MODIFIED NUCLEOSIDES AND OLIGONUCLEOTIDES AS LIGANDS FOR ASYMMETRIC REACTIONS

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A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

2010

Modified Nucleosides and Oligonucleotides as Ligands for Asymmetric Reactions

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April 2010

Thesis submitted to the University of St Andrews in application for the degree of Doctor in Philosophy

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Abstract

Development of chiral ligands capable of achieving high selectivity for various asymmetric catalytic reactions has been an important aim of both academia and industry. Nature is capable to selectively catalyze chemical reactions by using enzymes. An ideal catalyst would combine the selectivity of nature and the reactivity of man-made catalysts based on transition metal complexes. The two biomolecules chosen to achieve this are DNA and PNA. DNA is a chiral molecule with high binding selectivity towards small molecules and has been used as ligand for asymmetric catalysis. PNA is an achiral structural analogue of DNA that can form duplexes with DNA. To produce DNA based catalysts it is necessary to introduce a ligand such as a phosphine that will strongly coordinate to transition metals. To achieve this, functionalized linkers need to be introduced into a DNA strand, to covalently couple the phosphine moiety at a specific location of the DNA strand. Amine linkers and several modified nucleosides have been prepared containing thiol and amine functionalities and some of them were successfully introduced into DNA strands to function as linkers for the introduction of phosphine functionalities. Those strands were purified and an adequate procedure was developed for their analysis by MALDI-TOF. Diphenylphosphino carboxylic acids have been coupled to amine modified deoxyuridines by amide bond formation. The same coupling method has been used for oligonucleotides. DNA strands containing phosphine moieties were characterized by MALDI-TOF and ^{31}P NMR spectrometry. ^{31}P NMR spectroscopy was also used to confirm coordination of a phosphine modified 15-mer to $[PdCl(\eta^3\text{-allyl})]_2$. The phosphine modified nucleobases were also tested as ligands for palladium catalyzed allylic alkylation and allylic amination with diphenylallyl acetate as substrate although no enantioselectivity was observed. A PNA monomer was also modified with a bidentate sulfur protected phosphine and successfully introduced into a short PNA strand using manual solid phase synthesis. This strand was analyzed by MALDI-TOF. Moreover, preliminary studies were performed to test the use of aptamers as scaffolds for targets containing a ligand functionality.

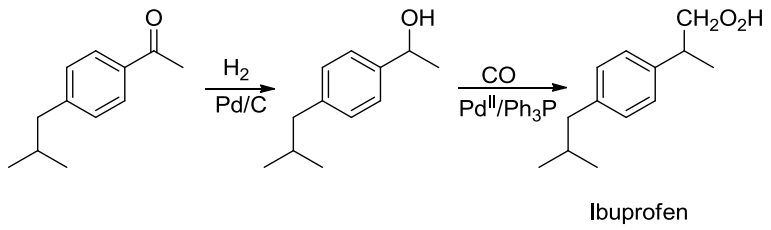
Chapter 1: General Introduction

1. Introduction

1.1 Need for catalysis

Fine chemical manufacturers rely on catalytic reactions for the production of the majority of their chemicals, although many chemicals are still made by using stochiometric amounts of activators. Those stochiometric reactions produce large amounts of waste per kilogram of product synthesized and the majority of those waste products are inorganic salts. For a sustainable development it is necessary to minimize the environmental damage by eliminating waste formation and the use of toxic materials during chemical production. This concept is known as green chemistry and was first introduced by Anastas in 1994 at the Environmental Protection Agency (EPA) in the United States.[1] To quantify the efficiency and environmental impact of a chemical reaction two parameters have been defined. The E factor is defined as the mass ratio of waste to desired product, the higher the E factor the larger the waste production[1]. The atom economy is calculated by dividing the molecular weight of the desired product by the sum of the molecular weight of all the products of the reaction.[2-4] A large scale process like oil refining has an E factor of <0.1, the E factor of Bulk chemicals increases to <5 while fine chemicals industries can reach an E factor of 50 whereas the production of pharmaceuticals can reach an E factor larger than 500.[3] Those two measurements for waste production only take into account the amount of waste produced. Since the environmental impact of this waste depends on its toxicity and potential danger, Sheldon *et al* have introduced the environmental quotient (EQ) which is the E factor multiplied by an unfriendliness quotient Q.[5] For example, NaCl can have a value of 1 compared to a heavy metal salt which would have a Q of 100-1000. Those values are only an estimate and are meant to give a general idea about the toxicity of a substance and its environmental damage. To reduce the EQ and increase the atom economy chemical industries are striving to develop processes based on the use of H_2, O_2, H_2O_2, CO, CO_2 and NH_3 as sources of H, O, C and N.[1] Catalysts can decrease the amount of waste production, prevent the use of dangerous chemicals and decrease the use of starting material. Figure 1 shows the drastic improvement in atom economy for several representative reactions by going from the use of a stoichiometric system to a catalytic one. Different types of catalysis are distinguished comprising organocatalysis, biocatalysis, homogeneous

and heterogeneous catalysis. Transition metal complexes have been used for catalytic processes because of their ability to activate organic molecules. An example is the synthesis toward the commonly used analgesic ibuprofen which has an annual production of several thousand tons. This drug is produced from *p*-isobutylacetophenone by hydrogenation and carbonylation and it is produced with an atom economy of 100% (Scheme 1).[1]



Scheme 1. Hoechst Celanese process for ibuprofen, 99% conv., 96% selectivity, 100% atom economy.[1]

	Reduction:	Oxidation:	C-C Formation:
STOCHIOMETRIC	$4\ PhCOCH_3 + NaBH_4 + 4\ H_2O$ ↓ $4\ PhCH(OH)CH_3 + NaB(OH)_4$ 120/145 = 82%	$3\ PhCH(OH)CH_3 + 2CrO_3 + 3\ H_2SO_4$ ↓ $3PhCOCH_3 + Cr_2(SO_4)_3 + 6H_2O$ 120/270 = 44%	$PhCH(OH)CH_3 + HCl$ ↓ $PhCH(CH_3)Cl + H_2O$ 1.Mg ↓ 2.CO_2 3.HCl ↓ $PhCH(CH_3)CO_2H + MgCl_2$ 148/243 = 61%
CATALYTIC	$PhCOCH_3 + H_2$ ↓ $PhCH(OH)CH_3$ 100%	$PhCH(OH)CH_3 + 1/_2O_2(H_2O_2)$ ↓ $PhCOCH_3 + H_2O\ (2H_2O)$ 120/138 =87% (120/156 = 77%)	$PhCH(OH)CH_3 + CO$ ↓ $PhCH(CH_3)CO_2H$ 100%

Figure 1. Atom economy of stochiometric process versus catalytic process for various reactions.[2]

1.2 Hybrid Catalysts

1.2.1 Artificial metalloenzymes

Enzymes, nature's catalysts, are essential molecules present in all living organisms that are able to largely increase the speed of a reaction. They have been thought to be exclusively proteins but only a few decades ago it was discovered that RNA can also function as a catalyst. They stabilize the transition states of the various reactions performed in a living organism.[6] Enzymes are composed of L-amino acids and have

an active binding site where a substrate is catalytically transformed. Enzymes are highly specific towards the identity of the substrate, the section of the substrate to be transformed and the course of the reaction toward the formation of a specific product. An enzyme generally binds the molecules undergoing the catalytic change and can distinguish the two sides of a prochiral molecule to give exceptionally high enantiomeric excess (ee).[7] Those chemical transformations are performed in the active site of the enzyme. An assembly of strategically placed amino acids in the active site weakly binds to a specific substrate and makes the desired reaction occur. This binding is possible through various noncovalent interactions such as hydrogen bonding, van der Waals forces, electrostatic bonds, and hydrophobic interactions.[6] Because of their high selectivity, specificity and control of both first and second coordination spheres, enzymes have a high potential for being used as catalysts for asymmetric reactions. The drawback of enzymatic catalysis is that enzymes are made for specific reactions that do not include many of the various chemical reactions performed in industry. The combination of biocatalysis with transition metal catalysis would ideally give life to the perfect catalyst for highly selective asymmetric reactions. Whitesides and Wilson were the first to combine the selectivity of enzymes with transition metal catalysis. Whitesides synthesized a modified biotin with a diphosphine ligand complexed to rhodium(I) to noncovalently bind it to the protein avidin (Figure 2). This new biocatalyst was then used for the asymmetric hydrogenation of α-acetamidoacrylic acid to *N*-acetylalanine (Scheme 2). Modified biotin with avidin gave ee up to 40% (*S*) while biotin alone gave no enantioselectivity proving that the protein was capable of transferring its chirality to the catalytic reaction.[8]

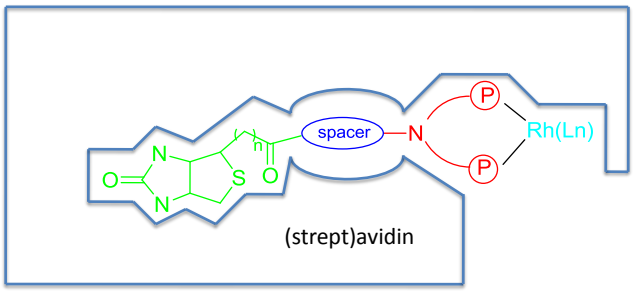
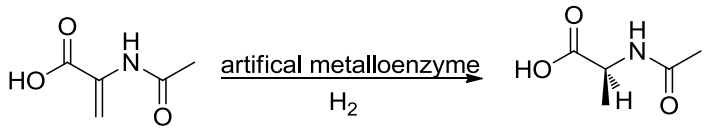
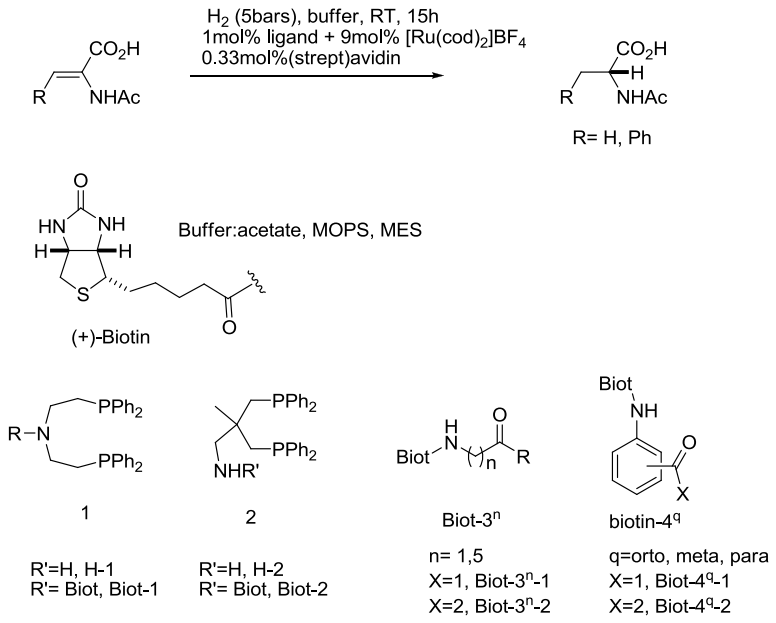


Figure 2. Biotin-(stept)avidin catalyst engineered by Whitesides for enantioselective hydrogenation reactions.[9] Reprinted from J. Steinreiber and T. R. Ward, *Coordination Chemistry Reviews*, 2008, **252**, 751-766. Copyright 2008, with permission from Elsevier.



Scheme 2. Enantioselective hydrogenation of α-acetamidoacrylic acid to *N*-acetylalanine using a protein based catalyst.[9]

Inspired by Whitesides' work, Thomas Ward has further investigated the use of the biotin-avidin biocatalyst for enantioselective hydrogenation reactions. By changing avidin to streptavidin Ward was able to obtain ees as high as 94% (*R*) with 85% conversion for the hydrogenation of acetamidoacrylic acid and acetamidocinnamic acid. The advantage of streptavidin over avidin is a deeper binding pocket. For the purpose of optimizing the selectivity of the catalyst, Ward has used the "chemogenetic approach" which involves modifying both the biotin and the protein parts. The biotin was modified by changing the metal, the phosphine ligands and the spacer between ligand and protein. The protein itself was modified by using saturation mutagenesis at a specific position of the scaffold.[9] Eight spacers between ligand and biotin were tested; five alkylamino acids and three arylamino acids and they were used for both steptavidin and avidin (Scheme 3). Avidin Biot-3-2 represented in Scheme 3 gives 80% ee (*S*) for the production of amidoalanine while the biotin 1 gives only 39% ee. The protein modification increased slightly the enatioselectivity of both enantiomers.[9,10] By changing the ligand scaffold to amino-sulfonamide ruthenium and rhodium complexes Humbert *et al* were able to catalyze the oxidation of secondary alcohols[11] and the reduction of ketones by transfer hydrogenation.[12] Figure 3 shows how the protein and the metal complex are positioned in the appropriate orientation towards the substrate for the hydrogenation reaction. The protein has a cavity where the biotin anchor is bound, this anchor contains a spacer and a bidentate for metal coordination. The substrate is introduced in the cavity and the hydrogen is transferred from the metal complex to the substrate without any coordination of the substrate with the metal to give enantioselectivities up to 97% (R).[13] Further modifications of the active site slightly increased the enantioselectivities to 98% (R).[14]



Scheme 3. Enantioselective hydrogenation with eight different ligands.[9] Reprinted from J. Steinreiber and T. R. Ward, *Coordination Chemistry Reviews*, 2008, **252**, 751-766. Copyright 2008, with permission from Elsevier.

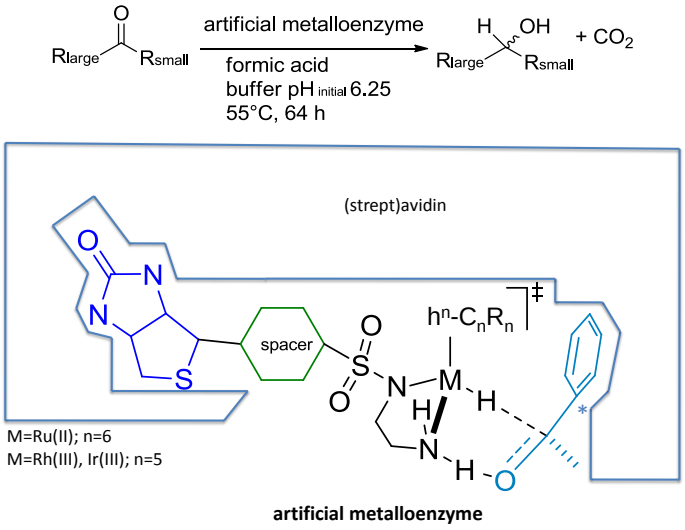


Figure 3. Artificial metalloenzymes used for the enantioselective transfer hydrogenation reactions.[13] Reprinted with permission from C. Letondor *et al*. *Journal of the American Chemical Society*, 2006, **128**, 8320-8328. Copyright 2006 American Chemical Society.

Streptavidin was also used with biotin modified with manganese-salen complexes to catalyze the aqueous sulfoxidation of thioanisole using hydrogen peroxide. Although enantioselectivities were not high (up to 56%) those findings show the transfer of chirality from the protein to the catalytic reaction.[15] Those examples show how ligand

design combined with biomolecular tuning can produce a large number of catalysts with varying activities in a short period of time.

Reetz and co-workes have used a Darwinistic approach to genetically modify proteins to better fit their enantioselective reactions and to be more stable in the presence of non-natural substrates. This method is called directed evolution and implies random mutagenesis of the gene of an enzyme, giving a library of numerous genes which are then expressed into enzymes. The size of the library, *n*, can be calculated by using the formula $n=19^{M} \times 285/[(285-M) \times M]$, where M is the number of amino acids substituted in the enzyme. Once the enzymes are produced, they are ready to be screened as enantioselective catalysts. Once a few improved mutants have been detected they go through the same cycle until the optimal enzyme is produced (Figure 4).[16] Several other approaches were used for proteins mutation and another common method is the combinational active-site saturation test (CAST). This method uses the 3D structure of the wild type protein containing a substrate to locate the amino acids that can participate to the substrate binding. Depending on the secondary structure of the protein the spatially close amino acids can be located in locations such as a loop, β–sheet or α helix. This method consists of randomly mutating specific pairs of amino acids to enhance the enantioselectivity and catalytic properties of the protein.[17,18]

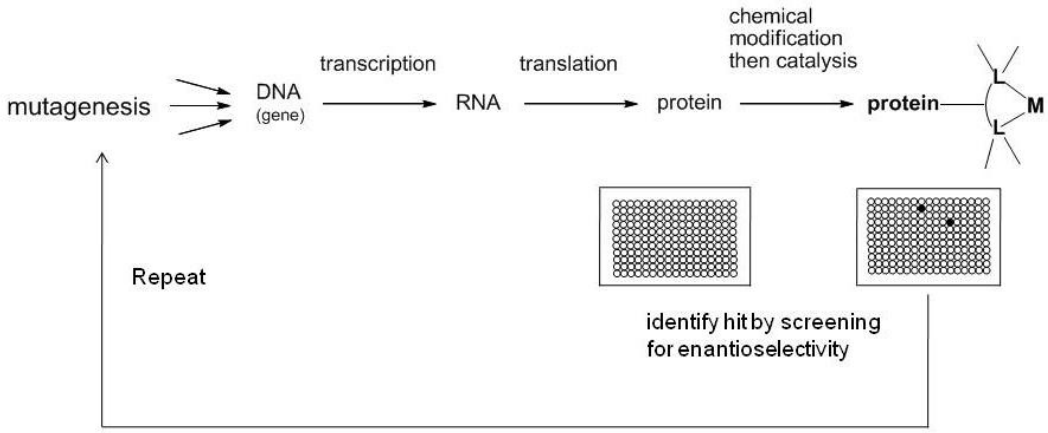
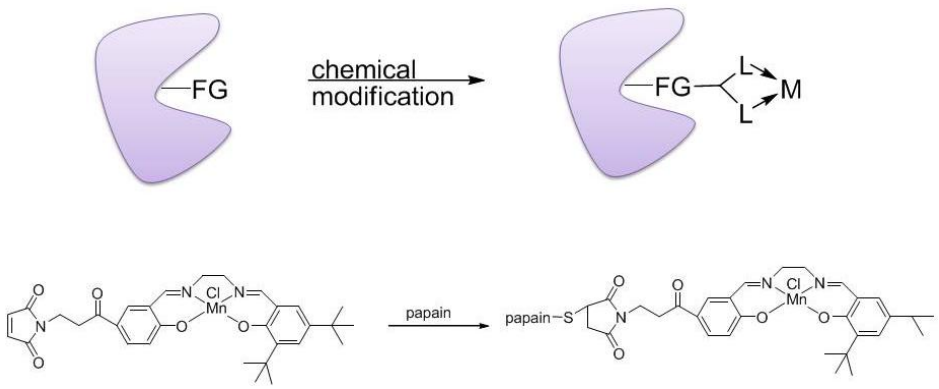


Figure 4. Directed evolution of an enantioselective enzyme.[19,20] Reprinted from M. T. Reetz *et al*. *Tetrahedron* 2007, **63**, 6404-6414. Copyright 2007, with permission from Elsevier.

