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Asymmetric organocatalytic synthesis of dinucleoside phosphorothioates. Towards *antisense* therapy.

Síntesi asimètrica organocatalítica de dinucleòsids-fosforotioats. Aproximació a la teràpia *antisense*.

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June 2017

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A little learning is a dangerous thing;
Drink deep, or taste not the Pierian spring.

Alexander Pope

I would like to first thank my supervisor, Professor Alan Spivey for his help and guidance throughout this project. His support and enthusiasm during the numerous discussions and group meetings have made me grow as a chemist. I would also like to thank Dr. Felix Urpí for his supervision during the writing of this report.

I would also like to thank the incredible people in Spivey group. Especially, I would like to thank Chris for his dedicated supervision and early morning coffees; as well as Christine for her help with the writing of this report. Also, I would like to thank Wouter for our discussions in front of the whiteboard; Lukas for his passion for Zürich; Richard for our chats abouts politics and British culture and Adrian, for his timeless music playlists. I would also like to thank Dr. Mohan, for his advice and contagious passion for chemistry.

Last, but not least, I would like to thank my family and friends. My parents and sister, for their unconditional support from a distance; my friends at home and in London; and Marta, for always being there.

REPORT

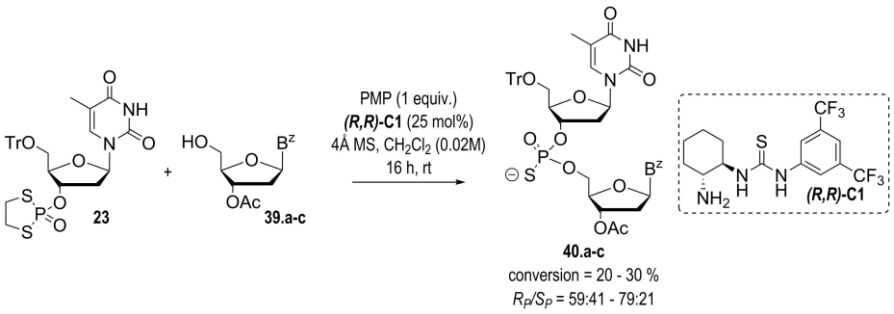
CONTENTS

1. SUMMARY

Phosphorothioate oligonucleotides have been deeply studied as *antisense* therapy drugs. Their stereoselective synthesis, however, still depends on the use of chiral auxiliaries or modifications on the individual nucleosides, which slow their synthesis and increase costs. A catalytic asymmetric synthesis of these oligonucleotides would therefore be of great interest.

Herein the catalytic asymmetric synthesis of dinucleoside phosphorothioates is attempted. After failing to optimise the conditions for the coupling using 4-DMAP-*N*-oxides as nucleophilic catalysts, hydrogen bond donors were envisaged as potential catalysts.

Through the use of bifunctional thioureas containing a tethered amine, adequate conditions for the coupling were found and diastereoselectivity could be measured. Although low conversions were obtained, moderate selectivities were achieved for the synthesis of these dinucleosides.



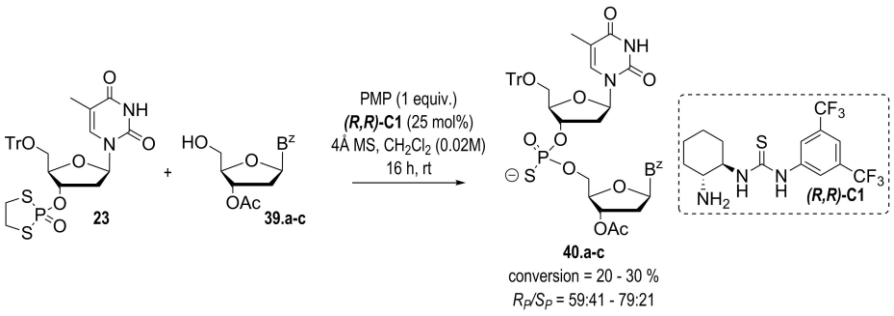
Keywords: dinucleoside phosphorothioates, asymmetric catalysis, bifunctional thioureas, *antisense* therapy.

2. RESUM

Els oligonucleòtids fosforotioats han estat intensament estudiats com a medicaments en teràpia *antisense*. Tanmateix, la seva síntesi estereoselectiva depèn encara de l'ús d'auxiliars quirals o modificacions en els nucleòsids, que alenteixen la síntesi i n'incrementen els costos. La síntesi asimètrica catalítica d'aquests oligonucleòtids desperta doncs un gran interès.

Al present estudi s'assaja la síntesi asimètrica catalítica de dinucleòsids fosforotioats. Després de l'optimització fallida de les condicions de la reacció quan s'empraren 4-DMAP-*N*-òxids com a catalitzadors nucleofílics, els donadors de ponts d'hidrogen van ser avaluats com a potencials catalitzadors.

A través de l'ús de tiourees bifuncionals amb un grup amina, es van aconseguir condicions adequades per a la reacció i la diastereoselectivitat es va poder mesurar. Malgrat que les conversions obtingudes van ser baixes, es van obtenir selectivitats moderades en la síntesi d'aquests dinucleòtids.



Paraules clau: dinucleòsids fosforotioats, catàlisi asimètrica, tiourees bifuncionals, teràpia *antisense*.

3. INTRODUCTION

3.1. *ANTISENSE* THERAPY. THE CONCEPT

According to the so-called *central dogma of molecular biology*, first stated by Crick in 1958, genetic information is found in DNA, and it is expressed through its transcription into mRNA, before being translated into proteins.[1]

In the "classic" target-based drug discovery approach, molecules are screened in biochemical assays in order to find "hits", which are compounds that bind to a specific protein known to have an important role in the metabolic route of a disease. These proteins are mainly ion channels, receptors or enzymes. This approach is therefore mainly focused on the interaction of the drug with the last product in the genetic information flow: proteins.

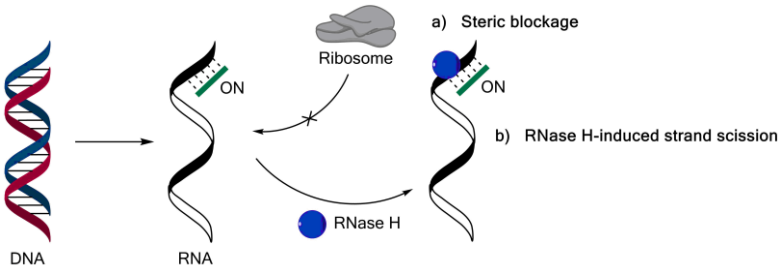
However, Zamecnik and Stephenson proposed an alternative approach in 1978.[2,3] They proved that a single, relatively short strand of unmodified DNA was capable of inhibiting the translation of proteins in the *Rous sarcoma* virus. Instead of inhibiting the activity of certain proteins, it was possible to avoid their synthesis through the interaction of a single strand oligonucleotide (ON) with their corresponding mRNA. This approach was later coined *antisense* therapy.

Zamecnik and Stephenson's discovery was hailed as a therapeutic approach with almost no limitations.[4] Via Watson-Crick base pairing, specific interactions would be easily designed, and diseases with genetic targets or factors could be tackled. However, reality did not meet the expectations, mainly due to the unexpected obstacles encountered during its development. The most important drawbacks in *antisense* therapeutics development were (i) poor stability against nucleases, (ii) inefficient intracellular delivery, (iii) low affinity toward the target and (iv) potential off-target toxicity.[5] As such only two *antisense* oligonucleotides have achieved approval by the FDA: fomivirsen (Vitravene, 1998, discontinued due to low demand) and mipomersen (Kynamro, 2013).[6]

However, the field appears to have now matured with more than 130 clinical trials listed and around 20 *antisense* drugs in phase II or III in clinical trials.[6,7] It is clear then that oligonucleotide therapeutics have gained new momentum and will surely provide new drugs in the near future.

3.2. THE MECHANISM OF ACTION OF *ANTISENSE* ONS

Despite the simplicity of the concept behind *antisense* therapy, no simple mechanisms have been proposed to explain the gene expression disruption caused by ONs. Two major mechanisms are suspected of regulating gene expression: steric blockage and RNase H-induced strand scission (Scheme 1).[7]



Scheme 1. Mechanism of action of antisense ONs. Adapted from Future Med. Chem. 2011, 3 (3), 339–365

Steric blockage is likely to be involved when modified ONs are poor activators of RNase H. Drug occupation of the target RNA hinders the factors involved in translation, blocking the binding of the ribosome to the mRNA. This generates a translational arrest of the target sequence. An alternative form of steric blockage takes place when the drug binds to pre-mRNA, which is an immature mRNA that requires further processing, called splicing. This also blocks its translation.

Alternatively, modified ONs coupling to specific sequences of mRNA can lead to RNase H-induced strand scission.[8] RNase H is a ribonuclease that binds to certain DNA-RNA complexes, degrading only the RNA strand. This is therefore the preferred mechanism, since after degradation of the mRNA the modified ON is released, closing a formal catalytic cycle. However, it should be noted that both mechanisms contribute in variable proportions to the expression of the pharmacological effects of *antisense* therapy.[7]

3.3. CHEMICAL ASPECTS OF *ANTISENSE* ONS

In order to overcome the aforementioned limitations of *antisense* ONs (see section 3.1), numerous modifications to nucleic bases, ribose or deoxyribose, as well as within the phosphate linkage have been explored. However, the most common and successful have been modifications in the backbone structure of ONs. Some of these are the replacement of one of the non-bridging oxygen atoms with a sulfur atom (PS-ONs), a selenium atom (PSe-ONs), a methyl (PMe-ONs) or a borane group (PBH_3-ONs) (Figure 1).[9]

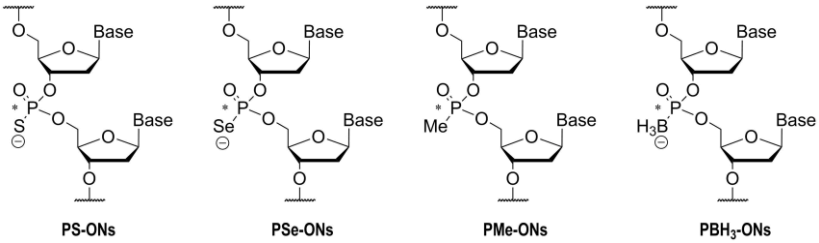


Figure 1. Most common backbone modifications in ONs

Nonetheless, the introduction of the phosphorothioate moiety by Eckstein in 1967[10] has been arguably the most important contribution to the field of *antisense* therapy. Substitution of the O-atom by an isoelectronic S-atom causes an increase in hydrophobicity and acidity, as well as geometric changes due to the more sterically demanding sulfur atom.

Further to these properties, PS-ONs are soluble in water, interact readily with wild-type nucleic acids and maintain the anionic character of oligonucleotides.[7,11] This modification has been proven to increase their nuclease resistance, but also to extend circulation times due to their unspecific binding to serum proteins.[12,13] Moreover, in 2007, Wang and co-workers made an unforeseen discovery: they found a segment of bacterial DNA that contained phosphorothioate linkages.[14] As such, PS-ONs could no longer be called 'unnatural' or 'synthetic', since such molecular architecture is present in Nature.

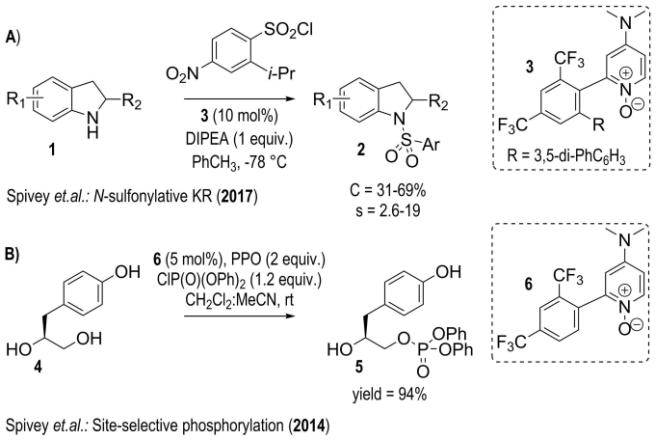
Despite the great improvement in pharmacokinetic properties afforded with PS-ONs, this backbone modification also implies the creation of new P-chiral centres. ONs with *n* nucleoside units include *n*-1 P-chiral centres, thus they exist as a mixture of 2^{n-1} diastereoisomers. This polydiastereoisomerism entails that the measured activity of these compounds is a distribution weighted average of the activities of all isomers, which have similar but different properties. This

is the reason why efforts during the last decades have been focused on the synthesis of oligonucleotides with stereochemically defined internucleotide linkages.[9]

Several approaches have been explored throughout the years in order to obtain enantiopure PS-ONs: separation of diastereoisomers by column chromatography[15] and the use of P-diastereopure phosphorylating reagents.[16–21] A recent publication by Davies[22] reported the first catalytic stereoselective method for the installation of phosphorus-stereogenic phosphoramidates to nucleosides through a dynamic stereoselective process. The catalytic diastereoselective synthesis of dinucleoside phosphorothioate, however, has not yet been reported. Therefore, the final aim of this project can be summarised as such: the catalytic diastereoselective coupling of nucleosides to yield PS-ONs.

