **B** by TLC and LCMS.



Scheme 98: First attempt at using a solid supported coupling reagent.

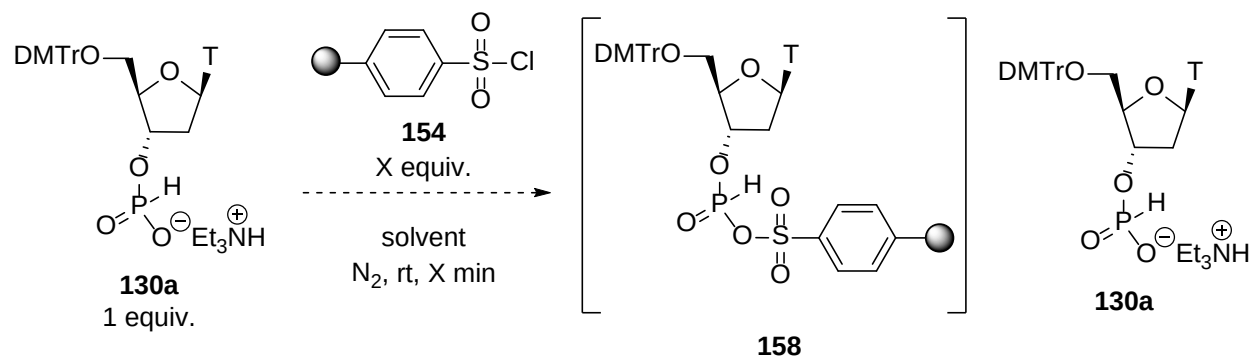
It appeared as though the solid supported reagent was preferentially reacting with the 5'OH of **137** instead of the H-phosphonate **130a**. Therefore, we changed the reaction set-up so that the H-phosphonate **130a** was premixed with the solid-supported reagent before adding the alcohol, Scheme 99.



Scheme 99: Reaction with pre-mixing of reagents.

Unfortunately when monitoring this reaction by LCMS, all that was seen was the H-phosphonate and alcohol, indicating that either the intermediate **158** was not forming, or that if it was, it wasn't reacting with the alcohol to form the desired product.

To see if the intermediate **158** was forming, we reacted the H-phosphonate with the solid-support, and then ran a ³¹P NMR on the reaction mixture to see how much of the H-phosphonate was remaining in the solution.

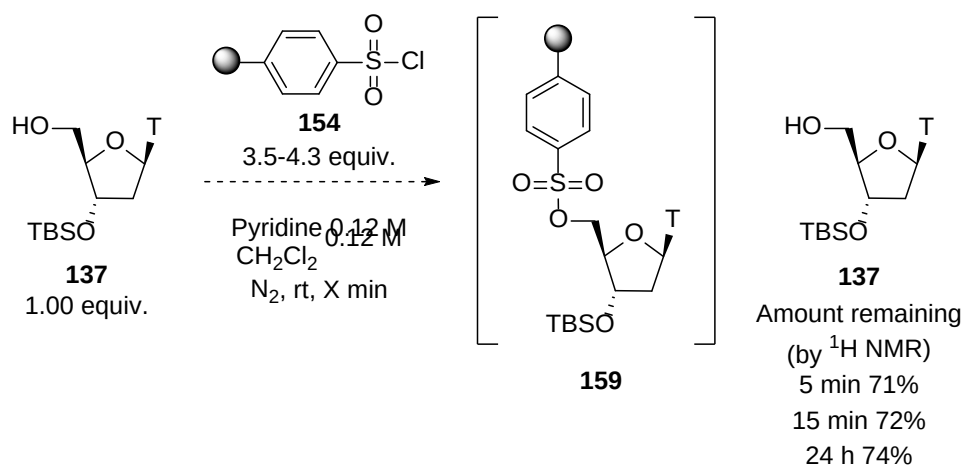


Scheme 100: Reaction of H-phosphonate and solid supported benzene sulfonyl chloride.

Entry	130a mmol	Solid support equiv.	Pyridine %total vol	MeCN %total vol	DCM %total vol	Total Molarity	% Remain 130a by ³¹ P NMR			
							5 min	15 min	30 min	60 min
1	0.022	2.18-2.82	16	84	-	0.034	77	73	82	81
2*	0.022	2.18-2.82	16	84	-	0.034	70	72	70	77
3	0.044	2.18-2.82	16	84	-	0.069	62	63	71	90
4	0.044	1.09-1.34	16	84	-	0.069	64	76	-	-
5	0.022	2.18-2.82	13	84	3	0.034	73	77	-	75
6**	0.030	3.50-4.30	50	-	50	0.060	54	56	54	47
7	0.030	7.00-9.00	50	-	50	0.060	56	51		
8	0.022	2.18-2.82	25	25	50	0.034	69	67		
9	0.022	3.50-4.30	16	84	-	0.034	75	73		
10	0.022	2.18-2.82	50	-	50	0.034	78	68		

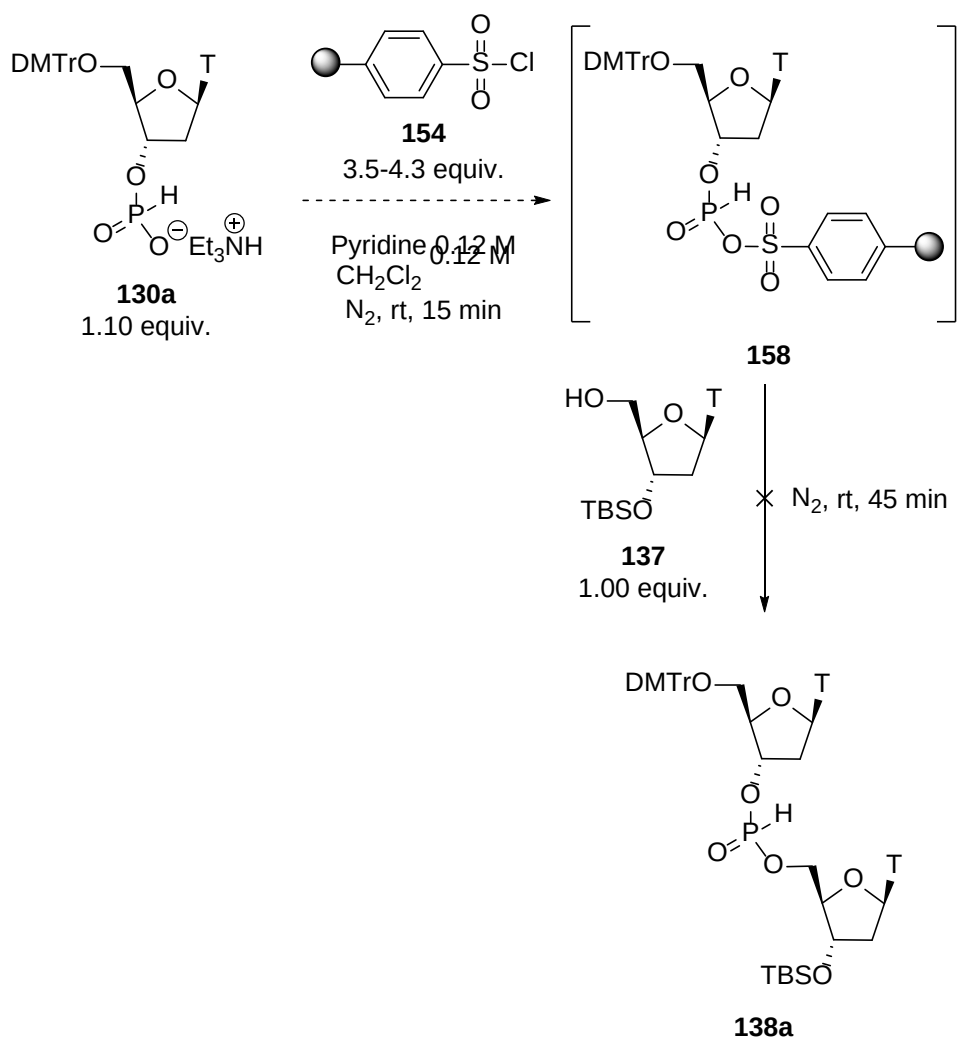
Table 14: Amount of H-Phosphonate **130a** remaining when reacting with solid support under various reaction conditions. Ph₃PO used as an internal standard. * fresh bottle of solid supported reagent used ** Reaction condition used by Morvan *et al.*⁵⁷

Table 14 shows the various solvent systems and equivalents of solid supported reagent tried. We found that using the literature conditions previously used by Morvan *et al.* for acyl solid supported reagents, resulted in the highest consumption of H-phosphonate. However, the conversion was still disappointingly only around 50%. We thought that if the alcohol was added to the reaction mixture after this reaction, this may cause the intermediate **158** to be used and therefore shift the equilibrium, eventually leading to full consumption of the alcohol. However, as we would still have excess solid supported reagent present in the reaction mixture we thought it prudent to check how much alcohol would react with the solid support under these conditions, Scheme 101.



Scheme 101: Consumption of alcohol **137** by benzene sulfonyl chloride on polystyrene. Homopiperonylnitrile used as an internal standard.

Pleasingly there was only a relatively small amount of consumption of the alcohol, even after 24 hours. We then attempted to apply these “optimised” conditions to the real system.



Scheme 102: Optimised conditions

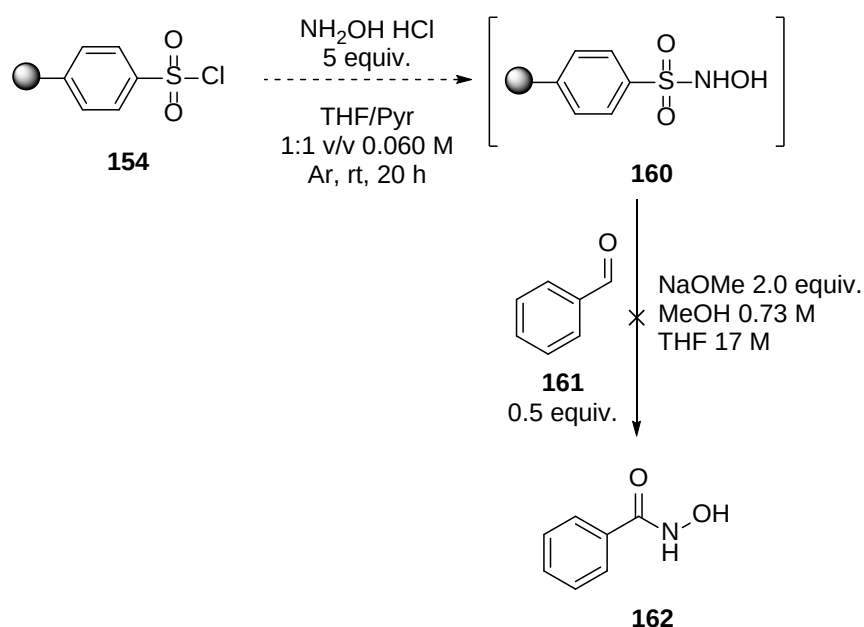
Entry	Alcohol mmol	% 138a by ³¹ P NMR	% 130a Remain by ³¹ P NMR
1	0.03	0	58%
2*	0.03	0	59%
3**	0.03	0	70%
4***	0.03	0	61%

Table 15: Results with optimised conditions. Ph₃PO used as an internal standard. * NMI 2 equivalents added. ** 40 °C used as temperature. ***

Reaction left stirring for 5 days.

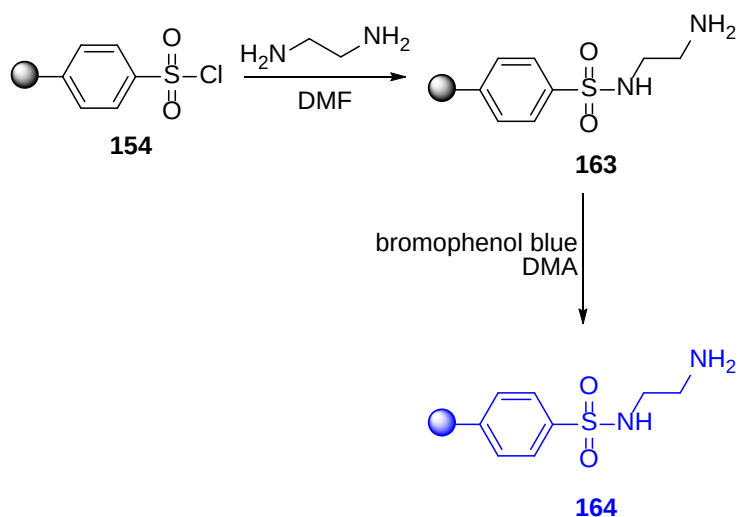
Unfortunately, we were unable to detect any of the desired product by ³¹P NMR in the reaction mixture. Because of this, we began to

wonder if the solid support was actually benzene sulfonyl chloride, and whether the loading of this on the solid support was as described on the bottle. There has been another reaction in which this solid support has been successfully used, by Porcheddu and Giacomelli for the synthesis of Hydroxamic Acids.⁵⁸ In this paper, polystyrene sulfonyl chloride is used to prepare a solid-supported N-hydroxybenzenesulfonamide reagent, which is then used in the Angeli-Rimini's reaction.



Scheme 103: Attempted Angeli-Rimini's reaction using solid supported reagent.⁵⁸

However when we attempted to repeat this with our supply of solid support we did not observe the formation of the hydroxamic acid **162**. In the paper, Porcheddu and Giacomelli check the formation of **160** by performing a "simple bead staining test". In this bromophenol blue test (Scheme 104), they detect the presence of unreacted sulfonyl chloride.



Scheme 104: Bromophenol blue colourimetric assay.

In this protocol “a few beads” are treated with 5% ethylenediamine in DMF for 5 min to form the sulfonamide-linked primary amine **163**, and then these are stained with a few drops of 1% bromophenol blue in DMA.⁵⁸ However when we attempted this with “a few beads” we did not stain the beads blue (Figure 23A). When we repeated this but reacted the beads with ethylenediamine for 2 hours we were able to observe staining of the beads (Figure 23B)



Figure 23: Attempted staining of beads after reacting with ethylenediamine for (A) 5 mins and (B) 2 hours.

This indicated that it was difficult for the ethylenediamine to react with the sulfonyl chloride, this could be due to lower than expected loading of the sulfonyl chloride, which could be in conjunction with it being sterically difficult to react with.

In order to fully test the hypothesis of whether using a solid-supported benzene sulfonyl chloride would lead to higher yields and a higher purity of the H-phosphonate dinucleotides would require synthesis of the solid-support.

Chapter 3

Conclusions and Future Work

In the first part of this thesis we attempted to synthesise H-phosphinates using catalysis. We found that using H-phosphinic acid was not applicable for the synthesis of nucleotides due to the high acidity, leading to removal of the alcohol protecting groups. We developed conditions for the synthesis of H-phosphinates using copper catalysis and anilinium hypophosphite, however were unable to use this with nucleosides as the alcohol due to the insolubility in the solvent system. Other solvent systems were tried, however no reaction took place.

In the second part of this thesis we have successfully synthesised menthyl H-phosphonate using palladium catalysis from anilinium hypophosphite and have shown the reaction pathway goes through an esterification to the H-phosphinate and then slow oxidation to the H-phosphonate. We found that there was a competing reaction of the oxidation of the anilinium hypophosphite to mono-anilinium phosphite, and when using a more sterically demanding alcohol such as 3'OBn Thymidine this would out compete the esterification.

We then optimised Stawinski's previously published synthesis of H-phosphonates from phosphonic acid (which we synthesised from H-phosphinic acid), for the synthesis of a variety of H-phosphonates in good yield (87-98 %) as well as using this for the synthesis of a 2'F RNA H-phosphonate (**119**).⁵³ We then attempted the synthesis of dinucleotide H-phosphonates and optimised the coupling reagent and isolation procedure to obtain a variety of purine and pyrimidine containing dimers in good yields (59-85 %). We found that when we oxidised the crude dimers we could obtain the phosphonate dimers in

excellent yields (88-98 %), including the synthesis of a dimer using the 2'F RNA H-phosphonate. We found the best yields were obtained when the H-phosphonate dimers were sulfurized to the corresponding phosphorothioate dimers, and obtained these in excellent yields (96-98 %). We then attempted the synthesis of a trimer, however encountered difficulties due to the difficulty in purifying the H-phosphonate dimers, and proposed the synthesis of these using solid-supported benzene sulfonyl chloride. We attempted to optimise these conditions, but found that our available supply of solid-supported reagent was not suitable.

We can conclude that the synthesis of oligonucleotides from a phosphorus source other than PCl_3 is feasible, but will only be useful for the large scale needed for medicinal chemistry purposes if the purification and isolation can be improved. Therefore it will be prudent to attempt to synthesise other solid-supported coupling reagents, so this reaction can be optimised in flow.

Chapter 4

Experimental

4.1 General Considerations

Starting materials were obtained from commercial suppliers and were used without further purification apart from hypophosphorous acid. Pre-dried hypophosphorus acid was obtained by concentrating Aqueous hypophosphorous acid (50 wt %) under vacuum for 5 hours at 60 °C. Anilinum hypophosphite was prepared as previously described.⁵⁹ All reactions were carried out in flame dried glassware and were carried out under atmospheric conditions at room temperature unless otherwise stated.

Anhydrous CH_2Cl_2 and MeCN were distilled from CaH_2 and DMF under reduced pressure over CaSO_4 and stored over 4Å molecular sieves according to literature procedures. Dry and degassed Toluene and THF were obtained by passing through anhydrous alumina columns using an Innovative Technology Inc. PS- 400-7 solvent purification system. The solvents used were all analytical grade reagents. The term 'petrol' refers to petroleum ether (40-60 °C fraction) and 'ether' refers to diethyl ether solvent. All aqueous solutions were prepared using deionized water. Brine refers to aqueous saturated sodium chloride. TEAB refers to Triethyl ammonium buffer, which was prepared by bubbling CO_2 through a aqueous solution of triethylamine until pH 7 was obtained.

Thin layer chromatography (TLC) was carried out using Merck silica gel precoated glass plates as the stationary phase. The TLC plate was then visualized under UV light at 254 and/or 360 nm then stained with either basic potassium permanganate solution. The flash column

chromatography was carried out using Merck silica gel 60, 35-70 μm as the stationary phase according to the procedure of Still *et al.* Infra-red spectra were obtained using a Perkin Elmer 1600 FTIR spectrometer. Spectra were acquired as dilute sample solutions in CHCl_3 or as thin films on a NaCl disc.

Mass spectra were obtained using a Bruker MicroTOF (Time of Flight) mass spectrometer using ElectroSpray Ionisation (ESI).

Melting points were measured using a Gallenkamp variable heater melting point apparatus.

NMR spectra were obtained at 298 K unless otherwise stated at the stated frequency using a Bruker DPX-400 (400 MHz), Bruker AV-400 (400 MHz), Bruker AV3-400 (400 MHz) or Bruker AV3-400hd (400 MHz). The samples were acquired using dilute solutions in CDCl_3 unless otherwise stated. The samples were referenced to a residual solvent peak (CHCl_3 7.26 ppm, 77.16 ppm). The ^{31}P NMR spectra are recorded with H_3PO_4 as an external standard unless otherwise stated, intermediates and final products were run with longer relaxation times to confirm NMR yields were not affected by NOE. All coupling constants are reported in Hertz (Hz) and the multiplicities of the signals labelled are abbreviated as singlet (s), doublet (d), double doublet (dd), dt (doublet of triplets) and t (triplet), br (broad), m (multiplet) and sometimes apparent (app) or obscured (obs). The protons of the furanose ring of the sugars are labelled *H*-1', *H*-2', *H*-3' etc. to distinguish them from the protons of the heterocyclic

nucleobases (5-H, 6-H etc.). Assignments were made using 2-dimensional NMR spectroscopy techniques.

4.2 General Procedures for Reaction Conditions Screenings, by Table

Tables 3 and 4

To a stirred solution of catalyst (X mol%) in anhydrous toluene or THF was added anilinium hypophosphite (X mmol) and menthol (1.6 mmol) and reaction mixture was heated at reflux under argon atmosphere for X hours. NMR sample was then taken directly.

Table 5

To a stirred solution of CuI (5 mol%) in anhydrous solvent (3.8 mL, 0.42 M) was added anilinium hypophosphite (1.6 mmol) and **110** (1.6 mmol) and reaction mixture was heated at X °C under argon atmosphere for X hours. NMR sample was then taken directly.

Tables 7 and 8

To a stirred solution of catalyst (X mol%) in solvent (10 mL, 0.32 M) was added pre-dried H₃PO₂ (317 mg, 1.5 equiv., 4.8 mmol), menthol (499 mg, 1.0 equiv., 3.2 mmol) and alkene/alkyne, and reaction mixture was heated at X °C under nitrogen atmosphere over X hours. After cooling down, the reaction was filtered through Celite®, washed with EtOAc and concentrated *in vacuo*. The resulting oil was dissolved in EtOAc and washed with brine. The organic layer was extracted with 0.5 M aqueous NaHCO₃, then water. The combined aqueous layers were acidified with 2 M aqueous HCl until pH 1, then extracted with EtOAc. The combined organic layers were washed with brine, dried

over MgSO_4 and concentrated in *vacuo* to afford the H-menthyl phosphonate in the acid form.

Table 9

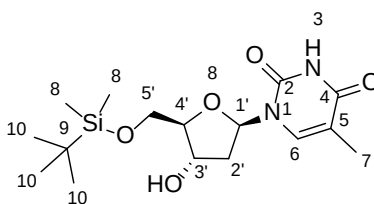
To a stirred solution of Pd/C (94.5 mg, 10 wt%, 2.5 mol%, 0.080 mmol) in anhydrous toluene : MeCN (10 mL, 1:1, v.v, 0.32 M) was added one of the salts **88b-d** (3.2 mmol, 1 equiv.), menthol (499 mg, 1.0 equiv., 3.2 mmol) and cyclohexene (0.65 mL, 2.0 equiv., 6.4 mmol) and reaction mixture was heated at 85 °C under nitrogen atmosphere over 24 hours. After cooling down, the reaction was filtered through Celite®, washed with EtOAc and concentrated *in vacuo*. The NMR was then taken on this material.

Table 14

To a stirred solution of benzenesulfonyl chloride, polymer-supported, 1.8-2.2 mmol/g on polystyrene in dry MeCN, dry pyridine and dry CH_2Cl_2 was added a solution of H-Phosphonate in dry MeCN, dry pyridine and dry CH_2Cl_2 . Reaction mixture was stirred for X min/hour then dissolved in deuterated DMSO, filtered and mixed with 1 equivalent of PH_3PO before an NMR was ran.