Reetz *et al* have used the CASTing approach for the optimization of streptavidin and the Rh(diphosphine) modified biotin cofactor to improve the 23% enantioselectivity

for (*R*) achieved by Thomas Ward in the hydrogenation of α–acetamido-acrylic acid. The protein cavity was modified in the proximity of Rh (I) and further away from the metal to give an enantioselectivity of 65% for the (*R*) product. Although the selectivity improved with respect to Ward's catalyst, not all 200-300 clones that were synthesized could be tested for catalysis because of the low clone yield. In theory, the mutant with the highest enantioselectivity might not have been the best mutant of the screening process.[20,21] Nevertheless, this study clearly shows the proof of principle that advanced techniques such as directed evolution combined with screening can be applied to improve the selectivity of a hybrid catalyst.

Kaiser *et al* in 1988 were the first to covalently modify the active site of a protein.[22] This approach was later used by Reetz to covalently introduce a coenzyme in a protein's scaffold. In this case papain was modified by addition of the thiol of cys25 to a maleimide containing a Mn/salen ligand (Scheme 4). This hybrid catalyst was used in asymmetric epoxidation which gave only 10% ee.[20,23]



Scheme 4. Schematic representation of covalently bonded ligand to a protein and Mn/Salen ligand coupled to papain.[20]

In 1996 Distefano *et al* covalently bonded a phenantroline moiety to a cysteine located inside the active site of Adipocyte Lipid Binding Protein (ALBP) for the coordination with Cu (II). This metalloenzyme was used for the enantioselective hydrolysis of various unactivated amino acid esters. High enantioselectivity was achieved with the racemic mixture of alanine isopropyl ester where the L enantiomer was hydrolyzed 13 times as faster than the D isomer giving 86% ee.[24,25] Several other groups have used anchoring methods to bind their ligand to a protein for catalytic purposes. Lu has used a methane thiosulphonate group for dual anchoring of a

Mn/Salen complex into apo sperm whale myoglobin for the enantioselective sulfoxidation of thioanisole. Figure 5 shows the dual anchoring by formation of disulfide bridges between the ligands and the protein. With his dual anchoring strategy, Lu achieved 51% ee without any need of protein modification. By using a double anchor, conformational states available for the metal complex are limited leading to better reaction rate and higher enantioselectivity.[26]

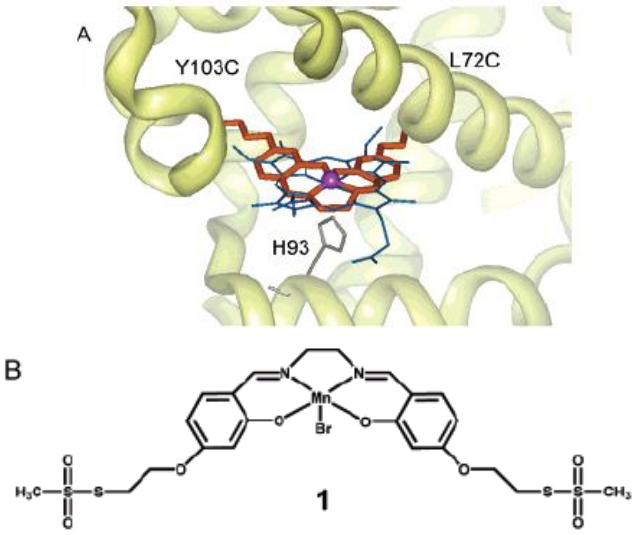


Figure 5. A) computer model of ligand 1 attached to myoglobin, B) Mn/Salen ligand.[26]
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1.2.2 Peptides as catalysts

Proteins are not the only biomolecules used for catalytic reactions. In 1970 the amino acid proline was used for the first time as a catalyst for the Robinson annulation reaction.[27,28] Since then other researchers have been using proline and small peptide molecules as biocatalysts.[29] Miller at al. have been using small and large oligopeptides for various asymmetric reactions. An example is an oligopeptide with a β-Turn and a stereogenic centre that can catalyze asymmetric aziridation.[30] Longer peptides have also been used for regioselective reactions such as the acylation of carbohydrate compounds[31] and even peptide containing phosphines have been used for enantioselective cycloadditions.[32] It is important to underline that all the catalysts used by Miller are organocatalysts that can produce high selectivities without the presence of transition metals. Gilbertson combined the special properties of amino acids with metal based catalysis producing metal-peptide complexes as catalysts for

asymmetric reactions. Since 1994, Gilbertson and co-workers have been studying the use of phosphine modified amino acids-metal complexes for asymmetric catalysis. Their first studies were focused on the addition of amino acids containing a protected phosphine moiety (diphenylphoshino-alanine sulfide) into a peptide chain with high helix content. Two phosphine moieties were placed with a four amino acid distance from each other in a solid supported peptide and later deprotected with Raney nickel. Owing to the helical structure of the peptide the two phosphine moieties were at the optimum distance from each other to coordinate as a bidentate to a metal, such as rhodium, forming a metal-peptide complex. (Figure 6)[33]

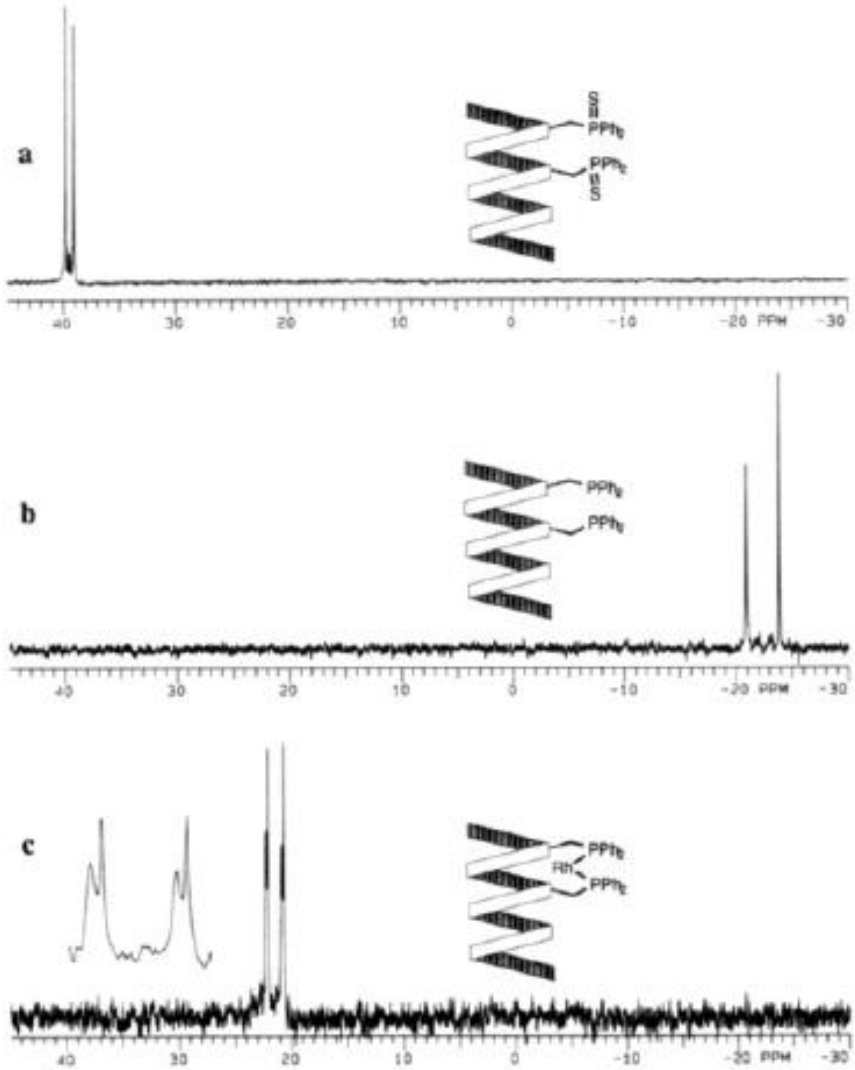


Figure 6. ^{31}P NMR representing: a) sulfur protected phosphine moiety introduced in a peptide, b) deprotectedPhosphine moiety, c) Rhodium complex of the phosphine modified peptide.[33] Reprinted withpermission from S. R.Gilbertson, G. Chen and M. McLoughlin, *Journal of the American Chemical Society*, 1994, **116**, 4481-4482. Copyright 1994 American Chemical Society.

The peptide ligand can be modified giving a large variety of catalysts in a relatively short time. In fact, Gilbertson has continued his study in peptide catalysis by introducing two electronically different phosphine moieties into a peptide. Since the peptide favours only one conformation, it is not necessary to use phosphine ligands with C_2 symmetry and it is possible to introduce (dicyclohexylphosphino)-alanine sulfide and the previously used diphenylphosphino-alanine sulphide ligands in the peptide. (Figure 7)[34]

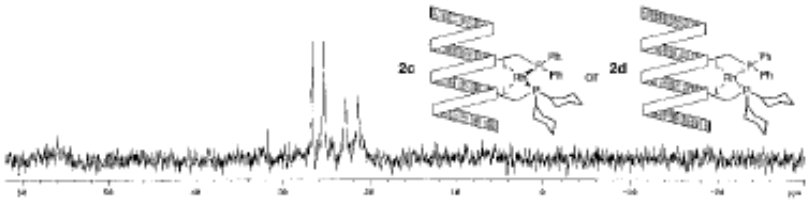


Figure 7. ^{31}P NMR of rhodium complex with two different phosphine moieties.[34] Reprinted with permission from S. R. Gilbertson and X. Wang, *J. Org. Chem.* 1996, **61**, 434-435. Copyright 1996 American Chemical Society.

The fist attempt of Gilbertson to use the metal complex in catalysis was in 1996 with peptide-rhodium complexes on solid support.[35, 36] 63 different phosphine modified peptides were synthesized on solid support. The phosphines were located at three different distances from each other (i+4, i+3, i+1, i=modified peptide) and tested for the hydrogenation of an enamide but enantioselectivities were lower than 20%. The *S* enantiomer reached an ee higher than 10% when dicyclohexylphosphinoalanine was present and when the distance between the two phosphines was i+3. For the *R* enantiomer an ee greater than 10 % was only achieved when the distance between the two phosphines was i+1.[36] More recently, Gilbertson has studied different asymmetric catalytic reactions obtaining higher enantiomeric excesses. The catalyst that he used for those reactions is a peptide with a derivative of proline inside a β-turn motive between the two phosphine groups. These turns increased the vicinity of the two phosphine moieties and favoured hydrogen bonding between the amino acids near the turns. (Figure 8)

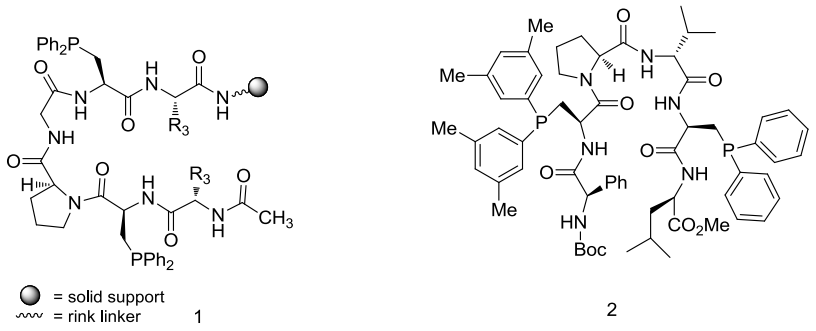
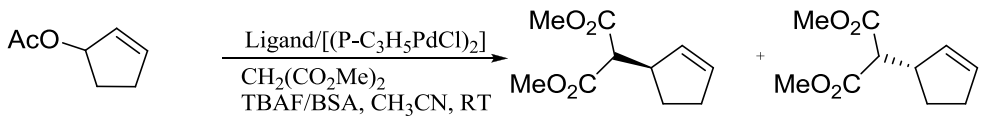


Figure 8. Peptides containing a proline inside β-turns between the two phosphine groups.[37,38]

The addition of dimethyl malonate to cyclopentenyl acetate (Scheme 5) with a palladium complex of the proline modified peptide with two diphenylphosphines as ligands (#1 in Figure 8) gave ee ranging from 34 to 80%.[37] For the same catalytic reaction it was possible to achieve up to 95% ee by using a different phosphine moiety on a peptide in solution (#2 in Figure 8).[38,39]



Scheme 5. Addition of dimethyl malonate to cyclopentenyl acetate.

1.3 DNA

As stated before, the presence of a chiral ligand is a prerequisite for asymmetric catalysis. Chirality is also present in all life forms. The chiral molecule DNA contains genes which have the hereditary information that is carried on from generation to generation in all living organisms. Deoxyribonucleic acid (DNA) consists of a series of coupled deoxynucleotides. A nucleotide is composed of a base, a ribose, and a phosphoric acid residue. The bases are purine bases, adenine (A) and guanine (G), and pyrimidine bases, cytosine (C) and thymine (T). Following the well known Watson-Crick rules for double helix formation, adenine pairs with thymine and cytosine pairs with guanine through hydrogen bonding. Two single complementary strands form a double helix because of interactions between complementary bases and π-π stacking. Base pairing is perpendicular to the helix central axis and the two

strands are antiparallel to each other, one going from 3' to 5' and the other going from 5' to 3' (Figure 9).

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Figure 9. DNA double helix with base pairings. Taken from the U. S. National Library of Medicine.

There are three main types of double helices. A, B and Z-DNA, (Figure 10).

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Figure 10. A, B, and Z-DNA double helix. Taken from www.oregonstate.edu

The B-DNA helix is the most common form of DNA found in nature and it contains a major and a minor groove which give the shape of the spiral double helix. The width of the double helix is around 20 Å (2 nm), the distance between bases is 3.4 Å, and one turn of the double helix consists of 10 to 10.5 bases or about 3.5 nm.[40] In A-DNA, the base pairs lie at a 20° angle with respect to the helix central axis. Both A and B DNA helixes wind upwards going from the left to the right and therefore are called right handed helices (Figure 11) In contrast Z-DNA is left handed and occurs when G-C base pairs frequently repeat each other. In fact, the base sequences in the strand can influence several parameters such as curvature, helical twist, rigidity of the duplex and groove width. An example is the propeller twist which happens when a base rotate between 5° and 25° with respect to their complementary base. This is common when a series of A residues are located next to each other.[41]

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Figure 11. Right and left handed double helixes. Taken from web.uconn.ed

In addition to these helices, formation of triple and quadruple helical DNA is also possible.[42] In triple or quadruple stranded DNA the third strand is placed in the major groove of a double helix. Hydrogen bonds between the purines and pyridines of the double helix and single strand give stable triplexes. Two types of triplexes are

possible. The first type is a py(pu-py) (py:pyrimidines, pu: purines) triplex where the additional strand is pyrimidine rich. The most stable triplexes of this kind are T(A-T) and C(G-C), often amines are required to overcome charge repulsion of phosphates. The base interactions between the triplexes are of the Hoogsteen type which means that the third strand is parallel to the purine strand of the double helix and forms hydrogen bonds between the pyrimidines of the third strand and the N7 group of the purine strand of the double strand (Figure 12a). The second type is a pu(pu-py) triplex where G(G-C) and A(A-T) triplexes stabilize the triple strand. The third strand is also antiparallel but to the purine strand of the duplex and it forms reverse Hoogsteen pairs (Figure 12b).[43] The stability of the triple helix not only depends on the base interactions but also on the DNA length, temperature, pH, and salt concentration.[44]

The unique proprieties of DNA have been used not only in biochemical research but also in nanotechnology to form DNA strands with supramolecular structures such as branches and 3-dimensional shapes.[40,45,46] DNA has also been manipulated to design origami type shapes.[47] The engineered DNA strands with three-dimensional shapes and ramifications can give rise to pockets that could be used as hosts or nanoreactors for catalytic reactions.

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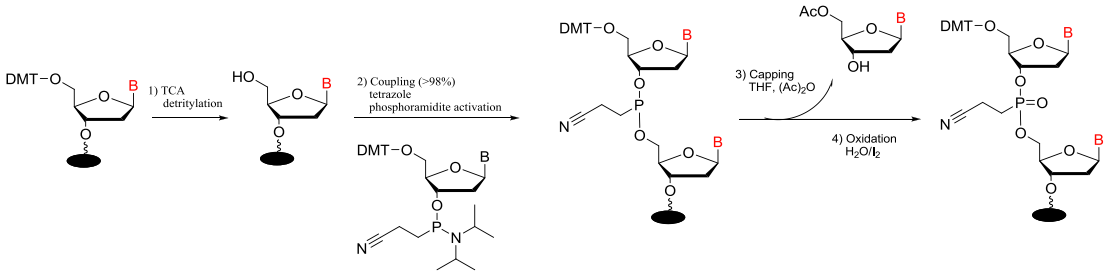
Figure 12. Represenation of Hoogsteen and Watson-Creek base pairs.[43]

Artificial DNA using the phosphoramidite method can be synthesized manually or by using a DNA synthesizer. The automated synthesis of DNA begins with a 3'-hydroxyl nucleotide attached to a solid support by a succinate ester bond. All 5'-OH of the sugar are protected with DMT, therefore the first step during the synthesis is the deprotection of DMT by TCA (trichloroacetic acid). The phosphoramidite nucleotide is added with tetrazole as the activating agent. All the unreacted nucleotides are capped with acetic anhydride and 1-methylimidazole and flushed away with acetonitrile. Finally $P^{(III)}$ is oxidized with iodine and water to give a phosphatetriester bond. This procedure is repeated until the desired sequence is formed. The DNA strand is cleaved from the solid support by using a strong base that will also cleave the cyanoethyl group of the phosphodiester groups and all other protecting groups of the bases (Scheme 6). The DNA synthesizer calculates the

coupling efficiency of the synthesis by measuring the DMT cation release by conductivity. The more modern DNA synthesizers can synthesize sequences of up to 175 bases. The positions and the frequency of the bases in the strand are inserted into the DNA synthesizer by the operator[48] so that the strand can be engineered to better fit its purpose.



Scheme 6. Automated DNA synthesis.