3.4. PREVIOUS WORK. DMAP-*N*-OXIDE CATALYSIS

Several chiral DMAP-*N*-oxides have been developed in Spivey group in order to catalyse acylation,[23–25] sulfonylation,[25,26] silylation[25] and phosphorylation reactions[27,28] (Scheme 2). These catalysts have also been employed in the kinetic resolution of indolines[26] and *sec*-alcohols.[24]



Scheme 2. Applications of 4-DMAP-N-oxide catalysts developed by Spivey et. al.[26,28]

Asymmetric phosphorylation has not yet been reported by the group, but 4-DMAP-*N*-oxides have been used as organocatalysts in the site-selective phosphorylation of polyols and amino acid derivatives, with excellent results.[28]

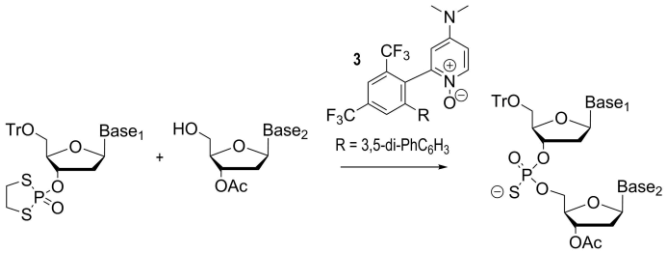
Fessner had previously worked on the asymmetric synthesis of phosphorothioate-nucleosides,[29] following the work by Okruszek[30] in order to prepare the phosphorylating reagent. However, diastereoselectivity was not evaluated for the coupling reaction in the presence of catalyst **3**.[29]

Drawing analogies with previous reports from the group, 4-DMAP-*N*-oxide catalysts are potential catalysts for the coupling between a nucleoside and a phosphorothionucleoside. Therefore, atropisomeric 4-DMAP-*N*-oxide catalyst **3** previously designed by Spivey and co-workers will be the starting point for the development of a stereoselective coupling reaction.

4. OBJECTIVES

The final aim of this project is to catalyse diastereoselective coupling of a nucleoside and a phosphorothionucleoside to form a dinucleoside phosphorothioate (Scheme 3). An atropisomeric 4-DMAP-*N*-oxide catalyst previously reported by the group was selected as a potential catalyst for the reaction. Hence, the initial objectives of the project are:

- Synthesis of the atropisomeric 4-DMAP-*N*-oxide catalyst **3**
- Synthesis of the phosphorylating reagent
- Optimisation of the conditions of the coupling between the phosphorylating reagent and a protected nucleoside.
- Evaluation of the diastereoselectivity of the catalysed coupling reaction



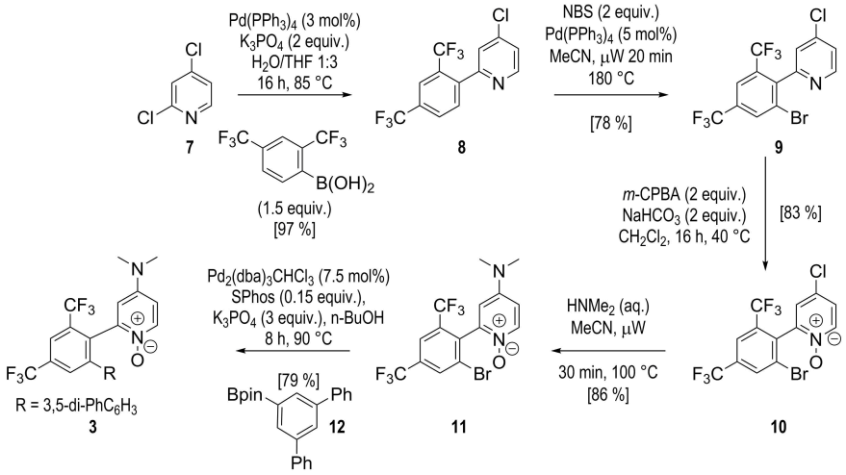
Scheme 3. Nucleoside coupling reaction to yield a dinucleoside phosphorothioate

5. RESULTS AND DISCUSSION

5.1. SYNTHESIS OF THE ATROPISOMERIC 4-DMAP-*N*-OXIDE CATALYST 3

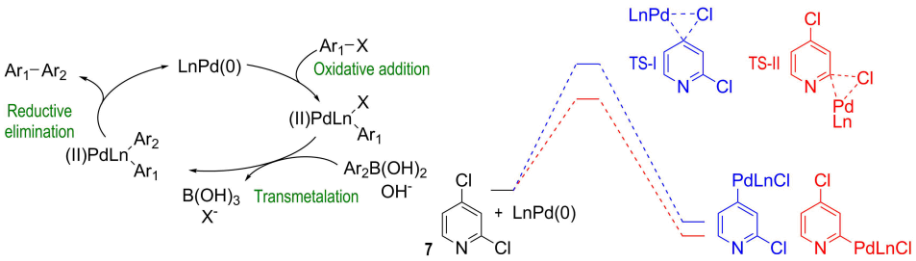
Atropisomeric 4-DMAP-*N*-oxide **3** was envisioned to be a potential catalyst for the diastereoselective coupling to yield a dinucleoside phosphorothioate (*vide supra*). This presumed reactivity was due to previous success for similar reactions in the group.[23,24,26–28]

Therefore, the first aim of this project was to prepare the atropisomeric 4-DMAP-*N*-oxide catalyst **3** as a racemate, in order to later resolve it through preparative chiral HPLC. Its synthesis consisted of 5 steps (Scheme 4):



Scheme 4. Synthesis of the atropisomeric 4-DMAP-N-oxide catalyst 3

In the first step, pyridine **7** was coupled to 2,4-bis(trifluoromethyl)phenylboronic acid via a Suzuki-Miyaura coupling reaction with excellent yields and regioselectivity. The reaction takes place mainly in C2 due to the weaker C–Cl bond, which decreases the transition state energy of the rate-determining step, the oxidative addition (Scheme 5).[31]



Scheme 5. Mechanism of the Suzuki-Miyaura coupling and transition state energy diagram (adapted from Chem. Soc. Rev. , 2007, 36, 1036–1045)

The following step was a palladium-catalysed directed C–H bromination using *N*-bromosuccinimide (NBS) as both a brominating reagent and an oxidant, which yielded the product in also good yields (77% average). The regioselectivity of the reaction is due to the directing effect of the nitrogen in the pyridine ring, which binds to the Pd centre to form a palladacycle (Figure 2).[32] The σ-arylpalladium bond in this complex is reactive and undergoes bromination with NBS.

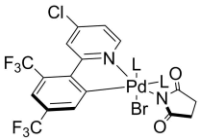


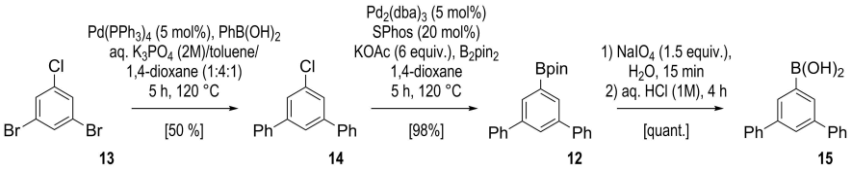
Figure 2. Reactive palladacycle in the bromination of compound 8

The third step involved the oxidation of pyridine **9** with *m*-CPBA to form a pyridine-*N*-oxide. ^{1}H NMR showed full conversion of the starting material, and good isolated product yields were achieved (75 %).

Next, the dimethylamino group was installed at the 4 position of the pyridine-*N*-oxide ring through a nucleophilic aromatic substitution. The reaction was carried out under microwave irradiation, in order to increase the rate of the reaction but also to ensure its regioselectivity. Under microwave activation, if the polarity of a system is enhanced from the ground state to the transition state, such a change results in an acceleration of that particular reaction path.[33] Therefore, substitution of the chloride is the preferred pathway, since the polarity of the C–Cl bond contributes more strongly towards the overall molecular dipole.

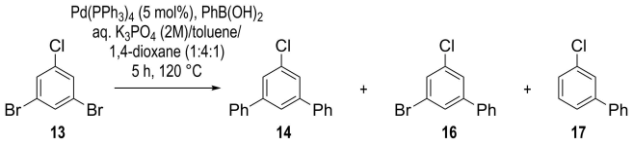
The following step was a Suzuki-Miyaura coupling between compound **11** and *meta*-terphenyl boronic acid. This boronic acid is commercially available and had been sourced for previous preparations of the catalyst. However, this time a route to prepare *meta*-terphenyl boronic acid was explored to reduce costs in the synthesis of the catalyst.

Following a literature search, a protocol towards the synthesis of boronic acid **15** was found and summarized in scheme 6.[34]



Scheme 6. Synthesis of meta-terphenylboronic acid by Kang et. al.[34]

In the first step, a double Suzuki-Miyaura coupling between phenylboronic acid and chlorobenzene **13** was carried out. After 5 hours, no starting material could be detected either by TLC or ^{1}H NMR. However, the purification of compound **14** was challenging, since two side-products with very similar polarities to the product were formed (Scheme 7). Both side-products were identified through ^{1}H NMR and MS: compound **16** was the monocoupling product, and compound **17** the reduction product.



Scheme 7. Product and side-products of the Suzuki-Miyaura coupling

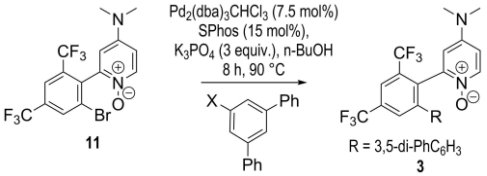
The following reaction was a Miyaura coupling between compound **14** and bispinacolatodiboron using SPhos as a ligand. SPhos is a ligand designed by Buchwald *et. al.* to increase reactivity of the palladium complex and enable the coupling of hindered substrates.[35] The premixing of the ligand and the palladium precatalyst was found essential for the reduction of reaction times (from eight to five hours),[34] as well as a concomitant increase in the isolated yield of product (98% vs 67% reported by Kang).[34] Compound **12** was purified by a very fast chromatography column, since it is prone to hydrolysis on silica.

Finally, the hydrolysis of boronic ester **12** was carried out using sodium periodate and hydrochloric acid to give boronic acid **15** in quantitative yield with no need for further purification. Pinacol, the alcohol released during hydrolysis, undergoes oxidative cleavage with sodium periodate to produce acetone.

Therefore, and after three steps, *meta*-terphenyl boronic acid **15** was obtained with a 49% overall yield.

Once boronic acid **15** was available, the last step of the synthesis of the catalyst **3** could be carried out. However, this reaction had been reported to be a challenging step, since no yields higher than 50% had been reported in the group.[26,29]

As the synthesis of boronic acid **15** went *via* boronic ester **12**, a comparison of the performance of the ester and acid was conducted in an attempt to shorten the overall synthesis. The ligand and palladium adduct were premixed, and after 8 hours (instead of the reported 72 hours[26]) no starting material could be detected in the reaction containing the boronic ester. After isolation, yields could be compared and, unexpectedly, boronic ester **12** performed better with no need for optimisation of the conditions (Table 1).



Entry	X	Isolated yield 3 (%)
1	$B(OH)_2$	50
2	Bpin	79

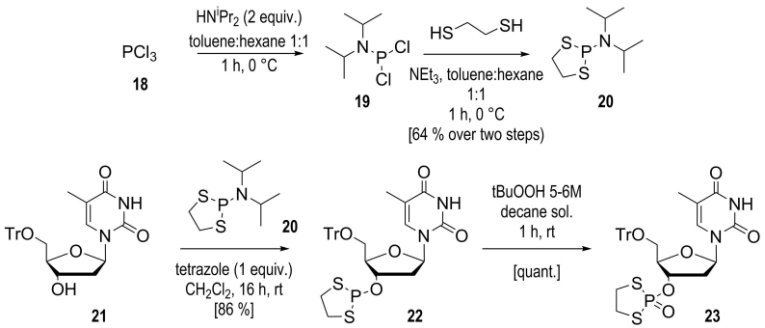
Table 1. Comparison of the performance of boronic ester 12 and boronic acid 13 in the coupling reaction.

Thanks to this increase in yield, catalyst **3** was synthesized in 5 steps with 43% overall yield.

5.2. SYNTHESIS OF THE PHOSPHORYLATING REAGENT

In order to prepare dinucleoside phosphorothioates a phosphorylating reagent is needed. This reagent has to contain a prochiral phosphorus, as well as some moiety that will yield the negatively charged sulphur atom. Following Okruszek's work, compound **23** was chosen to be the object of the nucleophilic attack,[30] and it was prepared according to their protocol

(Scheme 8). The deoxynucleoside of choice for the synthesis of the phosphorylating reagent was thymidine, since it has no functionalities to be protected.



Scheme 8. Synthesis of phosphorylating reagent 23 by Okruszek[30]

Okruszek described the synthesis of dithiaphospholane **20** from phosphorus trichloride, diisopropylamine and 1,2-ethanedithiol (Scheme 8).[30] However, the described procedure involved distillation of phosphorodichloridite **19**, which is pyrophoric and explosive when dry. For safety reasons, the protocol was modified in order to maintain compound **19** in solution.

Following this modified procedure (see section 6.3) dithiaphospholane **20** was prepared with good yields (64%) with no further purification. Both steps were simple substitutions on the phosphorus trichloride starting material, producing insoluble salts (diisopropylammonium chloride and triethylammonium chloride) that were filtered to yield a relatively pure solution of the product. Due to its intrinsic instability, compound **20** was stored under argon in a sealed vial, and its purity monitored periodically by ^{31}P NMR.

For the coupling between nucleoside **21** and dithiaphospholane **20**, a one-pot synthesis was explored, since it was hypothesised that P(III) compound **22** would be relatively unstable and prone to hydrolysis. Hence, by avoiding purification of compound **22**, higher yields would be obtained. Unfortunately, even though the coupling reaction proceeded smoothly, the oxidation of P(III) compound **22** to yield phosphorylating reagent **23** was not successful. The proposed explanation being that the presence of tetrazole in solution may distort the oxidation reaction, since a complex ^{31}P NMR spectrum was obtained in all attempts.