4.3 Protected Nucleosides

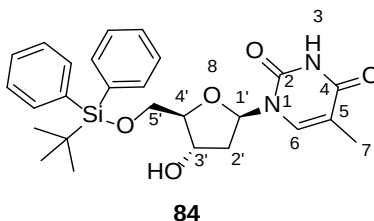
5'-OTBS thymidine, 103



Thymidine (3.00 g, 12.4 mmol) and imidazole (1.65 g, 28.7 mmol) were dissolved in anhydrous DMF (45.0 mL) under argon. To this was added *tert*-butyldimethylsilyl chloride (2.25 g, 14.9 mmol) and reaction mixture was stirred under argon at room temperature for 23 hours. The colourless and clear solution was poured into crushed ice/water (60.0 mL) at which point a white precipitate formed, this was collected by vacuum filtration and the resulting white precipitate was azeotroped with toluene (3 × 30 mL) and dried to give the title product (4.31 g, 12.1 mmol, 98%) as a white powder. m.p. 195-198 °C; R_f 0.44 (10% MeOH in CH₂Cl₂); $\nu_{\max}/\text{cm}^{-1}$ (CHCl₃ soln) 3394, 3101, 2957, 2930, 2859, 2742, 1687 (C=O), 1507, 1259, 1127; $[\alpha]_D^{25} + 5.21$ (c 1.00, CHCl₃); ¹H NMR (270 MHz, CDCl₃) 9.65 (1H, br. s., NH), 7.53 (1H, s, H-6), 6.38 (1H, dd, $J=8.5, 5.5$ Hz, H-1'), 4.42 (1H, app. d, $J=5.5$ Hz, H-3'), 4.07-4.05 (1H, m, H-4'), 3.76 - 3.92 (2H, m, H-5'), 2.38 (1H, ddd, $J=13.5, 5.5, 2.0$ Hz, one of H-2'), 2.05 (1H, ddd, $J=13.5, 8.0, 6.0$ Hz, one of H-2'), 1.88 (3H, s, H-7), 0.88 (9H, s, H-10), 0.08 (6H, s, H-8); ¹³C NMR (68 MHz, CDCl₃) 164.2 (C, C-4), 150.8 (C-C2), 135.6 (C,C-6), 111.1 (C, C-5), 87.5 (CH, C-4'), 85.2 (CH, C-1'), 72.6 (CH, C-3'), 63.7 (CH₂, C-5'), 41.2 (CH₂, C-2'), 26.0 (CH₃, C-10), 18.4 (C, C-9), 12.7 (CH₃-C5) -5.3 (CH₃, C-8) -5.4 (CH₃,

C-8); HRMS (ESI+) calculated for $C_{16}H_{29}N_2O_5Si$ ($M+H$)⁺ 357.5015, found 357.5009.

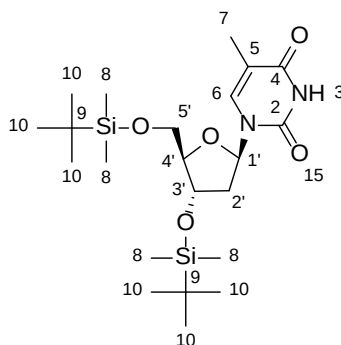
5'-OTBDPS thymidine, 119



Imidazole (204 mg, 6.60 mmol) was added to thymidine (726 mg, 3.00 mmol) in dry DMF (6.00 mL) under argon. To this was then added TBDPS-Cl (0.940 mL, 3.60 mmol) dropwise. Reaction was stirred under argon at room temperature for 2 days before adding TBDPS-Cl (0.160 mL, 0.200 equiv) and stirred for an additional 4 days. Water (20 mL) was added and this was extracted with ether (3 × 20 mL). Combined organic extracts were then washed with brine (2 × 20 mL), dried over $MgSO_4$ and solvent evaporated in *vacuo* to give the crude product. Purification by column chromatography (Ether:Petrol 2:1 to Ether:Petrol 3:1) gave the title product (830 g, 1.73 mmol, 58%) as a white powder. m.p. 163-165 °C; ν_{max}/cm^{-1} ($CHCl_3$ soln) 3611, 3395, 3073, 3009, 2961, 2933, 2896, 2861, 1687, 1590, 1471, 1428, 1267; ^1H NMR (400 MHz, $CDCl_3$), 9.49 (1H, br.s., NH), 7.66 (4H, d, $J = 8, 1.5$ Hz, H -Ph), 7.50 (1H, s, H -6), 7.48-7.36 (6H, m, H -Ph), 6.43 (1H, dd, $J = 8.5, 5.5$ Hz, H -1'), 4.59 – 4.54 (1H, m, H -3'), 4.06 – 4.03 (1H, m, H -4'), 3.98 (1H, dd, $J = 11.5, 2.5$ Hz, one of H -5'), 3.86 (1H, dd, $J = 11.5, 2.5$ Hz, one of H -5'), 2.91 (1H, br.s., OH), 2.49 – 2.39 (1H, m, one of H -2'), 2.24 – 2.13 (1H, m, one of H -2'), 1.61 (3H, s, H -7), 1.08 (9H, s, H -Si(CH_3)₃); ^{13}C NMR (101

Mhz, CDCl₃) 164.1 (C, C-2), 150.8 (C, C-4), 135.4 (CH, C-6), 135.4, 133.0, 132.5, 130.1, 128.1 (C-Ph), 111.4 (C, C-5), 87.3 (CH, C-4'), 84.9 (CH, C-1'), 72.4 (CH, C-3'), 64.3 (CH₂, C-5'), 41.1 (CH₂, C-2'), 27.1 (CH₃, SiC(CH₃)₃), 19.5 (C, SiC), 12.2 (CH₃, C-7); HRMS (ESI) calculated for C₂₆H₃₂N₂NaO₅Si (M+Na)⁺ 503.1973, found 503.1986.

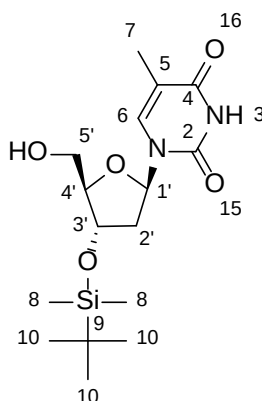
3'-OTBS, 5'-OTBS Thymidine



Imidazole (5.62 g, 82.5 mmol) was added to a solution of thymidine (5.00 g, 20.6 mmol) in dry DMF (30.0 mL). To this was then added TBS-Cl (6.54 g, 43.3 mmol) and reaction stirred at room temperature for 1 day before adding TBS-Cl (1.56 g, 10.3 mmol) and stirred for an additional day. The colourless and clear solution was poured into crushed ice/water (60.0 mL) at which point a white precipitate formed, this was collected by vacuum filtration and the resulting white precipitate was azeotroped with toluene (3 × 30 mL) and dried to give the title product (9.67 g, 20.6 mmol, 100 %) as a white powder. m.p 130-132 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2952, 2929, 2886, 2857, 1703, 1662, 1471, 1462, 1361, 1322, 1273, 1252, 1195, 1134, 1091, 1061, 1034, 1018, 1004. ¹H NMR (400 MHz, CDCl₃) 7.46 (1H, s, H-6), 6.32 (1H, dd, *J* = 7.9, 5.8 Hz, H-1'), 4.39 (1H, dt, *J* = 5.9, 2.6 Hz, H-4'), 3.92 (1H, q, *J* = 2.7 Hz, H-3'), 3.80 (2H, 2xdd, *J* = 11.4, 2.5 Hz, H-5'),

2.28 – 2.19 (1H, m, one of H-2'), 2.04 – 1.94 (1H, m, one fo H-2'), 1.90 (3H, s, H-7), 0.91 (9H, s, H-10 or H-13), 0.88 (9H, s, H-10 or H-13), 0.09 (6H, s, H-8 or H-11), 0.06 (6H, d, $J = 2.7$ Hz, H-8 or H-11). ^{13}C NMR (101 MHz, CDCl_3) 164.2 (C), 150.6 (C), 135.60 (CH, C6), 110.9 (C, C5) , 87.9 (CH, C3'), 84.9 (CH, C1'), 72.3 (CH, C4'), 63.1 (CH_2 , C5'), 41.5 (CH_2 , C2'), 26.04 (CH_3 , C10 or C13), 25.8 (CH_3 , C10 or C13), 18.5 (C, C9 or C12). 18.1 (C9 or C12) 12.7 (CH_3 , C7), -4.5 (CH_3 , C8 or C11), -4.7 (CH_3 , C8 or C11), -5.3 (CH_3 , C8 or C11), -5.3 (CH_3 , C8 or C11). HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{42}\text{N}_2\text{NaO}_5\text{Si}_2$ ($\text{M}+\text{Na}$) $^+$ 493.2524, found 493.2522.

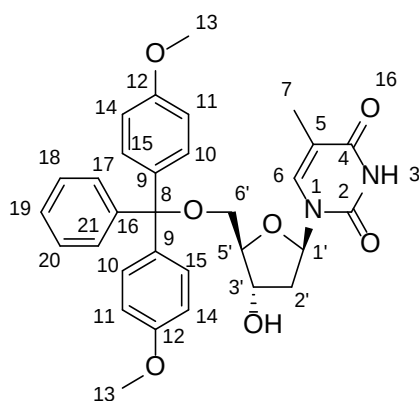
3'-OTBS Thymidine, 137



TFA/ H_2O (1.30 mL, 10:1, v/v) was added to a solution of 3'OTBS, 5'OTBS Thymidine (1.00 g, 2.12 mmol) in dry CH_2Cl_2 (11.0 mL). Reaction mixture was stirred at room temperature for 45 min then 0 °C for 1.5 hours. Reaction mixture was then diluted with ice-cold CH_2Cl_2 (2.6 mL), then organic layer was washed with ice-cold water (10 mL), sat. aq. NaHCO_3 (5.2 mL) and brine (2.6 mL), dried over MgSO_4 , filtered and concentrated in *vacuo* to give the crude product. Purification by column chromatography (EtOAc:Petrol 1:1) gave the

title product (434 mg, 1.22 mmol, 58 %) as a white powder. m.p. 80-83 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2952, 2929, 2887, 2857, 1702, 1664, 1471, 1402, 1361, 1323, 1274, 1251, 1196, 1134, 1093, 1060, 1034, 1005. ^1H NMR (400 MHz, CDCl_3) 9.30 (1H, s, NH), 7.39 (1H, s, H-6), 6.15 (1H, t, $J = 6.5$ Hz, H-1'), 4.48 (1H, dd, $J = 6.5, 3.5$ Hz, H-3'), 3.92 – 3.88 (2H, m, H-4' and one of H-5'), 3.82 – 3.70 (1H, m, one of H-5'), 2.98 (1H, br s, OH), 2.32 (1H, dt, $J = 13.5, 6.5$ Hz, one of H-2'), 2.20 (1H, ddd, $J = 13.5, 6.5, 3.5$ Hz, one of H-2'), 1.88 (3H, s, H-7), 0.88 (9H, s, H-10), 0.07 (6H, s, H-8). ^{13}C NMR (101 MHz, CDCl_3) 164.2 (C, C4), 150.6 (C, C2), 137.2 (CH, C6), 111.1 (C, C5), 87.7 (CH, C4'), 86.7 (CH, C1'), 71.7 (CH, C3'), 62.0 (CH_2 , C5'), 40.6 (CH_2 , C2') 25.8 (CH_3 , C10), 18.07 (C, C9), 12.6 (CH_3 , C7), -4.58 (CH_3 , C8), -4.73 (CH_3 , C8). HRMS (ESI) calculated for $\text{C}_{16}\text{H}_{28}\text{N}_2\text{NaO}_5\text{Si}$ ($\text{M}+\text{Na}$)⁺ 379.1660, found 379.1661

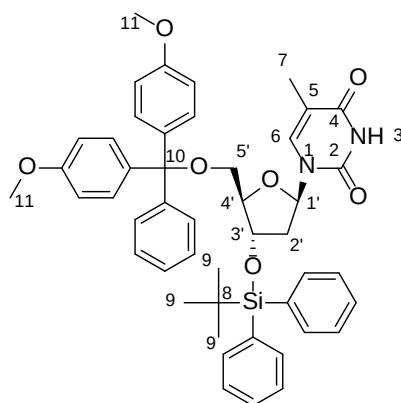
5'O-DMTr Thymidine, 53



Thymidine (5.00g, 20.6 mmol) was azeotroped with pyridine (3 x 30 mL) and was then dissolved in dry pyridine (30.0 mL) then cooled to 0 °C and DMTrCl (7.32 g, 21.6 mmol) was added in 4 portions over 30 minutes. Reaction mixture stirred under argon at RT for 15.5 hours

and was then quenched by addition of sat. aq. NaHCO₃ (50 mL) and water (50 mL). The aqueous layer was then extracted with CH₂Cl₂ (3 x 50 mL), then combined organic extracts were washed with water (2 x 50 mL) and brine (50 mL), dried over MgSO₄, filtered and concentrated *in vacuo* and then azeotroped with toluene (2 x 15 mL). Crude material was then dissolved in CH₂Cl₂ (20 mL) and hexane (75 mL) added, and this was heated until precipitate started to form. Filtration and further drying under vacuum afforded 5'ODMTr Thymidine as an off white solid (10.9 g, 20.1 mmol, 98%). m.p. 118-121 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3481, 3163, 3009, 2925, 2836, 1669, 1606, 1508, 1470, 1436, 1250, 1175, 1095. ¹H NMR (500 MHz, CDCl₃) 9.76 (1 H, s, NH), 7.62 (1 H, s, H6), 7.44 – 7.36 (2 H, m, DMTr), 7.31 – 7.18 (7 H, m, DMTr), 6.96 – 6.58 (4 H, m, DMTr), 6.45 (1 H, ddd, J 8.2, 6.0, 2.5, H-1'), 4.58 (1 H, dt, J 5.5, 2.5, H-3'), 4.10 (1 H, app p, J 2.5, H-4'), 3.77 (6 H, s, OMe DMTr), 3.50 (1 H, br s, OH), 3.45 (1 H, dt, J 10.6, 2.5, one of H-5'), 3.36 (1 H, dt, J 10.6, 2.5, one of H-5'), 2.45 (1 H, ddd, J 13.7, 5.5, 2.5, one of H-2'), 2.32 (1 H, dtt, J 13.7, 8.2, 5.5 one of H-2'), 1.44 (3 H, s, H7). ¹³C NMR (126 MHz, CDCl₃) 164.3 (C, C4), 158.7 (C, DMTr), 150.9 (C, C2) 144.4 (C, DMTr), 135.9 (CH, C6), 135.5 (C, DMTr), 135.4 (C, DMTr), 130.2 (CH, DMTr), 129.2 (CH, DMTr), 128.2 (CH, DMTr), 128.1 (CH, DMTr), 127.9 (CH, DMTr), 127.8 (CH, DMTr), 127.2 (CH, DMTr), 113.3 (CH, DMTr), 113.2 (CH, DMTr), 111.4 (C, C5) 87.0 (CH, C4'), 86.5 (C, DMTr), 84.9 (CH, C1'), 72.6 (CH, C3'), 63.8 (CH₂, C5'), 55.3 (CH₃, OMe DMTr), 41.0 (CH₂, C2'), 11.9 (CH₃, C7). HRMS (ESI) calculated for C₃₁H₃₂N₂NaO₇ (M+Na)⁺ 567.2102, found 567.2102.

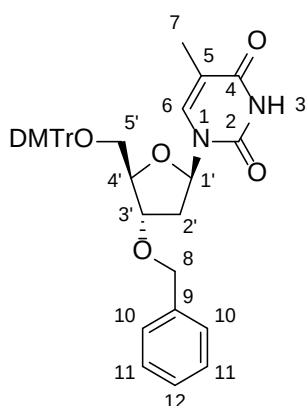
3'-OTBDPS, 5'-ODMTr Thymidine



Imidazole (1.38 g, 20.2 mmol) was added to a solution of 5'DMTr thymidine (5.00 g, 9.17 mmol) in dry DMF (11 mL) followed by TBDPS-Cl (2.60 mL, 10.1 mmol). Reaction mixture was stirred at RT for 18 hours, then partitioned between ether (60 mL) and water (60 mL). Aqueous layer was extracted with ether (3 x 60 mL), then combined organic extracts were washed with brine (2 x 80 mL), dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (3:1 Ether:Petrol) afforded the product as a white solid (4.96 g, 6.33 mmol, 69%). m.p. 91-96 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2930, 2857, 1684, 1607, 1507, 1462, 1427, 1364, 1292, 1273, 1247, 1175, 1103, 1063, 1031. ^1H NMR (400 MHz, CDCl_3) 8.88 (1H, s, NH) 7.76 – 7.08 (20H, m, ArH and H-6), 6.82-6.78 (4H, m, ArH) 6.56 (1H, dd, J = 8.5, 5.5 Hz, H-1'), 4.64 – 4.56 (1H, m, H-3'), 4.12 (1H, q, J = 2.5 Hz, H-4'), 3.83 (6H, s, H-11), 3.28 (1H, dd, J = 10.5, 2.5 Hz, one of H-5'), 2.94 (1H, dd, J = 10.5, 2.5 Hz, one of H-5'), 2.43 (1H, ddd, J = 13.0, 5.5, 2.0 Hz, one of H-2'), 2.13 (1H, ddd, J = 13.0, 8.5, 5.5 Hz, one of H-2'), 1.40 (3H, s, H-7), 1.09 (9H, s, H-9). ^{13}C NMR (101 MHz, CDCl_3) 163.9 (C), 158.7 (C), 158.7 (C), 150.5 (C), 144.4 (C), 135.8 (CH), 135.8 (CH), 135.7 (CH), 135.4 (CH), 135.4 (CH),

133.2 (CH), 133.2 (CH), 130.1 (CH), 130.1 (CH), 130.1 (CH), 128.2 (CH), 128.0 (CH), 128.0 (CH), 127.9 (CH), 127.1 (CH), 113.3 (CH), 111.2 (C) 86.9 (CH, C-4'), 85.0 (CH, C-1'), 74.1 (CH, C-3'), 63.4 (CH₂, C-5'), 55.4 (CH₃, C-11), 41.3 (CH₂, C-2'), 27.0 (CH₃, C-9), 19.1 (C), 11.8 (CH₃, C-7). HRMS (ESI) calculated for C₄₇H₅₀N₂NaO₇Si (M+Na)⁺ 805.3279, found 805.3277.

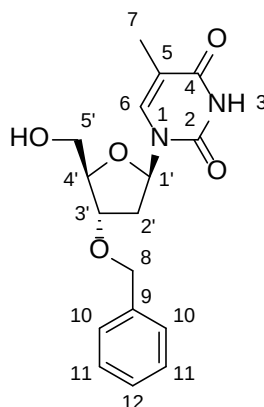
3'-OBn, 5'-ODMTr Thymidine, 126



NaH (60% dispersion in mineral oil) (1.10 g, 27.5 mmol) was added to a solution of 5'ODMTr Thymidine (5.99 g, 11.0 mmol) in dry THF (100 mL) at 0 °C under a flow of argon. Reaction mixture stirred at 0 °C for 30 min, the room temperature for 45 min. Reaction mixture was then cooled to 0 °C and benzyl bromide (1.60 mL, 13.8 mmol) was added under a flow of argon. Reaction stirred at 0 °C for 30 min, RT for 30 min then 60 °C for 2.5 hours. Reaction mixture was then quenched with water (2.00 mL) and concentrated *in vacuo*, and then partitioned between CH₂Cl₂ (75 mL) and water (75 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL), then combined organic extracts were washed with brine (1 x 50 mL), dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography

(EtOAc:Petrol 1:1 with 1% Et₃N) afforded 3'OBn 5'ODMT_r Thymidine (5.43 g, 8.55 mmol, 78%). m.p 112-116 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl₃ soln) 3176, 3034, 2929, 2865, 1683, 1606, 1582, 1507. 1462, 1362, 1248, 1175, 1092, 1030. ¹H NMR (400 MHz, CDCl₃) 9.32 (1 H, s, NH), 7.63 (1 H, d, J 1.1, H-6), 7.42 – 7.26 (14 H, m, DMTr and H10-12), 6.92 – 6.79 (4 H, m, DMTr), 6.42 (1 H, dd, J 7.8, 5.9, H-1'), 4.53 (2 H, app q, J 25.0, 12.0, H8), 4.35 (1 H, dt, J 5.7, 2.7, H-3'), 4.22 (1 H, q, J 2.9, H-4'), 3.82 (6 H, s, DMTr OMe), 3.50 (1 H, dd, J 10.5, 2.9, one of H-5'), 3.34 (1 H, dd, J 10.5, 2.9, one of H-5'), 2.58 (1 H, ddd, J 13.7, 5.9, 2.7, one of H-2'), 2.24 (1 H, ddd, J 13.7, 7.8, 6.4, one of H-2'), 1.50 (3 H, d, J 1.1, H-7). ¹³C NMR (101 MHz, CDCl₃) 164.1 (C, C4) 158.8 (C, DMTr), 150.5 (C, C2), 144.4 (C, DMTr), 137.5 (CH, Ar), 135.7 (C, C6), 135.5 (C, DMTr), 130.1 (CH, DMTr), 130.1 (CH, DMTr), 128.6 (CH, Ar), 128.2 (CH, DMTr), 128.1 (CH, Ar), 128.0 (CH, DMTr), 127.7 (CH, Ar), 127.2 (CH, DMTr), 113.3 (CH, DMTr), 111.2 (C, C5), 86.9 (C, Ar), 84.9 (CH, C1'), 84.2 (CH, C4'), 78.7 (CH, C3'), 71.4 (CH₂, C8), 63.6 (CH₂, C5'), 55.3 (CH₃, DMTr OMe), 38.1 (CH₂, C2'), 11.9 (CH₃, C7). HRMS (ESI) calculated for C₃₈H₃₈N₂NaO₇ (M+Na)⁺ 657.2571, found 657.2569.