One draw back of solid phase synthesis is that it is limited to 15μm per preparation. Solution phase DNA synthesis is also possible for short sequences[49] and will be discussed later in Chapter 2.

1.3.1. Aptamers

DNA and RNA sequences have extremely high recognition properties towards small organic molecules. Those biomolecules are called aptamers which are single or double stranded oligonucleotides that bind to their target by folding around it through hydrogen bonding and π-π stacking. The molecular weight range of the target is typically between 100 to 1000 Dalton.[50] Polynucleotide library pools are used to generate aptamers which can vary in size and diversity. The more common length for an aptamer is about 70 nt (nucleotides) but smaller aptamers can still have the same functions. For instance, a 14nt aptamer that binds to flavin mononucleotide (FMN) still holds all the properties of its longer version of 35nt and it is still able to selectively recognize its target even in the presence of structurally similar molecules.[51] Aptamers can bind to a variety of molecules such as organic dyes, antibiotics, amino acids, peptides, proteins, and vitamins.[52,53] Because of their wide binding range, aptamers have been used in therapeutics,[54-56] as biosensors,[57-62] analytical tools,[63,50,58] antiviral agents,[64] and as catalysts.[65-67]

Aptamers are produced by a technique called the Systematic Evolution of Ligands by EXponential enrichment (SELEX) (Figure 13) developed by Joyce, Szostak, and Gold in the late 1980s early 1990s.[67]

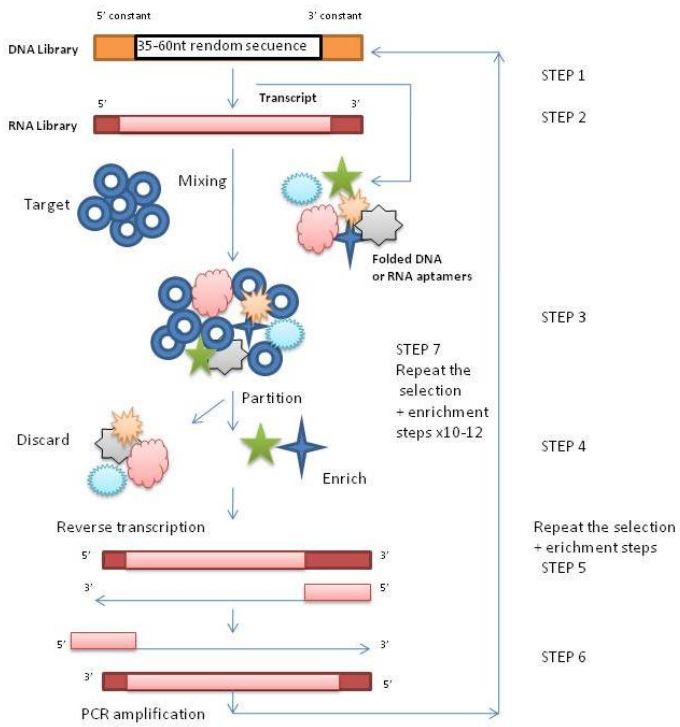


Figure 13. Systematic Evolution of Ligands by Exponential Enrichment. 1) synthesis of random DNA sequences, 2)conversion to RNA by transcription using T7 RNA polymerase, 3) introduction of target into the DNA pool, 4) removal of low affinity binders, 5) recovery of ssDNA and RNA strands, 6) PCR amplification.[68] Reprinted from T. Sampson, *World Pat. Inf.* 2003, **25**, 123-129.Copyright 2003, with permission from Elsevier.

Each oligonucleotide in the library is composed of a defined region containing primers and restriction sites at each end and a randomized region in the centre. Taking into account that 'n' nucleotides yield 4^n DNA molecules; the complexity of the library depends on the number of nucleotides used. Two different screening processes can be performed. The first screening process consists of incubating the random polynucleotide mixture and the target molecule in a buffer. The aptamers that bind to the target are filtered by using a nitrocellulose filter partitioning, the aptamers that are not selective to the target elute first, the aptamers bound to the target elute last. The second process to screen aptamers involves immobilizing the target molecule on a solid support. All the sequences that do not bind to the target are washed away. This step is less common because the aptamer can not always properly

fold around the molecule because one part of the molecules is attached to the solid support.[68-72] The bound sequences are eluted and used for the amplification step, during this step the previously selected aptamers are enriched for another cycle of selection and amplification. The affinity enrichment step is usually completed in 8-15 cycles depending on the binding target. Once the desired aptamer is selected it can be reproduced synthetically. It is often not necessary to use the whole aptamer length but it can be shortened to better fit its purpose. The entire cycle of SELEX manually would take 2 days but nowadays an automated system can complete 12 rounds of selection.[69,71] Aptamer-binding target studies done by Hermann and Patel[72] show that interaction between aptamers and aromatic ligands is highly specific. Hydrogen bonding together with π-π stacking interactions improves binding affinity and selectivity for these types of compounds. For example, Theophylline has the same molecular structure as caffeine except for an NH group instead of an N–methyl group and has 10,000 times more affinity to the aptamer than caffeine (Figure 14). This is because theophylline is stacked between two consecutive base-paired nucleotides and interacts with a cytosine base by hydrogen bonding. The methyl group in caffeine disrupts this interaction.

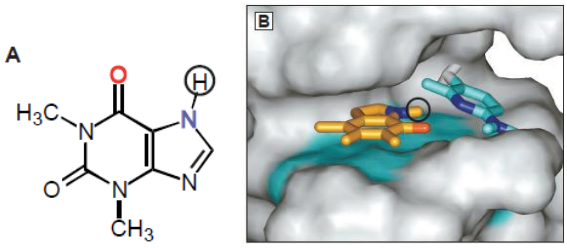


Figure 14. A) theophylline molecule, B) 3D image of theophylline in aptamer cavity.[72]
From T. Hermann and D. J. Patel, *Science (Washington, D. C.)* 2000, **287**, 820-825. Reprinted with permission from AAAS.

The selectivity of aptamers is not limited to cyclic compounds. Mann *et al*[73] have found that ssDNA aptamers that contained G-quadruplexes (guanine rich sequences) with potassium cations as stabilizers easily bind to ethanolamine located on magnetic beads or in solution. Ethanolamine is the smallest molecule used as an aptamer target and this discovery showed that aptamers can be used for clinical and environmental analysis. Another example of the high selectivity of aptamers is the RNA aptamer that recognizes L-arginine over D-arginine with a 12000-fold difference in binding constant.[74]

The selectivity of aptamers is improved by introducing small modifications to nucleotide structures that do not interfere with hydrogen bonding and aptamer folding. Those modifications have to be compatible with nucleic acids replicating enzymes or DNA and RNA polymerases. The preferred location for nucleotide modifications are the 5-position of pyrimidines, the 8-position of purines, and the 2'-position of all nucleotides.[75] Small chemical modifications such as the introduction of 2'-fluoro and 2'-amino can improve selectivity and sequence stability[53,76,77] An example is the aptamer developed for the endothelial growth factor (VEGF). This RNA aptamer has 2'-fluoro pyrimidine and 3'-*O*-methyl purine modifications. One isoform of the VEGF induces abnormal blood vessel growth and age related macular degradation which is a vascular disease that leads to blindness. VEGF is composed of a receptor binding domain and a hairpin binding domain. Its aptamer derived from the SELEX process specifically recognize this isoform and does not bind to any of the other four isoforms.[78]

The high selectivity of aptamers towards their target molecule combined with transition metal complexes can give rise to a hybrid catalyst capable of selectively recognizing the substrate by folding around the substrate by hydrogen and π-π interactions, thereby positioning it correctly for a selective modification.

1.3.3 DNA in Catalysis

DNA has been used in hybrid catalysis for a range of asymmetric reactions. Two methods have been used for the interaction of the ligand moiety with the DNA, the covalent and non covalent approach. The first method developed by Roelfes is the non covalent approach where the ligand is located on a DNA intercalator. This method places the metal complex in close proximity to the chiral environment of the helix without being coupled to a specific part of the DNA strands. This DNA/metal complex was used as catalyst for Diels-Alder reactions and Michael additions. For the Diels-Alder reaction the catalyst consisted initially of an intercalator such as 9-aminoacridine with a spacer and a metal binding group which gave enantioselectivities up to 90% for the reaction between cyclopentadiene and aza chalcone.[79] In more recent work the spacer has been removed and bipyridine ligands have been used to increase the proximity of the copper complex to the DNA for better transfer of chirality. Several intercalators with achiral bypyridine ligands were tested and the most selective was 4,4'-dimethyl-2,2'bypiridine give which gave 99% ee for

the *endo* isomer (Figure 15).[80,81] Roelfes and coworkers have also used α,β-unsaturated 2-acryl imidazoles as dienophile for its better solubility in water but this resulted in a slightly lower enantioselectivity (83-98% ee for the *endo* isomer).[82] The same imidazoles were also tested for enantioselective Michael reactions in water with the same bipyridine ligand (#5 in Figure 15) which resulted in 99% ee with nitromethane and dimethyl malonate as nucleophiles.[83] It has been also proven that for the Diels-Alder reactions changing the DNA sequence can result in a positive improvement of enantioselectivity and reactivity. Small changes such as shorter GC alternating sequences and two consecutive G's can improve the ee from 80% to more than 99% for the *endo* isomer. This is an illustrative example on how small changes in the microenvironment of the ligand can largely improve its properties.[84]

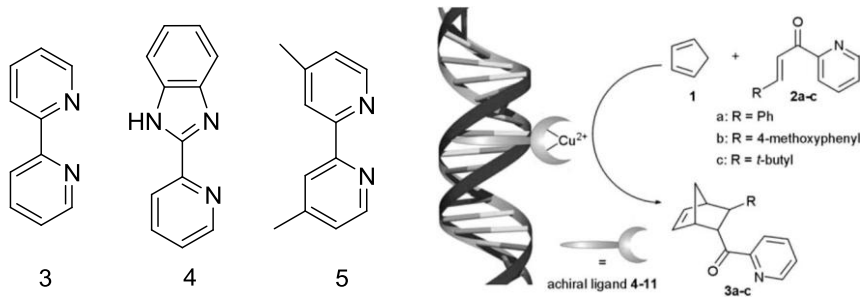
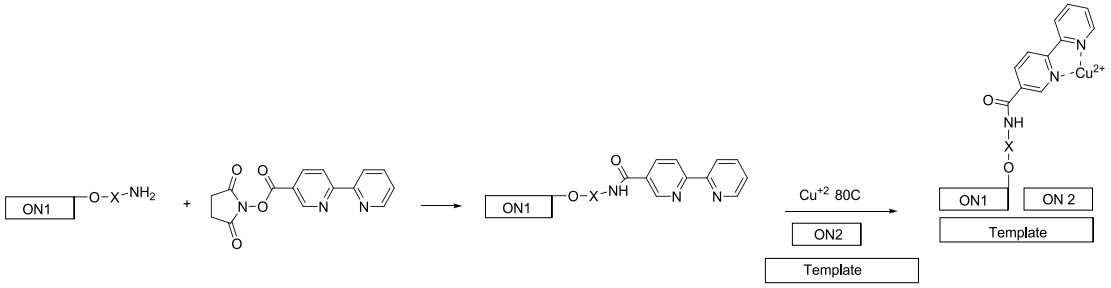


Figure 15. Asymmetric Diels-Alder reaction of cyclopentadiene (1) with aza-chalcone (2), using DNA metal complexes as catalysts. Dienophile 2b gave the highest enantioselectivity using ligands 7 (92% ee), 10 (95% ee), and 11 (>99% ee).[80] Reprinted with permission from G. Roelfes, A. J. Boersma and B. L. Feringa, *Chem. Commun. (Cambridge, U. K.)*2006, 635-637. Copyright 2006 The Royal Society of Chemistry.

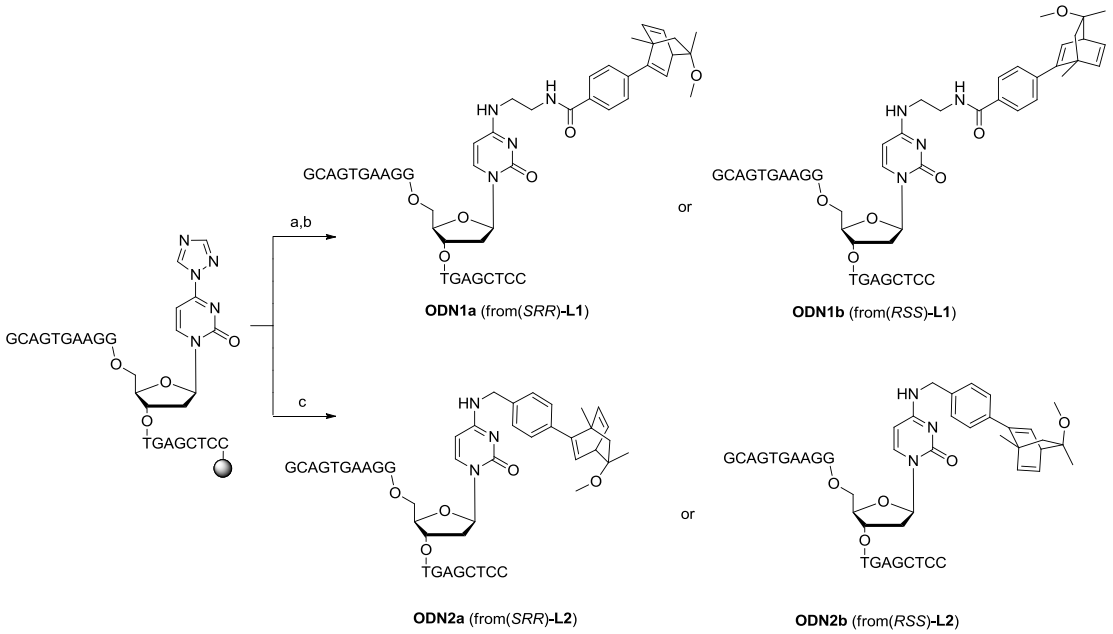
To understand the mode of action of a hybrid catalyst that uses the chirality and molecular recognition of DNA combined with the activity of transition metal catalysts it is important to know where the metal interacts with the DNA ligand. One way to introduce the ligand in a well defined position of the DNA strand is to have a covalent bond formation between the ligand and the DNA. More recently, Roelfes and Jäschke have explored the covalent approach. Roelfes has introduced an amine linker of different lengths into the terminal position of the DNA and has introduced a bipyridine ligand through amide bond formation. This oligonucleotide is then assembled with a second oligonucleotide on a complementary template strand that holds both oligonucleotides together. (Scheme 7) The bipyridine ligand was complexed with Cu and used for Diels-Alder reaction. Modification of the DNA

strands fine tuned the enantioselectivity and a three carbon linker also increased enantioselectivity suggesting that a close proximity to the chiral double helix improves the ee of the reaction. This great advantage of this method is that several ligands could be engineered just by changing the sequence of the complementary strand.[85]



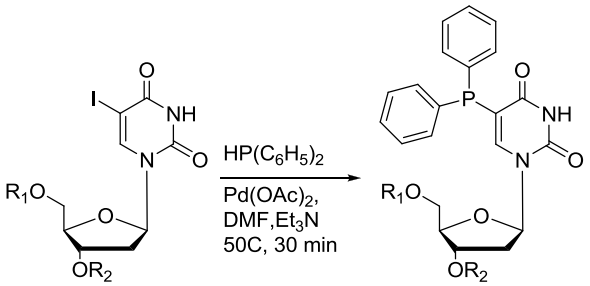
Scheme 7. Coupling of bipyridine ligand to DNA and its use in Diels-Alder.[85]

Jäschke *et al* explored DNA transition metal hybrid catalysts for asymmetric allylic substitution. An imidazole modified nucleoside was introduced in the central position of an oligonucleotide. The imidazole moiety was made to react with amine functionalities during the basic DNA cleavage from solid support giving DNA modification and cleavage in one pot. In this way amine linkers can be introduced into the DNA strand and those linkers can later be coupled to ligands containing a carboxylic acid moiety or alternatively ligands with an amine moiety can be directly introduced on the DNA strand (Scheme 8). Bicyclo[2.2.2]octadiene was modified with a carboxylic acid or an amine moiety and both methods were used for introduction of the diene ligands into the DNA strand. The complementary strand was varied forming loops in the opposite strand of the ligand or on the strand of the ligand. The diene ligand was complexed with iridium and used for asymmetric allylic alkylation. The enantioselectivity was slightly higher with the DNA ligand than only with the diene ligand, but it is important to mention that the engineering of the complementary strand gave definite changes in enantioselectivity[86] which was also observed by Roelfes.



Scheme 8. Coupling of diene ligand to DNA strands. a is the coupling of a diamine to the modified monomer and b is the coupling of a diene carboxylic acid to the amine functionality. C is the coupling of a diene amine modified directly on the modified monomer.[86] P. Fournier, R. Fiammengo and A. Jaeschke, *Angewandte Chemie, International Edition*, 2009, **48**, 4426-4429. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

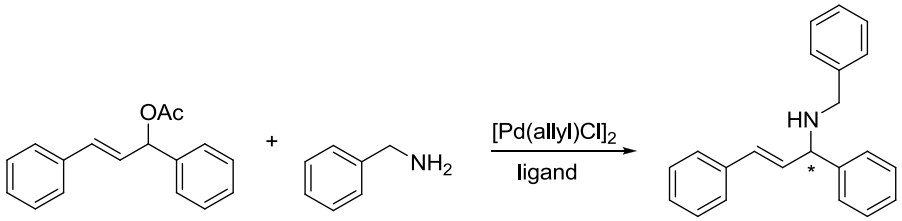
Phosphine modified nucleosides and a short oligonucleotide have also been used as ligands for asymmetric allylic substitution. Ropartz *et al*[87] have synthesized a trimer with a diphenylphosphine moiety. An 5-iodouridine has been introduced into the trimer using automated solid phase synthesis and diphenylphosphine has been introduced via palladium catalyzed phosphinylation (Scheme 9).