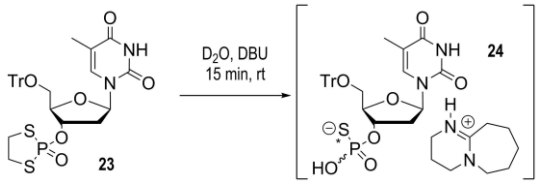
Once the one-pot synthesis was discarded, the procedure for the coupling and oxidation as described by Okruszek[30] was followed with no major modifications. Nucleoside **21** was coupled

to dithiaphospholane **20** using tetrazole as a nucleophilic catalyst with excellent yields (86% average). This was a major improvement on the results previously reported, since conversions had always been reported lower than 50%.[29] A plausible reason for this may be differences in the purity of compound **20**. Despite the fact of being a P(III) compound, nucleoside **22** was purified by column chromatography without apparent loss of product due to decomposition.

Finally, compound **22** was oxidized through the addition of *tert*-butyl hydroperoxide. After 1 hour at room temperature no starting material could be detected by ^{31}P NMR or TLC, and through the addition of anhydrous hexane the product was obtained as a white precipitate. In contrast to P(III) compound **22**, which was relatively stable in silica, P(V) compound **23** hydrolysed rapidly on the TLC plate. Fortunately, centrifugation of the suspension was enough to obtain pure phosphorylating reagent **23,** since the main side-product of the reaction was *tert*-butanol, which stayed in solution.

During characterization of compound **23**, a second peak in the ^{31}P NMR was always present in variable proportions (^{31}P NMR: δ 82.7, 81.7 ppm, $CDCl_3$). Since its intensity increased from the moment the sample was prepared, it was thought to be the product of hydrolysis – even though a markedly upfield shift would be expected for a phosphorothioic acid.

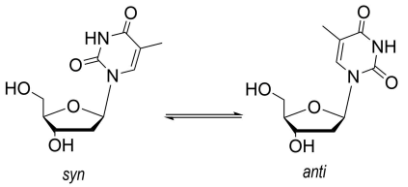
In order to confirm or reject this hypothesis, a simple ^{31}P NMR experiment was carried out. The phosphorylating reagent **23** was mixed in a NMR tube with deuterium oxide and DBU as a non-nucleophilic base. After 15 minutes, no starting material could be detected by ^{31}P NMR, and two new peaks appeared at δ 15.8 and 16.1 ppm. These corresponded to both diastereomers of the hydrolysis product **24**, which exist as DBU salts (Scheme 9).



Scheme 9. DBU-catalysed hydrolysis of phosphorylating reagent 23.

This experiment proved that the second peak at δ 81–83 ppm was not the hydrolysed starting material, but did not help towards its identification. After conducting a literature search, a reasonable explanation was found: nucleosides like thymidine have two main conformers (*syn*

and *anti*) depending on the relative position of the nucleic base and the deoxyribose (Scheme 10).



Scheme 10. Conformational equilibrium of thymidine in solution[36–38]

These conformations have been deeply studied,[36–39] and it is known that their relative populations depend on the solvent and their state, crystalline or in solution. In a solid form, thymidine exists as an individual high energy conformer, the *anti*. However, when the substance is in solution both conformers tend to equilibrate towards ratios that vary from 8:5 to 3:5 depending on the temperature.[37] Despite the difference in structure between the phosphorylating reagent and thymidine, these conformations are compatible with both and hence this is the proposed explanation for the appearance of the second peak in the δ 80 ppm region.

5.3. MODEL REACTION: COUPLING BETWEEN PHOSPHORYLATING REAGENT 23 AND BENZYL ALCOHOL

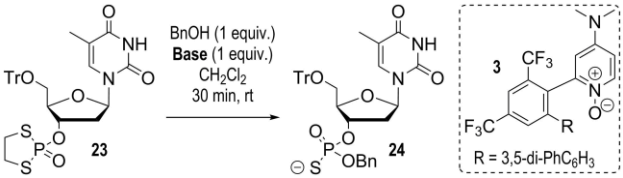
5.3.1 Screening of bases

Before facing the formation of dinucleoside phosphorothioates, the coupling between compound **23** and benzyl alcohol was studied as a model reaction. Benzyl alcohol was chosen since it is UV active, a primary alcohol and analysis of the reaction outcome would be relatively simple.

In order to achieve high levels of diastereoselectivity the difference in rate of the catalysed and non-catalysed reaction is crucial. Therefore, at this stage of the project, reaction conditions were optimised to achieve this difference of reactivity.

Since DBU is known to catalyse the reaction between compound **23** and a second nucleoside[30] –and keeping in mind the necessity to minimise the non-catalysed reaction– a

screening of milder bases was carried out (Table 2). Propylene oxide (PPO) appeared an ideal candidate for the catalysed coupling, since no reaction took place in its presence (Table 2, entry 2). Moreover, Spivey *et. al.* have reported its use as a proton scavenger for the catalysed phosphorylation of alcohols.[28] Triethylamine, 1,2,2,6,6-pentamethylpiperidine (PMP) and 2,2,6,6-tetramethylpiperidine (TMP) showed conversions to the desired product between 25 and 60%, while the reaction in the presence of DBU went almost to completion in agreement with the results reported by Okruszek.[30]

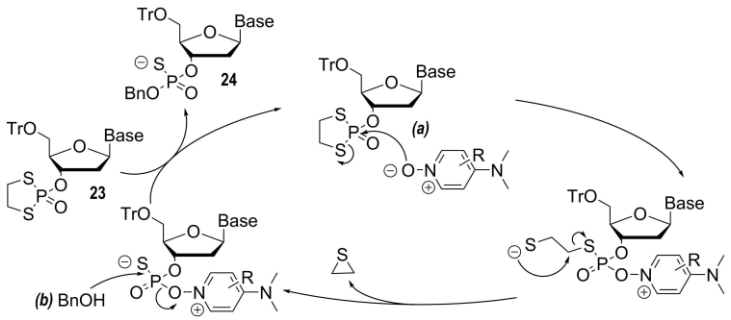


Entry	Base	Conv. (%)[a]
1	DBU	90
2	PPO	nr
3	NEt_3	55
4	PMP	25
5	TMP	60
6	-	nr
7[b]	PPO	nr

(a) Conversion to the desired product, determined by ^{31}P NMR; (b) Reaction carried out in the presence of 25 mol% catalyst **3**

Table 2. Impact of the base on the reaction conversion

Taking this into account, and following the work by Spivey[28], a reaction was set using PPO as a proton scavenger and 4-DMAP-*N*-oxide catalyst **3**. Propylene oxide would enable the reaction to occur without promoting background reaction. Unfortunately, no reaction was detected (Table 2, entry 7).



Scheme 11. Proposed mechanism for the 4-DMAP-N-oxide catalysis

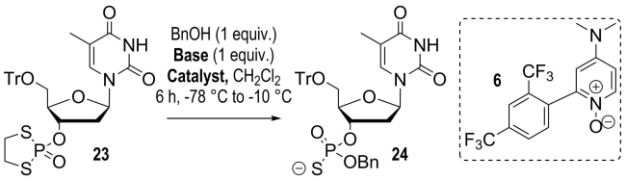
The absence of reactivity could have two main reasons: (a) lack of nucleophilic attack of the DMAP-*N*-oxide on the phosphorus centre or (b) absence of the subsequent attack of the alcohol (Scheme 11).

The presence of the catalyst had been proven to be necessary for the hydrolysis of compound **23** to occur. Therefore, hypothesis (a) was discarded, since almost quantitative catalysed hydrolysis of compound **23** implied turnover of the catalytic cycle.

5.3.2 Basic activation of the nucleophile

Phosphorylating reagent **23** was not as reactive as expected towards a nucleophilic attack. Therefore, efforts were now focused on the screening of conditions or additives that could activate either the nucleophile or electrophile, enhancing the reactivity of the system. The achiral catalyst **6** was used during the optimisation process as a model catalyst.

The first approach, taking into the account results in table 2, was to use a base in order to increase the nucleophilicity of benzyl alcohol. A very mild base at low temperature would be a good candidate, since it would increase the reactivity of the system while attenuating the background reaction. A number of reactions were set, in order to explore the potential of this approach (Table 3). Unfortunately, at low temperatures no reaction could be achieved with PMP or Cs_2CO_3, and at higher temperatures the differential rate of the background reaction compared to catalysed reaction would be unsatisfactory (Table 3, entry 5).



Entry	Base (1 equiv.)	Catalyst (20 mol%)	Conv. (%)[a]
1	PMP	-	nr
2	PMP	**6**	nr
3	Cs_2CO_3	-	nr
4	Cs_2CO_3	**6**	nr
5[b]	PMP	-	25

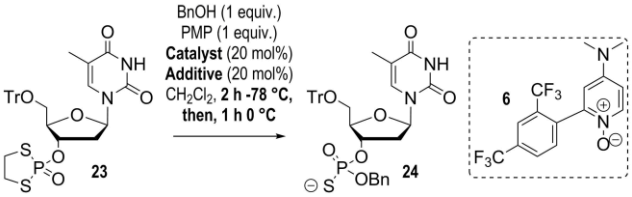
(a) Determined by ^{31}P NMR; (b) Reaction carried out 30 min at room temperature

Table 3. Basic activation of the reaction

5.3.3 Lewis acid activation of the electrophile

A second approach was the activation of the phosphorylating reagent through the decrease of electron density on the phosphorus. The most common method to do this is the addition of a Lewis acid as an additive. The coordination of a Lewis acid to the phosphorylating reagent was expected to increase the electrophilicity of the phosphorus making it more reactive towards the alcohol/alkoxide. This strategy was supported also by the fact that Sculimbrene and coworkers have reported the Lewis acid catalysed phosphorylation of alcohols.[40]

In Sculimbrene's work, different Lewis acids were screened in order to catalyse the phosphorylation of a secondary alcohol by a diphosphate. Titanium and scandium salts were reported as catalysts for this reaction, so they were both included in our screening towards an adequate additive (Table 4, entries 5-8). Unfortunately, in the presence of boron trichloride diethyl ethereate or scandium triflate no conversion could be detected with or without catalyst **6**. On the contrary, titanium tetraisopropoxide promoted the reaction in both the catalysed and non-catalysed route, which would be incompatible with high levels of diastereoselectivity. Therefore, milder conditions for the activation of the substrate needed to be explored.



Entry	Catalyst (20 mol%)	Additive (20 mol%)	Conv. 2h (%)[a]	Conv. 3h (%)[a]
1	-	-	nr	nr
2	**6**	-	nr	nr
3	-	$BCl_3{\cdot}Et_2O$	nr	nr
4	**6**	$BCl_3{\cdot}Et_2O$	nr	nr
5	-	$Ti(OiPr)_4$	nr	50
6	**6**	$Ti(OiPr)_4$	nr	80
7	-	$Sc(OTf)_3$	nr	nr
8	**6**	$Sc(OTf)_3$	nr	nr

(a) Determined by ^{31}P NMR

Table 4. Additives effect on the coupling reaction

5.3.4 Hydrogen bond donor cocatalysis

Since the results after using mild bases or Lewis acids were not promising, a completely different approach was proposed: using a hydrogen bond donor as a cocatalyst in the reaction.

Hydrogen bond donors, particularly ureas and thioureas, have been deeply studied as organocatalysts.[41–45] They have been shown to activate a broad scope of substrates, including carbonyls,[46–52] phosphates[53] and imines[54,55] and a large number of chiral analogues of the thiourea scaffold have been designed with great success.[43,45] Moreover, Seidel *et. al*. reported the use of these chiral thioureas in combination with 4-DMAP to catalyse enantioselective acyl transfer reactions, that have enabled the kinetic resolution of several amines,[56,57] including 1,2-diaryl-1,2-diaminoethanes,[58] meso-diamines,[59] propargylic[60] and allylic amines.[61]

Thus, phosphorylating reagent **23** was envisaged to be a good substrate for this kind of activation, since hydrogen bonding should decrease electron density on the phosphorus and therefore increase its electrophilicity (Figure 3). Schreiner's thiourea catalyst **30** was the additive of choice, since it has been demonstrated to be especially active in a variety of transformations.[53,62]

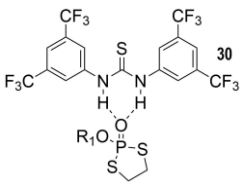
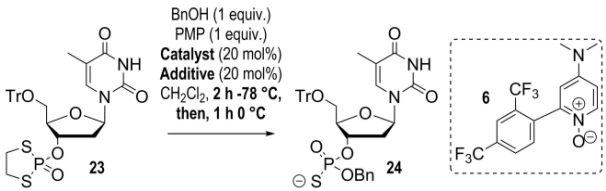


Figure 3. Proposed hydrogen bond donation of thiourea 30 to the substrate

A new set of reactions were carried out in order to determine whether thioureas were adequate additives for the coupling (Table 5). The results obtained were promising: Schreiner's thiourea appeared to be activating the phosphorylating reagent through hydrogen bond donation. However, in the absence of 4-DMAP-*N*-oxide the reaction also proceeded due to this activation even at very low catalyst loadings of Schreiner's catalyst (Table 5, entries 3-4). Thus, the use of an achiral thiourea as a cocatalyst was discarded, due to the unsatisfactory ratio between the catalysed and non-catalysed reaction. However, these same experiments supported a different approach: the use of a chiral thiourea alone as an organocatalyst for the reaction.



Entry	Catalyst (20 mol%)	Additive (20 mol%)	Conv. 2h (%)[a]	Conv. 3h (%)[a]
1	-	**30**	30	na
2	**6**	**30**	80	na
3	-	**30**[b]	nr	40
4	**6**	**30**[b]	nr	90

(a) Determined by ^{31}P NMR; (b) 1 mol%.

Table 5. Effect of the addition of Schreiner's thiourea 30 to the reaction

Schreiner's thiourea catalyst, without any complementary additive, enabled the coupling to take place. Moreover, it had been also proven that in its absence no conversion could be detected (Table 4, entry 1). Therefore, chiral thioureas were finally defined as the potential catalysts for the studied coupling. Hence, the next step would be to find an adequate chiral thiourea that would catalyse a diastereoselective reaction between the phosphorylating reagent **23** and a second nucleoside.