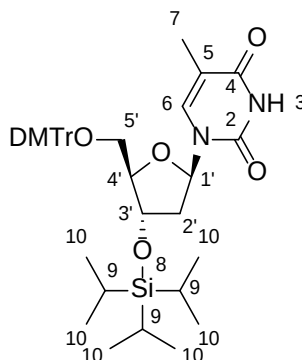
3'-OBn Thymidine, 110



5'ODMTTr 3'OBn Thymidine (635 mg, 1.00 mmol) was added to a stirring solution of pTSA monohydrate (561 mg, 2.95 mmol) in MeOH (25 mL) and CH₂Cl₂ (56 mL) at 0 °C. Reaction mixture was stirred at 0 °C for 25 minutes after which time sat. aq. NaHCO₃ (100 mL) and reaction stirred for 10 min. Layers were separated, and aqueous layer extracted with CH₂Cl₂ (3 x 50 mL) and then combined organic extracts were then washed with brine (1 x 100 mL). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (100% EtOAc) afforded 3'OBn Thymidine as a white solid (243 mg, 0.730 mmol, 73 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl₃ soln) 3485, 3156, 3089, 3013. 2917, 2877, 1713, 1659, 1477, 1396, 1282, 1259. ¹H NMR (400 MHz, CDCl₃) 9.09 (1 H, s, NH), 7.37 – 7.29 (5 H, m, H10-12), 6.14 (1 H, dd, J 7.7, 6.3, H-1'), 4.64 – 4.45 (2 H, app q, 11.7, 9.6, H8), 4.29 (1 H, app d, J 6.1, 3.2, H-3'), 4.15 (1 H, app q, J 3.0, H-4'), 3.91 (1 H, dt, J 12.0, 3.0, one of H-5'), 3.75 (1 H, dq, J 12.0, 3.0, one of H-5'), 2.87 (1 H, br s, OH), 2.48 – 2.27 (2 H, m, H-2'), 1.89 (3 H, d, J 1.2, H7). ¹³C NMR (101 MHz, CDCl₃) 164.0 (C, C4), 150.5 (C, C2), 137.6 (C, C9), 137.2 (CH, Ar), 128.7 (CH, Ar), 128.1 (CH, Ar), 127.8 (CH, Ar), 111.2 (C, C5), 87.4 (CH, C1'), 85.3 (CH,

C4'), 78.7 (CH, C3'), 71.7 (CH₂, C8), 62.9 (CH₂, C5'), 37.3 (CH₂, C2'), 12.6 (CH₃, C7). HRMS (ESI) calculated for C₁₇H₂₀N₂NaO₅ (M+Na)⁺ 355.1264, found 355.1255.

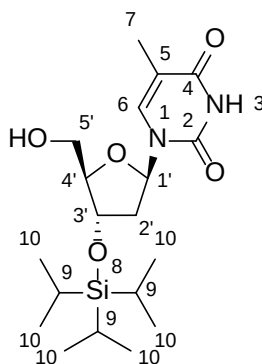
3'-OTIPS, 5'-ODMTr Thymidine



Imidazole (974 mg, 14.3 mmol) was added to a solution of 5'ODMTr thymidine (3.00g, 5.50 mmol) in dry DMF (5.50 mL). Reaction cooled to 0 °C and TIPSCI (1.53 mL, 7.15 mmol) was added dropwise. Reaction mixture left to reach RT and stirred for 22 hours before adding Imidazole (749 mg, 11.0 mmol), cooling to 0 °C, adding TIPSCI (1.20 mL, 5.50 mmol) and stirred for an additional 19.5 hours. Reaction mixture was then portioned between EtOAc (50 mL) and water (50 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and then the combined organic extracts washed with brine (3 x 50 mL). The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (ether:pentane 2:1 with 1% Et₃N) afforded 3'OTIPS 5'ODMTr Thymidine as a white solid (2.36 g, 3.37 mmol, 61 %). m.p. 58-61 °C, ν_{max} /cm⁻¹ (CHCl₃ soln) 2942, 2865, 2837, 1686, 1607, 1508, 1461, 1300, 1248, 1174, 1151, 1099, 1065, 1033. ¹H NMR (400 MHz, CDCl₃) 8.88 (1 H, br s, NH), 7.70 (1 H, app d, J 1.2, H6), 7.45 – 7.39

(2 H, m, DMTr), 7.36 – 7.23 (7 H, m, DMTr), 6.94 – 6.79 (4 H, m, DMTr), 6.43 (1 H, dd, J 7.9, 5.9, H-1'), 4.61 (1 H, dt, J 5.4, 2.4, H-3'), 4.07 (1 H, q, J 2.4, H-4'), 3.81 (6 H, s, DMTr OMe), 3.52 (1 H, dd, J 10.8, 2.7, one of H-5'), 3.32 (1 H, dd, J 10.8, 2.8, one of H-5'), 2.42 (1 H, ddd, J 13.2, 5.9, 2.4, one of H-2'), 2.26 (1 H, ddd, J 13.5, 7.8, 5.9, one of H-2'), 1.53 (3 H, app d, J 1.1, H7), 1.01 (21 H, m, Si(CH₃)₃(CH₃)₆). ¹³C NMR (101 MHz, CDCl₃) 164.0 (C, C4), 158.8 (C, DMTr), 150.4 (C, C2), 144.4 (C, DMTr), 135.8 (CH, C6), 135.5 (C, DMTr), 135.5 (C, DMTr), 130.2 (CH, DMTr), 128.2 (CH, DMTr), 128.1 (CH, DMTr), 127.2 (CH, DMTr), 113.4 (CH, DMTr), 113.3 (CH, DMTr), 111.2 (C, C5), 87.5 (CH, C4'), 87.0 (C, DMTr), 85.1 (CH, C1'), 73.0 (CH, C3'), 63.5 (CH₂, C5'), 55.4 (CH₃, OMe DMTr), 42.1 (CH₂, C2'), 18.0 (CH₃, C10), 12.1 (CH, C9), 12.0 (CH₃, C7). HRMS (ESI) calculated for C₄₀H₅₂N₂NaO₇Si (M+Na)⁺ 723.3436, found 723.3434.

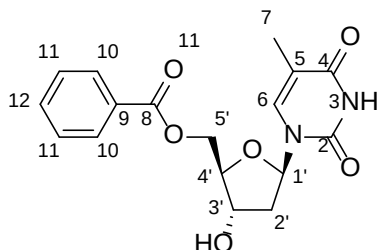
3'-OTIPS Thymidine, 112



3'OTIPS 5'ODMTr Thymidine (2.00 g, 2.86 mmol) in CH₂Cl₂ (59.0 ml) was added to a solution of pTSA monohydrate (1.60 g, 8.44 mmol) in MeOH (72.0 ml) and CH₂Cl₂ (100 ml) at 0 °C slowly. Reaction mixture stirred at 0 °C for 45 min after which time sat. aq. NaHCO₃ (60 mL) was added and reaction stirred for 10 min. Layers were then

separated, and aqueous extracted with CH₂Cl₂ (3 x 50 mL) and then combined organic extracts washed with brine (1 x 50 mL). The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc:petrol 1:1) afforded 3'OTIPS Thymidine as a white solid (861 mg, 2.16 mmol, 76%). m.p. 81-84 °C, $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl₃ soln) 2941, 2865, 1676, 1463, 1273, 1132, 1096, 1059, 1032, 1012, ¹H NMR (400 MHz, CDCl₃) 9.24-9.14 (1 H, br s, NH), 7.40 (1 H, s, H-6), 6.16 (1 H, dd, J 7.5, 6.4, H-1'), 4.59 (1 H, dt, J 6.0, 2.9, H-3'), 3.99 (1 H, q, J 2.9, H-4'), 3.91 (1 H, dd, J 12.0, 2.9, one of H-5'), 3.77 (1 H, dd, J 12.0, 2.9, one of H-5'), 2.41 – 2.34 (1 H, m, one of H-2'), 2.24 (1 H, ddd, J 13.1, 6.4, 3.0), 1.89 (3 H, s, CH₃-5), 1.15 – 0.93 (21 H, m, Si(CH)₃(CH₃)₆). ¹³C NMR (101 MHz, CDCl₃) 164.1 (C, C4), 150.6 (C, C2), 137.4 (CH, C6), 111.1 (C, C5), 88.3 (CH, C4'), 87.4 (CH, C1'), 72.5 (CH, C3'), 62.5 (CH₂, C5'), 40.9 (CH₂, C2'), 18.0 (CH₃, C10), 12.6 (CH, C9), 12.1 (CH₃, C7). HRMS (ESI) calculated for C₁₉H₃₄N₂NaO₅Si (M+Na)⁺ 421.2129, found 421.2129.

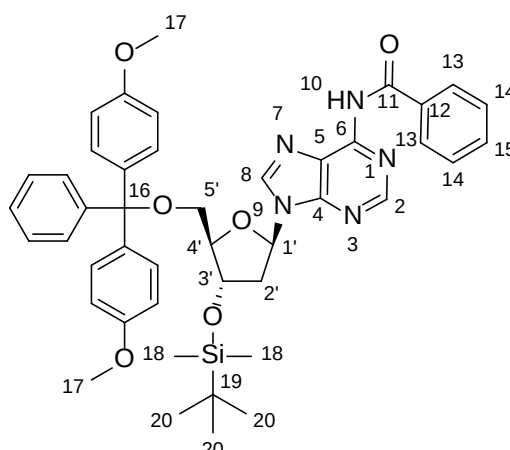
5' Benzoyl Thymidine



Thymidine (242 mg, 1.00 mmol) was azeotroped with dry pyridine (3 x 6.0 mL), dissolved in dry pyridine (6 mL) and then cooled to 0 °C. A solution of benzoyl chloride (0.13 mL, 1.10 mmol) in pyridine (6.0

mL) was then added dropwise over 2.75 h, then the reaction was stirred for an additional hour before quenching with MeOH (5 mL) and stirring for 15 min before reaction was then concentrated *in vacuo*. Purification by column chromatography (EtOAc) afforded 5' Benzoyl Thymidine as a white solid (168 mg, 0.486 mmol, 49%). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3366, 3183, 2977, 1714, 1654, 1476, 1441, 1361, 1315, 1262. ^1H NMR (400 MHz, DMSO- d_6) 11.31 (1 H, s, NH), 7.99 (2 H, dd, J 8.4, 1.3, H-10), 7.71 – 7.65 (1 H, m, H-12), 7.58 – 7.51 (2 H, m, H-11), 7.39 (1 H, app d, J 1.3, H-6), 6.21 (1 H, t, J 6.9, H-1'), 5.48 (1 H, br s, OH), 4.55 (1 H, dd, J 12.0, 3.7, H-3'), 4.42 (2 H, dt, J 10.9, 5.4, H-5'), 4.05 (1 H, dt, J 5.4, 3.7, H-4'), 3.33 (1 H, s), 2.30 – 2.12 (2 H, m, H-2'), 1.59 (3 H, s, H-7). ^{13}C NMR (101 MHz, DMSO) 165.6 (C, C8), 163.6 (C, C4), 150.4 (C, C2), 135.6 (CH, C6), 133.6 (CH, C12), 129.2 (CH, C10), 128.9 (CH, C11), 109.8 (C, C5), 83.8 (CH, C4'), 83.6 (CH, C1'), 70.2 (CH₂, C5'), 64.4 (CH, C3'), 38.8 (CH₂, C2'), 11.9 (CH₃, C7). HRMS (ESI) calculated for C₁₇H₁₉N₂O₆ (M+H)⁺ 347.1238, found 347.1239.

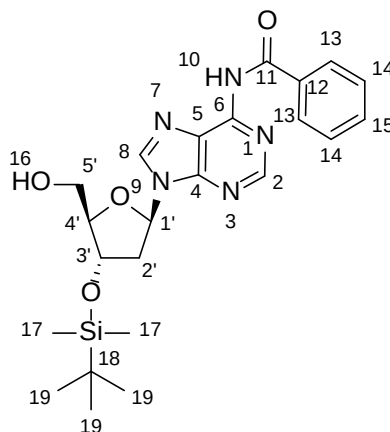
6-N-benzoyl-5'-ODMTr-3'-OTBS-2'-deoxyadenosine



5'-ODMTr A^{Bz} (1.97 g, 3.00 mmol) was dissolved in dry DMF (3 mL) under argon. Imidazole (898 mg, 13.2 mmol) was added, the reaction mixture was cooled to 0 °C and then TBS Cl (997 mg, 6.60 mmol) was added. Reaction was stirred for 21.5 hours, and then dissolved in EtOAc (10 mL) and washed with water (10 mL) and brine (2 x 10 mL). The combined aqueous washings were then extracted with EtOAc (3 x 10 mL), and the combined organic extracts were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc:petrol 4:1) afforded 5'-ODMTr 3'-OTBS A^{Bz} as a white solid (1.75 g, 2.27 mmol, 76%). $\nu_{\max}/\text{cm}^{-1}$ (ATR) 2952, 2928, 2855, 1700, 1607, 1579, 1508, 1454, 1413, 1327, 1298, 1247, 1175, 1092, 1031, 1004. ¹H NMR (400 MHz, CDCl₃) 9.19 (1 H, s, H-10), 8.77 (1 H, s, H-2), 8.24 (1 H, s, H-8), 8.10 – 8.00 (2 H, m, H-13), 7.65 – 7.58 (1 H, m, H-15), 7.53 (2 H, dd, *J* 8.2, 6.8, DMTr), 7.45 – 7.40 (2 H, m, H-14), 7.37 – 7.18 (8 H, m, DMTr), 6.82 (3 H, d, *J* 8.7, DMTr), 6.51 (1 H, t, *J* 6.5, H-1'), 4.64 (1 H, dt, *J* 5.8, 3.5, H-3'), 4.16 (1 H, td, *J* 4.5, 3.5, H-4'), 3.80 (6 H, s, H-17), 3.44 (1 H, dd, *J* 10.4, 4.5, one of H-5'), 3.33 (1 H, dd, *J* 10.4, 4.5, one of H-5'),

2.83 (1 H, ddd, J 13.0, 6.5, 5.8 one of H-2'), 2.49 (1 H, ddd, J 13.0, 6.5, 3.5), 0.92 – 0.87 (9 H, m, H-20), 0.11 – 0.01 (6H, m, H-18). ^{13}C NMR (101 MHz, CDCl_3) 164.7 (C, C11), 158.6 (C, DMTr), 152.7 (CH, C2), 151.5 (C, C6), 149.6 (C, C4), 144.6 (C, DMTr), 141.7 (CH, C8), 135.8 (C, DMTr), 133.8 (C, C12), 132.8 (CH, C15), 130.1 (CH, DMTr), 128.9 (CH, C13), 128.2 (CH, DMTr), 128.0 (CH, C13), 127.0 (CH, DMTr), 123.5 (C, C5), 113.3 (CH, DMTr), 87.2 (CH, C4'), 86.6 (C, DMTr), 85.0 (CH, C1'), 72.6 (CH, C3'), 63.3 (CH_2 , C5'), 55.3 (CH_3 , C17), 40.8 (CH_2 , C2'), 25.9 (CH_3 , C18), 18.1 (C, C19), -4.7 (CH_3 , C20). HRMS (ESI) calculated for $\text{C}_{44}\text{H}_{50}\text{N}_5\text{O}_6\text{Si}$ ($\text{M}+\text{H}$) $^+$ 772.3525, found 772.3529.

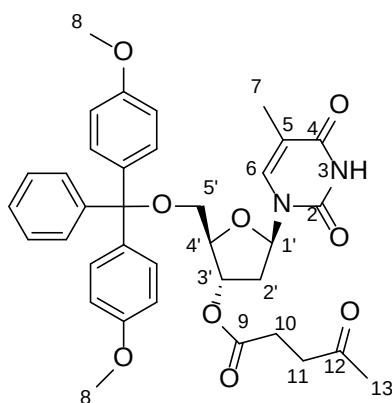
6-N-benzoyl-3'-OTBS-2'-deoxyadenosine



5'-ODMT 3'-OTBS A^{Bz} (2.30 g, 3.00 mmol) was dissolved in CH_2Cl_2 (40 mL) and then dichloroacetic acid (1.30 mL, 15 mmol) was added. The reaction was stirred for 15 min, then quenched with sat. aq. NaHCO_3 until pH 7, then aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL), combined organic extracts were then washed with brine, dried over MgSO_4 , filtered and solvent removed under reduced pressure. The crude product was purified with silica gel

chromatography 100% EtOAc to give the product as a white solid (850 mg, 1.81 mmol, 60 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3275, 2929, 2856, 1697, 1609, 1581, 1511, 1454, 1330, 1296, 1249, 1096, 1024. ^1H NMR (400 MHz, CDCl_3) 9.09 (1 H, s, H-10), 8.77 (1 H, s, H-8), 8.09 (1 H, s, H-2), 8.04 – 8.00 (2 H, m, H-14), 7.64 – 7.59 (1 H, m, H-15), 7.56 – 7.49 (2 H, m, H-13), 6.36 (1 H, dd, J 9.5, 5.5, H-1'), 4.71 (1 H, d, J 5.0, H-3'), 4.16 (1 H, app s, H-4'), 3.97 (1 H, d, J 12.0, one of H-5'), 3.77 (1 H, t, J 12.0, one of H-5'), 3.04 (1 H, ddd, J 13.0, 9.5, 5.0, one of H-2'), 2.26 (1 H, dd, J 13.0, 5.5, one of H-2'), 1.81 (1 H, br s, H-16), 0.93 (9 H, s, H-19), 0.12 (6 H, s, H-17). ^{13}C NMR (101 MHz, CDCl_3) 164.6 (C, C11), 152.2 (CH, C8), 150.8 (C, C6), 150.4 (C, C4), 142.7 (CH, C2), 133.6 (C, C12), 133.1 (CH, C15), 129.1 (CH, C13), 128.0 (CH, C14), 124.8 (C, C5), 90.6 (CH, C4'), 88.1 (CH, C1'), 74.1 (CH, C3'), 63.4 (CH_2 , C5'), 41.5 (CH_2 , C2'), 25.9 (CH_3 , C19), 18.2 (C, C18), -4.6 (CH_3 , C17). HRMS (ESI) calculated for $\text{C}_{23}\text{H}_{32}\text{N}_5\text{O}_4\text{Si}$ ($\text{M}+\text{H}$) $^+$ 470.2218, found 470.2223.

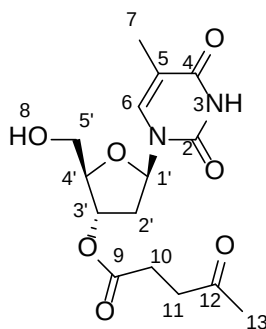
5'-O-DMTr-3'-O-levulinoyl-thymidine ⁶⁰



DMT T (1.6 g, 3.0 mmol) was dissolved in 1-4 dioxane (18 mL) under argon, the EDC·HCl (1.2 g, 6.3 mmol) was added, followed by DMAP

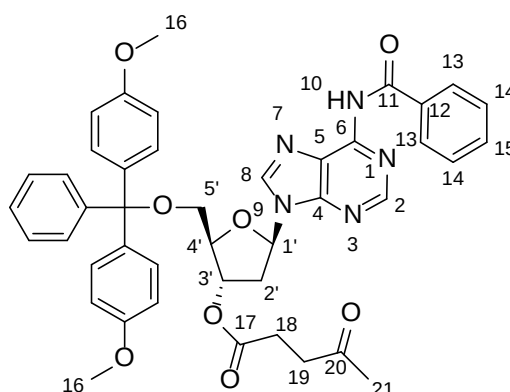
(8.0 mg, 0.070 mmol) then levulinic acid (0.61 mL, 6.0 mmol). Reaction mixture was stirred at ambient temperature for 23 hours then solvent was removed under reduced pressure, the residue was partitioned between CH₂Cl₂ (25 mL), and sat. aq. NaHCO₃ (25 mL). Aqueous layer was then extracted with CH₂Cl₂ (2 x 25 mL), combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified with silica gel chromatography, using 100 % EtOAc to give the product as a white solid (1.8 g, 2.8 mmol, 94 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3179, 3010, 2931, 2837, 1683, 1607, 1508, 1463, 1359, 1248, 1175, 1153, 1104, 1072, 1030. ¹H NMR (400 MHz, CDCl₃) 8.75 (1 H, br s, H-3), 7.63 (1 H, app d, *J* 1.3, H-6), 7.44 – 7.37 (2 H, m, DMTr), 7.37 – 7.22 (7 H, m, DMTr), 6.90 – 6.82 (4 H, m, DMTr), 6.46 (1 H, dd, *J* 8.8, 5.7, H-1'), 5.48 (1 H, dt, *J* 5.7, 1.7, H-4'), 4.20 – 4.14 (1 H, m, H-4'), 3.81 (6 H, s, H-8), 3.53 – 3.41 (2 H, m, H-5'), 2.78 (2 H, td, *J* 6.2, 1.8, H-11), 2.60 (1 H, t, *J* 6.2, H-10), 2.53 – 2.40 (2 H, m, H-2'), 2.21 (3 H, s, H-13), 1.38 (3 H, app d, *J* 1.2, H-7). ¹³C NMR (101 MHz, CDCl₃) 206.4 (C, C12), 172.4 (C, C9), 163.7 (C, C4), 158.9 (C, DMTr), 158.9 (C, DMTr), 150.5 (C, C2), 144.3 (C, DMTr), 135.6 (CH, C6), 135.4 (C, DMTr), 135.3 (C, DMTr), 130.2 (CH, DMTr), 130.2 (CH, DMTr), 128.3 (CH, DMTr), 128.2 (CH, DMTr), 127.3 (DMTr), 113.4 (CH, DMTr), 111.8 (C, C5), 87.3 (C, DMTr), 84.5 (CH, C1'), 84.1 (CH, C4'), 75.8 (CH, C3'), 63.9 (CH₂, C5'), 55.4 (CH₃, C8), 38.0 (CH₂, C11), 37.9 (CH₂, C2'), 29.9 (CH₃, C13), 28.1 (CH₂, C10), 11.7 (CH₃, C7). HRMS (ESI) calculated for C₃₆H₃₈N₂NaO₉ (M+Na)⁺ 665.2470, found 665.2464.