Scheme 9. Synthesis of modified monomers and trimers, in dppdU R_1 and R_2 = H, in AcdppdU R_1 and R_2 = Ac, in dAdppdUdT R_1= deoxyadenosine-3-phosphate and R_2 = deoxythymidine-5-phosphate.[87]

The monomer was tested with acetyl protected and free hydroxyl groups. Table 2 shows that the unprotected monomer in THF results in the highest enantioselectivity

(80%) and that when the hydroxyl groups are protected or the solvent is changed the enantioselectivity drastically decreases even resulting in change of absolute product configuration. This effect could be explained by possible hydrogen bonding interactions of the hydroxyl groups with the structure of the ligand. Trimers show much lower selectivity and conversion but they represent a good study for the use of larger DNA sequences with modified phosphine moieties.[87]



Scheme 10. Assymetric Allylic amination using monomers and tirmers as ligands.[87]

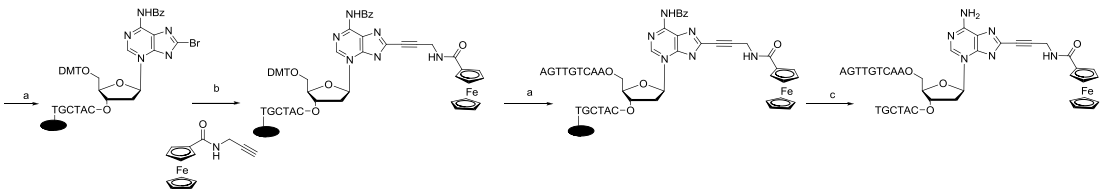
Ligand (L)	Solvent	L:Pd ratio	Incubation time/h	Conversion (%)	ee (%)	Absolute Configuration
dppdU[a]	THF	0.5	0.5-4	>99	62	S
dppdU[a]	THF	2	2	>99	72	S
dppdU[a]	THF	2	4-16	>99	80	S
dppdU[a]	THF	4	4	>99	79	S
dppdU[a]	CH3CN-THF	2	4	>99	16	R
dppdU[a]	DMF	2	4	>99	14	R
AcdppdU[a]	THF	2	2	>99	8	S
AcppdU[a]	DCM	2	2	>99	21	R
AcppdU[a]	DCM	2	2	83	23	R
dAdppdUdT[b]	THF-H_2O 3:1	4	8-24	15	8-12	R
dAdppdUdT[b]	H_2O	4	24	30	-	-

[a]Reaction conditions: 7.5 μmol $[Pd(allyl)Cl]_2$, benzylamine:propenyl acetate: Pd = 120:40:1, solvent 2 cm^3, 25 ˚C, 24 h. [b]Reaction conditions: 0.5μmol $[Pd(allyl)Cl]_2$, benzylamine:propenyl acetate: Pd: K_2CO_3 = 120:40:1:120, solvent 1 cm^3, 25 ˚C, 36 h.

Table 2. Allylic amination of 1,3-diphenyl-2-propenyl acetate with benzylamine with monomers and trimers as ligands.[87]

1.3.4 Introduction of transition metals into DNA

Linkers in DNA can be placed at different locations of the DNA. Amine and thiol linkers have been introduced directly on a nucleoside,[88-94] or as a carbon linker at the terminal positions of a DNA strand.[95] Propargyl linkers have been introduced on thymidine and have also been used to couple moieties through click chemistry.[96] Covalent bonding with DNA has been used in the past to introduce ligands for transition metals. Ferrocene ligand has been introduced into DNA strands as a tool for labelling by two different methods. It can be introduced on the monomer prior to DNA synthesis or during DNA synthesis. A ferrocene with a propargyl functionality can be coupled to iodo/bromo-uridine[97] or a 5' amine modified thymidine can be coupled by amide bond formation with a carboxylic acid modified ferrocene.[98] Introduction of ferrocene during DNA synthesis is achieved by introducing iodo-uridine or bromo-adenosine monomers directly on the DNA. DNA Synthesis is interrupted after the introduction of the halogenated monomer and the ferrocene ligand is attached using sonagashira coupling. All the excess reagents are easily washed away while the DNA stays attached on solid support. After that DNA synthesis is continued using the standard procedure (Scheme 11).[98,99]



Scheme 11. a) ODN (oligodeoxynucleotide) synthesis; b) $Pd(Ph_3)_4$, CuI, DMF; c) ODN deprotection.[98] Reprinted from A. E. Beilstein and M. W. Grinstaff, *Journal of Organometallic Chemistry*, 2001, **637-639**, 398-406. Copyright 2001, with permission from Elsevier.

Ruthenium complexes have also been introduced into DNA during solid phase synthesis on amine modified bases at the 3' position[100] or directly on the modified base prior to DNA synthesis.[101,102] Ligands coordinated to ruthenium have been widely used to probe the electron transfer capabilities of DNA.[103] They are known to give photo cross-linking with guanine bases of complementary strands. Guanine is the most reductive base present in the DNA and when the metal complex is excited by illumination it is capable to abstract an electron from dG. Those metal complexes have potential for a wide range of applications in therapeutics. To covalently attach

the Ru complex to DNA, aliphatic chains with terminal amine have been introduced into the 5' and 3' terminal position of DNA strands and nucleotides have been modified also with amine functionalities at the central position. The desired Ru complex is attached to the DNA via amide bond formation.[100,104-110]

Ligands typically used to coordinate to the metal are phenanthrene diimine, phenanthroline, and bipyridyl derivatives, some examples are shown in Figure 16.

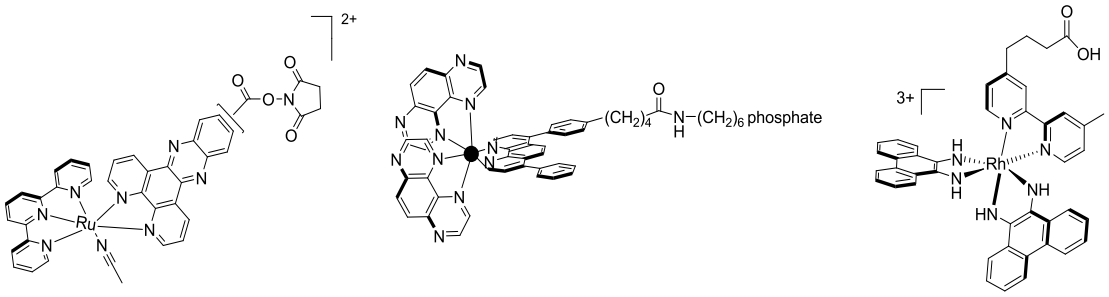
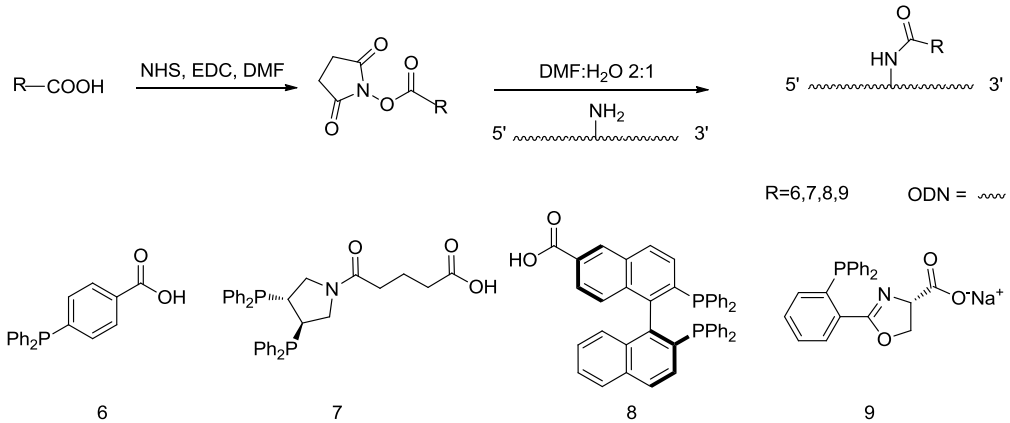


Figure 16. Ru and Rh complexes with bipyridine ligands.[100,105,106]

Bipyridyl ligands are not only used with Rh metal[111,112] but also with Cu as shown in Roelfes work.[79] Several of those methods used for ligand binding to DNA can be adopted for the introduction of phosphine moieties into DNA, two very good examples are the work done by Ropartz *et al.*[87] explained in the previous section (Scheme 9) and Jäschke.[113] Jäschke was capable of introducing several phosphine moieties into longer DNA strands using the same method used for the introduction of diene ligands. With this method four different phosphine moieties shown in Scheme 12 were introduced in the DNA strand by amide bond formation using EDC and NHS as the coupling agents under basic conditions (Scheme 12).[113]



Scheme 12. Introduction of phosphine moieties into DNA after its synthesis.[113]

1.4 Peptide Nucleic Acids (PNA)

After the discovery of DNA several artificial nucleic acids mimics have been synthesized. One of them is Peptide Nucleic Acids (PNA) developed in 1991 by Nielsen *et al* PNA has an *N*-(2-aminoethyl)glycine backbone with a DNA base attached to the central amine.[114] Those bases can form Watson-Crick and Hoogsteen duplexes with DNA single and double strands (Figure 17) or with other PNA strands.[115]

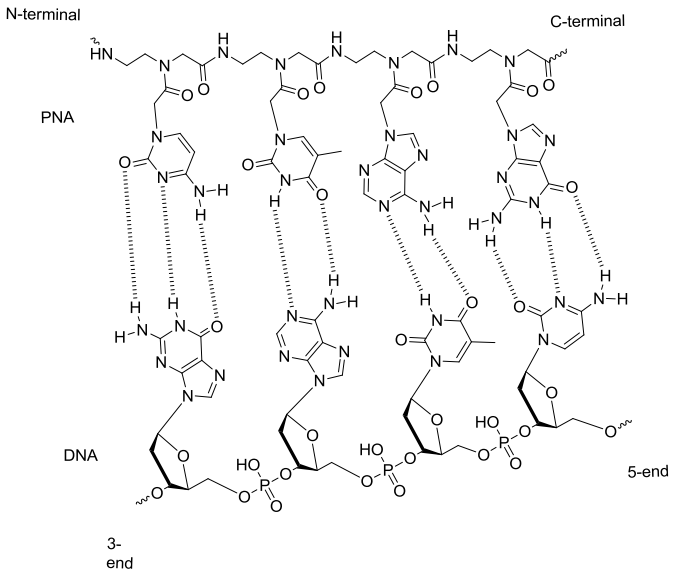


Figure 17. Example of a PNA DNA duplex.[114] Reprinted from L. Bialy *et al*. *Tetrahedron* 2005, **61**, 8295-8305. Copyright 2005, with permission from Elsevier.

Since its first preparation, PNA has been extensively used in several different scientific fields such as in molecular biology as genome cutter, in drug discovery as an antigen[116] or as mRNA inhibitor for therapeutic drugs; and in detection as biosensor for nucleic acids.[117]

Because of its similarity with DNA, PNA has been used to make chimeras by itself or with DNA if better solubility in water is needed. This field has been studied by Messere and co-workers who produced chimeras for the recognition of nucleic acids and proteins. Chimeras are composed of two parallel strands of PNA or DNA and PNA connected at the ends with an alkyl chain forming a closed circle. (Figure 18)[118] With this shape the PNA or DNA could be modified to covalently introduce metal complexes in the chimeras to benefit the chirality and steric hindrance of those modified biomolecules.

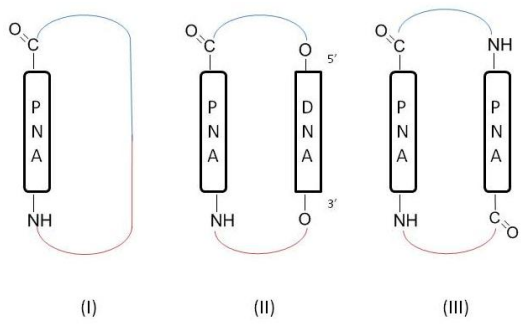


Figure 18. PNA chimeras, (I) only PNA strand, (II) PNA and DNA, (III) two PNA strands.[118] Reprinted with permission from L. Moggio *et al*. *Org. Lett.* , 2006, **8**, 2015-2018. Copyright 2006 Chemical Society.

PNA has also been used to form duplexes and triplexes with DNA.[115,119-121] PNA has the same ability to recognize DNA as DNA has with itself. Not only the base pairing between the two different strands for duplex formation is similar but also the neutral backbone of PNA gives less repulsion between the two strands in comparison to two negatively charged DNA strands. This high affinity of PNA to DNA results in duplex formation with a helical structure similar to type A or B form of DNA which means that a single DNA strand is able to transfer its chirality to an achiral PNA strand.[119-121] Further work by Nielsen showed that PNA-DNA duplexes are even more stabilized when PNA strands contain a relatively large number of purines.[122] Double stranded PNA can also form a helical structure because of the helical stacking of the nucleobases although due to the non chiral backbone the helical structure can interchange between the right and left handed structures. It is possible to induce chirality in a PNA double strand by introducing a chiral amino acid instead of glycine at the terminal monomer of the PNA single strand. The amino acid usually used is L-lysine. This approach eliminates the need for a DNA strand to produce chirality but it has its limitations. With a chiral amino acid the chirality can be transferred only to a strand that has a maximum number of ten nucleobases and the choice of the nucleobase nearest the amino acid is very important to obtain only one helical structure. Guanine or cytosine need to be near the chiral amino acid. Because of the different structure of the two bases it is believed that is the thermal stability of their G-C base pair that induces a specific handedness and not their possible interaction with the amino acid.[123] Chirality also depends on where the monomer with the introduced amino acid is located in the strand and the hydrophilicity of the amino acid side chains. Modified monomers introduced in the central position of the strand give

the strongest effect for chirality and hydrophobic amino acid side chains give one handedness while hydrophilic chains give the other handedness.[124]

PNA is not only capable of forming duplexes and triplexes with DNA but it can also invade a DNA double helix to form a triplex, duplex or a double duplex invasion. (Figure 19)

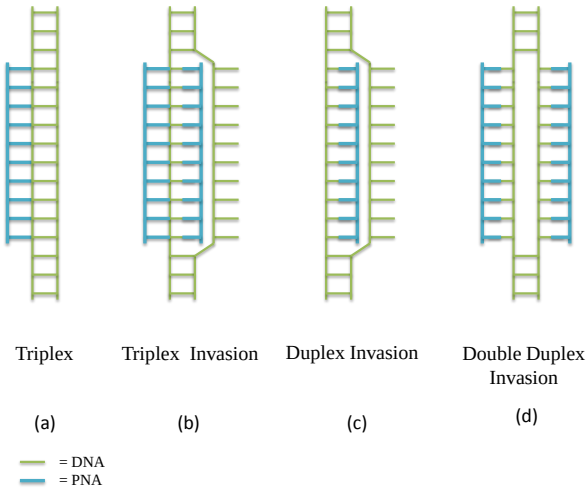


Figure 19. Representative drawing of PNA invasion of a DNA double strand.[125] P. E. Nielsen, *Methods in Enzymology*, 2001, **340**, 329-340. Copyright 2008, with permission from Elsevier.

For triplex invasion one PNA strand binds to one strand of a DNA duplex through Watson-Crick base pairing while the other PNA strand binds to the same DNA strand through Hoogsteen base pairing (b). A single stranded homopurine PNA strand can give rise to duplex invasion (c) although it is not as strong an interaction as the triplex invasion. Modifications of adenine and thymine to form diaminopurine and thiouracil have improved DNA invasion especially for a double duplex invasion (d) where the two PNA strands prefer to bind to the target DNA over binding each other due to the steric hindrance formed by the altered adenine and thymine. This ability to invade DNA strands makes PNA highly valuable in molecular biology and medicinal applications.[125]

The chirality and selectivity of DNA and the vast use of PNA are very important qualities for ligands to make selective transition metal complexes. As previously showed DNA has been already successfully used together with transition metals as catalyst for several asymmetric reactions.

1.5 Asymmetric Catalysis

A chiral compound is defined as a compound whose mirror image can not be superimposed to the original molecule. Chirality can be attributed to the presence of one or more stereogenic elements which can be centres, axes, and planes. A carbon with four different substituents is a chiral centre. When two optical isomers are mirror images they are called enantiomers which have the same properties but can behave differently in a chiral environment. In contrast, diastereomers have different physicochemical properties and are not mirror images of each other. An example of different behaviour of enantiomers is the pharmaceutical drug thalidomide of which its *S* isomer appeared to be teratogenic while his *R* isomer is an effective sedative. In spite of this many drugs are still sold in their racemic form because of the laborious production of enantiopure compounds and high costs involved. About 80% of industrial processes use catalysts to increase the yield of their products, the reaction rates and the selectivity to the desired compound. The products resulting from the catalytic reaction can be chiral or not. There are three different techniques to produce only one enantiomer of chiral compounds: resolution of racemic mixtures, stereoselective synthesis and asymmetric catalysis.[126] This section will focus on asymmetric homogeneous catalysis.

A catalyst does not alter the equilibrium constant of a reaction but lowers its activation energy making the reaction faster or creates another reaction pathway.[7] Generally, several types of selectivity can be distinguished for a chemical reaction (Table 2). In homogeneous catalysis the reagents and the catalyst are in the same phase usually the liquid phase. For asymmetric catalysis, transition metal complexes have proved to be highly efficient and have been widely studied by both industry and academia. To design efficient catalysts for asymmetric transformations it is necessary to have metal complexes which create a chiral environment. This is possible by using chiral ligands. It is important to emphasise that the main challenging goal in homogeneous catalysis is to design complexes capable to selectively produce only one isomer of the desired compound.

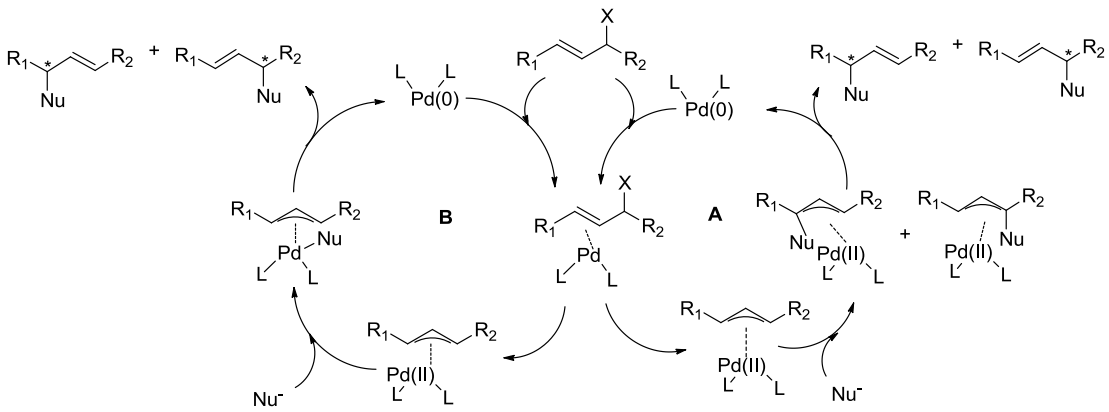
Reaction selectivity	Definition
Chemoselectivity	One functional group of the substrate is altered over the other functional groups present in the substrate
Regioselectivity	The alteration is preferred at one location of the substrate over other possible locations.
Diastereoselectivity	Only one diastereoisomer is formed out of an enantiomer
Enantioselectivity	The formation of one enantiomer over the other

Table 2. Definition of the four possible types of selectivity.[127]

Metal-catalysed allylic substitution is an indispensable tool in organic synthesis and has mainly been used at laboratory scale. Because of the extensive studies done on this reaction and its complexity it was chosen as a catalytic model reaction. A brief overview of this reaction is given in the next section.