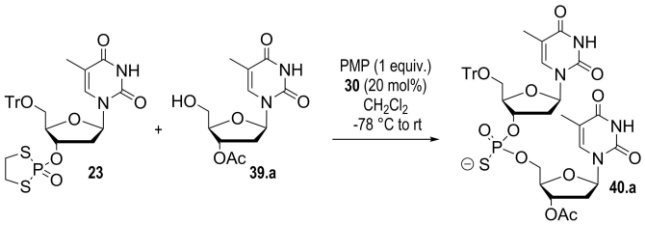
5.4. COUPLING BETWEEN PHOSPHORYLATING REAGENT 23 AND A NUCLEOSIDE

5.4.1 Selection of the hydrogen bond donor catalyst

Once chiral thioureas had been set as the lead catalysts, benzyl alcohol was no longer used in the coupling reactions. Instead, 3'-*O*-acetylthymidine was used as the nucleophile for the

coupling. Thymidine was chosen as the nucleophile, since a homodinucleoside was thought to be the simplest model for the reaction.

Before facing the challenging task of finding an adequate chiral thiourea for the reaction, a set of experiments were carried out with the achiral thiourea **30**, in order to gain an insight into the reactivity of the phosphorylating reagent **23** with protected thymidine **39.a** (Scheme 12).



Scheme 12. Coupling between phosphorylating reagent 23 and nucleoside 39.a

Surprisingly, no reaction could be detected for any of the conditions tested (see appendix, entries 4-9) despite the fact that benzyl alcohol had been reactive as a nucleophile under some of these conditions (Table 5). The protected nucleoside appeared to be a much less reactive nucleophile than benzyl alcohol, which had been used as a model. This difference in reactivity may well be due to the existence of an intramolecular hydrogen bond between the reacting hydroxyl group and a hydrogen of the heterocycle,[63] which increases its steric hindrance (Figure 4).

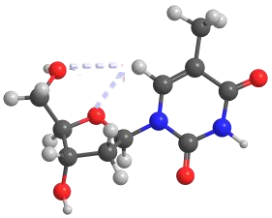


Figure 4. Lowest energy conformer of thymidine. Adapted from J. Phys. Chem. B 2007, 111, 9655-9663

One simple way to increase the nucleophilicity of the second nucleoside would be to increase the number of equivalents of base, or even to increase the concentration of all reactants. However, both these methods were proven to cause the appearance of undesired background reaction by general base catalysis (see appendix, entries 14, 16, 18).

A second approach was proposed and applied: through the addition of a tethered amine to the catalyst, the local concentration of base would increase notably, while keeping the rate of the background reaction low. Therefore, a literature search was conducted in order to find a thiourea catalyst with a base present within the scaffold (Figure 5).

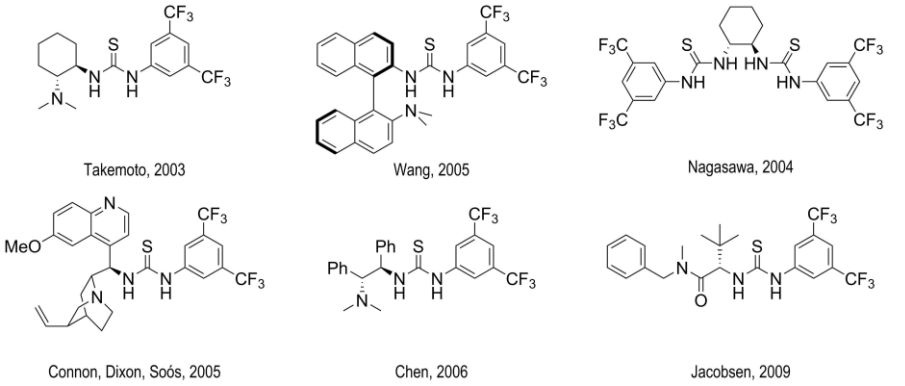


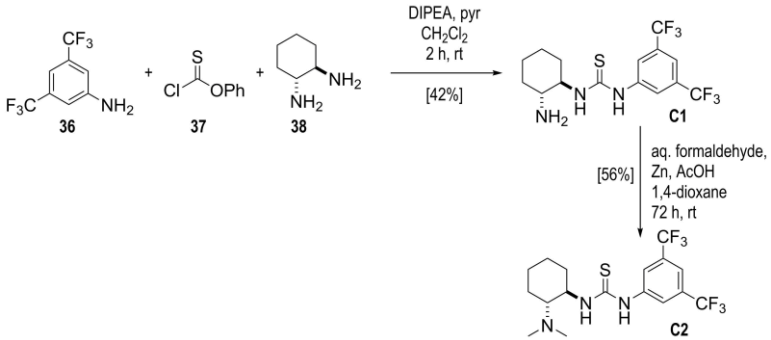
Figure 5. Some popular chiral thiourea organocatalysts[49,64–68]

Further to this, the ideal structure would have: (i) chirality near to the thiourea moiety; (ii) presence of a tethered amine; (iii) electron deficient ring bound to the thiourea moiety, which increases its hydrogen bond donation[62] and (iv) tuneable structure. Following these criteria, Takemoto catalyst was chosen to be the most suitable candidate for the asymmetric synthesis of dinucleoside phosphorothioates.

5.4.2 Synthesis of Takemoto's catalyst analogues and optimisation of the coupling conditions

Berkessel reported a particularly interesting simplified synthesis of Takemoto's catalyst that yields primary amine **C1** as an intermediate (Scheme 13). This synthesis consisted on the sequential coupling of aniline **36**, chlorothioformate **37** and diaminocyclohexane **38**; followed by reductive amination with formaldehyde to provide catalyst **C2**. Primary amine **C1**, apart from being a potential catalyst for the coupling, is also the starting material for a broad number of modifications to yield a scope of catalysts readily available after one or two steps, through *e.g.* alkylation or acylation.

Following this stereospecific route, **(±)-C1** and **(±)-C2** were prepared before the optimisation of the conditions for the coupling.

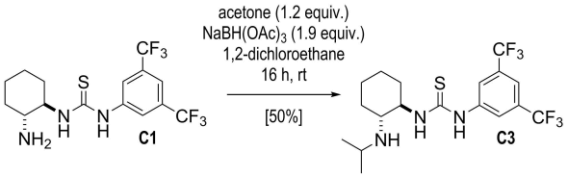


Scheme 13. Simplified synthesis of Takemoto's catalyst by Berkessel[69]

Concentration, temperature and time were the key parameters for the optimisation, since they would determine the difference in rate of the catalysed and background reactions. After an initial screening (see appendix, entries 10-25), optimal conditions for the coupling were determined in which no conversion could be detected without any catalyst, but reaction took place in its presence. Unfortunately, low conversions were always obtained irrespective of the reaction conditions. This may be due to the fast hydrolysis of the starting material to form PMP salt **24** or because of the inhibition caused by the hydrogen bonding between the catalyst and dinucleoside **40.a** or **24**. In order to increase the conversion, 4Å molecular sieves were added to the reaction mixture yielding better but still low conversions (see appendix, entries 22-25).

5.4.3 Evaluation of the diastereoselectivity of the coupling

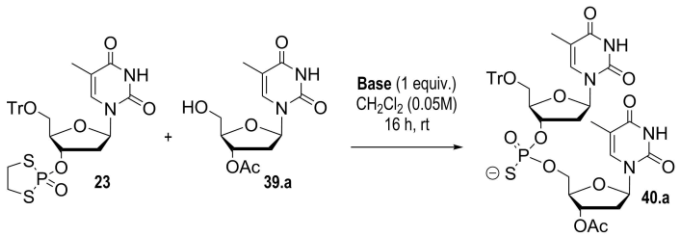
Once reaction conditions had been optimised, a set of homochiral catalysts were prepared in order to measure their chirality induction. Following Berkessel's protocol, ***(R,R)*-C1**, ***(S,S)*-C1** and ***(R,R)*-C2** were synthesised. Moreover, and in order to shed some light on the reaction transition state, the monoalkylated and bulkier thiourea ***(R,R)*-C3** was also synthesized through reductive amination of ***(R,R)*-C1** (Scheme 14).[55]



Scheme 14. Reductive amination of primary amine C1 to secondary amine C3.

Diastereomeric ratios were measured by ^{31}P NMR, and assignment was carried out following work by Okruszek and co-workers.[30] The assignment was based upon analysis of the ^{31}P NMR chemical shifts and RP-HPLC retention times and their comparison with original samples prepared independently by a phosphoramidite methodology.[70] Using these methods, they established that R_P isomers of all dinucleoside phosphorothioates appear at higher frequencies in the ^{31}P NMR and lower retention times in RP-HPLC.

In order to evaluate the diastereoselectivity of the catalysed reaction, the intrinsic selectivity due to the chirality in the reactants had to be first measured. Because of the existence of a chiral environment around the reactive sites of both the electrophile and the nucleophile, natural selectivity needs to be measured. In order to do so, blank experiments were carried out at higher concentrations – known to enable the general basic catalysed reaction (see section 6.6). Also, to shed light on the actual role of the base in the reaction, these blank experiments were carried out using PMP, TMP and DBU as a base (Table 6).



Entry	Base	Natural R_P/S_P(%)[b]
1	DBU	45:55[c]
2	TMP	55:45
3	PMP	71:29

a) Determined by ^{31}P NMR; (b) Determined by ^{31}P NMR, and assigned following Okruszek's work[30]; (c) In agreement with Okruszek's data[30]

Table 6. Natural selectivities obtained in the presence of different bases

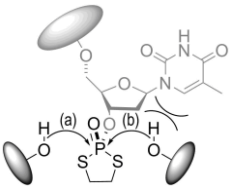
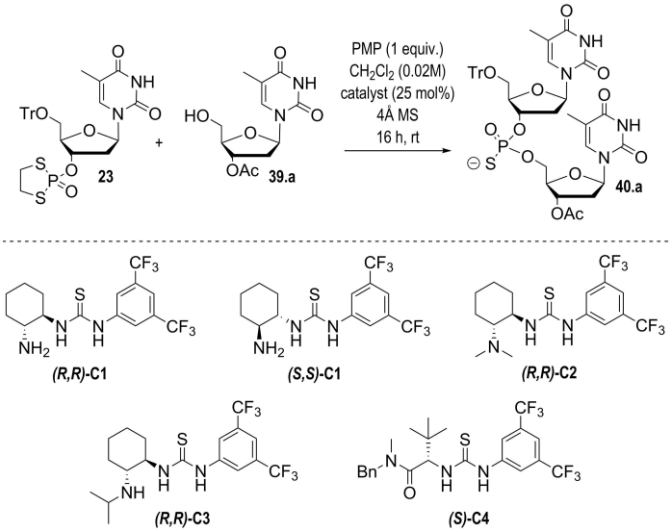


Figure 6. Proposed geometry of the transition state of the coupling.

Surprisingly, the natural selectivity was found to be highly base dependant. This confirmed the role of the base as a general base catalyst; however, it also raised questions as to the nature of the transition state. One hypothesis to explain the preference towards the R_P isomer is that if the rotation of the C3-O bond is restricted through repulsion of the lone pairs of the phospholane and the furan oxygen, then the two possible trajectories for the nucleophilic attack of the incoming alcohol nucleophile would be energetically very different. Trajectory (*a)*, which yields the R_P isomer, would be more favoured than trajectory (*b)*, which yields the S_P isomer, because the latter leads the incoming nucleophile to clash with pyrimidine (Figure 6). This is in agreement with the results obtained with PMP, but does not explain the base dependence or the results obtained using DBU as a base. Computational studies and a set of experiments using different bases, nucleophiles and phospholanes would help in the elucidation of this transition state geometry.

Once the natural selectivity had been determined, evaluation of the induced diastereoselectivity by the H-bonding/base dual-catalysts could be measured. Applying the optimised conditions (see section 6.5) and using the previously prepared chiral catalysts ***(R,R)*-C1**, ***(S,S)*-C1**, ***(R,R)*-C2** and ***(R,R)*-C3**, as well as the commercial Jacobsen's thiourea catalyst ***(S)*-C4**, R_P/S_P ratios were determined by ^{31}P NMR (Table 7).



Entry	Catalyst	Conv. (%)[a]	*RP/SP* (%)[b]
1	*(R,R)*-C1	29	79:21
2	*(S,S)*-C1	27	59:41
3	*(R,R)*-C2	14	72:28
4	*(R,R)*-C3	12	61:39
5	*(S)*-C4	39	70:30

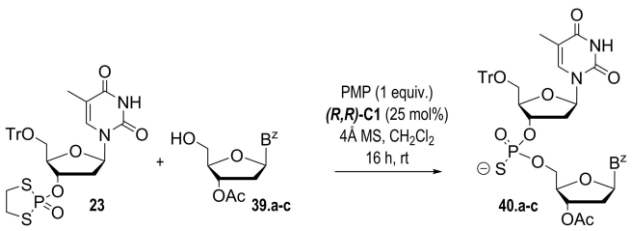
(a) Determined by ^{31}P NMR; (b) Determined by ^{31}P NMR, and assigned following Okruszek's work[30]

Table 7. Evaluation of induced selectivities using different thiourea catalysts

The results obtained proved that chiral thioureas that contain a tethered amine are potential catalysts for the synthesis of dinucleoside phosphorothioates. Even though high levels of diastereoselectivity proved elusive, these catalysts do affect the ratios of diastereomers formed and so must be influencing the energies of the preceding diastereomeric transition states.