3'-O-Levlinoylthymidine, 56a ⁶¹



DMT Lev T (4.25 g, 6.00 mmol) was dissolved in dry CH₂Cl₂ (68 mL), cooled to 0 °C and a 6% trichloroacetic acid in CH₂Cl₂ (w/v 68 mL) was added over 1 hour. After reaction completion MeOH (20 mL) added, reaction stirred for 5 min then solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography 2:1 EtOAc:Petrol to 100% EtOAc. The product was obtained as a white foam (1.22 g, 3.59 mmol, 60%). ¹H NMR (400 MHz, CDCl₃) 8.72 (1 H, s, H-3), 7.50 (1 H, app d, J 1.3, H-6), 6.22 (1 H, dd, J 8.2, 6.0, H-1'), 5.35 (1 H, dt, J 6.0, 2.5, H-3'), 4.09 (1 H, q, J 2.5, H-4'), 3.96 – 3.85 (2 H, m, H-5'), 2.80 – 2.76 (2 H, m, H-11), 2.65 (1H, br S, H-8), 2.61 – 2.56 (2 H, m, H-10), 2.48 – 2.34 (2 H, m, H-2'), 2.20 (3 H, s, H-13), 1.92 (3 H, app d, J 1.3, H-7). ¹³C NMR (101 MHz, CDCl₃) 206.7 (C, C12), 172.7 (C, C9), 163.7 (C, C4), 150.5 (C, C2), 136.5 (CH, C6), 111.5 (C, C5), 86.3 (CH, C1'), 85.1 (CH, C4'), 75.0 (CH, C3'), 62.7 (CH₂, C5'), 38.0 (CH₂, C11), 37.2 (CH₂, C2'), 29.9 (CH₃, C13), 28.1 (CH₂, C10), 12.7 (CH₃, C7). HRMS (ESI) calculated for C₁₅H₂₁N₂O₇ (M+H)⁺ 341.1343, found 341.1345.

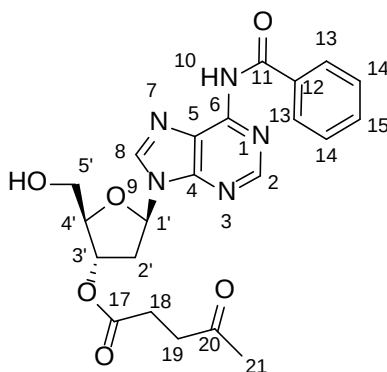
5'-O-DMTr-N⁶-benzoyl-3'-O-levuliny-2'-deoxyadenosine



DMTr A^{Bz} (1.9 g, 3.0 mmol) was dissolved in 1,4 dioxane (18 mL) under argon, the EDC·HCl (1.2 g, 6.3 mmol) was added, followed by DMAP (8.0 mg, 0.070 mmol) then levulinic acid (0.61 mL, 6.0 mmol). Reaction mixture was stirred at ambient temperature for 17.5 hours then solvent was removed under reduced pressure, the residue was partitioned between CH₂Cl₂ (25 mL), and sat. aq. NaHCO₃ (25 mL). Aqueous layer was then extracted with CH₂Cl₂ (2 x 25 mL), combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified with silica gel chromatography, using 100 % EtOAc to give the product as a white solid (1.9 g, 2.6 mmol, 86 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3003, 2934, 2837, 1714, 1607, 1580, 1508, 1454, 1298, 1246, 1176, 1154, 1072, 1031. ¹H NMR (400 MHz, CDCl₃) 9.24 (1 H, s, H-10), 8.74 (1 H, s, H-2), 8.20 (1 H, s, H-8), 8.10 – 7.99 (2 H, m, H-12), 7.65 – 7.57 (1 H, m, H-15), 7.57 – 7.48 (2 H, m, H-14), 7.44 – 7.37 (2 H, m, DMTr), 7.33 – 7.17 (7 H, m, DMTr), 6.85 – 6.77 (4 H, m, DMTr), 6.54 (1 H, dd, J 8.5, 5.6, H-1'), 5.57 (1 H, dt, J 6.0, 1.8, H-3'), 4.34 (1 H, td, J 4.0, 1.8, H-4'), 3.78 (6 H, s, H-16), 3.46 (2 H, d, J 4.0, H-5'), 3.04 (1 H, ddd, J 14.3, 8.6, 6.0, one of H-2'), 2.81 (2

H, ddd, J 6.5, 5.6, 1.7, H-19), 2.73 – 2.60 (3 H, m, one of H-2' and H-18), 2.22 (3 H, s, H-21). ¹³C NMR (101 MHz, CDCl₃) 206.4 (C, C20), 172.1 (C, C17), 164.7 (C, C11), 158.6 (C, C9), 152.6 (CH, C2), 151.6 (C, C6), 149.6 (C, C4), 144.4 (C, DMTr), 141.3 (CH, C8), 135.5 (C, DMTr), 133.7 (C, C12), 132.8 (CH, C15), 130.0 (C, DMTr), 130.0 (C, DMTr), 128.8 (CH, C14), 128.1 (CH, C13), 127.9 (CH, DMTr), 127.9 (CH, DMTr), 127.0 (CH, DMTr), 123.4 (C, C5), 113.2 (CH, DMTr), 86.7 (C, DMTr), 84.5 (C, DMTr), 84.4 (CH, C1'), 75.5 (C, C3'), 63.6 (CH₂, C5), 55.2 (CH₃, C16), 38.1 (CH₂, C2'), 37.9 (CH₂, C19), 29.8 (CH₃, C21), 28.0 (CH₂, C18). HRMS (ESI) calculated for C₄₃H₄₂N₅O₈ (M+H)⁺ 756.3028, found 756.3020.

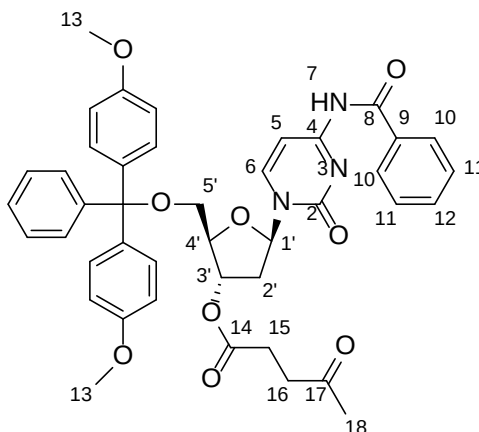
N⁶-benzoyl-3'-O-levuliny-2'-deoxyadenosine, 56b ⁶²



5'-ODMTr-3'-OLev- A^{Bz} (1.94 g, 2.57 mmol) was dissolved in CH₂Cl₂ (35 mL), and to this was added DCA (1.10 mL, 12.9 mmol). Reaction mixture was stirred for 5 min, then sat aq. NaHCO₃ was added with vigorous stirring until pH 7. The layers were separated, the aqueous extracted with CH₂Cl₂ (3 x 50 mL). Combined organic extracts were dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified with silica gel chromatography, using 2% MeOH 98% EtOAc to give the product as

a white solid (518 mg, 1.14 mmol, 44 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3188, 2965, 2922, 2844, 1693, 1609, 1580, 1059, 1455, 1418, 1360, 1247, 1177, 1154, 1099, 1058, 1033, 1018. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.22 (1 H, s, H-10), 8.76 (1 H, s, H-2), 8.71 (1 H, s, H-8), 8.14 – 7.93 (2 H, m, H-13), 7.75 – 7.63 (1 H, m, H-15), 7.55 (2 H, dd, J 8.2, 6.8, H-14), 6.50 (1 H, dd, J 8.5, 6.0, H-1'), 5.40 (1 H, dt, J 6.0, 1.8, H-3'), 5.20 (1 H, OH), 4.11 (1 H, td, J 4.3, 1.8, H-4'), 3.75 – 3.52 (2 H, m, H-5'), 3.05 (1 H, ddd, J 14.3, 8.5, 6.0, one of H-2'), 2.78 (2 H, t, J 6.4, H-18), 2.55 (3 H, m, one of H-2' and H-17), 2.14 (3 H, s, H-20). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) 206.9 (C, C19), 172.0, (C, C16) 152.0 (C, C11), 151.6 (CH, C2), 150.5 (C, C6), 143.0 (CH, C8), 133.3 (C, C12), 132.5 (CH, C15), 128.5 (CH, C14), 128.5 (CH, C13), 125.9 (C, C5), 85.3 (CH, C4'), 83.8 (CH, C1'), 75.0 (CH, C3'), 61.5 (CH_2 , C5'), 37.5 (CH_2 , C18), 36.4 (CH_2 , H-2), 29.6 (CH_3 , C20), 27.8 (CH_2 , C17). HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{24}\text{N}_5\text{O}$ ($\text{M}+\text{H}$) $^+$ 454.1721, found 454.1719.

5'-O-DMTr-N⁴-3'-O-levuliny-2'-deoxycytidine



DMTr C^{Bz} (3.17 g, 5.00 mmol) and DMAP (50.0 mg, 0.410 mmol) were dissolved in dry CH_2Cl_2 (25 mL) under argon. Triethylamine (1.00 mL,

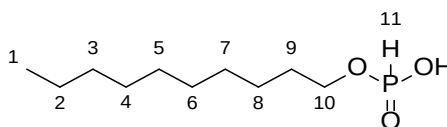
7.50 mmol) was then added followed by levulinic anhydride (1.30 g, 6.00 mmol) and the reaction mixture was stirred at ambient temperature for 16 hours, then at 40 °C for 24 hours. Levulinic anhydride (535 mg, 2.50 mmol) and triethylamine (0.35 mL, 2.5 mmol) were then added and reaction stirred at 40 °C for 1 hour. Reaction mixture was then quenched at with sat. aq. NaHCO₃ (25 mL), organic layer separated and the aqueous layer extracted with CH₂Cl₂ (3 x 25 mL), combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was purified with silica gel chromatography 100% EtOAc to give the product as a white foam (3.40 g, 4.65 mmol, 93%).

$\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2928, 2836, 1606, 1554, 1479, 1394, 1298, 1243, 1174, 1243, 1174, 1152, 1111, 1069, 1028. ¹H NMR (400 MHz, CDCl₃) 8.66 (1 H, s, H-7), 8.17 (1 H, d, *J* 7.5, H-6), 7.96 – 7.87 (2 H, m, H-10), 7.67 – 7.60 (1 H, m, H-12), 7.54 (2 H, dd, *J* 8.4, 6.9, H-11), 7.43 – 7.36 (2 H, m, DMTr), 7.37 – 7.22 (8 H, m, DMTr and H-5), 6.88 (4 H, dd, *J* 8.9, 3.0, DMTr), 6.34 (1 H, dd, *J* 7.4, 5.8, H-1'), 5.45 (1 H, dt, *J* 6.2, 2.4, H-3'), 4.36 – 4.24 (1 H, m, H-4'), 3.81 (6 H, app d, *J* 3.3, H-13), 3.55 – 3.44 (2 H, m, H-5'), 2.89 – 2.75 (3 H, m, one of H-2' and H-16), 2.64 – 2.57 (2 H, m, H-15), 2.37 (1 H, ddd, *J* 14.0, 7.5, 6.2, one of H-2'), 2.22 (3 H, s, H-18). ¹³C NMR (101 MHz, CDCl₃) 206.4 (C), 172.3 (C), 162.2 (C), 158.8 (C), 158.8 (C), 144.2 (C), 135.4 (C), 135.2 (C), 133.3 (CH), 130.2 (CH), 130.1 (CH), 129.2 (CH), 128.2 (CH), 128.2 (CH), 127.6 (CH), 127.3 (CH), 113.5 (CH), 113.5 (CH), 87.3 (CH), 87.3 (CH), 84.8 (CH), 74.9 (CH), 63.3 (CH₂), 55.4 (CH₃), 39.6 (CH₂), 37.9 (CH₂), 29.9 (CH₃), 28.1 (CH₂).

HRMS (ESI) calculated for $\text{C}_{42}\text{H}_{42}\text{N}_3\text{O}_9$ $(\text{M}+\text{H})^+$ 732.2916, found 732.2907.

4.4 H-phosphonate Synthesis

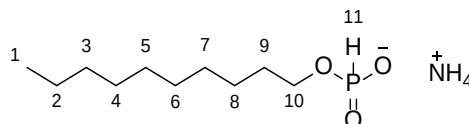
H-Decyl phosphonate, 99



Pre-dried hypophosphorus acid (320 mg, 1.50 equiv, 4.80 mmol), was added to a solution of Pd/C (188 mg, 10 wt %, 5.00 mol %, 0.160 mmol) in anhydrous toluene/MeCN (10.0 mL, 1:1, v/v) and to this was added cyclohexene (0.650 mL, 2.00 equiv, 6.40 mmol) and then decanol (0.610 mL, 1.00 equiv, 3.20 mmol) and reaction mixture was heated at 85 °C under nitrogen atmosphere over 24 hours. After cooling, the reaction was filtered through Celite and concentrated *in vacuo*. The resulting oil was dissolved in ethyl acetate (25 mL) and washed with brine (3 X 10 mL). The organic layer was extracted with saturated aqueous NaHCO₃ (0.5 M, 10 mL), then water (2 X 10 mL). The combined aqueous layers were acidified with 2M aqueous HCl until pH 1, then extracted with ethyl acetate (3 X 25 mL). The combined organic layers were washed with brine (5 mL), dried over MgSO₄ and concentrated in *vacuo* to afford the title product as an oil (353 mg, 1.60 mmol, 50%). $\nu_{\max}/\text{cm}^{-1}$ (CHCl₃ soln.) 3011, 2928, 2856, 2432, 1652, 1468, 1379, 1240, 1086, 1007; ¹H NMR (400 MHz, CDCl₃) δ 8.78 (1H, s, OH), 6.78 (1H, d, *J* = 704 Hz, H-11), 4.05 (2H, dd, *J* = 14.9, 6.5 Hz, H-10), 1.80 – 1.64 (2H, m, H-9), 1.47 – 1.19 (14H, m, H-2 – H-8), 0.88 (3H, t, *J* = 6.5 Hz, H-1); ¹³C NMR (101 MHz, CDCl₃) 65.87 (d, *J* = 5.7 Hz, CH₂), 32.01 (CH₂), 30.45 (CH₂), 29.64 (CH₂), 29.42 (CH₂), 29.25 (CH₂), 25.60 (CH₂), 22.80 (CH₂),

14.22 (CH₃); ³¹P NMR (162 MHz, CDCl₃) δ 9.08 (d, *J* = 704 Hz); HRMS (ESI+) calculated for C₁₀H₂₃NaO₃P (M+Na)⁺ 245.1277, found 245.1277.

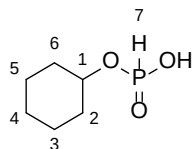
Ammonium decyl phosphonate, 114



Pre-dried hypophosphorus acid (634 mg, 1.50 equiv, 9.60 mmol), decanol (1.22 mL, 1.00 equiv, 6.40 mmol), Pd/C (378 mg, 10 wt %, 5 mol % Pd, 0.320 mmol) in distilled toluene/acetonitrile (20.0 mL, 1:1, v/v) were heated at 85°C under nitrogen atmosphere over 24 hours. After cooling down, the reaction was filtered through Celite®, washed with EtOAc (30 mL) and concentrated *in vacuo*. The resulting oil was dissolved in EtOAc (50 mL) and washed with brine (3 × 20 mL). The organic layer was then dried over MgSO₄ and concentrated *in vacuo* to afford *H*-Decyl phosphonate. Ammonium hydroxide (25 wt %, 30 mL) was added to the resulting oil and the resulting mixture again concentrated *in vacuo*. Cold ether (20 mL) was added to the resulting oil and triturated to offer ammonium salt of *H*-Decyl phosphonate (397 mg, 1.66 mmol, 26 %). ¹H NMR (400 MHz, CDCl₃) 7.62 (4H, br s), 6.70 (1H, d, *J*_{HP} = 620.0 Hz), 3.77 (2H, q, *J* = 7.0 Hz), 1.60 (2H, quin, *J* = 8.0 Hz), 1.38 – 1.20 (14H, m), 0.88 (3H, t, *J* = 6.8 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) 64.5 (d, *J*_{COP} = 4.0 Hz, CH₂), 32.1 (CH₂), 31.0 (d, *J*_{CCOP} = 6.5 Hz, CH₂), 30.0 (CH₂), 29.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 26.1 (CH₂), 22.9 (CH₂), 14.2 (CH₃); δ_P

^{31}P NMR (162 MHz, CDCl_3) δ 4.05 (dt, $J_{\text{PH}} = 620, 8$ Hz); HRMS (ESI+) calculated for $\text{C}_{10}\text{H}_{23}\text{NaO}_3\text{P}$ ($\text{M}+\text{Na}$) $^+$ 245.1277, found 245.1283.

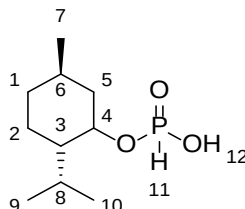
H-cyclohexyl phosphonate, 117



Pre-dried hypophosphorus acid (317 mg, 1.50 equiv, 4.80 mmol), was added to a solution of Pd/C (189 mg, 10 wt %, 5 mol %, 0.160 mmol) in distilled toluene/acetonitrile (10 mL, 1:1, v/v) and to this was added cyclohexene (0.650 mL, 2.00 equiv, 6.40 mmol) and then cyclohexanol (0.390 mL, 1.00 equiv, 3.20 mmol) and reaction mixture was heated at 85°C under nitrogen atmosphere over 24 hours. After cooling down, the reaction was filtered through Celite®, washed with EtOAc (20 mL) and concentrated *in vacuo*. The resulting oil was dissolved in ethyl acetate (25 mL) and washed with brine (3 × 10 mL). The organic layer was extracted with NaHCO_3 (0.5 M, 10 mL), then water (2 × 10 mL). The combined aqueous layers were acidified with 2M aqueous HCl until pH 1, then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine (5 mL), dried over MgSO_4 and concentrated *in vacuo* to afford the title product (212 mg, 1.29 mmol, 40 %) as a clear oil. $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl_3 soln.) 3011, 2942, 2862, 2434, 1661, 1452, 1377, 1241; ^1H NMR (400 MHz, CDCl_3) 10.87 (1H, s, OH), 6.84 (1H, d, $J_{\text{HP}} = 701.5$ Hz, H-7), 4.61 – 4.32 (1H, m, H-1), 2.04 – 1.86 (2H, m), 1.86 – 1.69 (2H, m), 1.63 – 1.51 (2H, m), 1.46 – 1.19 (4H, m); ^{13}C NMR (101 MHz, CDCl_3) 76.2 (d, $J_{\text{COP}} = 6.1$ Hz, CH), 33.7 (d, $J_{\text{CCOP}} = 4.3$ Hz, CH_2),

25.2 (CH₂), 23.6 (CH₂); δ_p ³¹P NMR (162 MHz, CDCl₃) 7.05 (dd, J_{PH} = 702, 7 Hz); HRMS (ESI+) calculated for C₆H₁₃O₃PNa (M+Na)⁺ 187.0495, found 187.0497.

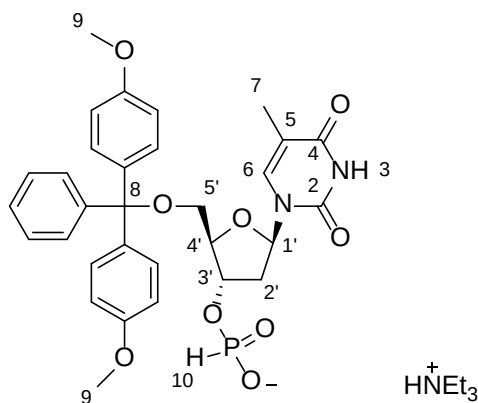
H-Menthyl phosphonate, 116



Pre-dried hypophosphorus acid (320 mg, 1.5 equiv, 4.8 mmol), was added to a solution of Pd/C (189 mg, 10 wt %, 5 mol %, 0.16 mmol) in anhydrous toluene/MeCN (10.0 mL, 1:1, v/v) and to this was added cyclohexene (0.650 mL, 2.00 equiv, 6.40 mmol) and then (-)-Menthol (499 mg, 1.00 equiv, 3.20 mmol) and reaction mixture was heated at 85 °C under nitrogen atmosphere over 24 hours. After cooling down, the reaction was filtered through Celite®, washed with EtOAc (20 mL) and concentrated *in vacuo*. The resulting oil was dissolved in ethyl acetate (25 mL) and washed with brine (3 × 10 mL). The organic layer was extracted with saturated aqueous NaHCO₃ (0.5 M, 10 mL), then water (2 × 10 mL). The combined aqueous layers were acidified with 2 M aqueous HCl until pH 1, then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine (5 mL), dried over MgSO₄ and concentrated *in vacuo* to afford the title product as a clear oil (422 mg, 1.90 mmol, 60 %). $\nu_{\max}/\text{cm}^{-1}$ (CHCl₃ soln.) 2960, 2929, 2873, 2726, 2433, 2360, 2186, 1731, 1671, 1457, 1373, 1242; ¹H NMR (400 MHz, CDCl₃) 6.81 (1H, d, J = 699.0 Hz, H-11), 4.18 (1H, ddd, J = 20.0, 10.5, 4.5 Hz,

H-4), 2.22 – 1.99 (3H, m, H-5 and H-6), 1.65 (2H, d, $J = 10.5$ Hz, H-1), 1.49 – 1.30 (2H, m, H-3 and H-8), 1.26 – 1.16 (1H, m, H-2) 1.07 – 0.94 (1H, m, H-2) 0.92 (6H, dd, $J = 6.8, 4.6$ Hz, H-9 and H-10), 0.81 (3H, d, $J = 7.0$ Hz, H-7); ^{13}C NMR (101 Mhz, CDCl_3) 78.1 (d, $J = 6.9$ Hz, CH), 48.3 (d, $J = 6.4$ Hz, CH), 43.0 (CH_2), 34.0 (CH_2), 31.7 (CH), 25.7 (CH), 23.0 (CH_2), 22.0 (CH_3), 21.0 (CH_3), 15.8 (CH_3); ^{31}P NMR (162 Mhz, CDCl_3) 6.86 (d, $J_{\text{PH}} = 699$ Hz); HRMS (ESI+) calculated for $\text{C}_{10}\text{H}_{21}\text{NaO}_3\text{P}$ ($\text{M}+\text{Na}$) $^+$ 243.1121, found 243.1122.