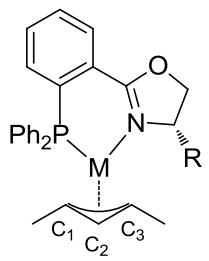
1.5.1 Allylic Substitution

Stoichiometric allylic substitution was first discovered by Tsuji in 1965[128] and in 1977 Trost[129] developed the first asymmetric Pd catalysed version. This reaction consists of the replacement of a leaving group in an allylic position by a nucleophile. The most common transition metal used for those reactions is palladium but many others such as Cu, Ir, Pt and Mo have also been employed. The general cycle of palladium catalyzed allylic substitution for a soft and hard nucleophile is represented in Scheme 13. The first step of a palladium catalyzed allylic substitution is coordination of the allyl substrate to the metal, the second step is an oxidative addition of the allylic substrate to the metal, in this case Pd(0), to give rise to a π-allyl-Pd(II) complex.[127] The third step is the nucleophilic attack, which happens directly on the coordinated allyl group outside the primary coordination sphere of the metal for soft nucleophiles (**A**).[132] The forth step is dissociation of the desired product and reduction to Pd(0), which can re-enter the catalytic cycle.[127] When hard nucleophiles are used (**B**), the nucleophilic attack takes place on the metal instead of the allyl ligand and the product is formed by reductive elimination.[131] This section will focus on soft nucleophiles because they are most commonly used in allylic substitution reactions.



Scheme 13. General catalytic cycle for Pd catalyzed allylic substitution with soft (**A**) and hard (**B**) nucleophiles.[133]

The mechanism of regio- and enantioselection for this reaction depends on the substrate and nucleophile used and is controlled by the chiral ligands coordinated to the metal. For example, the enantioselectivity of products which are generated from an asymmetric allylic fragment depends among other things on the differentiation between the two carbons on the allyl group.[134] This selectivity is controlled by the ligands via steric and electronic effects. Successful examples are the chiral phosphino-oxazoline ligands synthesized by Pfaltz as in the metal allyl complexes represented in Figure 20.[135]



R = CH_3, CH_2Ph, *i*Pr, Ph, *t*Bu

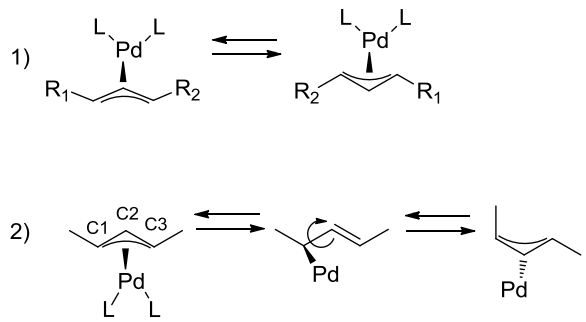
Figure 20. allyl metal complex using phosphino-oxazoline ligands[135]

This metal complex contains P and N donor atoms which create electronic and steric dissymmetry into the coordinated allylic group. Consequently, the C3-M bond length of the allyl complex is increased compared to the C1-M bond length therefore favouring the nucleophile attack on the carbon *trans* to the P atom. This is also called "trans influence" which is defined as "the extent to which a ligand weakens the bond

trans to itself".[136] The phosphorous atom makes a strong π-bond with the metal resulting in an increase in electrophilicity in the carbon at its *trans* position. In addition to this electronic effect steric effects can also apply. An example is the ligand shown in Figure 20 where the *cis* position to the nitrogen atom is less sterically hindered.[133]

For monosubstituted allylic substrates, the less hindered termini of the allyl-metal complex is more prone to nucleophilic attack for steric reasons resulting in the mostly linear product which is achiral. In spite of that, nucleophilic attack can be directed toward the more substituted termini by choosing the appropriate catalyst. Pfaltz *et al* prepared chiral phosphite-oxazoline ligands favouring the branched product. That was achieved by introducing electronegative substituents at the phosphorus atom and changing the R group of the oxazoline ligand to adjust the steric repulsions. With the optimized system, it was possible to achieve a branched:linear ratio of 76:24 with 90% ee.[137] Depending on the catalyst employed it is also possible to direct the regioselectivity of the linear product toward the *E* or *Z* regioisomer.[127,133] Many other types of substrates are used which require specific ligand design to accommodate the desired regio and enantioselectivities.[133]

To produce enantiopure products an additional complication found in allylic substitution reactions are the rearrangements of the π-allyl-Pd intermediates.



Scheme 14. 1) π-rotation, 2) π-σ-π isomerization.[127]

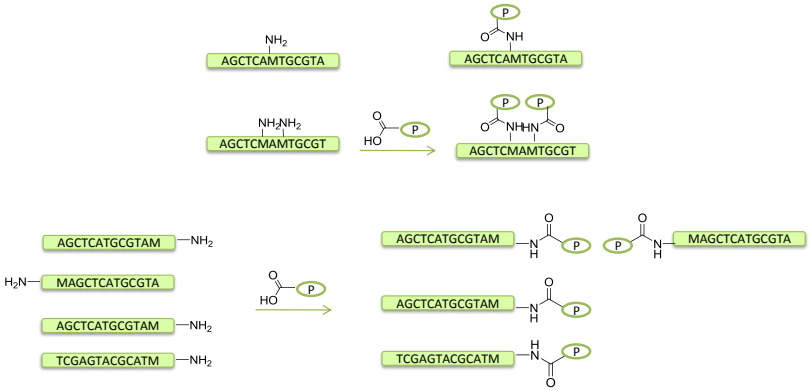
Two principal mechanisms of rearrangement have been described; the first one (Scheme 14, 1)) consists of a formal allyl rotation, which interchanges only the terminal substituents without changing the face of the allyl group which is coordinated to the metal although it changes the enantioselectivity of the reaction. The second mechanism (Scheme 14, 2)) known as π-σ-π isomerization occurs

thorough a σ-bound intermediate and implies a change in the face of the allyl group coordinated to the metal. Once the σ-bond is formed between C1 and Pd the alkene rotates at the C1-C2 bond and coordinates again to the Pd at the opposite face. Moreover, the methyl substituent at the C1 carbon changes from a *syn* position to an *anti* position.[127,138]

The mechanistic examples of asymmetric allylic substitution explained above demonstrate the complexity of this reaction and the demand of ligands capable of modifying different types of substrates without the need of elaborate changes in the structure of the ligand.

1.6 Project Aim

To reduce the amount of industrial chemical waste it is mandatory to design catalysts with high selectivity and efficiency for a range of chemical reactions. It is well known that metal atoms bound to a chiral organic molecule can form effective catalysts for the production of enantiomerically pure compounds.[7] Nature uses enzymes which are the most efficient and selective catalysts known. If nature's properties could be combined with transition metals reactivity it would be possible to form highly selective hybrid catalysts. Because of their chirality and selectivity those catalysts could be applied for asymmetric reactions. The two biomolecules chosen for this hybrid catalyst are DNA and PNA which is a peptide mimic of DNA that forms duplexes with DNA. DNA is chosen because of its chirality and selective binding toward small molecules. The first step towards the production of DNA based catalysts is to introduce a moiety such as phosphine ligands that will strongly coordinate to transition metals. To achieve this, functionalized nucleosides will have to be introduced into a DNA strand, which can be covalently linked to the phosphine moiety in a specific location of the DNA strand. (Figure 21)



M= modified monomer, P= phosphine moiety, A=adenine, G= guanine, T= thymidine, C= cytosine

Figure 21. Introduction of phosphine moieties into DNA.

As can be seen in Figure 21 the modified nucleotide can be introduced in the central or terminal position of a DNA single strand. By changing the position and characteristics of the phosphine moiety and by changing the complementary strands it could be possible to produce a variety of modified double helix DNA. An amine or thiol linker can be introduced into a modified oligonucleotide for the introduction of the phosphine moiety. This approach can be used for both DNA and PNA and because of their affinity to each other it would be possible to combine PNA and DNA single strands therefore increasing the variety of ligands produced. Once the strands have been functionalized with phosphine moieties they will be complexed to a transition metal and their enantioselective properties will be tested in asymmetric reactions such as hydrogenation, hydroformylation, and allylic substitution.

A noncovalent approach is also possible. As seen in the work of Thomas Ward it is possible to couple the ligand to a molecule that is well recognized by the biomolecule of interest. In our case the biomolecule of interest is DNA and the well recognized molecule is L-arginine. In the literature several aptamers of arginine have been studied and we plan to couple a bipyridine ligand to arginine and use its aptamer as the source of chirality. Those types of ligands can be tested as catalysts for Michael additions or Diels-Alder reactions.

1.7 References

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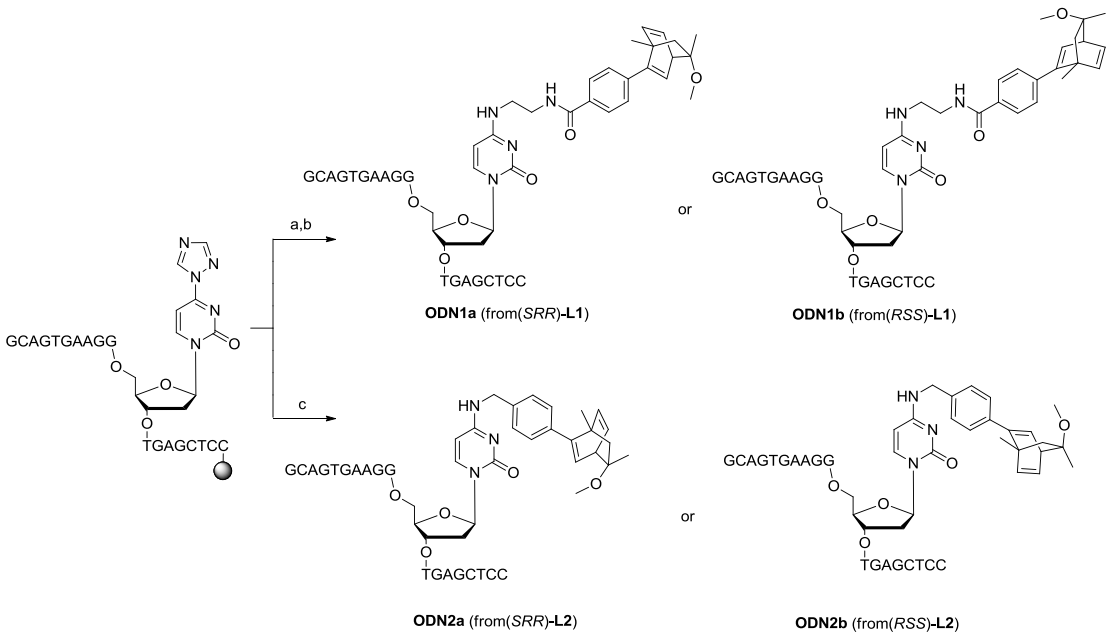
Chapter 2: Synthesis of Thiol Modified Monomers

2.1 Introduction

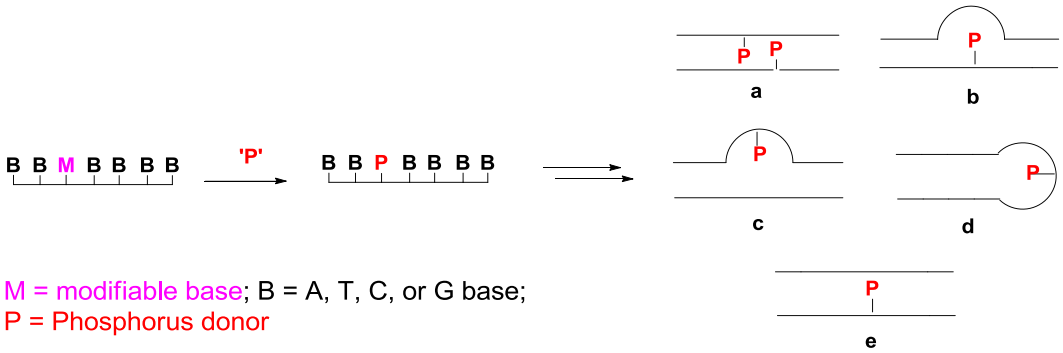
Life as we know it would be impossible without enzymes, which are biocatalysts often containing metallic ions in their structure. Enzymes are used by nature to catalyze a wide range of reactions resulting in the formation of the desired product in high efficiency. Artificial catalysts have also been used for centuries and since the twentieth century this field has been rapidly growing in both industry and academia. In particular, homogeneous catalysis with transition metal complexes is nowadays a very important area in chemistry. A countless number of ligands has been prepared and screened in the search for catalysts achieving the selectivity and reactivity needed in many reactions. Despite all the effort and knowledge gained over the years, many reactions in industry still lack a proper catalytic system, forcing the use of stoichiometric amounts of reagents resulting in the accumulation of large quantities of chemical waste. To reduce the environmental damage caused by the chemical industry it is necessary to build catalysts which combine the selectivity of nature and the reactivity of transition metal moieties.[1-3] In academia several research groups have combined the molecular recognition of proteins with transition metal catalysis[4-6,7] and in more recent years the focus has also spread to the use of DNA combined with transition metal catalysis. Two methods have been used to attach the transition metal fragment to DNA: the covalent and the non covalent approaches. Roelfes *et al*[2, 8] developed the non covalent approach, which consists of the use of random DNA sequences (from commercial salmon testes or calf thymus DNA) together with a bipyridine intercalator and Cu(II) salts to catalyze asymmetric Diels-Alder and Michael addition reactions. The results are impressive with enantioselectivities reaching up to 99%. Those findings were pivotal to prove the concept of DNA as an efficient chiral inductor for highly selective transformations. A disadvantage of this non covalent approach is that the location of the intercalator in the DNA (and therefore of the catalytic centre) is not known and hence optimisation of the catalyst is difficult. For this reason the same group has more recently shifted to the covalent approach.[9] Bipyridine Cu(II) complexes have been introduced into specific locations of a double stranded DNA via amide bond formation. This covalent approach has also been pursued by other groups. Ropartz *et al*[10] introduced phosphine moieties onto a mononucleoside and short oligonucleotides and used them as ligands for Pd-catalyzed

asymmetric allylic substitution reactions. An important contribution in this field has been made by Jäschke *et al*,[11] who introduced bicyclo-[2.2.2]octadiene in the central position of a 19-mer as a ligand for asymmetric allylic alkylation with Ir (Scheme 1) The enantioselectivities were not high (up to 27% ee) although it was found that the catalytic results were sequence dependent. The same finding was observed by Roelfes *et al* in both covalent and non covalent approaches.[12]



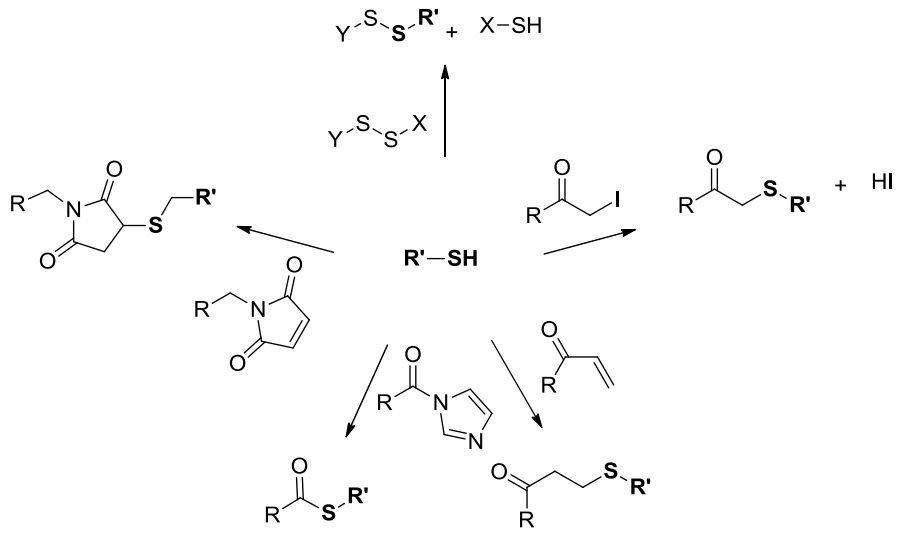
Scheme 1. Coupling of diene ligands to DNA strands. a is the coupling of a diamine to the modified monomer and b is the coupling of a diene carboxylic acid to the amine functionality. C is the coupling of a diene amine modified directly on the modified monomer.[11] P. Fournier, R.Fiammengo and A. Jaeschke, *Angewandte Chemie, International Edition*, 2009, **48**, 4426-4429. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

This discovery shows a powerful advantage of DNA as ligand because simple changes in structure of the DNA such as the sequence of the complementary strands can give a completely new catalyst that can place the substrate of interest in a very different environment. Scheme 2 represents an example of how changing the complementary strand of a modified DNA strand can result in a very different environment.



Scheme 2. Coupling of a phosphine moiety to a DNA strand and introduction of complementary strands. Introduction of a complementary strand also containing a phosphine moiety results in a bidentate (**a**), mismatches in the complementary strand sequence can give rise to loops in the complementary strands (**b**) in the modified strand (**c**), or in a self complementary strand (**d**), addition of a fully complementary strand (**e**) produces yet another environment.

Phosphine ligands are very powerful for asymmetric catalysis because they form stable complexes with catalytically interesting metals and their structure can be modulated with relative ease. Our aim is to functionalize DNA strands with phosphine units at specific positions, with the objective of applying them in homogeneous catalysis. A diphenylphosphine unit was previously introduced in our laboratory[10] into monomers and trimers via Pd-catalyzed phosphine introduction to commercially available 5-iodo-2'-deoxyuridine. This method worked well for monomers and trimers and was also tested for longer strands such as nonamers although the phosphine coupling reactions were very low yielding, requiring high Pd loadings resulting in phosphine oxidation during purification by FPLC (Fast Protein Liquid Chromatography). Therefore, it was decided to change the method of phosphine introduction into the DNA strands. Sulfhydryl compounds are often used in biomolecules as linkers. Thiols are better nucleophiles than amines because of their high energy nonbonding lone pairs. Therefore, thiol groups allow fast couplings and are often used in molecules such as proteins which contain several amine functionalities. Several reagents have been used and a small selection is depicted in Scheme 3.