The highest selectivity was obtained in the presence of catalyst ***(R,R)*-C1**, which contains a primary amine as a tethered base. However, there was no obvious relationship between the results obtained with catalysts ***(R,R)*-C2** and ***(R,R)*-C3**, which contain a tertiary and a secondary amine, respectively. No link was found between the basicity or bulkiness of the base and the measured selectivity. It is possible that more subtle effects may be responsible for this non-intuitive behaviour; so Sigman analysis[71,72] would likely be informative for optimisation of the catalyst.

Finally, and in order to further understand the effect of the structure of the nucleosides in the coupling, natural and induced selectivity were also evaluated for the coupling of the phosphorylating reagent with deoxyadenosine and deoxycytidine using ***(R,R)*-C1** as a catalyst (Table 8).



Entry	B	Z	Conv. (%)[a]	*RP/SP* (%)[b,c]	Natural *RP/SP* (%)[b,d]
1	dAde	-	nr	na	63:37
2	dCyt	*N4*-acetyl	14	59:41	77:23

(a) Determined by ^{31}P NMR; (b) Determined by ^{31}P NMR, and assigned following Okruszek's work[30]; (c) Following procedure in section 6.5; (d) Following procedure in section 6.6

Table 8. Evaluation of natural and induced selectivities with different nucleosides

The results obtained for the natural selectivity of the coupling of phosphorylating reagent **23** and protected nucleosides **39.b-c** were similar to the ones obtained for the coupling with thymidine **39.a**. For the catalysed reaction, however, adenosine **39.b** did not yield the desired product but the product of *N*-nucleophillic attack to the phosphorylating reagent **41** (Figure 7). This was due to inadequate protection of the amino group at the 6 position the adenine ring. Interestingly, ***(R,R)*-C1** which increased selectivity towards the *Rp* isomer in the coupling with **39.a** (natural: 71:29; measured: 79:21), did the opposite when deoxycytidine **39.c** was used as a nucleophile (natural: 77:23; measured: 59:41)(Table 8).

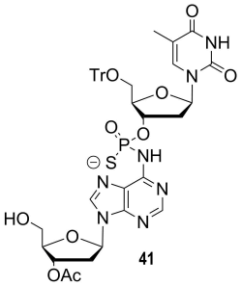


Figure 7. N-nucleophillic attack product 41

In summary, bifunctional thioureas which bear an amine group are potential catalysts for the asymmetric synthesis of dinucleoside phosphorothioates; however, the catalysts evaluated herein provided low conversions and low to moderate selectivities.

5.5. FUTURE WORK

Bifunctional thioureas have been proven to be potential catalysts for the asymmetric synthesis of nucleoside phosphorothioates. However, results obtained in this study are far from optimal and need to be improved. In order to do so, conversion and selectivity of the coupling need to be increased.

Low conversions have been identified to be due to hydrolysis of the starting material, which is also catalysed by the thiourea catalyst and takes place simultaneously to the coupling reaction. In order to minimise this side-reaction, apart from thorough drying of the solvents and reactants, a modification to the phosphorylating reagent may be useful. Modification of the 5-membered ring dithiaphospholane to a less strained six-membered ring (Figure 8) may reduce the instability of the phosphorylating reagent towards hydrolysis. This, together with other modifications on the reaction conditions should be explored in order to achieve higher conversions and isolated yields.

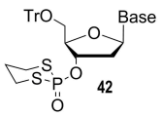


Figure 8. Modified phosphorylating reagent

In order to achieve higher levels of diastereoselectivity, further screening of catalysts – including bifunctional squaramides – and conditions are needed. Moreover, elucidation of the

transition state of the reaction and sources of chirality induction may be also useful, so rational design of the catalyst can be performed. In order to gain an insight in these, computational studies or Sigman analysis (*vide supra*) could be performed.

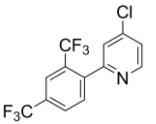
6. EXPERIMENTAL SECTION

6.1. GENERAL DIRECTIONS

All reactions were performed under nitrogen using oven-dried glassware unless stated otherwise. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogenous materials, unless otherwise indicated. CH_3CN, CH_2Cl_2, THF, hexane and toluene were dried and deoxygenated with a Grubbs Pure-Solv 400 solvent purification system. All other materials were obtained from commercial suppliers and used without further purification unless stated otherwise. Flash chromatography (FC) was performed on silica gel (Merck Kieselgel 60 F254 230-400 mesh) unless otherwise stated. Thin Layer Chromatography was performed on Merck aluminium-backed plates pre-coated with silica (0.2 mm, 60 F254) which were visualized either by quenching of ultraviolet fluorescence (λ_{max}= 254 and 366 nm) or by charring with 10% $KMnO_4$ in 1 M H_2SO_4. Melting points were determined on a Stanford Research System OptiMelt. Optical rotations were measured with a Bellingham Stanley APP440+ Polarimeter. Standard infra-red spectra were recorded on Perkin-Elmer Two Spectrum ATR-IR spectrometer and Shimadzu IRAffinity-1S. Only selected absorbances are reported. 1H NMR spectra were recorded at 400 MHz on a Bruker DRX-400 instrument. Chemical shifts (δ_H) are quoted in parts per million (ppm), referenced to the residual solvent peak as an internal standard. Coupling constants (J) are reported to the nearest 0.1 Hz. ^{13}C NMR spectra were recorded at 100 MHz on Bruker AMX- 400 instrument. Chemical shifts (δ_C) are quoted in ppm, referenced to the residual solvent peak as an internal standard. High-resolution mass spectra (m/z) were recorded on Micromass Autospec Premier spectrometer with magnetic sector detector. High Resolution Mass Spectrometry (HRMS) measurements are valid to ±5ppm.

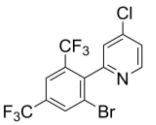
6.2. PREPARATION OF THE 4-DMAP-*N*-OXIDE CATALYST 3

2-(2,4-Bis(trifluoromethyl)phenyl)-4-chloropyridine (8)[26]



According to the method of Spivey,[26] 2,4-bis(trifluoromethyl)phenylboronic acid (2.2 g, 8.5 mmol, 1.5 equiv.), potassium phosphate (2.4 g, 11 mmol, 2.0 equiv.) and $Pd(PPh_3)_4$ (0.39 g, 6 mol%) were dissolved in a 1:3 mixture of degassed water:THF (133 mL). After the addition of 2,4-dichloropyridine (0.84 g, 5.7 mmol, 1.0 equiv.) the mixture was heated (85 °C) for 16 h under a nitrogen atmosphere. The reaction mixture was then allowed to cool down to room temperature and 40 mL of ethyl acetate were added. The organic layer was separated and washed with brine (2 x 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by column chromatography (5-15% EtOAc in hexane) and provided the product as an off-white powder (1.8 g, 97%). m.p. = 67.4 – 70.8 °C; R_f = 0.35 (EtOAc/hexane, 1:4); IR (neat, cm^{-1}) 1574, 1555, 1345, 1282, 1071; ^1H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, *J* = 5.4 Hz, 1H), 8.04 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.39 (dd, *J* = 5.4, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.7, 150.3, 144.3, 142.2, 132.3, 131.3 (q, *J* = 33.7 Hz), 129.3 (q, *J* = 32.2 Hz), 128.5, 124.5, 124.2, 123.8, 123.6, 121.8; HRMS (m/z +ES): Found: M+H, 326.0171. $C_{13}H_6{}^{35}ClF_6N^+$ requires M+H, 326.0179; Δ = 2.5 ppm. Spectroscopic data in agreement with literature.[26]

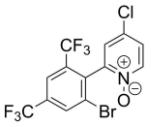
2-(2-Bromo-4,6-bis(trifluoromethyl)phenyl)-4-chloropyridine (9)[26]



According to the method of Spivey,[26] pyridine **8** (0.76 g, 2.5 mmol, 1.0 equiv.), *N*-bromosuccinimide (0.89 g, 5.0 mmol, 2 equiv.) and $Pd(PPh_3)_4$ (0.220 g, 7.5 mol%) were dissolved in acetonitrile (17 mL) in a 20 mL microwave vial. The reaction mixture was then heated to 180 °C for 20 min in a microwave reactor. The solution was then allowed to cool down and the solvent evaporated *in vacuo*. The resulting oil was purified by column chromatography (5% EtOAc in hexane) to provide 2-(2-bromo-4,6-bis(trifluoromethyl)phenyl)-4-chloropyridine as a yellow solid (0.78 g, 78%). m.p. = 42.0 – 44.7 °C; R_f = 0.45 (EtOAc/hexane, 1:4); IR (neat, cm^{-1}) 1576, 1556, 1329, 1284, 892; ^1H NMR (400 MHz, $CDCl_3$): δ 8.64 (d, *J* = 5.4 Hz, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 7.42 (dd, *J* = 5.4, 2.0 Hz, 1H), 7.33 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3, 150.3, 144.4, 142.2, 133.3, 132.5 (q, *J* = 34.1 Hz), 131.6 (q, *J* = 32.1 Hz), 126.2, 124.9, 124.0, 123.6, 122.4, 120.9;

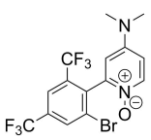
HRMS (m/z +ES): Found: M+H, 403.9276. $C_{13}H_6{}^{79}Br^{35}ClF_6N^+$ requires M+H, 403.9295; Δ = 4.7 ppm. Spectroscopic data in agreement with literature.[26]

2-(2-Bromo-4,6-bis(trifluoromethyl)phenyl)-4-chloropyridine-*N*-oxide (10)[26]



According to the method of Spivey,[26] pyridine **9** (0.50 g, 1.2 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C in an ice bath. Then, a solution of $NaHCO_3$ (0.21 g, 2.5 mmol, 2.0 equiv.) in 4.0 mL of water and *m*-CPBA (<77% m/m, 0.57 g, 2.5 mmol, 2.0 equiv.) were added to the solution. The resulting mixture was heated to reflux (40 °C) for 16 h. The reaction was then quenched with 25 mL of saturated Na_2SO_4 solution, the phases separated and the resulting organic layer washed with saturated $NaHCO_3$ solution (10 mL) and brine (20 mL) adding CH_2Cl_2 before every wash (3 x 20 mL). The resulting solution was then dried over $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (65% EtOAc in petroleum ether) providing 2-(2-bromo-4,6-bis(trifluoromethyl)phenyl)-4-chloropyridine-*N*-oxide as an off-white powder (0.43 g, 83%). m.p. = 126.3 – 128.6 °C; R_f = 0.35 (65% EtOAc in petroleum ether); IR (neat, cm^{-1}) 3106, 1402, 1335, 1252, 1132; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, *J* = 7.0 Hz, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.43 (dd, *J* = 7.0, 3.0 Hz, 1H), 7.30 (d, *J* = 3.0 Hz 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.2, 140.5, 135.2, 133.8, 133.4, 132.7 (q, *J* = 32.2 Hz), 132.1 (q, *J* = 32.3 Hz), 130.9, 127.5, 127.0, 123.4, 122.7, 120.8; HRMS (m/z +ES): Found: M+H, 419.9245. $C_{13}H_6{}^{79}Br^{35}ClF_6NO^+$ requires M+H, 419.9225; Δ = 4.8 ppm. Spectroscopic data in agreement with literature.[26]

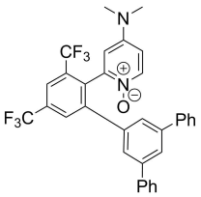
2-(2-Bromo-4,6-bis(trifluoromethyl)phenyl)-4-dimethylaminopyridine-*N*-oxide (11)[26]



According to the method of Spivey,[26] pyridine-*N*-oxide **10** (0.30 g, 0.71 mmol, 1.0 equiv.) was dissolved in 3.0 mL of acetonitrile in a microwave vial. Dimethylamine (60% aqueous solution, 4.3 mL) was added and the reaction mixture was then heated in a microwave reactor for 30 min (100 °C). The resulting yellow solution was concentrated *in vacuo* and purified by column chromatography (2-propanol/EtOAc, 1:1) to provide a yellow solid (0.26 g, 86 %). m.p. = 150.0 – 156.3 °C; R_f = 0.20 (2-propanol/EtOAc, 1:1); IR (neat, cm^{-1}) 2927, 1634, 1619, 1508, 1129; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, *J* = 7.6 Hz, 1H), 8.13 (s, 1H), 7.98 (s, 1H), 6.63 (dd, *J* = 7.6, 3.5 Hz, 1H), 6.39 (d, *J* = 3.5 Hz, 1H), 3.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.4, 144.6, 139.7, 137.2,

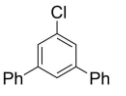
133.2, 132.8 (q, *J* = 34.2 Hz), 132.0 (q, *J* = 31.9 Hz), 127.2, 123.6, 122.6, 120.8, 108.6, 39.8; HRMS (m/z +ES): Found: M+H, 429.0035. $C_{15}H_{12}{}^{79}BrF_6N_2O^+$ requires M+H, 429.0037; Δ = 0.5 ppm. Spectroscopic data in agreement with literature.[26]

(±)-4-(Dimethylamino)-2-(5'-phenyl-3,5-bis(trifluoromethyl)-[1,1':3',1"-terphenyl]-2-yl)pyridine-*N*-oxide (3)[26]