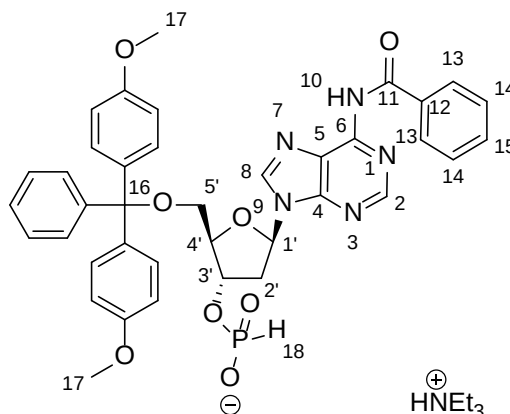
5'-ODMTr 3'H-phosphonate Thymidine Et₃N Salt, 130a



Phosphonic acid (6.56 g, 80.0 mmol) was dissolved in dry pyridine (19 mL) and then pivaloyl chloride (2.96 mL, 24.0 mmol) and this was stirred for 15 min under N_2 before adding dropwise to a solution of DMT T (2.18 g, 4.00 mmol) in dry pyridine (10 mL). Reaction was stirred at 30 °C under argon for 5 days, then quenched with 1 M TEAB (2 mL), before partitioning between CH_2Cl_2 (50 mL) and 0.1 M TEAB (50 mL). Aqueous was then extracted with CH_2Cl_2 (3 x 50 mL). Combined organic extracts were then dried over MgSO_4 , filtered and solvent removed under reduced pressure. The crude product was then purified using silica gel chromatography using the solvent system

CH₂Cl₂:MeOH:Et₃N 94:5:1 to 89:10:1 to give the product as a white solid (2.54 g, 3.59 mmol, 90 %). $\nu_{\max}/\text{cm}^{-1}$ (ATR) 2955, 2836, 2625, 2381, 2349, 2119, 1926, 1684, 1607, 1582, 1508, 1463, 1399, 1366, 1275, 1248, 1213, 1174, 1132, 1100, 1057, 1030. ¹H NMR (400 MHz, CDCl₃) 9.62 (2 H, br s NH) 7.60 (1 H, app d, *J* 1.4, H-6), 7.41 – 7.35 (2 H, m, DMTr), 7.27 (6 H, ddd, *J* 8.0, 4.1, 2.0, DMTr), 7.23 – 7.16 (1 H, m, DMTr), 6.87 (1 H, d, *J* 624.0, H-10), 6.84 – 6.77 (4 H, m, DMTr), 6.43 (1 H, dd, *J* 8.3, 5.5, H-1'), 5.02 (1 H, ddd, *J* 8.8, 5.6, 2.3, H-3'), 4.24 (1 H, q, *J* 2.8, H-4'), 3.77 (6 H, s, H-9), 3.45 (1 H, dd, *J* 10.6, 2.8, one of H-5'), 3.38 (1 H, dd, *J* 10.6, 2.8, one of H-5'), 2.98 (6 H, q, *J* 7.3, N(CH₃CH₂)₃), 2.59 (1 H, ddd, *J* 13.8, 5.6, 2.3, one of H-2'), 2.38 (1 H, ddd, *J* 13.8, 8.3, 5.6, one of H-2'), 1.34 (3 H, app d, *J* 1.2, H-7), 1.23 (9 H, t, *J* 7.3, N(CH₃CH₂)₃). ¹³C NMR (101 MHz, CDCl₃) 164.2 (C, C4), 158.7 (C, DMTr), 150.7 (C, C2), 144.4 (C, DMTr), 135.9 (CH, C-6), 135.5 (C, DMTr), 135.4 (C, DMTr), 130.2 (CH, DMTr), 130.2 (CH, DMTr), 128.3 (CH, DMTr), 128.0 (CH, DMTr), 127.1 (CH, DMTr), 113.3 (CH, DMTr), 111.2 (C, C5), 87.0 (C, C8), 85.3 (d, *J* 6.3, CH, C4), 84.6 (CH, C1'), 74.3 (d, *J* 3.8, CH, C3'), 63.5 (CH₂, C5'), 55.3 (CH₃, C9), 45.1 (CH₂, N(CH₃CH₂)₃), 39.9 (CH₂, C2'), 11.6 (CH₃, C7), 8.8 (CH₃, N(CH₃CH₂)₃). ³¹P NMR (162 MHz, CDCl₃) 2.90 (dd, *J* 624.0, 9.0). HRMS (ESI) calculated for C₃₁H₃₃N₂NaO₉P (M+Na)⁺ 631.1816, found 631.1793.

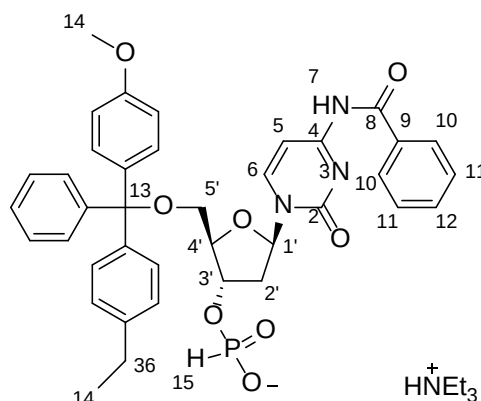
5'-ODMTr 3' H-Phosphonate 6-N-benzoyl 2'-deoxyadenosine Et₃N Salt, 130b



Phosphonic acid (3.24 g, 40.0 mmol) was dissolved in dry pyridine (20.0 mL) under N₂ and pivaloyl chloride (1.50 mL, 12.0 mmol) was added and the this was stirred for 15 min before adding dropwise to a solution of DMTr A^{Bz} (1.32 2.00 mmol) in dry pyridine (12.0 mL). Reaction was stirred at 30 °C under N₂ for 3 days, then quenched with 1 M TEAB (5 mL), before partitioning between CH₂Cl₂ (50 mL) and 0.1 M TEAB (50 mL). Aqueous was then extracted with CH₂Cl₂ (3 x 50 mL). Combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified using silica gel chromatography using the solvent system CH₂Cl₂:MeOH:Et₃N 94:5:1 to 89:10:1 to give the product as a white solid (1.59 g, 1.93 mmol, 97%). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2973, 2957, 2871, 2620, 2496, 2111, 1913, 1698, 1608, 1579, 1509, 1480, 1455, 1398, 1360, 1294. ¹H NMR (400 MHz, CDCl₃) 10.65 (2 H, br s, NH), 8.66 (1 H, s, H-2), 8.21 (1 H, s, H-8), 8.03 - 8.01 (2 H, m, H-13), 7.56 - 7.49 (1 H, m, H-15), 7.44 (2 H, m, H-14), 7.35 - 7.32 (2 H, m, DMTr), 7.25 - 7.09 (7 H, m, DMTr), 6.87 (1 H, dd, J 629.0, 1.7,

H-18), 6.78 – 6.65 (4 H, m, DMTr), 6.58 – 6.45 (1 H, m, H-1'), 5.07 (1 H, dd, J 6.2, 3.2, H-3'), 4.37 (1 H, p, J 2.0, H-4'), 3.71 (6 H, d, J 2.0, H-17), 3.38 – 3.31 (2 H, m, H-5'), 2.98 (6 H, qd, J 7.3, 1.4, N(CH₂CH₃)₃), 2.92 – 2.74 (2 H, m, H-2'), 1.18 (9 H, t, J 7.3, N(CH₂CH₃)₃). ¹³C NMR δ C (101 MHz, CDCl₃) 165.5 (C, C11), 158.5 (C, DMTr), 152.2 (CH, C2), 151.7 (C, C6), 149.8 (C, C5), 144.5 (C, DMTr), 141.6 (CH, C8), 135.7 (C, DMTr), 133.7 (C, C12), 132.6 (CH, C15), 130.1 (CH, DMTr), 128.5 (CH, DMTr), 128.4 (CH, C14), 128.1 (CH, C13), 127.9 (CH, DMTr), 126.9 (CH, DMTr), 123.3 (C, C5), 113.2 (CH, DMTr), 86.6 (C, DMTr), 85.9 (d, J 6.3, CH, C4'), 84.7 (CH, C1'), 74.2 (d, J 3.9, CH, C3'), 63.5 (CH₂, C5'), 55.2 (CH₃, C17), 44.9 (CH₂, N(CH₂CH₃)₃), 39.8 (CH₂, C2'), 8.6 (CH₃, N(CH₂CH₃)₃). ³¹P NMR (162 MHz, CDCl₃) 2.67 (dd, J 629.0, 8.8). HRMS (ESI) calculated for C₃₈H₃₇N₅O₈P (M+H)⁺ 722.2374, found 722.2368.

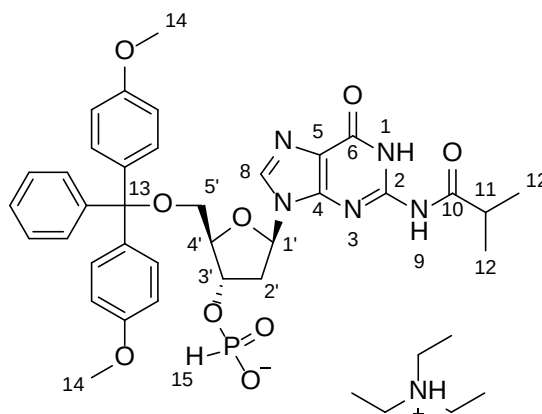
5'-ODMT 3' H-Phosphonate 4-N-benzoyl 2'-deoxycytosine Et₃N Salt, 130c



Phosphonic acid (820 mg, 10.0 mmol) was dissolved in dry pyridine (7.00 mL) under N₂ and pivaloyl chloride (370 μL, 3.00 mmol) was added and the this was stirred for 15 min before adding dropwise to

a solution of DMTr C^{Bz} (317 mg, 0.500 mmol) in dry pyridine (3.00 mL). Reaction was stirred at 30 °C under N₂ for 2 days, then quenched with 1 M TEAB (2 mL), before partitioning between CH₂Cl₂ (20 mL) and 0.1 M TEAB (20 mL). Aqueous was then extracted with CH₂Cl₂ (3 x 20 mL). Combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified using silica gel chromatography using the solvent system CH₂Cl₂:MeOH:Et₃N 94:5:1 to 89:10:1 to give the product as a white solid (378 mg, 0.474 mmol, 98%). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2959, 2871, 2630, 2516, 2106, 1696, 1608, 1558, 1507, 1481, 1397, 1362, 1298, 1251, 1173, 1103, 1058, 1031. ¹H NMR (400 MHz, CDCl₃) 10.84 (2 H, br s, NH), 8.26 (1 H, d, *J* 7.5, H-6), 8.06 – 7.96 (2 H, m, DMTr), 7.61 – 7.19 (13 H, m, DMTr, Bz, H-5), 6.87 (1 H, d, *J* 630.3, H-15), 6.85 (4 H, dd, *J* 9.0, 2.5, DMTr), 6.27 (1 H, t, *J* 6.0, H-1'), 5.00 (1 H, dq, *J* 9.4, 4.5, H-3'), 4.34 (1 H, q, *J* 3.3, H-4'), 3.79 (6 H, app d, *J* 2.2m H-14), 3.44 (2 H, tt, *J* 10.8, 5.8, H-5'), 3.04 (6 H, q, *J* 7.3, N(CH₂CH₃)₃), 2.84 (1 H, dt, *J* 13.8, 5.3, one of H-2'), 2.37 (1 H, dt, *J* 13.9, 6.0, one of H-2'), 1.24 (9 H, t, *J* 7.3, N(CH₂CH₃)₃). ¹³C NMR (101 MHz, CDCl₃) 183.9 (C), 163.1 (C), 158.7 (C), 154.6 (C), 145.3 (C), 144.2 (CH), 135.6 (C), 135.3 (C), 133.0 (CH), 130.2 (CH), 130.1 (CH), 128.7 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 127.1 (CH), 113.4 (CH), 96.7 (CH), 87.3 (CH), 87.0 (C), 85.8 (CH, d, *J* 6.6), 72.8 (d, *J* 4.2), 62.6 (CH₂), 55.3 (CH₃), 45.0 (CH₂), 41.1 (CH₂, d, *J* 2.9), 38.8 (C), 27.5 (CH₃), 8.6 (CH₃). ³¹P NMR 162 MHz, CDCl₃) 2.7 (dd, *J* 630.3, 9.0). HRMS (ESI) calculated for C₃₇H₃₇N₃O₉P (M+H)⁺ 698.2262, found 698.2248.

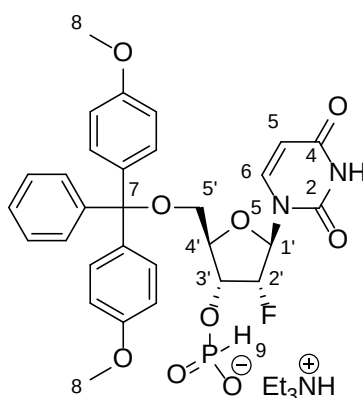
5'-ODMTr-3'-H-phosphonate 2N-isobutryl-2'-deoxyguanosine Et₃N
Salt, 130d



Phosphonic acid (4.10 g, 50.0 mmol) was dissolved in dry pyridine (10.0 mL) then pivaloyl chloride (1.90 mL, 15.0 mmol) and this was stirred for 15 min under N₂ before adding dropwise to a solution of DMT G^{iBu} (1.60 g, 2.50 mmol) in dry pyridine (10 mL). Reaction was stirred at 30 °C under argon for 7 days, then quenched with 1 M TEAB (2 mL), before partitioning between CH₂Cl₂ (50 mL) and 0.1 M TEAB (50 mL). Aqueous was then extracted with CH₂Cl₂ (3 x 50 mL). Combined organic extracts were then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified using silica gel chromatography using the solvent system CH₂Cl₂:MeOH:Et₃N 94:5:1 to 89:10:1 to give the product as a white solid (1.52 g, 2.17 mmol, 87 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2973, 2936, 2675, 1606, 1556, 1508, 1479, 1400, 1360, 1300, 1248, 1210, 1175, 1058. ¹H NMR (400 MHz, CDCl₃) 7.79 (1 H, s, H-8), 7.35 – 7.11 (10 H, m, DMTr), 6.83 (1 H, d, *J* 624.5, H-15), 6.71 (4 H, dd, *J* 8.9, 2.6, DMTr), 6.19 (1 H, t, 6.2, H-1'), 5.33 – 5.21 (1 H, m, H-3'), 4.24 (1 H, q, *J* 4.3, H-4'), 3.73 (6 H, app d, *J* 1.0, H-14), 3.40 – 3.27 (2 H, m, H-5'), 3.00 (6 H, q, *J* 7.3, N(CH₂CH₃)₃), 2.89 (1 H, dt, *J* 12.5, 6.0, one of H-

2'), 2.77 – 2.60 (2 H, m, one of H-2' and H-11), 1.22 (9 H, t, J 7.3, $N(CH_2CH_3)_3$), 1.17 – 1.10 (6 H, m, H-12). ^{13}C NMR (101 MHz, $CDCl_3$) 180.3 (C, C10), 158.4 (C, DMTr), 156.0 (C, C6), 148.1 (C4), 144.5 (C, DMTr), 138.2, (CH, C8), 135.7 (C, DMTr), 130.0 (CH, DMTr), 128.2 (CH, DMTr), 127.7 (CH, DMTr), 126.8 (CH, DMTr), 121.3 (C, C5), 113.0 (CH, DMTr), 86.3 (C, DMTr), 85.08 (CH, d, J 6.4, C4'), 84.5 (CH, C1'), 73.28 (CH, d, J 3.9, C3'), 63.5 (CH_2 , C5'), 55.2 (CH_3 , H-14), 45.6 (CH_2 , $N(CH_2CH_3)_3$), 39.1 (CH_2 , C2'), 35.7 (CH, C11), 19.0 (CH_3 , C12), 8.6 (CH_3 , $N(CH_2CH_3)_3$). HRMS (ESI) calculated for $C_{35}H_{39}N_5O_9P$ ($M+H$) $^+$ 704.2480, found 704.2467.

5'-ODMTr-2'-F-3'-H-phosphonate Uridine Et_3N Salt, 134

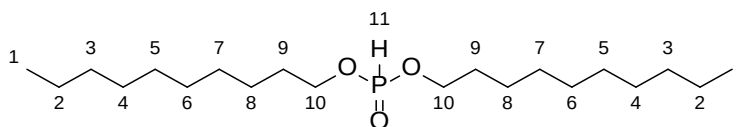


Phosphonic acid (1.64 g, 20.0 mmol) was dissolved in dry pyridine (5.0 mL) and then pivaloyl chloride (740 μ L, 6.00 mmol) was added, this was stirred under argon for 25 min at room temp, then was added slowly to a solution of 5'-ODMTr-2'-F Uridine (549 mg, 1.00 mmol) in dry pyridine (3 mL). Reaction was stirred at 30 $^{\circ}C$ under argon for 2 days, then quenched with 1 M TEAB (1 mL), before partitioning between CH_2Cl_2 (20 mL) and 0.1 M TEAB (20 mL). Aqueous was then extracted with CH_2Cl_2 (3 x 20 mL). Combined organic extracts were

then dried over MgSO₄, filtered and solvent removed under reduced pressure. The crude product was then purified using silica gel chromatography using the solvent system CH₂Cl₂:MeOH:Et₃N 94:5:1 to 89:10:1 to give the product as a white solid (99.0 mg, 0.140 mmol, 14%) and the recovered starting material (445 mg, 0.810 mmol, 81%). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2935, 2837, 2639, 2350, 1759, 1687, 1607, 1582, 1508, 1459, 1380, 1301, 1248, 1217, 1176, 1109, 1062, 1031, 983. ¹H NMR (400 MHz, CDCl₃) 7.93 (1 H, d, J 8.2, H-5), 7.44 – 7.36 (2 H, m, DMTr), 7.29 (6 H, m, DMTr), 7.25 – 7.17 (1 H, m, DMTr), 6.97 (1 H, dd, J 6.34, 1.4, H-9), 6.83 (4 H, dd, J 9.0, 1.0, DMTr), 6.05 (1 H, d, J 16 H-1'), 5.22 (1 H, dd, J 5.2, 4.0 H-2') 5.21 (1 H, d, J 8.2, H-6'), 5.01 – 4.86 (1 H, m, H-3'), 4.25 (1 H, d, J 8.7, H-4'), 3.77 (6 H, app d, J 1.7, H-8), 3.54 (2 H, ddd, J 13.9, 11.2, 2.2, H-5'), 3.00 (1 H, q, J 7.3, N(CH₃CH₂)₃), 1.25 (9 H, t, J 7.3, N(CH₃CH₂)₃). ¹³C NMR (101 MHz, CDCl₃) 163.6 (C), 158.8 (C), 158.7 (C), 150.4 (C), 144.3 (C), 139.6 (CH), 135.3 (C), 135.1 (C), 130.4 (CH), 130.3 (CH), 128.3 (CH), 128.1 (CH), 127.2 (CH), 113.4 (CH), 102.5 (CH), 94.2 (CH, C-2'), 88.00 (CH), 87.65 (CH), 87.1 (C), 80.85 (CH, d, J 8.1, C-4'), 69.30 – 68.92 (CH, m, C-3') 60.42 (CH₂), 55.34 (CH₃), 45.64 (CH₂), 8.83 (CH₃). ³¹P (162 MHz, CDCl₃) 3.50 (dd, J 6.34, 8.1). ¹⁹F (376 MHz, CDCl₃) -200.78 (ddd, J 5.2, 2.2, 1.6). HRMS (ESI) calculated for C₃₀H₂₉FN₂O₉P (M-H)⁻ 611.1600, found 611.1595.

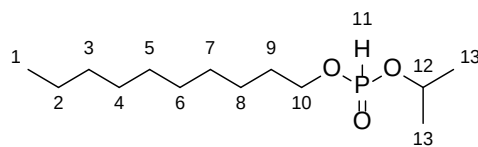
4.5 Diester Synthesis

Didecyl phosphonate, 101



Pre-dried hypophosphorus acid (990 mg, 1.00 equiv , 15 mmol) was added to a solution of decanol (2.90 mL, 1.00 equiv , 15.0 mmol) in anhydrous toluene (30.0 mL, 0.500 M) to a flask to which a Dean-Stark trap filled with excess toluene was fitted. The reaction mixture was heated under reflux for 52 hours, then allowed to cool before being partitioned between brine (30 mL) and EtOAc (20 mL). The organic layer was then washed with saturated aqueous sodium bicarbonate (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL) and combined organic extracts were then washed with brine (20 mL), dried over MgSO₄, concentrated in *vacuo* to give the title product (1.18 g, 3.25 mmol, 43 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl₃ soln.) 3625, 3010, 2928, 2867, 2441, 1467, 1379, 1252; ¹H NMR (400 MHz, CDCl₃) 6.76 (1H, d, *J* = 692 Hz, H-11), 4.03 (4H, dt, *J* = 15.0, 3.5 Hz, H-10), 1.65 (4H, quin, *J* = 15.0, 8.0 Hz, H-9), 1.40 – 1.11 (28H, m, H2-H7), 0.84 (6H, t, *J* = 7.0 Hz, H-1); ¹³C NMR (101 MHz, CDCl₃) 65.9 (d, *J* = 6.0 Hz, CH₂), 31.9 (CH₂), 30.5 (d, *J* = 6.0 Hz, CH₂), 29.6 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 25.9 (CH₂), 25.6 (CH₂), 22.7 (CH₂), 14.1 (CH₃); ³¹P NMR (162 MHz, CDCl₃) 7.73 (dquin, *J* = 693, 6.5 Hz); HRMS (ESI+) calculated for C₂₀H₄₄O₃P (M+H)⁺ 363.3029, found 363.3023.