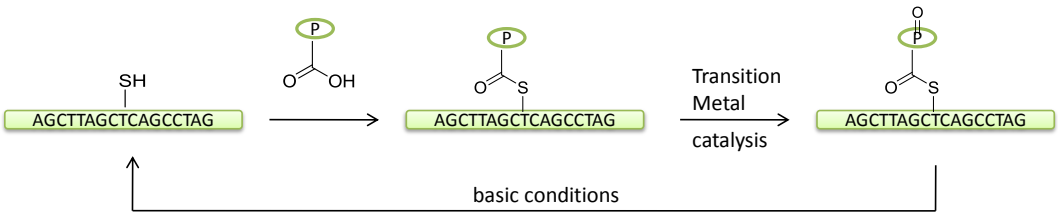


Scheme 3. Selected methods for coupling to a thiol functionality.[13, 14]

Thiol functionalities are often introduced in DNA strands for a variety of applications. The sulfhydryl functionality can be placed at different locations of the DNA strand. Most commonly, thiol aliphatic chains are placed at the 5' or 3' termini of DNA strands[15, 16] although they have been placed also at the 2' position of a nucleoside,[17] on the phosphate[18] and on purines and pyrimidines.[19-22] Thiol modified oligonucleotides have been grafted on gold nanoparticles,[23-26] glass[27] and polyethylene glycol surfaces.[28] They can potentially be used in clinical diagnosis for the specific recognition of certain analytes such as proteins and specific nucleic acid sequences. They have also been used to form cross linkages between two DNA strands to study their properties such as structure, folding and dynamics.[29] Thiol modifications have also been employed as a tool to form peptide-oligonucleotide conjugates;[15,30] and they have found applications in the attachment of oligonucleotides to resins for the use in affinity chromatography.[31,32] The two most common coupling methods used to modify oligonucleotides with thiol groups are via maleimides and disulfide bond formation.

Oligonucleotide modifications are essential for the introduction of phosphine moieties into a DNA strand. Thiols readily react with different functional groups and in some cases such as thioesters and disulfides the linkage formation is reversible. In our laboratory, phosphines containing a carboxylic acid functionality have been coupled to a protein via thioester bond formation and cleaved under basic conditions to reproduce the free thiol functionality.[14] This property could have high potential for the recycling of DNA ligands. Phosphine moieties are well known to be oxygen-sensitive

and once oxidized the DNA ligand looses its high affinity toward transition metals. We are aiming at the development of efficient DNA-based transition metal catalysts for selective conversions. We chose to couple phosphine moieties on thiol modified DNA strands as this is a well-developed selective and effective coupling reactions. Moreover, the expensive DNA ligand can potentially be recycled after its use by applying standard conditions for isolation and purification of synthetic DNA (Scheme 4). This chapter focuses on the synthesis of three thiol modified monomers and their introduction into DNA.



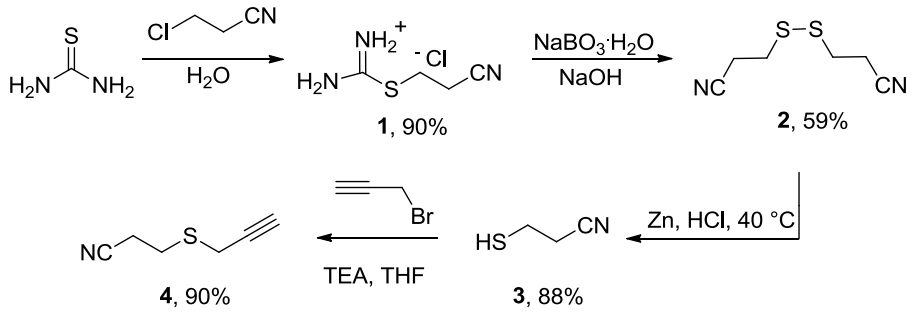
Scheme 4. Introduction of phosphine moieties (P) onto oligonucleotides and recycling of DNA strands.

2.2 Results and Discussion

2.2.1 Synthesis of cyanoethyl protected monomer

For the introduction of phosphine moieties into DNA a central modification in the oligonucleotide strand is preferred. By placing the catalytic site in the central position it could benefit from the chiral environment produced by the surrounding nucleotides possibly exerting high enantioselectivity in the catalytic reactions. The first step toward the synthesis of a thiol modified nucleoside was the preparation of a terminal alkyne bearing a thiol functionality which can be attached to 5-iodo-2-deoxyuridine (IdU) via a Sonogashira coupling. Protection of the thiol functionality is necessary to prevent side reactions such as formation of disulfides. A cyanoethyl group was initially chosen as a suitable protecting group because it is compatible with DNA synthesis and is easily removed during DNA cleavage from solid support. The propargyl linker was synthesized over four steps as depicted in Scheme 5. Commercially available thiourea and 2-chloropropionitrile reacted to afford compound **1**[33] in 90% yield, which was oxidized to compound **2** with $NaBO_3$ in 52% yield. The yield of this step was largely improved by ensuring a vigorous stirring of the reaction mixture. Reduction of the disulfide with Zn/HCl gave the free thiol[34] (**3**) in 88% yield. This thiol is a very good nucleophile and was easily reacted with

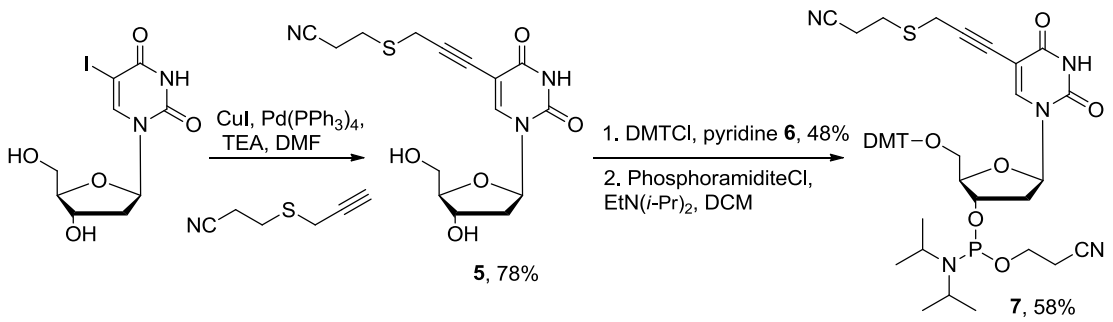
propargyl bromide under basic conditions resulting in the desired product (**4**) (Scheme 5).



Scheme 5. Synthesis of protected thiol linker.[33, 34]

Compound **4** was then coupled to 5-iodo-2-deoxyuridine by Sonagashira coupling resulting in 78% yield of the expected product **5** without any noticeable interference of the sulfur atom in this catalytic reaction. The next two steps to the target molecule were the standard protection of the primary hydroxyl group with 4,4'-Dimethoxytrityl chloride (DMT-Cl) giving compound **6** and phosphoramidite introduction to provide building block **7** (Scheme 6). The final product was found to be contaminated with 1 eq of hydrolyzed chlorophosphoramidite reagent, which resonated at 14 ppm in the $^{31}P\{^{1}H\}$ NMR spectrum. This compound is known not to interfere with DNA synthesis[35] and therefore compound 7 was used without any further purification.

A crystal structure was also obtained for compound 5 which proved that the desired compound had been made (Figure 1).



Scheme 6. Synthesis of thiol modified phosphoramidite.

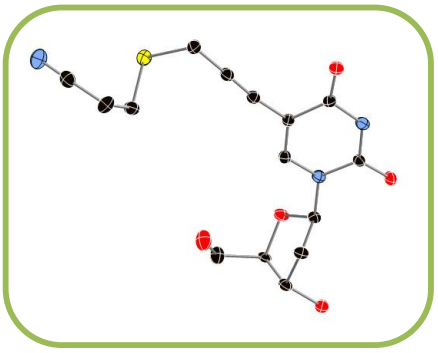
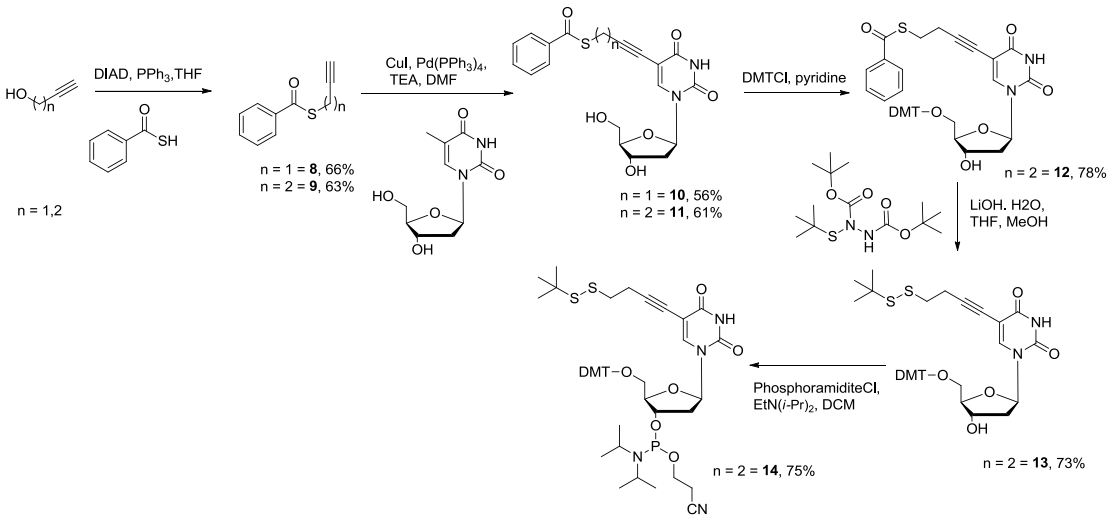


Figure 1. Ortep representation for **5**. Thermal ellipsoids are drawn at 50% probability.

2.2.2 Synthesis of tert-butyl protected monomer

Monomers with *tert*-butyl disulfide as protecting group were synthesized[22] in order to have a protecting group that would remain attached to the thiol functionality during cleavage from the solid support and purification of the oligonucleotide. C_4 and C_3 propargyl linkers were chosen to be coupled to 5-iodo-2-deoxyuridine. The thiol functionality is introduced via the use of thiobenzoic acid. This protecting group is compatible with Sonagashira coupling although it does not survive DNA synthesis conditions therefore later in the synthesis it will be exchanged with a disulfide tert butyl group. The linkers were synthesized from commercially available 3-butyn-1-ol or propargyl alcohol and thiobenzoic acid using Mitsunobu conditions giving 66 and 63% yield of the expected compounds **8** and **9** respectively. Although ^{1}H NMR showed higher conversion to the product, the lower yield is explained by an unidentified orange impurity that could not be efficiently separated from compound **9** because conventional column chromatography resulted in losses of product and vacuum distillation resulted in decomposition. In contrast, compound **8** could be purified by vacuum distillation although the yield was also only 66%. The remaining steps to reach the target compound are the same for both monomers and the resulting yields for each monomer are similar. Sonagashira coupling with 5-iodo-2-deoxyuridine afforded compound **11** in 61% yield. Protection of the primary alcohol with DMT-Cl resulted in compound **12** in 78% yield. Changing the thiol protecting group under basic conditions using di-*tert*-butyl(tert-buthylthio)-1,2-hyrozinedicarboxylate produced compound **13** in 73% yield. Phosphoramidite introduction afforded compound **14** (Scheme 7) along with hydrolyzed chlorophosphoramidite.



Scheme 7. Synthesis of thiol modified phosphoramidite containing a *tert*-butyl disulfide as protecting group.[22]

By crystallization from dichloromethane crystals suitable for X-ray diffraction were obtained for compound **11**. The structure confirms the formation of the desired compound and the expected absolute configuration of the stereogenic carbon atoms of the ribose ring (Figure 2).

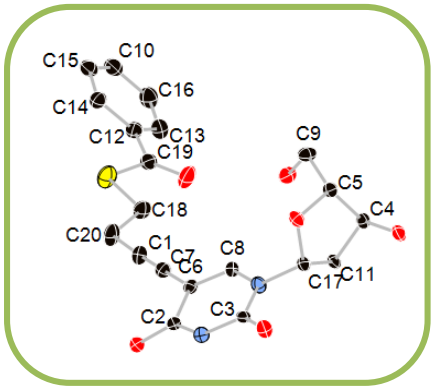


Figure 2. Ortep representation for **11**. Thermal ellipsoids are drawn at 50% probability.

2.2.3 Introduction of monomers into DNA strands

Modified base 7 was introduced into two DNA strands of 15 bases long (15-mers): 5'-CGCCTACMACCGAAT-3' and 5'-CTAGCTTMAAGCTAG-3'. A 15-mer is an adequate length to form double strands by strong enough Watson-Crick base pairing. In our experience longer strands might reduce the total yield of the synthesis to an unacceptably low level. Unexpectedly, purification of those two strands did not result in the desired product according to MALDI-TOF analysis and the chromatogram from

preparative HPLC showed several peaks suggesting that either the synthesis failed or DNA decomposition occurred before or during its purification. Thiols are known to dimerize in the presence of air. Therefore, during basic treatment and purification there is a risk that the DNA will form dimers or other side reactions could occur that might result in DNA degradation.

The difficulties encountered in obtaining a DNA 15-mer containing compound **7** in the central position brought us to change the protecting group used for the protection of the thiol functionality. As explained before the difference in the two protecting groups is that the disulfide *tert*-butyl group is much more robust and is expected to withstand the cleavage and purification steps. This protecting group will be eliminated shortly before coupling to the phosphine moiety. Compound **14** was introduced into a 15-mer using standard automated DNA synthesis and purified by HPLC. MALDI-TOF analysis confirmed the preparation of the desired product (Figure 3).

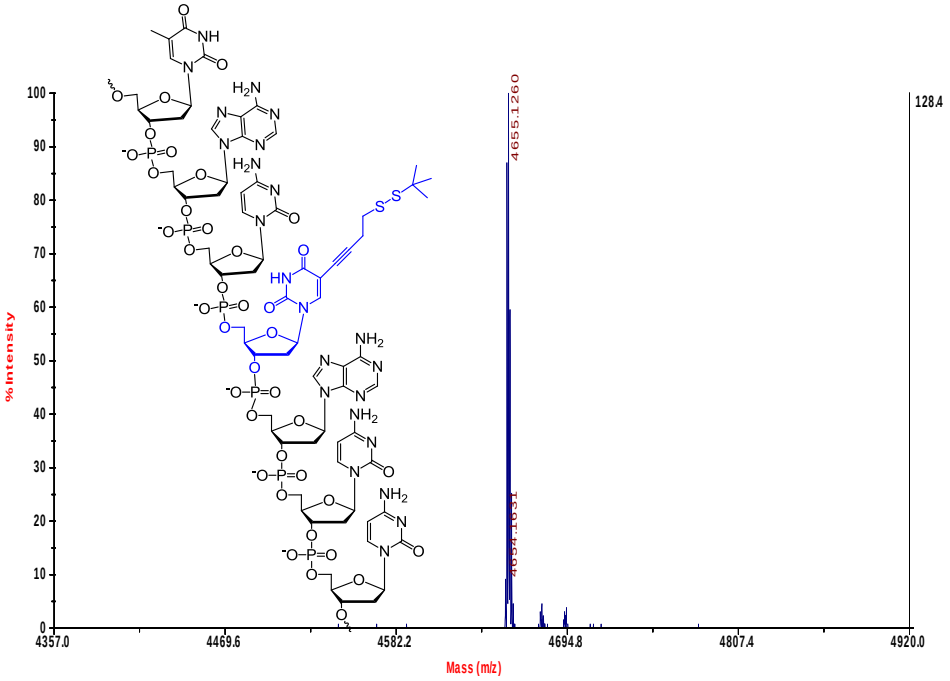
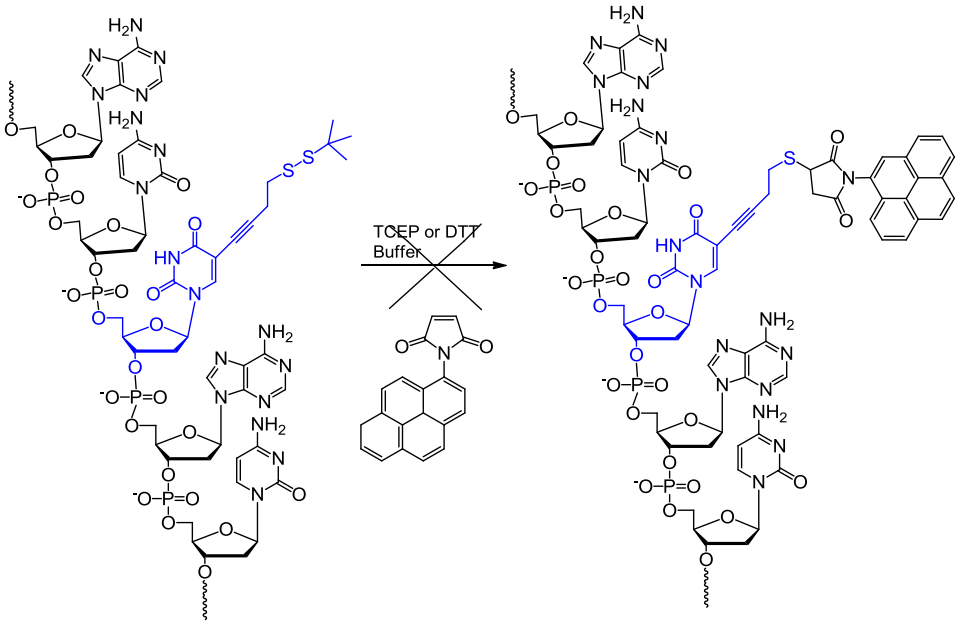


Figure 3. 5'-CGCCTACMACCGAAT-3', M = compound **14**, MW = 4657.20. The two adjacent peaks (4678 and 4695) correspond to desired molecular weight plus Na and K.

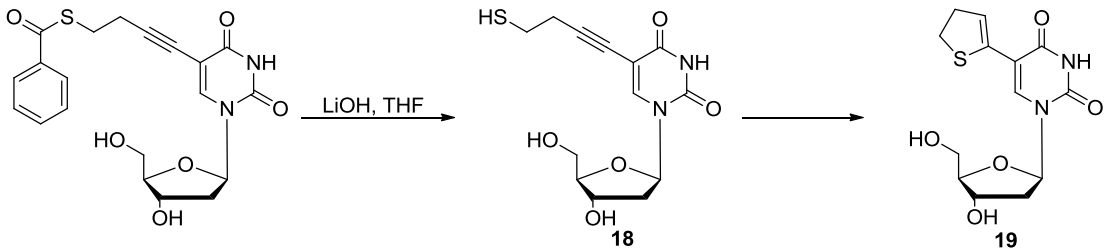
Once the desired DNA strand was obtained, deprotection of the thiol *tert*-butyl disulfide functionality was attempted with TCEP (*tris*(2-carboxyethyl)phosphine hydrochloride) or DTT (DL-Dithiothreitol). Deprotection was checked by a coupling of a maleimide containing a fluorescence probe. Maleimides are known to couple to

thiols efficiently[13] and therefore this maleimide was used to test the coupling efficiency by fluorescence (Scheme 8).