Based on the procedure of Spivey,[26] $Pd_2(dba)_3{\cdot}CHCl_3$ (18 mg, 0.017 mmol, 7.5 mol%) and SPhos (15 mg, 0.035 mmol, 15 mol%) were added to a sealed vial and vacuum dried for 1 h. They were dissolved in 1.1 mL of *n*-butanol and allowed to stir at room temperature for 15 min. 4-DMAP-*N*-oxide **11** (0.10 g, 0.23 mmol, 1.0 equiv.), anhydrous potassium phosphate (0.15 mg, 0.70 mmol, 3.0 equiv.) and boronic ester **12** (0.17 g, 0.47 mmol, 2.0 equiv.) were added to a separate sealed vial and vacuum dried for 1 h before the addition of the catalyst solution. The brown mixture was then allowed to stir for 8 h at 90 °C. The resulting solution was concentrated *in vacuo* and purified by column chromatography (0-20% 2-propanol in EtOAc) to provide a yellow solid (0.10 g, 79 %). m.p. = 67.9 – 70.2 °C (dec.); R_f = 0.40 (10% 2-propanol in EtOAc); IR (neat, cm^{-1}) 2932, 1635, 1378, 1270, 1132; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 – 7.94 (m, 3H), 7.81 – 7.71 (m, 3H), 7.66 – 7.60 (m, 4H), 7.50 – 7.44 (m, 4H), 7.42-7.36 (m, 2H), 6.40 (dd, *J* = 7.5, 3.5 Hz, 1H), 6.27 (d, *J* = 3.5, 1H), 2.88 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.1, 141.6, 140.2, 139.2, 130.5, 128.9, 127.7, 127.1, 126.2, 125.6, 109.6, 108.4, 39.7; LRMS (m/z +ES): Found: M+H, 579.2. $C_{33}H_{25}F_6N_2O^+$ requires M+H, 579.2. Spectroscopic data in agreement with literature.[26]

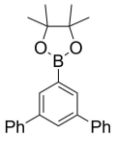
5'-Chloro-1,1':3',1"-terphenyl (14)[73]



According to the method of Kang,[34] after dissolving 1,3-dibromo-5-dichlorobenzene (0.15 g, 0.56 mmol, 1 equiv.), phenyl boronic acid (0.16 mg, 1.3 mmol, 2.4 equiv.) and $Pd(PPh_3)_4$ (45 mg, 0.039 mmol, 7 mol %) in a mixture solvent of 2M K_3PO_4 aqueous solution, toluene and 1,4-dioxane (1:4:1, 4.5 mL) the mixture was stirred under reflux at 120 °C for 5 hours. The resulting mixture was then diluted with 5 mL of EtOAc, the aqueous phase extracted with EtOAc and dried over $MgSO_4$. The residue was concentrated *in vacuo* and purified by column chromatography (petroleum ether) to provide a

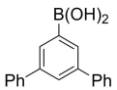
white solid (78 mg, 53%). m.p. = 90.1 – 92.3 °C; R_f = 0.35 (petroleum ether); IR (neat, cm^{-1}) 3035, 1685, 1596, 1566, 754; ^1H NMR (400 MHz, $CDCl_3$): δ 7.69 (t, *J* = 1.6 Hz, 1H), 7.66 – 7.62 (m, 4H), 7.58 (d, *J* = 1.6 Hz, 2H), 7.52 – 7.46 (m, 4H), 7.45 – 7.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.6, 140.0, 135.2, 129.1, 128.1, 127.4, 126.2, 124.5; HRMS (m/z +EI): Found: M+, 264.0701. $C_{18}H_{13}{}^{35}Cl^+$ requires M+, 264.0706; Δ = -0.5 ppm. Spectroscopic data in agreement with literature.[73]

2-([1,1':3',1''-Terphenyl-5')-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (12)[74]



According to the method of Kang,[34] $Pd_2(dba)_3{\cdot}CHCl_3$ (97 mg, 0.093 mmol, 5 mol%) and SPhos (0.15 mg, 0.37 mmol, 20 mol%) were added to a sealed vial and vacuum dried for 1 h. They were dissolved in 3.0 mL of anhydrous 1,4-dioxane and allowed to stir at room temperature for 15 min. Terphenyl **14** (0.50 g, 1.9 mmol, 1.0 equiv.), potassium acetate (1.1 g, 11 mmol, 6.0 equiv.) and bis(pinacolato)diboron (1.4 g, 5.7 mmol , 3.0 equiv.) were added to a separate sealed vial and vacuum dried for 1 h, before the addition of 20 mL of anhydrous 1,4-dioxane and the catalyst solution. The resulting red solution was then allowed to stir at 120 °C for 2 h. After this time, the reaction mixture was poured into 20 mL of water, extracted with CH_2Cl_2 (3 x 5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by column chromatography (petroleum ether/EtOAc, 10:1) to provide the product as a pale yellow solid (0.67 g, 98%). R_f = 0.80 (petroleum ether/EtOAc, 4:1); IR (neat, cm^{-1}) 2973, 1589, 1463, 1371, 1140; ^1H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, *J* = 1.9 Hz, 2H), 7.91 (t, *J* = 1.9 Hz, 1H), 7.71 – 7.66 (m, 4H), 7.49 – 7.42 (m, 4H), 7.39 – 7.33 (m, 2H), 1.38 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.3, 141.3, 132.7, 129.1, 128.8, 127.5, 127.5, 25.1; LRMS (m/z +EI): Found: M+H, 357. $C_{24}H_{26}BO_2^+$ requires M+H, 357. Spectroscopic data in agreement with literature.[74]

1,1':3',1''-Terphenyl-5'-boronic acid (15)

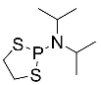


Based on the procedure of Hartwig,[75] boronic ester **12** (50 mg, 0.14 mmol, 1.0 equiv.) and sodium periodate (89 mg, 0.42 mmol, 3.0 equiv.) were dissolved in a water/THF mixture (5:4, 0.27 mL) and allowed to stir for 15 min. Then, 0.30 mL of aqueous solution of hydrochloric acid (1M) were added, and the mixture was stirred for 4 h at room temperature. The resulting solution was extracted with EtOAc (3 x 2 mL), washed with

water (2 mL) and brine (2 x 2 mL), dried over $MgSO_4$ and concentrated *in vacuo* to provide a white solid (31 mg, 81 %). m.p. = 288-290 °C; IR (film, cm^{-1}): 3034, 1419, 1363, 1261, 887; ^1H NMR (400 MHz, DMSO-d_6): δ 8.26 (s, 2H), 8.08 (d, J = 1.9 Hz, 2H), 7.92 (t, J = 1.9 Hz, 1H), 7.77 (d, J = 7.6 Hz, 4H), 7.50 (t, J = 7.6 Hz, 4H), 7.39 (t, J = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 141.0, 140.4, 132.2, 129.4, 127.9, 127.4, 127.2; LRMS (m/z -ES): Found: M-H+HCO_2H adduct, 319.1. $C_{19}H_{18}BO_4^-$ requires M-H+HCO_2H adduct, 319.1

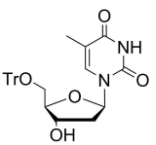
6.3. PREPARATION OF THE PHOSPHORYLATING REAGENT 23

2-*N*,*N*-Diisopropylamino-1,3,2-dithiaphospholane (20)[30]



According to the method of Okruszek,[30] into a solution of freshly distilled phosphorus trichloride (1 mL, 11.5 mmol, 1.0 equiv.) in anhydrous toluene/benzene (1:1, 10 mL), a solution of freshly distilled diisopropylamine (3.2 mL, 23 mmol, 2.0 equiv.) in 2.0 mL of toluene was added dropwise with vigorous stirring and cooling to 0 °C. The resulting suspension was then allowed to stir for 1 h at room temperature, filtered through a cannula under nitrogen, and the precipitate thoroughly washed with toluene/hexane (1:1, 15 mL). The combined fractions were cooled to 0 °C and a solution of 1,2-ethanedithiol (1,1 mL, 13 mmol, 1.1 equiv.) and freshly distilled triethylamine (3.2 mL, 23 mmol, 2 equiv.) in 6.0 mL of dry toluene was added dropwise with vigorous stirring. The resulting mixture was then stirred for 1 h, filtered through a cannula under nitrogen and the precipitate thoroughly washed with toluene/hexane (1:1, 20 mL). The combined fractions were concentrated *in vacuo* to yield the product as a colourless oil (1.7 g, 64 %) with no need for further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 3.52 – 3.44 (m, 4H), 3.15 – 3.09 (m, 2H), 1.17 (d, J = 6.8 Hz, 12H); ^{31}P NMR (162 MHz, $CDCl_3$): δ 94.64. Spectroscopic data in agreement with literature.[30]

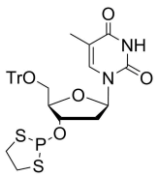
5'-*O*-Trityl-thymidine (21)[76]



According to the method of Takeshi,[77] trityl chloride (0.30 g, 1.1 mmol, 1.3 equiv.) was added to a solution of thymidine (0.20 mg, 0.83 mmol, 1 equiv.) in pyridine (2.0 mL) and allowed to stir at 70 °C for 18 h. The reaction mixture was then quenched with saturated $NaHCO_3$ solution (2.0 mL). The phases were separated and the aqueous layer extracted with CH_2Cl_2 (3 x 5 mL).

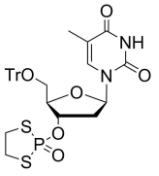
The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by column chromatography (0-3% MeOH in CH_2Cl_2) providing the product as a white solid (0.16 g, 43%). R_f = 0.30 (5% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ7.95 (s, 1H), 7.54 (s, 1H) , 7.46 – 7.28 (m, 15H), 6.39 (t, *J* = 6.8 Hz, 1H), 4.58 (br s, 1H), 4.03 (q, *J* = 3.1 Hz, 1H), 3.49 (dd, *J*= 10.5, 3.3 Hz, 1H), 3.39 (dd, *J* = 10.5, 3.1 Hz, 1H), 2.48 – 2.26 (m, 2H), 1.50 (s, 3H); LRMS (m/z -ES): Found: M-H, 483.2. $C_{29}H_{27}N_2O_5^-$ requires M-H, 483.2. Spectroscopic data in agreement with literature.[76]

5'-*O*-Tritylthymidine-3'-*O*-(1,3,2-dithiaphospholane) (22)[29]



According to the method of Okruszek,[30] 5'-*O*-trityl-thymidine (0.10 g, 0.21 mmol, 1.0 equiv.) and a tetrazole solution in acetonitrile (0.46 mL, 0.45 M, 0.21 mmol, 1.0 equiv.) were added to a sealed vial and vacuum dried for 8 h. Then, a solution of dithiaphospholane **20** (69 mg, 0.31 mmol, 1.5 equiv.) in CH_2Cl_2 (2 mL) was added dropwise, and the reaction mixture was allowed to stir for 16 h at room temperature. The resulting solution was purified by column chromatography (0-5 % MeOH in $CHCl_3$) to provide a white solid (0.11 g, 86 %). R_f = 0.55 (5% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ8.15 (s, 1H), 7.57 (d, *J* = 1.3 Hz, 1H), 7.42 – 7.27 (m, 15H), 6.33 (dd, *J* = 8.2, 5.7 Hz, 1H), 4.72 – 4.66 (m, 1H), 4.09 (m, 1H), 3.51 (dd, *J* = 10.7, 2.6 Hz, 1H), 3.36 (dd, *J* = 10.6, 2.6 Hz, 1H), 3.32 – 3.10 (m, 4H), 2.49 (ddd, *J* = 13.4, 5.7, 2.3 Hz, 1H), 2.37 – 2.28 (m, 1H), 1.43 (d, *J* = 1.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ163.5, 150.1, 143.3, 135.5, 128.8, 128.2, 127.7, 111.5, 85.3, 84.7, 63.0, 41.9, 40.0, 11.9; ^{31}P NMR (162 MHz, $CDCl_3$): δ 152.92; LRMS (m/z -ES): Found: M-H, 605.2. $C_{31}H_{30}N_2O_5PS_2^-$ requires M-H, 605.1. Spectroscopic data in agreement with literature.[29]

5'-*O*-Tritylthymidine-3'-*O*-(2-oxo-1,3,2-dithiaphospholane) (23)[30]



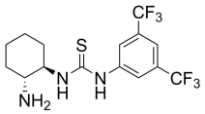
According to the method of Okruszek,[30] dithiaphospholane **22** (0.10 g, 0.17 mmol, 1.0 equiv.) was dissolved in anhydrous toluene (1.0 mL) before the addition of 5–6 M decane solution of *tert*-butyl hydroperoxide (40 µL, 0.18 mmol, 1.1 equiv.). The resulting solution was allowed to stir for 1 h under a nitrogen atmosphere at room temperature. The solution was then quenched by the addition of hexane at 0 °C, which caused the product to precipitate as a white solid. The

suspension was then centrifuged and the solid cleaned with cold hexane (92 mg, 90 %). R_f = 0.50 (5% MeOH in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) for both conformers: δ 8.38 (s, 1H), 7.57 (d, *J* = 1.4 Hz, 1H), 7.41 – 7.27 (m, 15H), 6.48 (dd, *J* = 8.6, 5.5 Hz, 1H, *major* conformer), 6.22 (dd, *J* = 7.7, 6.3 Hz, 1H, *minor* conformer), 5.37 – 5.31 (m, 1H), 4.35 – 4.30 (m, 1H), 3.71 – 3.35 (m, 4H), 2.71 (ddd, *J* = 14.2, 5.5, 1.9 Hz, 1H), 1.92 (m, 1H), 1.41 (d, *J* = 1.4 Hz, 3H); ^{31}P NMR (162 MHz, $CDCl_3$): δ 82.74 (*minor* conformer), 81.66 (*major* conformer); LRMS (m/z +ES): Found: M+NH4, 640.2. $C_{31}H_{35}N_3O_6PS_2^+$ requires M+NH4, 640.2. Spectroscopic data in agreement with literature.[30].

Note: 5'-O-tritylthymidine-3'-O-(2-oxo-1,3,2-dithiaphospholane) exists as a mixture of two conformers (syn and anti). Their relative populations vary with temperature and solvent.