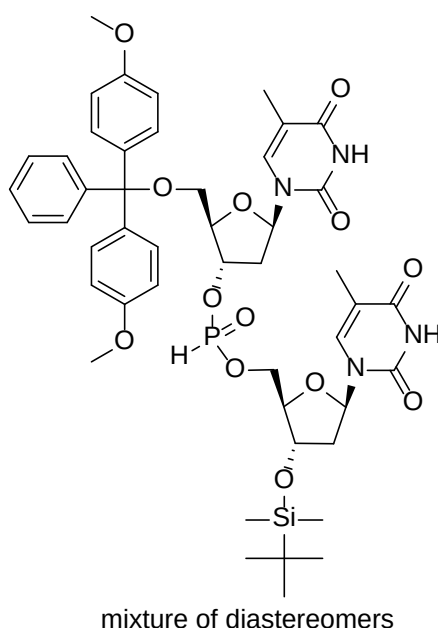
decyl isopropyl phosphonate



Pivaloyl chloride (70.0 μ L, 1.10 equiv, 0.539 mmol) was added to a solution of dry pyridine (50.0 μ L, 1.25 equiv, 0.613 mmol) in dry MeCN (3.00 mL, 0.210 M). To this was added **114** (117 mg, 1.00 equiv, 0.490 mmol) and after 5 minutes isopropanol (60.0 μ L, 1.50 equiv, 0.735 mmol) was added and then the reaction mixture was stirred at room temperature for 40 minutes. Reaction mixture was poured into H₂O (10 mL) and extracted with EtOAc (4 $\times$ 15 mL). Combined organic extracts then washed with 1M aqueous HCl (2 $\times$ 10 mL) and brine (10 mL), dried over MgSO₄ and concentrated in *vacuo* to give the title product (55 mg, 0.212 mmol, 43%). $\nu_{\text{max}}/\text{cm}^{-1}$ (CHCl₃ soln) 3617, 3010, 2929, 2856, 2441, 1716, 1467, 1378, 1241; ¹H NMR (400 MHz, CDCl₃) δ 6.81 (1H, d, J_{PH} = 691.0 Hz, H-11), 4.74 (1H, ddt, J = 12.5, 8.5, 6.0 Hz, H-12), 4.15 – 3.91 (2H, m, H-10), 1.77 – 1.61 (2H, m, H-9), 1.44 – 1.18 (20H, m, H-2 – H-8 and H-13), 0.87 (3H, t, J = 6.9 Hz, H-1); ¹³C NMR (101 MHz, C₆D₆) 70.5 (d, J_{COP} = 5.5 Hz, CH), 65.3 (d, J_{COP} = 5.5 Hz, CH₂), 32.3 (CH₂), 30.8 (d, J_{CCOP} = 6.0 Hz, CH₂), 29.9 (d, J_{CCOP} = 3.5 Hz, CH₂), 29.7 (CH₂), 29.5 (CH₂), 27.6 (CH₂), 25.9 (CH₂), 24.0 (d, J_{CCOP} = 4.0 Hz, CH₃), 23.7 (d, J_{CCOP} = 5.0 Hz, CH₃), 23.1 (CH₂), 14.4 (CH₃); δ_{P} ³¹P NMR (162MHz, CDCl₃) 6.21 (dq, J_{PH} = 690 Hz, J_{POCH} = 8 Hz); HRMS (ESI+) calculated for C₁₃H₃₀O₃P (M+H)⁺ 265.1927, found 265.1925.

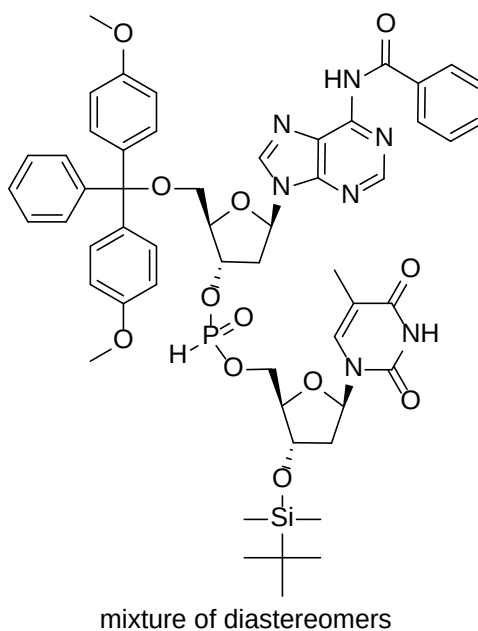
4.5.1 Dinucleotide H-Phosphonates

5'-ODMTr Thymidine 3'-5'-H-Phosphonate 3'-OTBS Thymidine, 138a⁵⁴



5'-ODMTr 3'-H-Phosphonate Thymidine (362 mg, 0.510 mmol) and 3'-OTBS Thymidine (178 mg, 0.500 mmol) were azeotroped with pyridine (3 x 5 mL) and then dissolved in dry MeCN/Pyridine (1:1 v/v 4 mL). Then a solution of PivCl (148 μ L, 1.20 mmol) in dry MeCN/Pyridine (95:5 v/v 12 mL) was added, reaction was stirred at room temperature for 15 min. Reaction mixture was then quenched with water (2 mL) and solvent evaporated *in vacuo*. Reaction mixture was then columned using a 12 g SNAP Ultra C18 column, using a gradient of 5-90% MeCN in 12 mM aqueous ammonium acetate, in two batches. The fractions containing pure product were combined to give the product as a mixture of diastereomers as a white solid (269 mg, 0.280 mmol, 56 %). ¹H and ³¹P NMR spectra matched those reported in the literature.⁵⁴ ³¹P NMR (162 MHz, CDCl₃) 8.52 (dq, J 717, 9), 7.16 (dq, J 717, 9).

5'-ODMTr N6-Benzoyl Adenosine 3'-5'-H-Phosphonate 3-OTBS
Thymidine, 138b⁵⁵



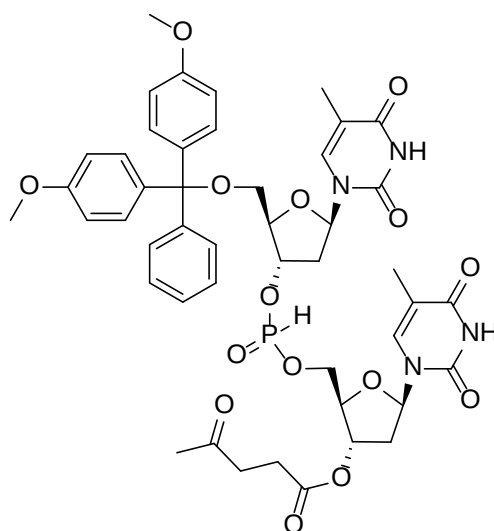
5'-ODMTr 3'-H-Phosphonate N6-Benzoyl Adenosine (453 mg, 0.550 mmol) and 3'-OTBS Thymidine (178 mg, 0.500 mmol) were azeotroped with pyridine (3 x 4 mL) and then dissolved in dry Pyridine (12.5 mL). Then PivCl (230 μ L, 4.20 mmol) was added, reaction was stirred at room temperature for 1 hour. Reaction mixture was then quenched with 1M TEAB (2 mL) and CH₂Cl₂ (5 mL). Reaction was vigorously stirred and then partitioned between CH₂Cl₂ (5 mL) and 0.1 M TEAB (10 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL), dried over MgSO₄ and solvent evaporated *in vacuo*. Reaction mixture was then columned using a 12 g SNAP Ultra C18 column, using a gradient of 15-85% MeCN in 12 mM aqueous ammonium acetate. The fractions containing pure product were combined to give the product as a mixture of diastereomers as a white solid (278 mg, 0.262 mmol, 53 %). ¹H and ³¹P NMR spectra matched those reported

in the literature.⁵⁵ ^{31}P NMR (162 MHz, CDCl_3) 8.40 (dq, J 717, 9), 7.10 (dq, J 717, 9).

4.5.2 Dinucleotide H-Phosphonates Table 11

Representative procedure

5'-ODMTr Thymidine 3'-5'-H-Phosphonate 3'-OLEv Thymidine, 145a

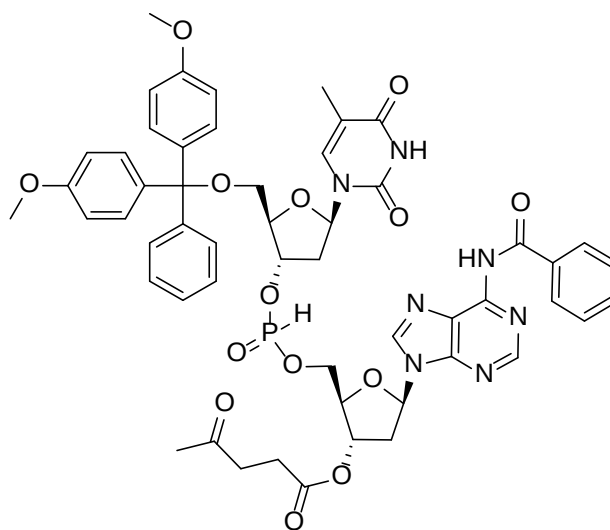


mixture of diastereomers

5-ODMTr 3'-H-Phosphonate Thymidine (157 mg, 0.220 mmol) and the 3'OLEv Thymidine (68.0 mg, 0.200 mmol) were co-evaporated in dry pyridine (3 x 2 mL), then dissolved in dry pyridine/MeCN (1:1 v/v 2 mL). A solution of TIPBSCI (145 mg, 0.480 mmol) in dry MeCN (5.75 mL) and dry pyridine (0.250 mL) was then added. Reaction mixture was stirred for at room temperature for 15 min, solvent evaporated *in vacuo*. The crude product was then purified using silica gel chromatography using the solvent system 5% MeOH in EtOAc with oven dried silica to give the product as a mixture of diastereomers as a white solid (135 mg, 0.145 mmol, 73 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3164, 3058, 3043, 2953, 2928, 2835, 1682, 1607, 1582, 1508, 1463, 1414, 1365, 1248, 1175, 1155, 1104, 1065, 1032, 936. ^1H NMR (400 MHz,

DMSO) 11.43 – 11.29 (1 H, m), 7.52 (1 H, dd, J 6.0, 1.2), 7.48 (1 H, s), 7.41 – 7.19 (5 H, m), 7.96 – 6.02 (1 H, m, P-H), 6.92 – 6.82 (3 H, m), 6.31 – 6.11 (2 H, m), 5.28 – 5.08 (2 H, m), 4.33 – 4.07 (2 H, m), 4.17 – 4.07 (1 H, m), 3.73 (6 H, app d, J 1.4), 3.29 – 3.17 (2 H, m), 2.78 – 2.68 (2 H, m), 2.42 – 2.29 (1 H, m), 2.28 – 2.18 (1 H, m), 2.10 (3 H, app q, J 3.6), 1.78 – 1.67 (2 H, m), 1.44 (2 H, dd, J 7.1, 0.9). ^{13}C NMR (101 MHz, DMSO) 206.9 (C), 172.0 (C), 163.6 (C), 158.2 (C), 150.4 (C), 150.4 (C), 150.4 (C), 150.3 (C), 135.7 (CH), 135.6 (CH), 135.6 (CH), 135.2 (C), 135.1 (C), 135.0 (C), 135.0 (C), 129.7 (CH), 127.9 (CH), 127.7 (CH), 126.7 (CH), 121.3 (CH), 113.26 (CH), 110.1 (C), 110.0 (C), 109.9 (C), 109.9 (C), 86.2 (CH), 86.1 (C), 84.0 (CH), 84.0 (CH), 84.0 (C), 83.6 (CH), 83.6 (CH), 83.50 (CH), 81.7 (CH), 81.5 (CH), 76.0 (CH), 76.0 (CH), 75.8 (CH), 73.6 (CH), 73.6 (CH), 65.1 (CH₂), 63.5 (CH₂), 55.0 (CH₃), 37.40 (CH₂), 35.42 (CH₂), 29.5 (CH₃), 27.6 (CH₃), 20.8 (CH₃), 14.1 (CH₃). ^{31}P NMR (162 MHz, DMSO) 9.58 (dq, J 722, 8.6), 8.74 (dq, J 724, 8.2). HRMS (ESI) calculated for C₄₆H₅₁N₄O₁₅PNa (M+Na)⁺ 953.2981, found 953.2992.

5'-ODMTr Thymidine 3'-5'-H-Phosphonate 3-OLev N⁶-benzoyl
Adenosine, 145b

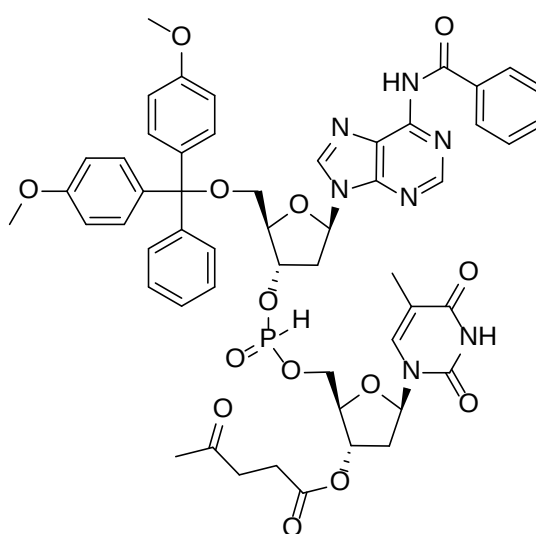


mixture of diastereomers

149 mg, 0.143 mmol, 72 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3057, 2955, 2867, 2837, 1737, 1689, 1606, 1581, 1509, 1492, 1461, 1423, 1359, 1248, 1202, 1175, 1156, 1103, 1084, 1069, 1031, 965. ^1H NMR (400 MHz, DMSO) 11.37 (1 H, app d, J 1.1), 11.20 (1 H, br s), 8.77 – 8.66 (2 H, m), 8.07 – 7.99 (2 H, m), 7.69 – 7.43 (4 H, m), 7.40 – 7.17 (9 H, m), 6.95 (2 H, s), 6.91 (1 H, PH, dd, J 7.22, 11), 6.91 – 6.82 (4 H, m), 6.54 – 6.48 (1 H, m), 6.24 – 6.15 (1 H, m), 5.47 – 5.35 (1 H, m), 5.13 (1 H, dd, J 7.2, 3.7), 4.38 – 4.20 (3 H, m), 4.09 (1 H, dd, J 6.5, 3.4), 3.75 – 3.67 (6 H, m), 2.82 – 2.73 (3 H, m), 2.59 – 2.53 (3 H, m), 2.15 – 2.12 (3 H, m). ^{13}C NMR (101 MHz, DMSO) 206.9 (C), 172.1 (C), 165.6 (C), 163.6 (C), 158.2 (C), 152.0 (C), 51.7 (C), 150.5 (C), 150.4 (C), 147.2 (C), 146.8 (C), 144.5 (C), 143.2 (C), 141.9 (C), 135.6 (CH), 135.2 (C), 135.1 (C), 133.3 (C), 132.5 (CH), 129.7 (CH), 128.9 (C), 128.6 (CH), 128.5 (CH), 127.9 (CH), 127.7 (CH), 126.8 (C), 125.9 (C), 121.3 (CH), 113.3 (C), 113.2 (CH), 112.8 (CH), 109.9

(C), 86.1 (CH), 83.8 (CH), 83.6 (CH), 74.0 (CH), 64.9 (CH₂), 64.7 (CH₂), 63.1 (CH₂), 63.0 (CH₂), 55.0 (CH₃), 37.4 (CH₂), 33.2 (CH), 29.5 (CH₃), 28.0 (CH₂), 27.7 (CH₃), 24.8 (CH₃), 23.85 (CH₃). ³¹P NMR (decoupled 162 MHz, DMSO) 9.24, 8.71. HRMS (ESI) calculated for C₅₃H₅₃N₇O₁₄P (M-H)⁻, 1042.3394 found 1042.3376.

5'-ODMTri N⁶-Benzoyl Adenosine 3'-5'-H-Phosphonate 3-OLev
Thymidine, 145c

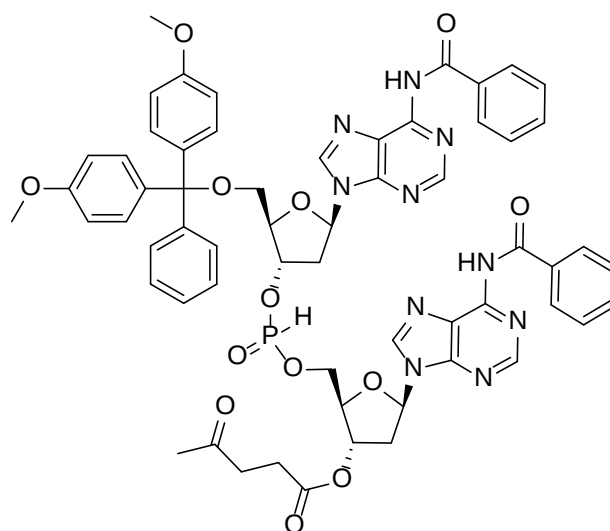


mixture of diastereomers

122 mg, 0.117 mmol, 59 %. $\nu_{\max}/\text{cm}^{-1}$ (ATR) 3055, 2955, 2867, 1687, 1606, 1581, 1553, 1509, 1491, 1461, 1422, 1380, 1360, 1297, 1246, 1174, 1155, 1069, 1052, 1032, 1011, 965. ¹H NMR (400 MHz, DMSO) 11.38 (1 H, app d, J 6.4), 11.20 (1 H, s), 8.59 (1 H, d, J 4.1), 8.13 (1 H, d, J 7.4), 8.04 (2 H, d, J 7.4), 7.96 (0.5 H, s), 7.69 – 7.49 (5 H, m), 7.34 (2 H, d, J 7.2), 7.27 – 7.14 (6 H, m), 6.96 (2 H, s), 6.81 (3 H, ddd, J 8.9, 6.8, 2.1), 6.52 (1 H, dd, J 10.1, 3.7), 6.27 – 6.11 (1.5 H, m), 5.37 (1 H, d, J 3.9), 5.21 (1 H, t, J 6.6), 4.38 – 4.22 (3 H, m), 4.14 (1 H, s), 3.71 (6 H, app t, J 1.5), 3.34 (2 H, s), 3.28 (3 H, d, J 4.1), 2.75 – 2.66 (3 H, m), 2.40 (1 H, ddd, J 16.3, 7.9, 2.0), 2.30 – 2.19 (1 H, m), 1.87 – 1.71 (3 H, m). ¹³C NMR (101 MHz, DMSO) 206.9

(C), 172.1 (C), 165.6 (C), 163.6 (C), 163.6 (C), 158.1 (C), 151.9 (C), 151.5 (C), 151.1 (C), 150.5 (C), 150.5 (C), 150.4 (C), 147.2 (C), 146.8 (C), 146.2 (C), 144.7 (C), 143.5 (C), 141.8 (C), 135.8 (CH), 135.4 (C), 135.3 (C), 133.3 (C), 132.8 (CH), 132.5 (CH), 129.7 (CH), 128.6 (CH), 128.5 (CH), 128.5 (CH), 127.8 (CH), 127.6 (CH), 126.7 (CH), 126.0 (C), 121.4 (CH), 114.4 (C), 113.1 (CH),, 110.1 (C), 110.1 (C), 85.7 (C) 85.7 (C), 84.0 (CH), 83.9 (CH), 81.7 (CH), 76.1 (CH), 65.1 (CH₂), 65.1 (CH₂), 65.0 (CH₂), 64.9 (CH₂), 63.2 (CH₂), 63.1(CH₂), 55.0 (CH₃), 37.40 (CH₂), 35.5 (CH₂), 33.3 (CH), 29.5 (C), 28.0 (CH) 27.7 (CH), 27.0 (CH₂), 12.1 (CH₃), 12.1 (CH₃). ³¹P NMR (162 MHz, DMSO) 9.51 (dq, J 720, 9) 8.66 (dq, J 723, 8). HRMS (ESI) calculated for C₅₃H₅₃N₇O₁₄P (M-H)⁻ 1042.3394, found 1042.3387.

5'-ODMTr N⁶-Benzoyl Adenosine 3'-5'-H-Phosphonate 3'-OLev N⁶-Benzoyl Adenosine



mixture of diastereomers

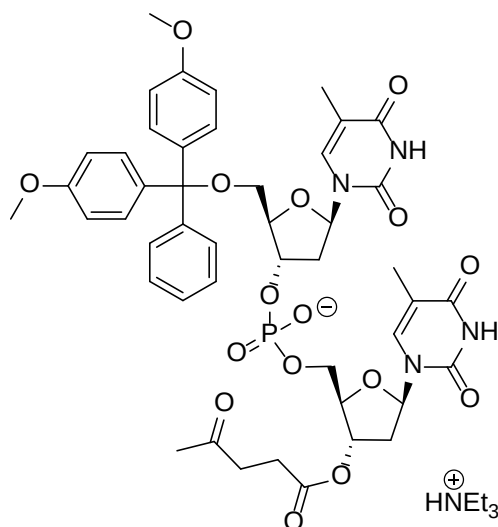
98.0 mg, 0.0847 mmol, 85% yield. ν_{max} /cm⁻¹ (ATR) 2955, 2867, 1738, 1690, 1605, 1581, 1509, 1491, 1461, 1422, 1382, 1359, 1303, 1243, 1155, 1084, 1069, 1052, 1032, 1011, 969. ¹H NMR (400 MHz,

DMSO) 11.20 (1 H, s), 8.84 – 8.63 (2 H, m), 8.59 (2 H, dd, *J* 7.8, 6.2), 8.04 (4 H, dd, *J* 7.1, 4.6), 7.69 – 7.48 (7 H, m), 7.39 – 7.28 (2 H, m), 7.28 – 7.10 (8 H, m), 6.98 (4 H, dd, *J* 7.21, 8), 6.85 – 6.72 (4 H, m), 6.57 – 6.45 (2 H, m), 5.47 (1 H, d, *J* 5.9), 5.33 (1 H, dd, *J* 5.0, 2.6), 4.43 – 4.17 (4 H, m), 3.69 (6 H, d, *J* 1.2), 3.31 – 3.10 (4 H, m), 2.84 – 2.73 (3 H, m), 2.70 – 2.52 (4 H, m), 2.11 (3 H, d, *J* 2.0). ¹³C NMR (101 MHz, DMSO) 206.9, 172.1 (C), 165.6 (C), 158.1 (C), 152.0 (C), 151.7 (C), 151.4 (C), 150.5 (C), 147.3 (C), 144.7 (C), 143.6 (C), 143.5 (C), 143.2 (C), 135.4 (C), 135.3 (C), 133.3 (C), 132.5 (CH), 129.7 (CH), 128.5 (CH), 128.5 (CH), 127.8 (CH), 127.6 (CH), 126.7 (CH), 126.1 (C), 125.9 (C), 113.1 (CH), 83.8 (CH), 76.1 – 75.8 (CH, m), 75.9 (CH), 74.1 (CH), 74.0 (CH), 65.0 – 64.7 (CH₂, m), 63.2 – 63.0 (CH₂, m), 55.0 (CH₃), 37.4 (CH₂), 36.5 – 36.1 (CH₂, m), 35.53 – 35.18 (CH₂, m), 29.51 (CH₃), 27.7 (CH₂). ³¹P NMR (162 MHz, DMSO) 9.20 (dq, *J* 7.21, 8.), 8.67 (dq, *J* 7.21, 8). HRMS (ESI) calculated for C₆₀H₅₈N₁₀O₁₃P (M+H)⁺ 1157.3907, found 1157.3902.