Scheme 8. Attempted coupling of a fluorescence probe to an oligonucleotide.

No fluorescence could be detected after coupling and MALDI-TOF analysis gave the mass which corresponds to the DNA 15-mer containing the deprotected free thiol. The same coupling was repeated using different buffers at different pH values but the same results were obtained. In order to understand the problems encountered with the 15-mer, the mononucleoside **11** was deprotected and purified by column chromatography. ^{1}H NMR and mass spectroscopy confirmed that the desired compound was obtained. Compound **18** was then tested for coupling to phosphine moieties unsuccessfully despite many attempts under different conditions. The cause of the failure of all those couplings was finally exposed by the X-ray crystal structure of compound **18** (Figure 4), which revealed cyclization of the thiol linker after deprotection which led to compound **19** (Scheme 9).



Scheme 9. Cyclization of compound **18**.

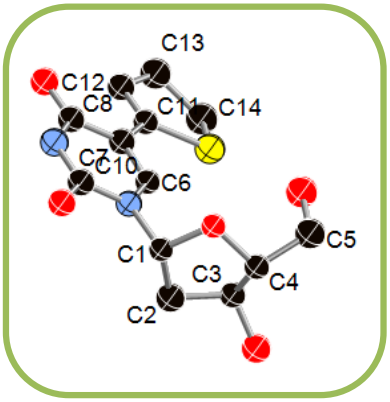


Figure 4. Ortep representation of **19**. Thermal ellipsoids are drawn at 50% probability.

It was concluded that a free thiol functionality is very reactive toward the triple bond of the linker giving a stable 2,3-dihydrothiophene ring. To prevent this cyclization, two approaches were considered: hydrogenation of the triple bond in compound **10** and the synthesis of a shorter linker, which is less likely to cyclise because of the formation of an unfavourable four-membered ring. Hydrogenation of the triple bond was tested with compound **6**. $NaBH_4/NiCl_2$, H_2/PtO_2 (5 and 20%), $H_2/Pd/C$, $H_2/[Rh(COD)(CNMe)_2]BF_4$ and $H_2/[RhCl(PPh_3)_3]$ were used under different conditions but in all cases the hydrogenated product was not formed. Hydrogenation was also tested on compound **11** giving the same results. It seems that the protected sulfur atom in the linker deactivates the hydrogenation catalysts. Instead of hydrogenating the triple bond prior to sulfur introduction it was decided to synthesize a monomer similar to compound **11** with one carbon less in the thiol linker to prevent cyclization. Compound **10** was synthesized and deprotected. The molecular weight of the desired product is 298 but mass spectroscopy analysis showed molecular weights in the range of dimeric structures.

2.3 Conclusion

This chapter reports the synthesis of thiol modified nucleosides with three different protecting groups and attempts to introduce them into DNA strands. A monomer with a cyanoethyl protecting group on the thiol linker was introduced into a 15-mer although it was not possible to confirm the identity of the desired strand during and after purification. One of the monomers was successfully introduced in a 15-mer DNA strand although studies with the monobase showed cyclization of the reactive

thiol functionality with the triple bond of the linker forming a stable 2,3-dihydrothiophene ring. To prevent this cyclization a similar monomer with a shorter carbon bridge was synthesized and deprotected, mass spectroscopy analysis suggests dimer formation. Another route to prevent the cyclization of the thiol linker is to hydrogenate the triple bond of the linker. This was attempted with the synthesized monomers although the thiol functionality deactivated the hydrogenation catalysts used. All the data obtained indicates that a triple bond and a free thiol functionality are not compatible in the systems studied here. Consequently, in future work monomers containing a fully hydrogenated thiol linker should be synthesized, a few examples of such monomers can be found in the work of Glick[36] and Eritja.[37]

The difficulties encountered during the synthesis of thiol modified DNA strands proved the task of introducing thiol functionalities into DNA to be more complex than expected and therefore, it was decided to change the strategy to amine modifications. Amine linkers have been widely applied in DNA chemistry by using terminal aliphatic linkers or amine modified monomers. The great advantage of using an amine group instead of a thiol functionality is that amines are less reactive and therefore are expected to give less unwanted reactions. On the other hand, the amide reaction is not reversible therefore this method will eliminate the possibility to recycle the DNA strand once used for catalytic reactions. Chapter 3 will focus on the synthesis of amine linkers into DNA strands.

2.4 Experimental

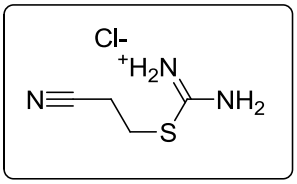
General procedures

All reactions were performed under argon using standard Schlenk techniques. Chemicals were purchased from Aldrich Chemical Company, Acros, Fluka, Link Technologies and Glen Research. CH_2Cl_2 and acetonitrile were distilled from CaH_2; ethanol and methanol were distilled from Mg/I_2; toluene was distilled from Na, THF and Et_2O were distilled from sodium /benzophenone and all the distillations were performed under nitrogen atmosphere. DMF was purchased “Extra dry” and stored over molecular sieves (4 Å) under argon. Aqueous solutions were degassed by three freeze/thaw cycles under vacuum and kept under argon. Thin Layer Chromatography (TLC) was performed on silica plates (Polygram 0.2 mm silica gel with fluorescent indicator UV_{254}). Ninhydrin spray was used for the detection of primary and secondary amines on TLC silica plates. Silica gel 60 particle size 0.063-0.2 mm from Fluka was used for flash chromatography. NMR spectra were recorded at room temperature on Bruker Avance spectrometers (300, 400, and 500 MHz). Positive chemical shifts (δ) are given (in ppm) for high-frequency shifts relative to a TMS reference (^1H and ^{13}C) or an 85% H_3PO_4 reference (^{31}P). ^{13}C, and ^{31}P spectra were measured with ^1H decoupling. The following abbreviations have been used in the description of the spectra: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were recorded on a Perkin-Elmer Spectrum GX spectrometer in KBr pellets, with a window between 4000 and 400 cm^{-1}. Mass spectrometry: MALDI-TOF mass spectra were recorded on a 4800 Plus MALDI TOF/TOF™

Analyzer using a 9:1 3-hydroxypicolinic acid (HPA): ammonium citrate matrix. The matrix is made with 50 mg/ml of HPA in 50:50 MeCN:H_2O and 50 mg/ml of ammonium citrate in H_2O. The desalting column used for the preparation of MALDI-TOF samples was a GE Healthcare Illustra® micron G-25 and the desalting tips were Millipore® zip-tip C18, the Dowex used for desalting was a 50WX4 200-400 mash resin. Oligonucleotides prepared at our laboratories were synthesized by an Applied Biosystems 392 DNA/RNA synthesizer using protocols obtained from Applied Biosystems. The reagents used for the synthesis of oligonucleotides were bought from Glen research and were: oxidizing solution 0.10 M I_2 in THF/pyridine/water; cap mix B (10% 1-methylimidazole in THF/pyridine); cap mix A (THF/pyridine/acetic anhydride); deblocking mix (3% trichloro acetic acid in DCM); activator (sublimed 1H-tetrazole in anhydrous acetonitrile); dA, dC, dG, dT phosphoramidites. Coupling yields for DNA synthesis are based on measurements of DMT release which is determined by conductivity measurements. Purification by ion exchange, size exclusion and desalting were carried out with a FPLC AktaBasic 100 (P-901), monitor UV-900. The column used for purification of the modified oligonucleotides was a HiLoad 16/10 Q Sepharose HP with the following buffers: buffer A = 1 M NaCl, 10 mM NaOH and buffer B = 10 mM NaOH. The column for the desalting was a HiPrep 26/10 Desalting with a linear gradient of 0.15 M NH_4HCO_3 buffer. HPLC purification of oligonucleotides was carried out in a Waters machine equipped of a Waters 2700 sample manager, Waters 600 controller and Waters 2487 dual absorption detector. The reverse phase column used was a Phenomenex, Clarity® C18 5 μ oligo-RP, 250 x 21.20 mm. Syringe filter used for DNA were WHATMAN 0.2 μm PTFE.

(2-Cyanoethyl)thiouronium hydrochloride (1)[33]

Compound **1** was synthesized following literature procedure. The desired product resulted in a white solid. Yield: 10.8 g, 90%



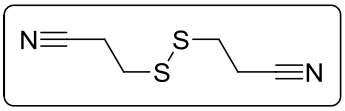
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.92 (t, 2H, J = 6.7 Hz), 5.47 (t, 2H, J = 6.7 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.2, 121.3, 28.7, 20.5

Di(2-cyanoethyl) disulfide (2)[34]

Compound **2** was synthesized following literature procedure. The remaining solid was crystallized from methanol to give a white solid. Yield: 13.7 g, 59%



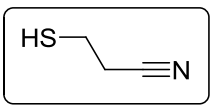
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.88 (t, 4H, 7.1 Hz), 2.75 (t, 4H, 7.1 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 116.8, 32.4, 16.9

3-Mercaptopropionitrile (3)[34]

Compound **3** was synthesized following literature procedure resulting in a colourless liquid. Yield: 3.5 g, 88%

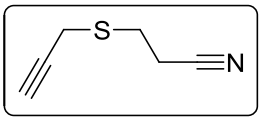


Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 Mz, $CDCl_3$): δ = 2.81-2.72 (m, 2H), 2.71-2.68 (m, 2H), 1.81 (t, 1H, J = 8.5 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 118.0, 20.6, 20.8

3-(2-Propynylthio)propanenitrile (4)



3-Mercaptopropionitrile (0.84 ml, 11 mmol, 1 eq) was dissolved in 15 ml of THF and triethylamine (3.2 ml, 23 mmol, 2 eq) was added to it. After stirring for 5 minutes propargyl bromide (2.5 ml, 23 mmol, 2 eq) was slowly added at 0 ˚C to the reaction mixture and a white precipitate was formed. After stirring for 1 h at room temperature the mixture was filtered and the filtrate concentrated *in vacuo*. The resulting slightly yellow oil was purified by column chromatography (SiO_2, ethyl acetate:petroleum ether, 30:70) resulting in a clear oil. Yield: 1.3 g, 90%

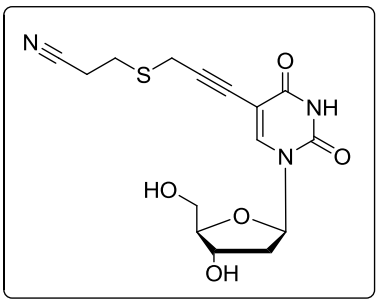
^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.28 (d, 2H, J = 2.6 Hz), 2.92 (t, 2H, J = 7.2 Hz), 2.67 (t, 2H, J = 7.2 Hz), 2.26 (t, 1H, J = 2.6 Hz)

^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 118.2, 78.7, 72.2, 26.9, 19.3, 18.5

IR (KBr), $\bar{\nu}$(cm^{-1}): 3584, 3288, 2963, 2250

MS (ES+): 163.93 (M+Na$^+$+O), 125.03 (M), HRMS calculated for C_6H_7NONaS; 164.0146, found 164.0144

5-(1-propynyl-3-thiopropanenitrile)-2'-deoxyuridine (5)



5-Iodo-2-deoxyuridine (3.6 g, 10.2 mmol, 1 eq) was dissolved in 60 ml of DMF and the flask was covered with aluminium foil. CuI (0.38 g, 2.04 mmol, 0.2 eq) was added to the reaction and it was stirred for 30 minutes. TEA (2.8 ml, 20.4 mmol, 2 eq), 3-(prop-2-ynylthio)propanenitrile (3.2 g, 25.6 mmol, 2.5 eq) and [Pd(PPh_3)$_4$] (1.18 g, 1.02 mmol, 0.1 eq) were added to the mixture and it was stirred for 16 h in the dark. The mixture was concentrated *in vacuo* at 60 ˚C resulting in a dark orange oil. The oil was purified by column chromatography (SiO_2, ethyl acetate:methanol (0-3%)) resulting in a yellow solid. The solid was heated in DCM at 50 ˚C for 10 minutes and filtered to obtain a slightly orange coloured solid. Yield: 2.8 g, 78%

^{1}H NMR (400 MHz, MeOD): δ = 8.21 (s, 1H), 6.12 (t, 1H, J = 6.6 Hz), 4.31-4.28 (m, 1H), 3.84-3.80 (m, 1H), 3.68 (ddd, 2H, J = 3.1 Hz, J = 12.2 Hz, J = 20.2 Hz), 3.51 (s, 2H), 2.91 (t, 2H, J = 6.9 Hz), 2.78 (t, 2H, J = 6.9 Hz), 2.14-1.94 (m, 2H)

^{13}C NMR (100.6 MHz, MeOD): δ = 164.5, 151.2, 145.1, 120.1, 100.2, 90.1, 89.2, 87.0, 75.9, 71.9, 62.6, 41.7, 28.2, 20.6, 19.0

Elemental analysis calculated for $C_{15}H_{17}N_3O_5S$: C 51.27, H 4.88, N 11.96:

Found: C 51.10, H 4.57, N 11.74

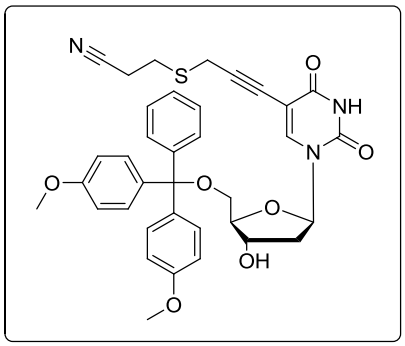
IR (KBr), $\bar{\nu}$(cm^{-1}): 3528, 3414, 3192, 2938, 2251, 1728, 1676, 1626

MS (ES+): 374.00 (M+Na$^+$), HRMS calculated for $C_{15}H_{17}N_3O_5NaS$, 374.0787; found 374.0801

Mp: 115-117 ˚C

X-ray diffraction Crystals suitable for X-ray diffraction were grown by evaporation of dichloromethane solution at room temperature.

5'-*O*-Dimethoxytrityl-5-(1-propynyl-3-thiopropanenitrile)-2'-deoxyuridine (6)



Compound **5** (2.0 g, 5.7 mmol, 1 eq) was coevaporated 3 times with dry pyridine and then dissolved in 20 ml of pyridine. 4,4'-Dimethoxytrityl chloride (2.7 g, 8 mmol, 1.4 eq) was dissolved in 20 ml of pyridine was slowly added to the former solution. After 4 h 15 ml of methanol was added to the reaction mixture and it was evaporated *in vacuo*. The resulting red oil was purified by column chromatography (SiO_2, dichloromethane:methanol 98:2) resulting in a yellow powder. Yield: 3.30 g, 48%

^{1}H NMR (400 MHz, MeOD): δ = 8.04 (s, 1H), 7.37-7.35 (m, 2H, Ar), 7.27-7.22 (m, 4H, Ar), 7.19-7.15 (m, 1H, Ar), 7.77-7.69 (m, 4H, Ar), 6.24 (t, 1H, J =6.8 Hz), 4.48-4.45 (m, 1H), 4.00-3.98 (m, 1H), 3.70 (s, 6H), 3.31 (ddd, 2H, J = 2.9 Hz, J = 10.8 Hz, J = 46.4 Hz), 3.14 (d, 2H, J = 2.4 Hz), 2.62 (dt, 2H, J = 2.8 Hz, J = 7.2 Hz) 2.45-2.40 (m, 1H) 2.38 (t, 2H, J = 6.8 Hz), 2.27-2.20 (m, 1H)

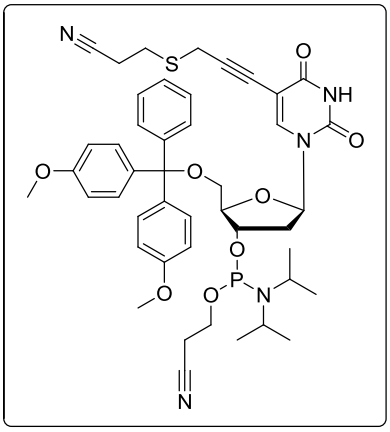
^{13}C NMR (100.6 MHz, MeOD): δ = 161.3, 158.7, 149.0, 144.6, 142.4, 135.5, 135.4, 130.0, 128.1, 127.9, 127.1, 118.5, 113.4, 99.9, 89.4, 87.0, 86.4, 85.6, 74.5, 72.1, 63.6, 55.3, 41.6, 27.0, 20.1, 18.3

MS (ES+): 675.78 ($M+Na^+$), HRMS calculated for $C_{36}H_{35}N_3O_7NaS$, 676.2093; found 676.2107

IR (KBr), $\bar{\nu}$(cm^{-1}): 3447, 3065, 2930, 1701, 1507, 1459, 1282, 1250

Mp: 83-90 ˚C

5'-*O*-Dimethoxytrityl-3'-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-5-(1-propynyl-3-thiopropanenitrile)-2'-deoxyuridine (7)



Compound **6** (1.3 g, 2 mmol, 1 eq) was dissolved in 25 ml of DCM and diisopropylethylamine (0.95 ml, 6 mmol, 3 eq) was added. The mixture was cooled to 70 ˚C and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.67 ml, 3 mmol, 1.5 eq) dissolved in 20 ml of DCM was slowly added to the reaction mixture. After 3 h of stirring at room temperature the mixture was diluted with 40 ml of dichloromethane and washed with 5% aqueous solution of $NaHCO_3$ (30 ml) and brine (30 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The compound was purified using a chromatotron (SiO_2, dichloromethane:methanol:TEA, 97:2:1) resulting in an oil. Yield : 1.0 g, 58%

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.09, 8.05 (2s, 1H), 7.39-7.36 (m, 2H), 7.30-7.14 (m, 7H), 7.80-7.76 (m, 4H), 6.26-6.20 (m, 1H), 7.57-4.51 (m, 1H), 4.13-4.07 (m, 1H), 3.80-3.35 (m, 11H), 3.24-3.17 (m, 1H), 3.11-3.08 (m, 2H), 2.63-2.48 (m, 4H), 2.40-2.20 (m, 4H), 1.11-1.08 (m, 9H), 1.00-0.95 (d, 3H, J = 7.2 Hz)