6.4. PREPARATION OF THE BIFUNCTIONAL CATALYSTS

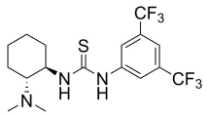
1-[(1*R*,2*R*)-2-Aminocyclohexyl]-3-[3,5-bis(trifluoromethyl)phenyl]thiourea (*(R,R)*-C1)[69]



According to the method of Berkessel,[69] phenyl chlorothioformate (0.12 mL, 0.88 mmol, 1.0 equiv.) was added to a solution of 3,5-bis(trifluoromethyl)aniline (0.14 mL, 0.88 mmol, 1.0 equiv.) and pyridine (78 µL, 0.96 mmol, 1.1 equiv.) in CH_2Cl_2 (3.0 mL) at room temperature. The resulting yellow solution was stirred for 2 h, then added dropwise to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane (0.10 mg, 0.88 mmol, 1.0 equiv.) in CH_2Cl_2 (0.50 mL), followed by the addition of *N,N*-diisopropylethylamine (0.15 mL, 0.88 mmol, 1.0 equiv.). Then, the mixture was allowed to stir at room temperature for 15 min, and quenched with a saturated $NaHCO_3$ solution (3.0 mL). The phases were separated, and the aqueous layer extracted with CH_2Cl_2 (3 x 5mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by column chromatography ($CHCl_3$/MeOH, 7:1) to obtain a pale yellow solid (0.14 g, 42%). R_f = 0.10 (MeOH/$CHCl_3$/NEt_3, 3:17:1); ^1H NMR (400 MHz, DMSO-d_6): δ 8.31 (s, 2H), 7.74 (s, 1H), 4.28 (br s, 1H), 3.01 (s, 1H), 2.04-1.93 (m, 2H), 1.70 (br s, 2H), 1.25 (br s, 4H); $[\alpha]_D^{23}$ +75.2 (*c* 0.93, $CHCl_3$); LRMS (m/z +ES): Found: M+H, 386.1. $C_{15}H_{18}F_6N_3S^+$ requires M+H, 386.1. Spectroscopic data in agreement with literature.[69]

*Note: **(S,S)-C1** was prepared following the same procedure.*

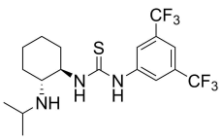
1-[3,5-Bis(trifluoromethyl)phenyl]-3-[(1*R*,2*R*)-2-(dimethylamino)cyclohexyl]thiourea (*(R,R)*-C2)[49]



According to the method of Berkessel,[69] zinc powder (17 mg, 0.26 mmol, 4.0 equiv.), acetic acid (30 µL, 0.52 mmol, 8.0 equiv.) and aqueous formaldehyde (37%, 23 µL, 3.0 equiv.) were added to a solution of ***(R,R)*-C1** (25 mg, 0.065 mmol, 1.0 equiv.) in 1,4-dioxane (0.15 mL). The resulting suspension was then allowed to stir for 72 h at room temperature. Aqueous NH_3 (80 µL) was added, the aqueous layer extracted with CH_2Cl_2 (3 x 1 mL) and the combined organic layers dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by column chromatography ($CHCl_3$/MeOH, 7:1) to obtain an off-white solid (15 mg, 56 %). R_f = 0.15 (MeOH/$CHCl_3$/NEt_3, 3:17:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 2H), 7.62 (s, 1H), 6.20 (br s, 1H), 3.77 (br s, 1H), 3.28 (br s, 1H), 2.74 (s, 6H), 2.44 – 2.41 (m, 1H), 1.91 – 1.78 (m, 4H), 1.33 – 1.16 (m, 4H). $[\alpha]_D^{23}$ -28.3 (*c* 1.0, $CHCl_3$); LRMS (m/z +ES): Found: M+H, 414.1. $C_{17}H_{22}F_6N_3S^+$ requires M+H, 414.1. Spectroscopic data in agreement with literature.[49]

Note: ***(S,S)*-C2** *was prepared following the same procedure.*

1-(3,5-Bis(trifluoromethyl)phenyl)-3-((1*R*,2*R*)-2-(isopropylamino)cyclohexyl) thiourea (*(R,R)*-C3)[78]

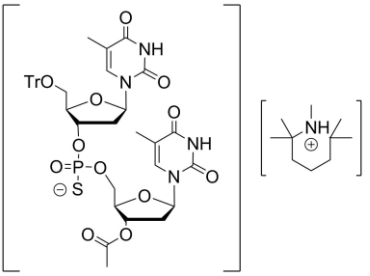


According to the method of Todd,[78] acetone (9.0 µL, 0.10 mmol, 1.2 equiv.) and $NaBH(OAc)_3$ (40 mg, 0.19 mmol, 1.9 equiv.) were added to a solution of ***(R,R)*-C1** (40 mg, 0.10 mmol, 1.0 equiv.) in 1,2-dichloroethane (1.6 mL). The resulting mixture was stirred at room temperature for 16 h. Then, 6.0 mL of saturated $NaHCO_3$ solution were added to the reaction mixture, which was then allowed to stir for 15 min. Layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases were dried over $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (5% MeOH in CH_2Cl_2) to provide an off-white solid (22 mg, 50%). R_f = 0.20 (MeOH/$CHCl_3$/NEt_3, 3:17:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 2H), 7.57 (s, 1H), 6.58 (br s, 1H), 3.40 (br s, 1H), 3.00 (br s, 1H), 2.38 (br s, 1H), 2.06 – 2.01 (m, 2H), 1.77 (br s, 2H), 1.40 – 1.19 (m, 4H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.11 (d, *J* = 6.0 Hz, 3H); $[\alpha]_D^{23}$ +95.3 (*c* 0.83, $CHCl_3$); LRMS (m/z -ES): Found: M-H, 426.1. $C_{18}H_{22}F_6N_3S^-$ requires M-H, 426.1. Spectroscopic data in agreement with literature.[78]

6.5. GENERAL PROCEDURE FOR THE SYNTHESIS OF DINUCLEOSIDE PHOSPHOROTHIOATES 40.A-C FROM DITHIAPHOSPHOLANE 23

Dithiaphospholane **23** (5 mg, 0.008 mmol, 1.0 equiv.), 3-*O*-acetyl-2-deoxyribonucleoside **39.a-c** (0.008 mmol, 1.0 equiv.), catalyst (25 mol%) and 4 Å molecular sieves (10 mg) were added to a sealed vial and vacuum dried for 8 h. Then, 0.10 mL of anhydrous CH_2Cl_2 was added to the mixture and the resulting suspension allowed to stir for 15 min at room temperature under an argon atmosphere. This was followed by the addition of 0.20 mL of PMP solution in CH_2Cl_2 (0.008 mmol, 1.0 equiv.). After 16 h at room temperature under an argon atmosphere, the mixture was filtered through celite and analysed by ^{31}P NMR.

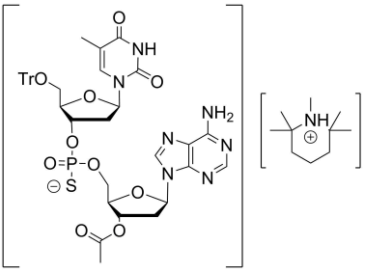
***SP*-(5'-*O*-Tritylthymidylyl)-3',5'-(3'-*O*-acetylthymidine) phosphoromonothiolate 1,2,2,6,6-pentamethylpiperidinium salt (40.a)**[30]



^{31}P NMR (162 MHz, $CHCl_3$): δ 54.22 (R_P) , 53.57 (S_P)

HRMS (m/z -ES): Found: M-PMPH$^-$, 845.2261. $C_{41}H_{42}N_4O_{12}PS^-$ requires M-PMPH$^-$, 845.2258; Δ = 0.3 ppm. Spectroscopic data in agreement with literature.[30]

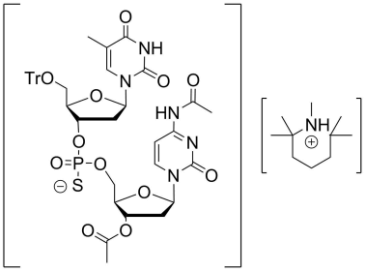
***SP*-(5'-*O*-Tritylthymidylyl)-3',5'-(3'-*O*-acetyl-2'-deoxyadenosine) phosphoromonothiolate 1,2,2,6,6-pentamethylpiperidinium salt (40.b)**[30]



^{31}P NMR (162 MHz, $CHCl_3$): δ 54.27 (R_P), 53.70 (S_P).

HRMS (m/z -ES): Found: M-PMPH$^-$, 854.2401. $C_{41}H_{41}N_7O_{10}PS^-$ requires M-PMPH$^-$, 854.2379; Δ = 2.6 ppm. Spectroscopic data in agreement with literature.[30]

_SP_-(5'-_O_-Tritylthymidylyl)-3',5'-(_N4_-acetyl-3'-_O_-acetyl-2'-deoxycytidine) phosphoromonothiolate 1,2,2,6,6-pentamethylpiperidinium salt (40.c)[30]



^{31}P NMR (162 MHz, $CHCl_3$): δ 54.24 (R_P), 53.69 (S_P). HRMS (m/z -ES): Found: M-PMPH$^-$, 872.2359. $C_{42}H_{43}N_5O_{12}PS^-$ requires M-PMPH$^-$, .872.2367; Δ = 0.9 ppm. Spectroscopic data in agreement with literature.[30]

6.6. GENERAL PROCEDURE FOR THE MEASUREMENT OF THE NATURAL SELECTIVITY IN THE SYNTHESIS OF DINUCLEOSIDE PHOSPHOROTHIOATES 40.A-C FROM DITHIAPHOSPHOLANE 23

Dithiaphospholane **23** (5 mg, 0.008 mmol, 1.0 equiv.) and 3-*O*-acetyl-2-deoxyribonucleoside **39.a-c** (0.008 mmol, 1.0 equiv) were added to a sealed vial and vacuum dried for 8 h. Then, a solution of the base of choice (0.008 mmol, 1.0 equiv.) in CH_2Cl_2 (0.1mL) was added to the vial and the resulting solution allowed to stir at room temperature for 16 h under an argon atmosphere. The reaction mixture was analysed by ^{31}P NMR.

7. CONCLUSIONS

In summary, the present study has found promising results which point towards the first asymmetric catalytic synthesis of dinucleoside phosphorothioates. Even though high levels of selectivity and conversion have not been achieved, it has been proved that these catalysts do affect the selectivity of the reaction. Further work is needed in order to find better catalysts and conditions for the coupling.

Moreover, an interesting base dependence on the diastereoselectivity of the coupling reaction has been found. The reasons for this switch in diastereoselectivity are not currently known; however, computational and Sigman analysis may shed some light on the subject through the study of its transition state.

Optimisation of the coupling conditions in the presence of the 4-DMAP-*N*-oxide catalyst **3** was not achieved, since an adequate additive was not found. However, an improvement in the synthesis of this catalyst was developed, enabling its preparation in 43% overall yield over 5 steps.

8. REFERENCES AND NOTES

(1) Crick, F. H. C. *Symp. Soc. Exp. Biol.* **1958**, *XII*, 138–163.
(2) Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75* (1), 280–284.
(3) Stephenson, M. L.; Zamecnik, P. C. *Proc Natl Acad Sci U S A* **1978**, *75* (1), 285–288.
(4) Pederson, T. *Nature* **2009**, *462*, 423.
(5) Moreno, P. M. D.; Pego, A. P. *Front. Chem.* **2014**, *2* (October), 1–7.
(6) Aartsma-Rus, A. *Mol. Ther.* **2016**, *24* (2), 193–194.
(7) Yamamoto, T.; Nakatani, M.; Narukawa, K.; Obika, S. *Future Med. Chem.* **2011**, *3* (3), 339–365.
(8) Bennett, C. F.; Swayze, E. E. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50* (1), 259–293.
(9) Guga, P.; Koziolkiewicz, M. *Chem. Biodivers.* **2011**, *8*, 1642–1681.
(10) Eckstein, F. *Tetrahedron Lett.* **1967**, *13*, 1157–1160.
(11) Oivanen, M.; Ora, M.; Almer, H.; Stromberg, R.; Lonnberg, H. *J. Org. Chem.* **1995**, *60* (12), 5620–5627.
(12) Crooke, S. T.; Graham, M. J.; Zuckerman, J. E.; Conklin, B. S.; Griffey, H. *J. Pharmacol. Exp. Ther.* **1996**, *277* (2), 923–937.
(13) Watanabe, T. A.; Geary, R. S.; Levin, A. A. *Oligonucleotides* **2006**, *16* (2), 169–180.
(14) Wang, L.; Chen, S.; Xu, T.; Taghizadeh, K.; S Wishnok, J.; Zhou, X.; You, D.; Deng, Z.; C Dedon, P. *Nat. Chem. Biol.* **2007**, *3* (11), 709–710.
(15) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2002**, *124* (18), 4962–4963.
(16) Oka, N.; Yamamoto, M.; Sato, T.; Wada, T. *J. Am. Chem. Soc.* **2008**, *130* (47), 16031–16037.
(17) Wada, T.; Oka, N.; Saigo, K. *Nucleic Acids Res. Suppl.* **2003**, No. 3, 109–110.
(18) Iyer, R. P.; Guo, M. J.; Yu, D.; Agrawal, S. *Tetrahedron Lett.* **1998**, *39* (17), 2491–2494.
(19) Iyer, R. P.; Yu, D.; Ho, N. H.; Tan, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, *6* (5), 1051–1054.
(20) Nawrot, B.; Rebowska, B.; Cieślińska, K.; Stec, W. J. *Tetrahedron Lett.* **2005**, *46* (39), 6641–6644.
(21) Almer, H.; Szabo, T.; Stawinski, J. *ChemInform* **2004**, *35* (22), 290–291.
(22) DiRocco, D. A.; Ji, Y.; Sherer, E. C.; Klapars, A.; Reibarkh, M.; Dropinski, J.; Mathew, R.; Maligres, P.; Hyde, A. M.; Limanto, J.; Brunskill, A.; Ruck, R. T.; Campeau, L.-C.; Davies, I. W. *Science.* **2017**, *356*, 426–430.
(23) Spivey, A. C.; Leese, D. P.; Zhu, F.; Davey, S. G.; Jarvest, R. L. *Tetrahedron* **2004**, *60* (20), 4513–4525.
(24) Spivey, A. C.; Arseniyadis, S.; Fekner, T.; Maddaford, A.; Leese, D. P. *Tetrahedron* **2006**, *62* (2-3), 295–301.
(25) Murray, J. I.; Spivey, A. C. *Adv. Synth. Catal.* **2015**, *357* (18), 3825–3830.
(26) Murray, J. I.; Flodén, N. J.; Bauer, A.; Fessner, N. D.; Dunklemann, D. L.; Bob-Egbe,