4.5.3 Dinucleotide Phosphonates Table 12

Representative Procedure

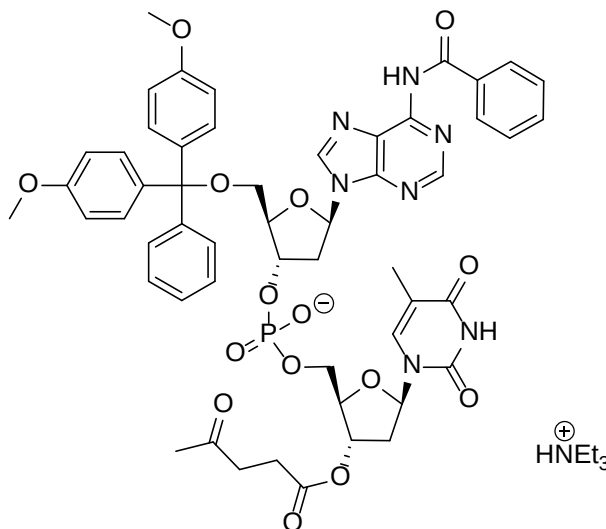
5'-ODMTr Thymidine 3'-5'-Phosphonate 3'-OLev Thymidine Et₃N Salt, 146a



5-ODMTr Protected H-Phosphonate Thymidine (78.1 mg, 0.110 mmol) and the 3'OLev Protected Thymidine (34.0 mg, 0.100 mmol) were co-evaporated in dry pyridine (2 x 2.0 mL), then dissolved in dry pyridine/MeCN (1:1 v/v 1.0 mL). A solution of TIPBSCl (72.5 mg, 0.240 mmol) in dry MeCN (2.9 mL) and dry pyridine (0.12 mL) was then added. Reaction mixture was stirred at room temperature for 15 min, solvent evaporated *in vacuo* and azeotroped with PhMe (2 x 4 mL). The crude product was then dissolved in dry pyridine (1.0 mL), then a 2% I2 solution in pyridine/water (96:4 v/v 1.0 mL) was added. Reaction was stirred at room temperature for 15 min, then diluted with CH₂Cl₂ (3.0 mL), washed with 10% aqueous Na₂S₂O₃ (1.0 mL) and then 1.0 M TEAB (1.0 mL). Organic layer was separated, solvent evaporated *in vacuo* and azeotroped with PhMe (3 x 3 mL). The crude product was then purified using silica gel chromatography using the

solvent system $\text{CH}_2\text{Cl}_2:\text{MeOH}:\text{Et}_3\text{N}$ 89:10:1 to 84:15:1 with oven dried silica to give the product as a white solid (93.0 mg, 0.0887 mmol, 89 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3061, 3034, 2808, 1684, 1607, 1583, 1508, 1465, 1401, 1363, 1275, 1247, 1176, 1156, 1102, 1059, 1031, 961. ^1H NMR (400 MHz, DMSO) 11.32 (2 H, app d, J 18.8), 7.86 (1 H, s), 7.50 (1 H, s), 7.36 (2 H, d, J 7.5), 7.29 (2 H, t, J 7.5), 7.23 (5 H, d, J 8.9), 6.87 (4 H, dd, J 8.9, 3.2), 6.21 (2 H, app dt, J 9.3, 5.1), 5.16 (1 H, app d, J 5.5), 4.75 (1 H, s), 4.13 (1 H, s), 4.01 (1 H, s), 3.82 (2 H, s), 3.72 (6 H, d, app J 1.2), 3.26 (1 H, dd, J 10.2, 4.0), 3.17 (1 H, dd, J 10.2, 2.4), 2.82 (8 H, br s), 2.72 (2 H, t, J 6.4), 2.49 – 2.46 (2 H, m), 2.38 – 2.15 (4 H, m), 2.14 – 2.07 (1 H, m), 1.75 (3 H, s), 1.35 (3 H, s), 1.08 (9 H, t, J 7.2). ^{13}C NMR (101 MHz, DMSO) 206.8 (C), 171.9 (C), 163.7 (C), 163.6 (C), 158.2 (C), 158.2 (C), 150.6 (C), 150.4 (C), 144.6 (C), 136.0 (CH), 135.5 (CH), 135.3 (C), 135.1 (C), 129.7 (CH), 129.7 (CH), 127.9 (CH), 127.7 (CH), 126.8 (CH), 113.2 (CH), 110.1 (C), 109.7 (C), 86.0 (C), 84.6 (d, J 5, CH), 83.8 (CH), 83.6 (CH), 83.2 (d, J 7.0, CH), 75.4 (CH), 74.9 (CH), 64.71 (d, J 5, CH_2), 64.0 (CH_2), 55.0 (CH_3), 45.5 (CH_2), 37.4 (CH_2), 36.2 (CH_2), 27.7 (CH_2), 12.00 (CH_3), 11.6 (CH_3), 9.5 (CH_3). ^{31}P NMR (162 MHz, DMSO) -2.30 (s). HRMS (ESI) calculated for $\text{C}_{46}\text{H}_{51}\text{N}_4\text{O}_{16}\text{P}$ (M-H)⁻ 945.2965, found 945.2949.

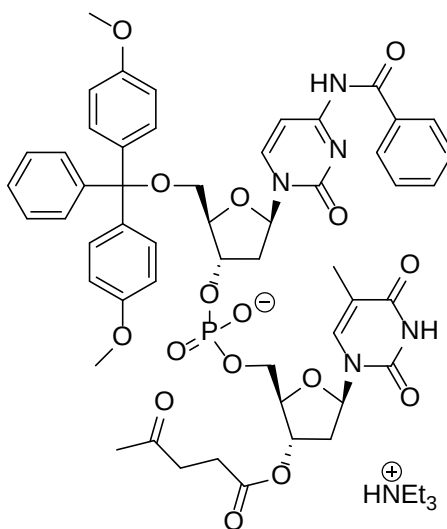
5'-ODMTri N6-Benzoyl Adenosine 3'-5'-Phosphonate 3-OLev
Thymidine Et₃N Salt, 146b



107 mg, 0.0922 mmol, 92 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3060, 2966, 2937, 2835, 2476, 2103, 1734, 1687, 1607, 1579, 1509, 1487, 1449, 1401, 1358, 1322, 1297, 1275, 1246, 1176, 1156, 1060, 1030, 946. ¹H NMR (400 MHz, DMSO) 11.29 (2 H, br s), 8.59 (1 H, s), 8.55 (1 H, s), 8.12 – 7.98 (2 H, m), 7.88 (1 H, s), 7.64 (1 H, t, *J* 7.4), 7.55 (2 H, t, *J* 7.5), 7.33 (2 H, d, *J* 6.7), 7.25 – 7.14 (6 H, m), 6.78 (4 H, t, *J* 9.4), 6.49 (1 H, d, *J* 7.2), 6.22 (1 H, dd, *J* 9.0, 5.8), 5.21 (1 H, d, *J* 5.5), 4.84 (1 H, s), 4.29 (1 H, s), 4.03 (1 H, s), 3.90 – 3.84 (2 H, m), 3.70 (6 H, d, *J* 2.6), 3.29 (1 H, dd, *J* 9.9, 6.4), 3.18 (1 H, dd, *J* 9.9, 3.6), 3.11 – 3.02 (1 H, m), 2.81 (8 H, br s), 2.72 (2 H, t, *J* 6.4), 2.62 – 2.54 (1 H, m), 2.50 – 2.45 (3 H, m), 2.31 – 2.20 (1 H, m), 2.18 – 2.06 (4 H, m), 1.08 (9 H, t, *J* 7.2). ¹³C NMR (101 MHz, DMSO) 206.8 (C), 171.9 (C), 165.7 (C), 163.7 (C), 158.0 (C), 158.0 (C), 151.9 (C), 151.3 (CH), 150.6 (C), 150.4 (C), 144.8 (C), 143.2 (CH), 136.0 (CH), 135.5 (C), 135.5 (C), 133.4 (C), 132.4 (CH), 129.7 (CH), 129.6 (CH), 128.5 (CH), 128.5 (CH), 127.7 (CH), 126.6 (CH), 126.0 (C), 113.0

(CH), 110.2 (C), 85.4 (C), 85.34 (d, J 5 CH), 84.2 (CH), 83.6 (CH), 83.3 (d, J 7.0, CH), 75.5 (CH), 74.9 (d, J 5, CH), 64.8 – 64.7 (m, CH₂), 64.3 (CH₂), 54.9 (CH₃), 45.5 (CH₂), 37.4 (CH₂), 37.2 (CH₂), 36.2 (CH₂), 29.5 (CH₃), 27.7 (CH₂), 9.6 (CH₃). ³¹P NMR (162 MHz, DMSO) -2.14. HRMS (ESI) calculated for C₅₃H₅₄N₇O₁₅P (M-H)⁻ 1058.3343, found 1058.3322.

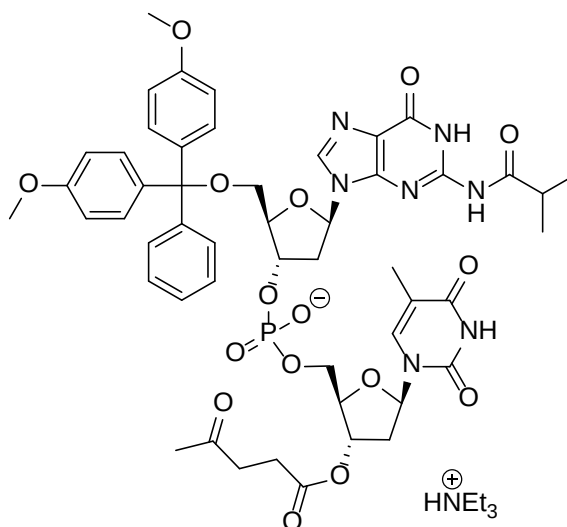
5'-ODMTTr 4-N-benzoyl 2'-deoxycytosine 3'-5'-H-Phosphonate 3-OLev
Thymidine, 146c



111 mg, 0.0980 mmol, 98 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3062, 2975, 2935, 2837, 2465, 2333, 1980, 1735, 1685, 1608, 1559, 1507, 1484, 1395, 1299, 1246, 1176, 1557, 1108, 1059, 1031, 958. ¹H NMR (400 MHz, DMSO) 11.30 (6 H, br s), 8.14 (1 H, d, J 7.5), 8.04 – 7.97 (2 H, m), 7.87 (1 H, s), 7.62 (1 H, t, J 7.5), 7.51 (2 H, t, J 7.5), 7.40 – 7.13 (10 H, m), 6.88 (4 H, d, J 9.0), 6.21 (1 H, dd, J 9.0, 5.8), 6.15 (1 H, t, J 6.2), 5.20 (1 H, d, J 5.5), 4.78 – 4.70 (1 H, m), 4.22 (1 H, dd, J 7.2, 3.0), 4.03 (1 H, br s), 3.84 (2 H, s), 3.73 (2 H, s), 3.34 (1 H, dd, J 10.4, 3.0), 3.24 (1 H, dd, J 10.4, 3.0), 2.86 (8 H, app d, J 6.3), 2.73 (2 H, t, J 6.4), 2.59 – 2.52 (1 H, m), 2.30 – 2.19 (2 H, m), 2.19

– 2.07 (4 H, m), 1.74 (3 H, s), 1.09 (9 H, t, J 7.2). ^{13}C NMR (126 MHz, DMSO) 206.8 (C), 171.9 (C), 167.5 (C), 163.7 (C), 163.0 (C), 158.1 (C), 154.2 (C), 150.6 (C), 144.5 (C), 144.3 (CH), 136.0 (CH), 135.3 (C), 135.2 (C), 133.2 (C), 132.7 (CH), 129.7 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 126.8 (CH), 113.2 (CH), 96.1 (CH), 86.3 (CH), 86.0 (C), 84.98 (d, J 6, CH), 83.7 (CH), 83.2 (d, J 7.0, CH), 75.5 (CH), 73.6 (d, J 4, CH), 64.7 (d, J 5, CH_2), 63.2 (CH_2), 55.0 (CH_3), 45.5 (CH_2), 37.4 (CH_2), 36.2 (CH_2), 29.5 (CH_3), 27.7 (CH_2), 9.3 (CH_3). δ_{P} ^{31}P NMR (162 MHz, DMSO) -2.30. HRMS (ESI) calculated for $\text{C}_{52}\text{H}_{54}\text{N}_5\text{O}_{16}\text{PNa}$ ($\text{M}+\text{Na}$) $^{+}$ 1058.3195, found 1058.3170.

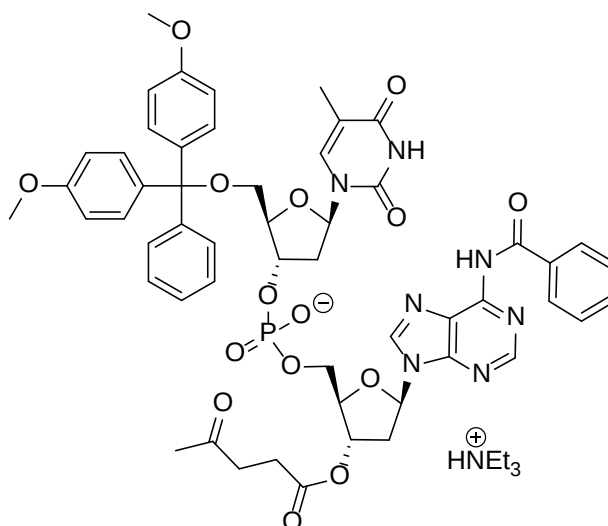
5'-ODMTr-2N-isobutryl-2'-deoxyguanosine 3'-5'-Phosphonate 3'-OLEv Thymidine Et_3N Salt, 146d



108 mg, 0.0945 mmol, 94 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2967, 2935, 2836, 2480, 2105, 1985, 1680, 1607, 1556, 1508, 1465, 1446, 1400, 1360, 1320, 1297, 1276, 1247, 1177, 1156, 1100, 1060, 1031, 945. ^1H NMR (400 MHz, DMSO) 11.53 (3 H, d, J 6.2), 8.04 (1 H, s), 7.84 (1 H, s), 7.35 – 7.27 (2 H, m), 7.25 – 7.14 (7 H, m), 6.78 (4 H, t, J 8.6), 6.29 (1 H, dd, J 8.2, 5.6), 6.20 (1 H, dd, J 9.0, 5.8), 5.17 (1 H, d, J

5.5), 4.74 (1 H, s), 4.27 – 4.19 (1 H, m), 4.02 (1 H, s), 3.85 (2 H, s), 3.71 (6 H, s), 3.31 – 3.23 (1 H, m), 3.11 – 3.06 (1 H, m), 2.83 – 2.67 (11 H, m), 2.46 (1 H, t, *J* 6.3), 2.28 – 2.17 (1 H, m), 2.15 – 2.06 (2 H, m), 1.72 (3 H, app d, *J* 0.8), 1.12 (6 H, d, *J* 6.8), 1.06 (9 H, t, *J* 7.2). ¹³C NMR (101 MHz, DMSO) 206.8 (C), 180.0 (C), 171.9 (C), 163.7 (C), 158.0 (C), 158.0 (C), 154.9 (C), 150.6 (C), 148.6 (C), 148.1 (C), 144.7 (C), 137.3 (CH), 136.0 (CH), 135.4 (C), 129.7 (CH), 129.6 (CH), 127.7 (CH), 126.6 (CH), 120.4 (C), 113.0 (CH), 110.2 (C), 85.5 (C), 85.1 (d, *J* 5, CH), 83.7 (CH), 83.3 (CH), 83.2 (CH), 83.2 (CH), 75.5 (CH), 74.9 (d, *J* 5, CH), 64.8 (d, *J* 5, CH₂), 64.4 (CH₂), 54.6 (CH₃), 54.9 (CH₃), 45.6 (CH₂), 37.8 (CH₂), 37.4 (CH₂), 36.1 (CH₂), 34.9 (CH), 29.5 (CH₃), 27.7 (CH₂), 18.9 (CH₃), 18.8 (CH₃), 12.0 (CH₃), 9.8 (CH₃). ³¹P NMR (162 MHz, DMSO) -2.83. HRMS (ESI) calculated for C₅₀H₅₅N₇O₁₆P (M-H)⁻ 1040.3448, found 1040.3455.

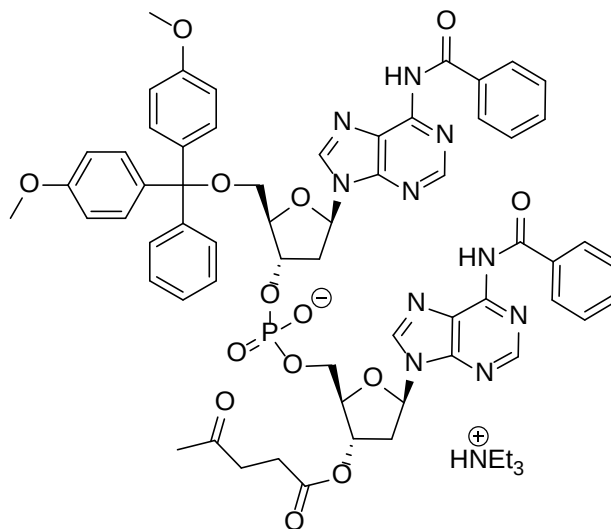
5'-ODMTTr Thymidine 3'-5' Phosphonate 3-OLev N6-benzoyl Adenosine Et₃N Salt, 146e



108 mg, 0.0930 mmol, 93 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3064, 2932, 2836, 2474, 2349, 2091, 1991, 1736, 1686, 1607, 1580, 1488, 1448, 1358,

1294, 1274, 1246, 1176, 1098, 1061, 1030, 962. ^1H NMR (400 MHz, DMSO) 11.34 (2 H, br s), 8.85 (1 H, s), 8.73 (1 H, s), 8.07 – 7.97 (2 H, m), 7.68 – 7.59 (1 H, m), 7.54 (2 H, t, J 7.6), 7.49 (1 H, d, J 7.8), 7.40 – 7.11 (9 H, m), 6.84 (4 H, dd, J 8.9, 3.7), 6.52 (1 H, dd, J 8.5, 6.3), 6.20 (1 H, dd, J 8.5, 5.8), 5.39 (1 H, d, J 5.5), 4.75 (1 H, s), 4.19 (1 H, t, J 4.0), 4.12 (1 H, d, J 4.0), 3.89 – 3.84 (2 H, m), 3.69 (6 H, d, J 2.8), 3.30 – 3.23 (1 H, m), 3.17 – 3.10 (1 H, m), 3.04 – 2.94 (1 H, m), 2.84 (6 H, d, J 7.2), 2.76 (2 H, t, J 6.5), 2.54 (2 H, t, J 6.8), 2.36 – 2.19 (2 H, m), 2.12 (3 H, s), 1.07 (9 H, t, J 7.2). ^{13}C NMR (101 MHz, DMSO) 206.9 (C), 171.9 (C), 163.6 (C), 158.1 (C), 158.1 (C), 152. (C), 151.6 (CH), 150.4(C), 150.3 (C), 144.6 (C), 142.9 (CH), 135.1 (C), 133.4 (C), 132.4 (CH), 129.7 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.6 (CH), 126.7 (CH), 125.5 (C), 113.2 (CH), 86.0 (C), 84.57 (d, J 5, CH), 83.8 (CH), 83.3 (CH), 75.7 (CH), 75.0 – 74.8 (m, CH), 64.7 (d, J 5, CH_2), 64.0 (CH_2), 55.0 (CH_3), 45.4 (CH_2), 38.2 (CH_2), 37.4 (CH_2), 36.5 (CH_2), 29.5 (CH_3), 27.8 (CH_2), 9.3 (CH_3). ^{31}P NMR (162 MHz, DMSO) -2.47. HRMS (ESI) calculated for $\text{C}_{53}\text{H}_{53}\text{N}_7\text{O}_{15}\text{P}$ (M-H) $^-$ 1058.3343, found 1058.3340.

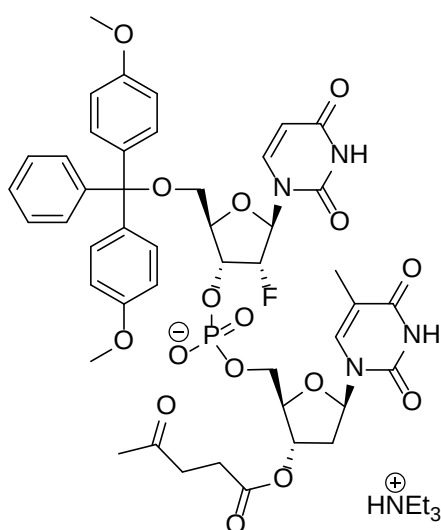
5'-ODMTr N6-Benzoyl Adenosine 3'-5'-Phosphonate 3-OLev N6-Benzoyl Adenosine Et₃N Salt, 146f



112 mg, 0.0879 mmol, 88 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3058, 2933, 2838, 2466, 2322, 2106, 1995, 1735, 1697, 1607, 1579, 1508, 1487, 1451, 1404, 1333, 1296, 1245, 1221, 1176, 1156, 1094, 1062, 1031, 935. ¹H NMR (400 MHz, DMSO) 11.18 (2 H, br s), 8.89 (1 H, s), 8.73 (1 H, s), 8.59 (1 H, s), 8.53 (1 H, s), 8.32 (1 H, s), 8.07 – 8.00 (4 H, m), 7.67 – 7.59 (2 H, m), 7.54 (4 H, dd, *J* 14.1, 7.4), 7.32 (2 H, d, *J* 7.3), 7.22 – 7.08 (6 H, m), 6.76 (3 H, t, *J* 9.0), 6.57 – 6.43 (2 H, m), 5.43 (1 H, d, *J* 5.3), 4.86 (1 H, s), 4.28 (1 H, s), 4.22 (1 H, s), 3.92 (2 H, s), 3.67 (6 H, d, *J* 2.1), 3.27 (1 H, dd, *J* 9.9, 6.3), 3.17 (1 H, dd, *J* 9.9, 3.5), 3.09 – 2.97 (2 H, m), 2.85 (3 H, dd, *J* 13.9, 6.8), 2.75 (2 H, t, *J* 6.3), 2.63 – 2.52 (3 H, m), 2.11 (3 H, s), 1.08 (9 H, t, *J* 7.2). ¹³C NMR (101 MHz, DMSO) 206.90, 171.94, 165.71, 157.97, 152.17, 151.93, 151.6 (CH), 151.4 (CH), 150.36, 144.79, 143.2 (CH), 143.0 (CH), 135.58, 135.45, 133.43, 133.39, 132.4 (CH), 129.7 (CH), 128.5 (CH), 128.5 (CH), 127.6 (CH), 126.6 (C), 125.9 (C), 125.5 (C), 113.1 (CH), 85.5 (CH), 85.26 (dd, *J* 6, 2, CH), 84.1 (CH), 83.8 (CH),

83.4 (CH), 75.7 (CH), 75.0 (dd, J 4, 2, CH), 64.8 (CH₂), 64.7 (CH₂), 64.2 (CH₂), 54.9 (CH₃), 45.5 (CH₂), 37.4 (CH₂), 36.5 (CH₂), 29.5 (CH₃), 27.8 (CH₂), 9.3 (CH₃). ³¹P NMR (162 MHz, DMSO) -2.53. HRMS (ESI) calculated for C₆₀H₅₆N₁₀O₁₄P (M-H)⁻ 1171.3681, found 1171.3661.