O.; Rzepa, H. S.; Bürgi, T.; Richardson, J.; Spivey, A. C. *Angew. Chemie Int. Ed.* **2017**, *56*, 5760–5764.
(27) Murray, J.; Woscholski, R.; Spivey, A. *Synlett* **2015**, *26* (07), 985–990.
(28) Murray, J. I.; Woscholski, R.; Spivey, A. C. *Chem. Commun.* **2014**, *50* (88), 13608–13611.
(29) Fessner, N. D. *MSci Thesis. Imperial College London*; 2015.
(30) Olesiak, M.; Okruszek, A. *Phosphorus. Sulfur. Silicon Relat. Elem.* **2009**, *184* (6), 1548–1560.
(31) Fairlamb, I. J. S. *Chem. Soc. Rev.* **2007**, *36* (7), 1036–1045.
(32) Kalyani, D.; Dick, A. R.; Anani, W. Q.; Sanford, M. S. *Tetrahedron* **2006**, *62* (49), 11483–11498.
(33) Hoz, A. de la; Diaz-Ortiz, A.; Moreno, A. *Chem. Soc. Rev.* **2005**, *34*, 164–178.
(34) Kang, H.-R.; Kang, H.-J.; Hong, J.-R.; Moon, D.-H.; Lim, Y.-M.; Kim, B.; Kim, N.-K. An organic electroluminescent compound and an organic electroluminescent device comprising the same. WO 2015/178731 A1, 2015.
(35) Barder, T. E.; Walker, S. D.; Martinelli, J. R.; Buchwald, S. L. *J. Am. Chem. Soc.* **2005**, *127* (13), 4685–4696.
(36) Follmann, H.; Pfeil, R.; Witzel, H. *Eur. J. Biochem.* **1977**, *77*, 451–461.
(37) Lemieux, R. U. *Can. J. Chem.* **1961**, *39*, 116–120.
(38) Yurenko, Y. P.; Zhurakivsky, R. O.; Ghomi, M.; Samijlenko, S. P.; Hovorun, D. M. *J. Phys. Chem. B* **2007**, *111* (32), 9655–9663.
(39) George, A. L.; Hruska, F. E.; Ogilvie, K. K.; Holy, A. *Can. J. Chem.* **1978**, *56*, 1170–1176.
(40) Fenton, O. S.; Allen, E. E.; Pedretty, K. P.; Till, S. D.; Todaro, J. E.; Sculimbrene, B. R. *Tetrahedron* **2012**, *68* (44), 9023–9028.
(41) De, C.; Klauber, E.; Seidel, D. *J. Am. Chem. Soc.* **2010**, *132*, 13624–13626.
(42) Fang, X.; Wang, C.-J. *Chem. Commun.* **2015**, *51* (7), 1185–1197.
(43) Schreiner, P. R. *Chem. Soc. Rev.* **2003**, *32* (5), 289–296.
(44) Takemoto, Y. *Org. Biomol. Chem.* **2005**, *3* (24), 4299–4306.
(45) Zhang, Z.; Schreiner, P. R. *Chem. Soc. Rev.* **2009**, *38* (4), 1187–1198.
(46) Mittal, N.; Sun, D. X.; Seidel, D. *Org. Lett.* **2014**, *16* (3), 1012–1015.
(47) Vecchione, M. K.; Li, L.; Seidel, D. *J. Am. Chem. Soc.* **2008**, *130* (37), 12248–12249.
(48) Wittkopp, A.; Schreiner, P. R. *Chem. - A Eur. J.* **2003**, *9* (2), 407–414.
(49) Okino, T.; Hoashi, Y.; Takemoto, Y. *J. Am. Chem. Soc.* **2003**, *125* (42), 12672–12673.
(50) Zhang, Z.; Lippert, K. M.; Hausmann, H.; Kotke, M.; Schreiner, P. R. *J. Org. Chem.* **2011**, *76* (23), 9764–9776.
(51) Enders, D.; Zhi, Y.; Zhao, K.; Wang, A.; Englert, U.; Raabe, G. *Adv. Synth. Catal.* **2017**.
(52) Han, X.; Ye, C.; Chen, F.; Chen, Q.; Wang, Y.; Zeng, X. *Org. Biomol. Chem.* **2017**, *15*, 3401–3407.
(53) Nödling, A. R.; Jakab, G.; Schreiner, P. R.; Hilt, G. *European J. Org. Chem.* **2014**, *2014* (29), 6394–6398.
(54) Jarvis, C. L.; Hirschi, J. S.; Vetticatt, M. J.; Seidel, D. *Angew. Chemie Int. Ed.* **2017**, 2670–2674.
(55) Amarasinghe, N. R.; Turner, P.; Todd, M. H. *Adv. Synth. Catal.* **2012**, *354* (16), 2954–2958.

(56) Mittal, N.; Sun, D. X.; Seidel, D. *Org. Lett.* **2012**, *14* (12), 3084–3087.
(57) Mittal, N.; Lippert, K. M.; De, C. K.; Klauber, E. G.; Emge, T. J.; Schreiner, P. R.; Seidel, D. *J. Am. Chem. Soc.* **2015**, *137* (17), 5748–5758.
(58) Min, C.; Mittal, N.; De, C. K.; Seidel, D. *Chem. Commun.* **2012**, *48* (88), 10853.
(59) De, C. K.; Seidel, D. *J. Am. Chem. Soc.* **2011**, *133* (37), 14538–14541.
(60) Klauber, E. G.; De, C. K.; Shah, T. K.; Seidel, D. *J. Am. Chem. Soc.* **2010**, *132* (39), 13624–13626.
(61) Klauber, E. G.; Mittal, N.; Shah, T. K.; Seidel, D. *Org. Lett.* **2011**, *13* (9), 2464–2467.
(62) Zhang, Z.; Bao, Z.; Xing, H. *Org. Biomol. Chem.* **2014**, *12* (20), 3151–3162.
(63) Bratek-Wiewiórowska, M. D.; Alejska, M.; Sarzyńska, J.; Surma, K.; Figlerowicz, M.; Wiewiórowski, M. *J. Mol. Struct.* **1992**, *275*, 167–181.
(64) Wang, J.; Li, H.; Yu, X.; Zu, L.; Wang, W. *Org. Lett.* **2005**, *7* (19), 4293–4296.
(65) Sohtome, Y.; Tanatani, A.; Hashimoto, Y.; Nagasawa, K. *Tetrahedron Lett.* **2004**, *45* (29), 5589–5592.
(66) McCooey, S. H.; Connon, S. J. *Angew. Chemie - Int. Ed.* **2005**, *44* (39), 6367–6370.
(67) Liu, T.-Y.; Long, J.; Li, B.-J.; Jiang, L.; Li, R.; Wu, Y.; Ding, L.-S.; Chen, Y.-C. *Org. Biomol. Chem.* **2006**, *4* (11), 2097.
(68) Zuend, S. J.; Coughlin, M. P.; Lalonde, M. P.; Jacobsen, E. N. *Nature* **2009**, *461* (7266), 968–970.
(69) Berkessel, A.; Seelig, B. *Synthesis.* **2009**, No. 12, 2113–2115.
(70) Wilk, A.; Stec, W. *Nucleic Acids Res.* **1995**, *23* (3), 530–534.
(71) Milo, A.; Neel, A. J.; Toste, F. D.; Sigman, M. S. *Science.* **2015**, *347* (6223), 737–743.
(72) Toste, F. D.; Sigman, M. S.; Miller, S. J. *Acc. Chem. Res.* **2017**, *50* (3), 609–615.
(73) Saednya, A.; Hart, H. *Synthesis.* **1996**, *12*, 1455–1458.
(74) Jiang, W.; Duan, L.; Qiao, J.; Zhang, D.; Dong, G.; Wang, L.; Qiu, Y. *J. Mater. Chem.* **2010**, *20* (29), 6131.
(75) Murphy, J. M.; Tzschucke, C. C.; Hartwig, J. F. *Org. Lett.* **2007**, *9* (5), 757–760.
(76) Windhorst, A. D.; Klein, P. J.; Eisenbarth, J.; Oeser, T.; Kruijer, P. S.; Eisenhut, M. *Nucl. Med. Biol.* **2008**, *35* (4), 413–423.
(77) Takeshi, I. Nucleoside analogues whose sugar moietes are bound in S-form and oligonucleotide derivatives comprising nucleotide analogues thereof. WO 2003/068794, 2004.
(78) Amarasinghe, N. R.; Turner, P.; Todd, M. H. *Adv. Synth. Catal.* **2012**, *354* (16), 2954–2958.

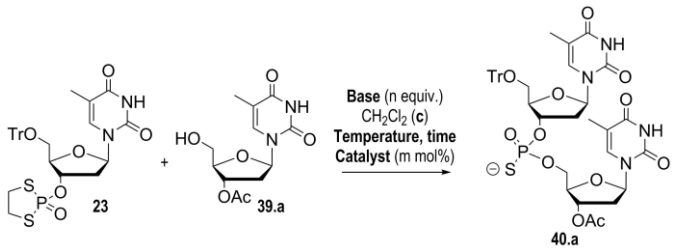
9. ACRONYMS

4Å MS	4 angstrom molecular sieves
4-DMAP	4-dimethylaminopyridine
AcOH	acetic acid
dAde	2'-*O*-deoxyadenosine
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
dCyt	2'-*O*-deoxycytidine
DIPEA	*N,N*-diisopropylethylamine
DNA	deoxyribonucleic acid
EI	electron ionization
ES	electrospray ionization
FDA	Food and Drug Administration
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IR	infrared spectroscopy
LRMS	low-resolution mass spectrometry
m.p.	melting point
m-CPBA	meta-chloroperoxybenzoic acid
mRNA	messenger RNA
MS	mass spectrometry
na	not available
NBS	*N*-bromosuccinimide
nr	no reaction
ON	oligonucleotide

PBH_3-ON	boranophosphate oligonucleotides
PMe-ON	methylphosphonate oligonucleotide
PMP	1,2,2,6,6-pentamethylpiperidine
PPO	propylene oxide
PSe-ON	phosphoroselenoate oligonucleotide
PS-ON	phosphorothioate oligonucleotide
pyr	pyridine
R_f	retention factor
RNA	ribonucleic acid
RP-HPLC	reverse-phase high-performance liquid chromatography
SPhos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
THF	tetrahydrofuran
Thy	thymidine
TLC	thin-layer chromatography
TMP	2,2,6,6-tetramethylpiperidine

APPENDICES

APPENDIX: SCREENING OF CONDITIONS FOR THE COUPLING BETWEEN DITHIAPHOSPHOLANE 23 AND THYMIDINE 39.A



Entry	T (°C)	t (h)	c (mol/L)	Base (n equiv.)	Catalyst (m mol%)	Conv. (%)[a]
1	-78	2	0.05	PMP (1 equiv.)	**6** (20%)	25
2	-78	2	0.05	PMP (1 equiv.)	**6** (20%)+ **30** (1%)	45
3	rt	0.5	0.05	DBU (1 equiv.)	-	100
4	-78	1	0.04	PMP (1 equiv.)	**30** (10 mol%)	nr
5	0	1.5	0.04	PMP (1 equiv.)	**30** (10 mol%)	nr
6	rt	1	0.04	PMP (1 equiv.)	**30** (10 mol%)	nr
7	-78	1	0.04	PPO (1 equiv.)	**30** (10 mol%)	nr
8	0	1.5	0.04	PPO (1 equiv.)	**30** (10 mol%)	nr
9	rt	1	0.04	PPO (1 equiv.)	**30** (10 mol%)	nr
10	-78	2	0.05	PMP (1 equiv.)	**(±)-C1** (25 mol%)	nr
11	0	3	0.05	PMP (1 equiv.)	**(±)-C1** (25 mol%)	24
12	-78	2	0.05	PMP (1 equiv.)	**(±)-C2** (25 mol%)	nr
13	0	3	0.05	PMP (1 equiv.)	**(±)-C1** (25 mol%)	19
14	0	3	0.15	PMP (3 equiv.)	-	25
15	0	3	0.15	PMP (3 equiv.)	**(±)-C1** (25 mol%)	25
16	0	3	0.15	PMP (1 equiv.)	-	25
17	0	3	0.15	PMP (1 equiv.)	**(±)-C1** (25 mol%)	25
18	0	2	0.05	PMP (1 equiv.)	-	20
19	0	2	0.05	PMP (1 equiv.)	**(±)-C1** (25 mol%)	50
20	-20	2.5	0.05	TMP (1 equiv.)	-	20
21	-20	2.5	0.05	TMP (1 equiv.)	**(±)-C1** (25 mol%)	30
22	rt	4	0.01	PMP (1 equiv.)	-	nr
23	rt	4	0.01	PMP (1 equiv.)	**(±)-C1** (25 mol%)	15
24[b]	rt	16	0.03	PMP (1 equiv.)	-	nr
25[b]	rt	16	0.03	PMP (1 equiv.)	**(±)-C1** (25 mol%)	25

(a) Determined by ^{31}P NMR; (b) Reaction carried out with 10mg 4Å MS