5'-ODMTr-2'-F Uridine-3'-5'-Phosphonate 3'-OLev Thymidine Et₃N Salt, 148



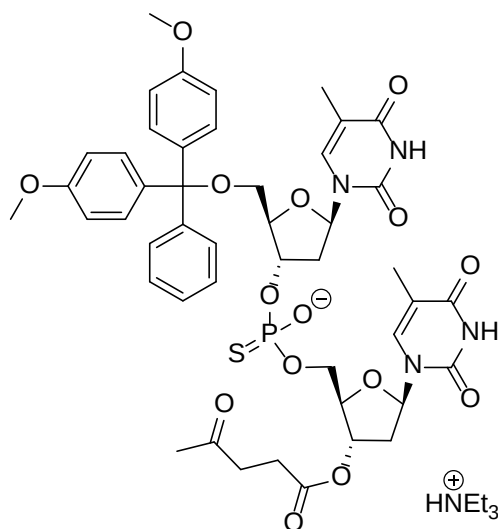
Using the procedure as above, the product was obtained as a white solid, 97.0 mg, 0.0922 mmol, 92 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3059, 2977, 2933, 2806, 1682, 1607, 1582, 1509, 1461, 1401, 1377, 1321, 1297, 1246, 1176, 1156, 1110, 1086, 1059, 1031, 1000, 963. ¹H NMR (400 MHz, DMSO) 11.31 (2 H, s), 7.86 (1 H, d, J 1.0), 7.77 (1 H, d, J 8.2), 7.40 – 7.36 (2 H, m), 7.32 – 7.19 (7 H, m), 6.91 – 6.83 (4 H, m), 6.20 (1 H, dd, J 9.0, 5.8), 5.88 (1 H, dd, J 16.4, 15.6), 5.29 – 5.07 (3 H, m), 4.76 (1 H, dtd, J 20.8, 8.0, 4.6), 4.13 (1 H, dd, J 7.8, 4.4), 3.99 (1 H, s), 3.82 – 3.75 (2 H, m), 3.73 (6 H, d, J 2.1), 3.42 – 3.28 (2 H, m), 2.89 (6 H, d, J 7.2), 2.73 (2 H, t, J 6.4), 2.49 – 2.46 (2 H, m), 2.30 – 2.20 (1 H, m), 2.18 – 2.12 (1 H, m), 1.74 (3 H, app d, J

1.0), 1.15 (1 H, d, J 6.9), 1.10 (9 H, t, J 7.2). ^{13}C NMR (101 MHz, DMSO) 206.9 (C), 171.9 (C), 163.7 (C), 163.0 (C), 158.2 (C), 158.1 (C), 150.6 (C), 150.1 (C), 144.6 (C), 140.6 (CH), 136.1 (CH), 136.1 (CH), 135.2 (C), 135.1 (C), 129.9 (CH), 129.9 (CH), 127.9 (CH), 127.8 (CH), 126.8 (CH), 123.9 (CH), 113.2 (CH), 110.1 (C), 101.5 (CH), 93.2 (CH), 91.3 (CH), 88.7 (CH), 88.4 (CH), 86.0 (C), 83.7 (CH), 83.1 (d, J 7, CH), 80.6 (d, J 7 CH), 75.4 (CH), 70.2 (dd, J 16.0, 5.0, CH), 64.8 (d, J 5, CH_2), 61.9 (CH_2), 55.0 (CH_3), 45.5 (CH_2), 37.4 (CH_2), 36.2 (CH_2), 27.7 (CH_2), 24.4 (CH_3), 9.0 (CH_3). ^{31}P NMR (162 MHz, DMSO) -2.17 (q, J 6). ^{19}F NMR (376 MHz, DMSO) -199.18 (dt, J 52, 20). HRMS (ESI) calculated for $\text{C}_{45}\text{H}_{47}\text{FN}_4\text{O}_{16}\text{P}$ 949.2714, found 949.2712.

4.5.4 Dinucleotide Phosphorothioates Table 13

Representative Procedure

5'-ODMTr Thymidine 3'-5'-Phosphorothioate 3'-OLev Thymidine Et₃N Salt, 149a

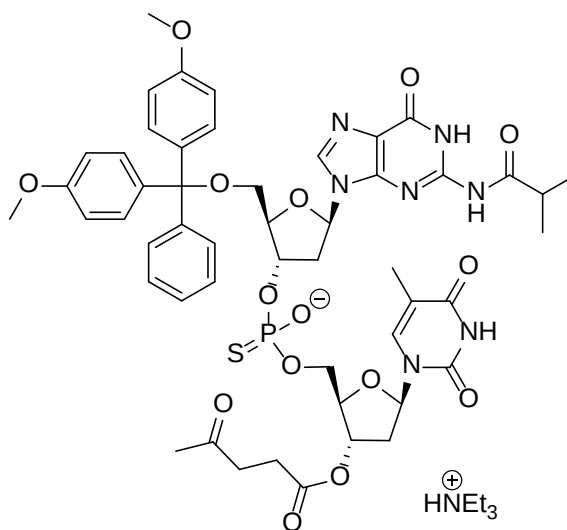


mixture of diastereomers

5-ODMTr Protected H-Phosphonate Thymidine (78.1 mg, 0.110 mmol) and the 3'OLev Protected Thymidine (34.0 mg, 0.100 mmol) were co-evaporated in dry pyridine (2 x 2.0 mL), then dissolved in dry pyridine/MeCN (1:1 v/v 1.0 mL). A solution of TIPBSCl (72.5 mg, 0.240 mmol) in dry MeCN (2.9 mL) and dry pyridine (0.12 mL) was then added. Reaction mixture was stirred at room temperature for 15 min, solvent evaporated *in vacuo* and azeotroped with PhMe (2 x 4 mL). The crude product was then dissolved in dry pyridine (4.0 mL), then sulfur (12.8 mg) was added. Reaction was stirred at room temperature for 2.5 h, then solvent evaporated *in vacuo* and azeotroped with PhMe (3 x 50 mL). The crude product was then purified using silica gel chromatography using the solvent system CH₂Cl₂:MeOH:Et₃N 89:10:1 with oven dried silica to give the product

as a white solid as a mixture of diastereomers (103 mg, 0.0968 mmol, 97 %). $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2956, 2930, 2868, 2687, 2504, 2332, 2237, 2110, 1991, 1687, 1603, 1564, 1509, 1462, 1422, 1400, 1381, 1295, 1275, 1249, 1215, 1155, 1109, 1082, 1067, 1050, 1033, 1008, 960. ^1H NMR (400 MHz, CDCl_3) 8.91 (2 H, br s), 7.77 (1 H, app d, J 1.1), 7.63 (1 H, d, J 1.1), 7.40 (2 H, dd, J 7.4, 1.6), 7.32 – 7.14 (7 H, m), 6.88 – 6.77 (4 H, m), 6.48 – 6.34 (2 H, m), 5.45 (1 H, d, J 5.6), 5.39 – 5.26 (2 H, m), 5.24 (1 H, d, J 5.3), 4.37 (1 H, s, J 1.4), 4.30 (1 H, s, J 7.3), 4.18 (1 H, s, J 7.2), 4.12 – 4.03 (2 H, m), 3.97 (1 H, ddd, J 11.2, 6.1, 2.6), 3.78 (6 H, d, J 5.1), 3.52 (1 H, dd, J 10.4, 2.5), 3.44 – 3.37 (2 H, m), 3.12 (6 H, q, J 7.3), 2.74 (2 H, t, J 6.6), 2.60 – 2.53 (2 H, m), 2.44 – 2.30 (4 H, m), 2.24 – 2.19 (1 H, m), 1.98 – 1.94 (6 H, m), 1.33 (9 H, t, J 7.3). ^{13}C NMR (126 MHz, CDCl_3) 206.5 (C), 172.2 (C), 163.9 (C), 158.7 (C), 158.7 (C), 150.6 (C), 150.5 (C), 144.4 (C) 136.4 (CH), 136.0 (CH), 135.7 (CH), 135.6 (C), 135.5 (C), 130.2 (CH), 129.2 (CH), 128.3 (CH), 128.0 (CH), 127.1 (CH), 113.3 (CH), 87.1 (C), 85.7 (d, J 6, CH), 85.2 (d, J 6.0, CH), 83.77 (d, J 8 (CH)), 83.6 (d, J 9, CH), 76.2 (CH), 75.6 (CH), 70.6 (CH), 65.83 (d, J 6.0, CH_2), 65.5 (d, J 7.0, CH_2), 55.3 (CH_3), 46.1 (CH_2), 37.2 (CH_2), 37.2 (CH_2), 28.1 (CH_2), 12.5 (CH_3), 12.5 (CH_3), 8.7 (CH_3). ^{31}P NMR (162 MHz, CDCl_3) 57.22 (dd, J 15, 8.), 56.83 (dd, J 15, 8). HRMS (ESI) calculated for $\text{C}_{46}\text{H}_{50}\text{N}_4\text{O}_{15}\text{PS}$ (M-H) $^-$ 961.2736, found 961.2710.

5'-ODMTr-2N-isobutryl-2'-deoxyguanosine 3'-5'-Phosphorothioate 3'-
 OLev Thymidine Et₃N Salt, 149b

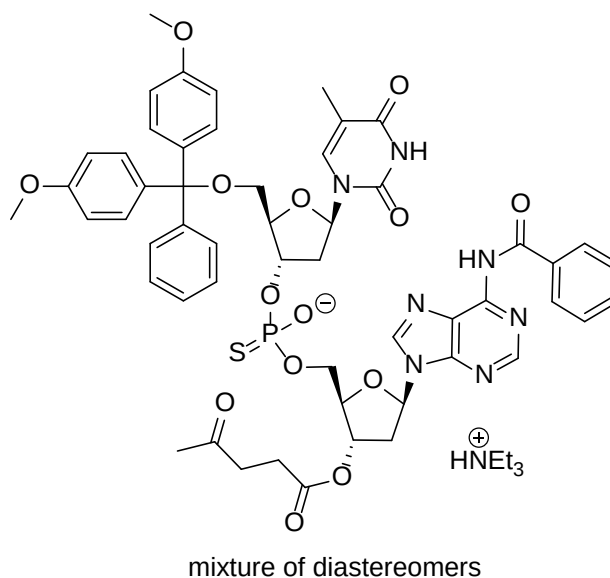


mixture of diastereomers

113 mg, 0.0975 mmol, 98 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2967, 2932, 2869, 2689, 2502, 2240, 2107, 1682, 1605, 1560, 1509, 1462, 1420, 1401, 1381, 1360, 1315, 1298, 1276, 1249, 1214, 1175, 1154, 1068, 1082, 1068, 1050, 1032, 1008, 912. ¹H NMR (400 MHz, CDCl₃) 12.14 (1 H, s), 9.84 (1 H, app d, *J* 63.6), 8.09 – 8.01 (1 H, m), 7.45 – 7.36 (1 H, m), 7.34 – 7.11 (10 H, m), 6.88 – 6.71 (4 H, m), 6.51 – 6.32 (1 H, m), 6.17 – 6.06 (1 H, m), 5.57 – 5.40 (2 H, m), 5.40 – 5.24 (1 H, m), 4.41 – 4.20 (2 H, m), 4.18 – 3.99 (3 H, m), 3.83 – 3.70 (6 H, m), 3.43 – 3.24 (2 H, m), 3.12 (6 H, q, *J* 7.3), 3.01 – 2.91 (1 H, m), 2.89 – 2.79 (1 H, m), 2.80 – 2.51 (6 H, m), 2.42 – 2.25 (2 H, m), 2.18 (3 H, app d, *J* 2.6), 1.32 (9 H, t, *J* 7.3), 1.20 – 1.15 (6 H, m). ¹³C NMR (126 MHz, CDCl₃) 206.5 (C), 206.5 (C), 179.9 (C), 179.7 (C), 172.3 (C), 165.0 (C), 164.9 (C), 158.6 (CH), 158.6 (CH), 155.8 (C), 155.7 (C), 150.7 (C), 149.2 (CH), 148.5 (C), 148.3 (C), 148.1 (C), 148.0 (C), 147.4 (C), 144.6 (C), 144.5 (C), 139.6 (C), 137.3

(CH), 137.2 (CH), 136.9 (CH), 135.7 (C), 135.7 (C), 135.6 (C), 135.6 (C), 130.2 (CH), 130.2 (CH), 129.2 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 127.9 (CH), 127.8 (CH), 127.1 (CH), 127.0 (CH), 113.2 (CH), 113.2 (CH), 113.2 (CH), 111.5 (C), 86.6 (C), 86.6 (C), 85.9 (d, *J* 5, CH), 85.7 (CH), 85.5 (d, *J* 6, CH), 84.8 (CH), 84.3 (CH), 84.3 (CH), 83.9 (CH), 83.8 (CH), 83.5 (CH), 81.4 (CH), 75.8 (CH), 70.6 (CH₂), 66.0 (d, *J* 5, CH₂), 65.1 (d, *J* 5, CH₂), 64.2 (CH₂), 64.1 (CH₂), 55.3 (CH₃), 46.2 (CH₂), 40.1 (d, *J* 3, CH₂), 39.4 (d, *J* 3, CH₂), 37.8 (CH₂), 37.7 (CH₂), 37.7 (CH₂), 35.9 (CH), 35.9 (CH), 34.1 (CH), 28.0 (CH₂), 28.0 (CH₂), 12.5 (CH₃), 12.5 (CH₃), 8.7 (CH₃). ³¹P NMR decoupled (162 MHz, CDCl₃) 57.6, 57.4. HRMS (ESI) calculated for C₅₀H₅₅N₇O₁₅PS (M-H)⁻ 1056.3220, found 1056.3193.

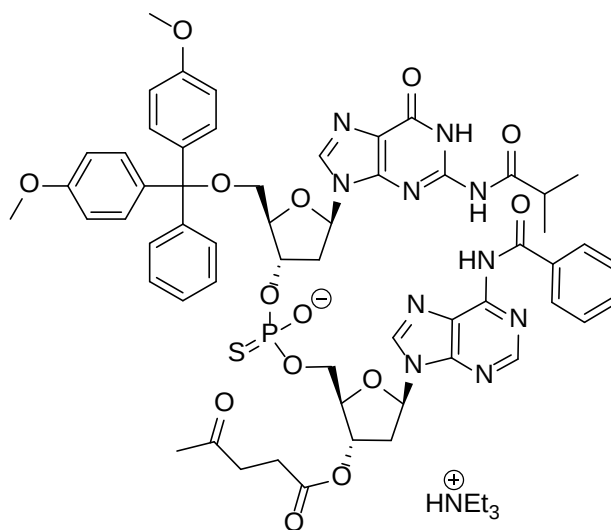
5'-ODMT Tr Thymidine 3'-5' phosphorothioate 3-OLev N6-benzoyl Adenosine Et₃N Salt, 149c



114 mg, 0.0968 mmol, 97 %. $\mu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2956, 2868, 2689, 2506, 2334, 1689, 1604, 1580, 1509, 1461, 1422, 1401, 1381, 1360, 1296, 1249, 1218, 1154, 1106, 1081, 1068, 1050, 1032, 1007, 961.

^1H NMR (400 MHz, CDCl_3) 9.46 (1 H, d, J 104.0), 9.01 – 8.70 (2 H, m), 8.07 – 7.81 (2 H, m), 7.65 – 7.36 (5 H, m), 7.36 – 7.11 (10 H, m), 6.88 – 6.75 (3 H, m), 6.66 (1 H, ddd, J 20.3, 8.8, 5.9), 6.59 – 6.43 (1 H, m), 5.65 – 5.32 (2 H, m), 4.38 – 4.25 (2 H, m), 4.25 – 3.95 (2 H, m), 3.74 (5 H, dd, J 8.0, 3.7), 3.60 – 3.42 (1 H, m), 3.40 – 3.25 (1 H, m), 3.12 (6 H, q, J 7.3), 2.92 – 2.75 (5 H, m), 2.72 – 2.47 (4 H, m), 2.41 – 2.29 (1 H, m), 2.19 (3 H, d, J 4.3), 1.32 (9 H, t, J 7.3). ^{13}C NMR (126 MHz, CDCl_3) 206.5 (C), 206.4 (C), 172.2 (C), 172.1 (C), 165.3 (C), 164.9 (C), 163.8 (C), 163.7 (C), 158.7 (C), 158.7 (C), 152.5 (CH), 152.0 (C), 151.9 (C), 150.4 (C), 149.8 (CH), 149.7 (C), 149.6 (C), 144.5 (C), 144.5 (C), 142.4 (CH), 142.2 (CH), 135.9 (CH), 135.9 (CH), 135.7 (C), 135.5 (C), 135.4 (C), 134.2 (C), 133.8 (C), 132.7 (CH), 132.5 (CH), 130.2 (CH), 130.2 (CH), 128.7 (CH), 128.7 (CH), 128.3 (CH), 128.2 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.1 (CH), 123.2 (C), 123.2 (C), 113.4 (CH), 113.3 (CH), 111.3 (C), 111.2 (C), 87.1 (C), 87.0 (C), 85.78 (d, J 5, CH), 85.27 (d, J 6, CH), 84.8 (CH), 84.7 (CH), 84.4 (d, J 8, CH), 84.3 (d, J 8, CH), 84.1 (CH), 84.0 (CH), 77.4 (CH), 76.4 (CH), 76.2 (CH), 66.2 (d, J 6, CH_2), 65.7 (d, J 6, CH_2) 64.1 (CH_2), 55.3 (CH_3), 46.1 (CH_2), 39.8 (d, J 4.0, CH_2), 39.3 (d, J 4 CH_2), 38.6 (CH_2), 38.6 (CH_2), 37.9 (CH_2), 29.9 (CH_3), 28.0 (CH_2), 28.0 (CH_2), 11.5 (CH_3), 11.5 (CH_3) 8.75 (CH_3). ^{31}P NMR decoupled (162 MHz, CDCl_3) 57.3, 57.3. HRMS (ESI) calculated for $\text{C}_{53}\text{H}_{53}\text{N}_7\text{O}_{14}\text{PS}$ (M-H) $^-$ 1074.3114, found 1074.3096.

5'-ODMTr-2N-isobutryl-2'-deoxyguanosine 3'-5'-Phosphorothioate 3-OLev N6-benzoyl Adenosine Et₃N Salt, 149d



mixture of diastereomers

122 mg, 0.0959 mmol, 96 %. $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 2957, 2934, 2869, 2689, 2504, 2239, 1736, 1681, 1605, 1581, 1562, 1509, 1459, 1421, 1401, 1382, 1360, 1315, 1299, 1249, 1215, 1154, 1104, 1082, 1050, 1032, 1008, 912. ¹H NMR (400 MHz, CDCl₃) 11.98 (1 H, br s), 9.62 (1 H, s), 9.27 (1 H, br s), 8.78 (2 H, app dd, J 15.4, 11.1), 8.02 – 7.91 (2 H, m), 7.78 (1 H, d, J 7.3), 7.59 – 7.36 (5 H, m), 7.30 – 7.26 (4 H, m), 7.21 – 7.09 (3 H, m), 6.76 – 6.69 (4 H, m), 6.61 (1 H, app td, J 8.6, 6.4), 6.14 (1 H, dt, J 12.1, 6.3), 5.83 – 5.44 (2 H, m), 4.36 – 4.15 (3 H, m), 4.15 – 3.96 (1 H, m), 3.74 – 3.68 (6 H, m), 3.40 – 3.24 (2 H, m), 3.10 (6 H, q, J 7.3), 2.76 (2 H, dd, J 12.3, 6.4), 2.68 – 2.38 (5 H, m), 2.17 (3 H, app d, J 5.6), 1.29 (9 H, t, J 7.3), 1.11 – 0.99 (6 H, m). ¹³C NMR (101 MHz, CDCl₃) 206.5 (C), 206.5 (C), 179.4 (C), 179.3 (C), 172.2 (C), 172.1 (C), 158.5 (CH), 158.4 (CH), 155.8 (C), 155.7 (C), 152.5 (CH), 151.8 (CH), 151.7 (C), 149.5 (C), 149.5 (C), 148.1 (C), 147.5 (CH), 147.4 (C), 144.7 (C), 144.7 (C).

142.4 (CH), 142.3 (CH), 138.4 (CH), 137.9 (CH), 135.8 (C), 135.7 (C), 133.5 (C), 132.7 (CH), 130.1 (CH), 130.1 (CH), 128.9 (CH), 128.8 (CH), 128.2 (CH), 128.2 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 126.8 (CH), 126.7 (CH), 113.1 (CH), 113.0 (CH), 86.3 (C), 86.2 (C), 85.0 (d, J 8, CH), 84.7 (d, J 8, CH), 84.6 (CH), 84.5 (CH), 84.4 (CH), 84.4 (CH), 84.3 (CH), 84.3 (CH), 84.1 (CH), 77.4 (CH), 76.3 (CH), 76.2 (CH), 75.7 (d, J 4, CH), 75.6 (d, J 4, CH), 65.9 (d, J 6, CH₂), 65.5 (d, J 6, CH₂), 63.8 (d, J 4, CH₂), 55.2 (CH₃), 46.1 (CH₂), 39.1(CH₂), 38.5 (CH₂), 37.8 (CH₂), 37.8 (CH₂), 36.1 (CH), 36.0 (CH), 19.1 (CH₃), 18.9 (CH₃) 18.8 (CH₃), 18.6 (CH₃), 8.8 (CH₃), 8.6 (CH₃).
31P NMR decoupled (162 MHz, CDCl₃) 57.3, 57.3. HRMS (ESI) calculated for C₅₇H₅₈N₁₀O₁₄PS (M-H)⁻ 1169.3598, found 1169.3594.

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