^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.8, 158.7, 149.3, 144.6, 142.4, 135.5, 130.1, 130.0, 128.1, 128.0, 127.9, 127.0, 113.4, 99.8, 86.9, 85.7, 85.6, 63.1, 63.0, 58.4, 58.3, 58.2, 58.1, 55.3, 43.4, 43.3, 43.2, 43.1, 40.7, 26.9, 24.6, 24.5, 20.2, 20.0, 18.3

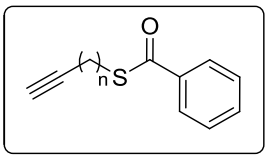
^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 149.2, 148. 8

MS (ES+): 875.79 ($M+Na^+$), HRMS calculated for $C_{45}H_{52}N_5O_8NaPS$, 876.3172; found 876.3145

IR (KBr), $\bar{\nu}$(cm^{-1}): 3199, 3061, 2967, 2932, 2837, 2360, 2251, 1695, 1608, 1509, 1561, 1281, 1180

Mp: 66-70 ˚C

2-propynyl benzothioate (8)[22] n = 1



Compound **8** was synthesized according to literature procedure and resulted in a yellow oil. Yield: 8.37 g, 66%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96-7.94 (m, 2H, Ar), 7.61-7.57 (m, 1H, Ar), 7.48-7.44 (m, 2H, Ar), 3.83 (d, 2H, J = 2.7 Hz), 2.23 (t, 1H, J = 2.7 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 190.1, 136.3, 133.8, 128.8, 127.4, 71.1, 17.5

3-butynyl benzothioate (9)[22] n = 2

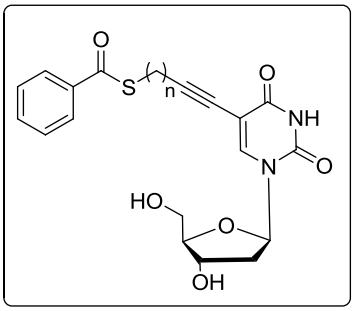
The same method as for **8** was employed using 3-butyn-1-ol. Yield: 3.40 g, 63%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (m, 2H, Ar), 7.50 (m, 1H), 7.37 (m, 2H, Ar), 3.17 (t, 2H, J = 7.1 Hz), 2.50 (dt, 2H, J = 2.7 Hz, J = 7.1 Hz), 1.98 (t, 1H, J = 2.7 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 191.4, 136.8, 135.5, 128.7, 127.3, 82.2, 69.7, 28.0, 19.6

5-(4-Benzoylthio-1- propynyl)-2'-deoxyuridine (10)[22] n = 1



Compound **10** was synthesized according to literature procedure resulting in a light yellow solid. The solid was precipitated in dichloromethane, filtered and concentrated *in vacuo*.Yield: 1.65 g, 56%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, MeOD): δ = 8.18 (s, 1H), 7.87-7.84 (m, 2H), 7.57-7.53 (m, 1H), 7.44-7.40 (m, 2H), 6.13 (t, 1H, J = 6.7 Hz), 4.30-4.27 (m, 1H), 3.99 (s, 2H), 3.83-3.80 (m, 1H), 3.67 (dd, 1H, J = 3.1 Hz, J = 12.1 Hz), 2.23-2.08 (m, 2H)

^{13}C NMR (101 MHz, MeOD): δ = 191.6, 164.4, 151.4, 145.5, 137.8, 135.0, 130.0, 128.2, 100.0, 89.4, 89.1, 87.0, 75.1, 72.0, 62.6, 41.7, 19.1

5-(4-Benzoylthio-1-butynyl)-2'-deoxyuridine (11)[22] n = 2

The same method as for **10** was employed. Yield: 1.6 g, 61%

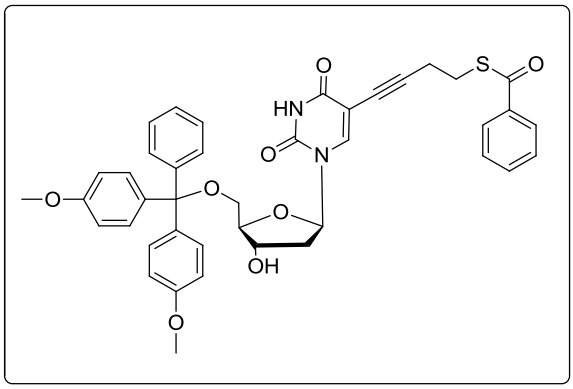
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, acetone-d_6): δ = 8.14 (s, 1H), 7.86-7.84 (m, 2H, Ar), 7.57-7.53 (m, 1H), 7.45-7.41 (m, 2H, Ar), 6.25 (t, 1H, J = 6.8 Hz), 4.40-4.37 (m, 1H), 3.86-3.83 (m, 1H), 3.72-3.64 (m, 2H), 3.14 (t, 2H, J = 7.3 Hz), 2.61 (t, 2H, J = 7.3 Hz), 2.18-2.14 (m, 2H)

^{13}C NMR (100.6 Hz, acetone-d_6): δ = 191.7, 162.1, 150.4, 144.2, 137.8, 134.6, 129.8, 127.9, 100.1, 91.5, 88.9, 86.2, 74.7, 72.0, 62.6, 41.6, 28.7, 21.1

5'-*O*-dimethoxytrityl-5-(4-Benzoylthio-1-butynyl)-2'-deoxyuridine (12)[22]

Compound **12** was synthesized according to literature procedure and resulted as a pale yellow solid. Yield: 1.26 g, 78%



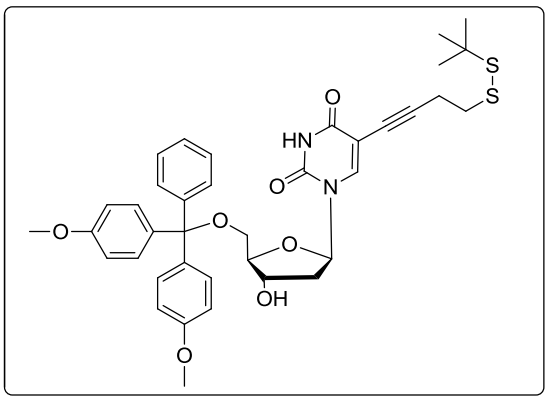
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (s, 1H), 7.83-7.81 (m, 2H, Ar), 7.49-7.45 (m, 1H, Ar), 7.36-7.18 (m, 10H, Ar), 7.14-7.11 (m, 1H, Ar), 6.76 (d, 4H, Ar, J = 8.8 Hz), 6.25 (t, 1H, J = 6.6 Hz), 4.47 (m, 1H), 4.03 (m, 1H), 3.68 (s, 6H) 3.27 (dt, 2H, J = 2.9 Hz, J = 10.8 Hz, 22.3 Hz), 2.75(t, 2H, J = 7.4 Hz), 2.46-2.34 (m, 3H), 2.25-2.18 (m, 1H)

^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.3, 160.7, 157.6, 148.3, 143.5, 141.2, 135.8, 134.5, 132.4, 128.9, 127.5, 127.0, 126.9, 126.2, 125.9, 112.3, 99.6, 91.7, 86.0, 85.6, 84.6, 71.3, 70.9, 62.5, 54.2, 40.5, 29.9, 26.5, 19.6

5'-*O*-dimethoxytrityl-5-(1-butynyl)-2'-deoxyuridine-*tert*-butyl disulfide (13)[22]

Compound **13** was synthesized according to literature procedure. The desired product was obtained as a yellow solid. Yield: 0.72 g, 73%

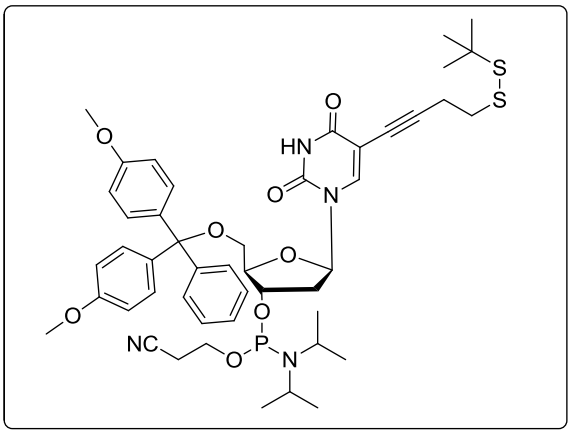


Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.63 (bs, 1H), 8.00 (s, 1H), 7.37-7.13 (m, 9H), 6.79 (d, 4H, J = 8.8 Hz), 6.26 (t, 1H, J = 6.6 Hz), 4.48-4.45 (m, 1H), 4.01-3.96 (m, 1H), 3.72 (s, 6H), 3.30 (ddd, 2H, J = 4.3 Hz, J = 8.7 Hz, J = 25.8 Hz), 2.45-2.17 (m, 6H), 1.98 (bd, 1H, J = 3.7 Hz) 1.18 (s, 9H)

^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.4, 158.7, 149.0, 144.5, 142.1, 135.5, 130.0, 128.1, 127.9, 127.0, 133.4, 100.6, 92.9, 87.1, 86.4, 85.5, 72.4, 71.6, 63.4, 55.3, 41.5, 37.8, 29.8, 20.4

5'-*O*-dimethoxytrityl-3'-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-5-(1-butynyl)-2'-deoxyuridine-*tert*-butyl disulfide (14)[22]



Compound **14** was synthesized according to literature procedure resulting in a yellow solid. Desired product contains hydrolyzed starting material. Yield: 0.56 g, 75%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, C_6D_6): δ = 8.13, 8.11 (2s, 1H), 7.69 (d, 2H, J = 8.0 Hz), 7.56 (d, 4H, J = 8.6 Hz), 7.33 (t, 2H, J = 7.7 Hz), 7.20-7.15 (m, 1H), 6.92 (d, 4H, J = 8.9 Hz), 6.41 (t, 1H, J = 6.4 Hz), 4.73-4.63 (m, 1H), 4.30, 4.24 (2m, 1H), 3.61-3.07 (m, 13H), 2.56-2.35 (m, 3H), 2.29-2.11 (m, 1H), 1.81-1.68 (m, 3H), 1.20, 1.19 (2s, 9H), 1.16-1.04 (m, 12H)

^{31}P NMR (121.5 Hz, C_6D_6): δ = 149.0, 148.2

2.5 References

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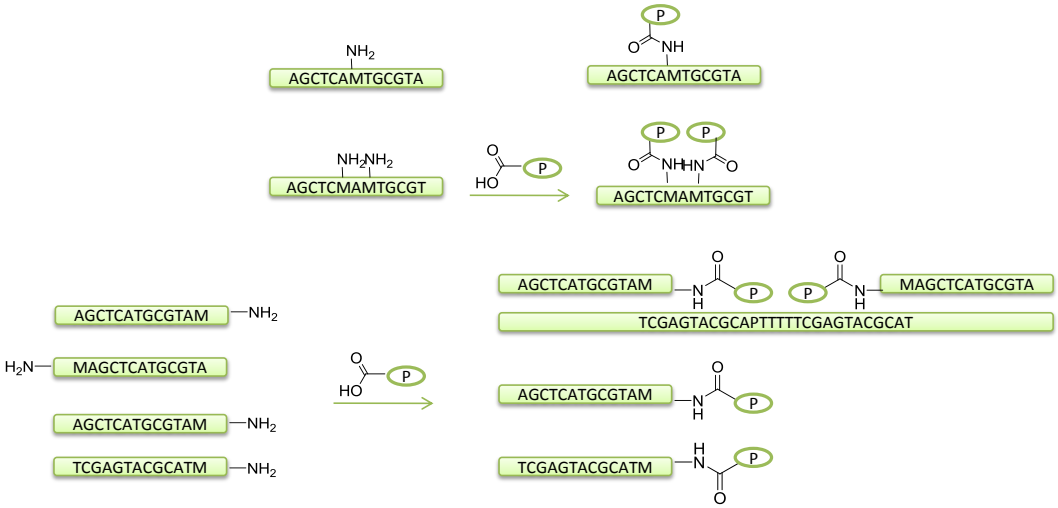
Chapter 3: Amine Modified Oligonucleotides

3.1 Introduction

As previously explained in chapter 2 our aim is to functionalize DNA strands with phosphine units at specific locations, with the objective of applying them in asymmetric homogeneous catalysis. After the failed attempts to introduce a thiol functionality in an oligonucleotide the coupling method was changed to amide bond formation. This method is compatible with water and results in high conversion. This method was also successfully used by Jäschke[1] and other groups[2-4] to introduce phosphine moieties into oligonucleotides. This chapter focuses on the introduction of amine linkers into nucleosides and nucleotides, the subsequent coupling of phosphine moieties on amino groups via amide bond formation and the use of new chiral phosphine modified nucleosides in asymmetric catalytic reactions. Several amine linkers of different length can be introduced at different locations of the DNA. Our focus is on the introduction of aliphatic linkers at the terminal and central positions of oligonucleotides (Figure 1).



M= modified monomer, P= phosphine moiety, A=adenine, G= guanine, T= thymidine, C= cytosine

Figure 1. Introduction of phosphine moieties into DNA.

Figure 2 shows the aliphatic linkers that have been synthesized to introduce in DNA strands.

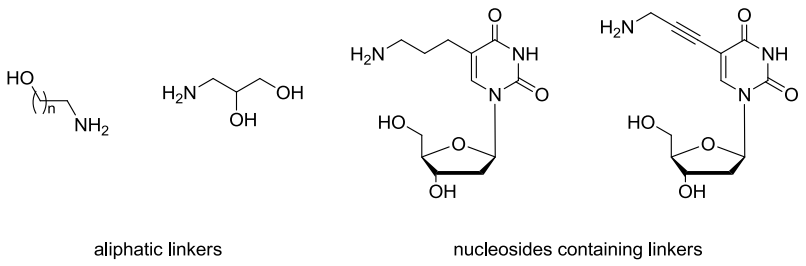


Figure 2. Amino linkers and modified nucleosides.

3.2 Results and Discussion

3.2.1 Aliphatic linker modifiers

Aliphatic amine linkers can be synthesized in less than three steps and are compatible with standard DNA synthesis. Four linkers have been synthesized for the introduction into DNA strands. To introduce a linker into DNA it is necessary to have a phosphoramidite unit and the amino and hydroxyl functionalities have to be protected to prevent unwanted side reactions during DNA synthesis. C_2, C_3 and C_5 linkers were synthesized (**1**, **2**, **3** Figure 3) for the 5' terminal position and a chiral amino diol linker (**4**) was synthesized as a nucleotide replacement for modification at a central position (Figure 3).

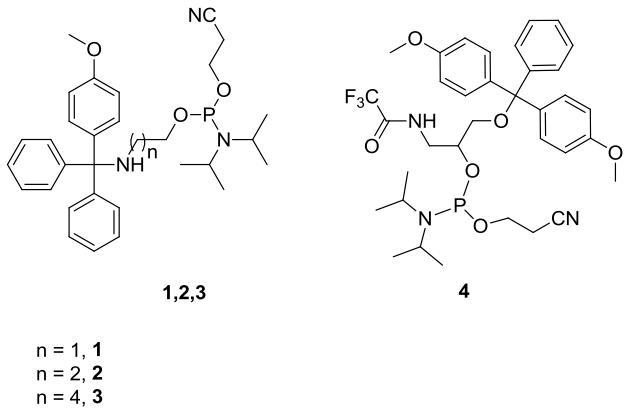
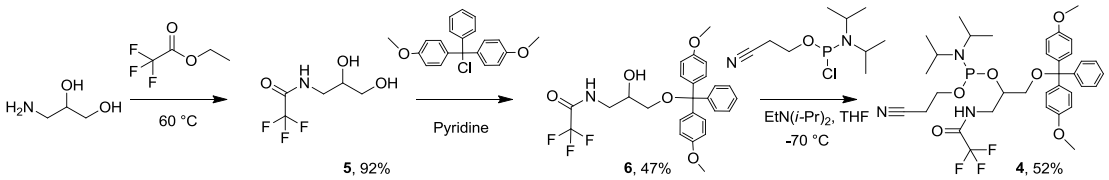


Figure 3. Protected aliphatic amine linkers.[5, 6]

3.2.1.1 Central linker

The central aliphatic modifier[6] was initially preferred over a modified nucleoside because of its easy and fast synthesis and because the amine functionality is embedded in the oligonucleotide therefore placing the phosphine moiety closer to the

chiral centres. The central linker was synthesized from the commercially available (*R*)-3-amino-1,2-propandiol. TFA (trifluoroacetic acid) was chosen as a more suitable protecting group (**5**, Scheme 1). It is a stable group during the linker synthesis and can withstand the conditions of DNA synthesis. Protection of the amino group with a trifluoro acetyl group was accomplished with neat trifluoroacetic acid ethylester at 60 ˚C, which gave high yields (92 %) and easy work up. DMT protection of the primary hydroxyl group gave relatively low yields (47%) (**6**). Phosphoramidite introduction to the secondary hydroxyl group was initially carried out with pyridine as the base needed to quench the equivalent of HCl produced. However, the pyridinium salt formed during the reaction is too acidic and cleaves off the diisopropylamino fragment of the phosphoramidite. When the base was changed to diisopropylethylamine (Hünig's base) the desired product (**4**) was obtained as confirmed by NMR analysis (^{1}H-^{13}C HMBC experiments) (Scheme 1).[6]

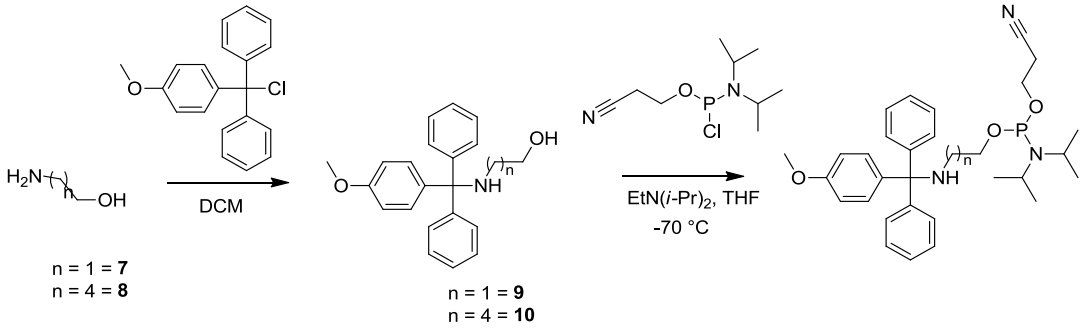


Scheme 1. Synthesis of central protected linker (**6**).[6]

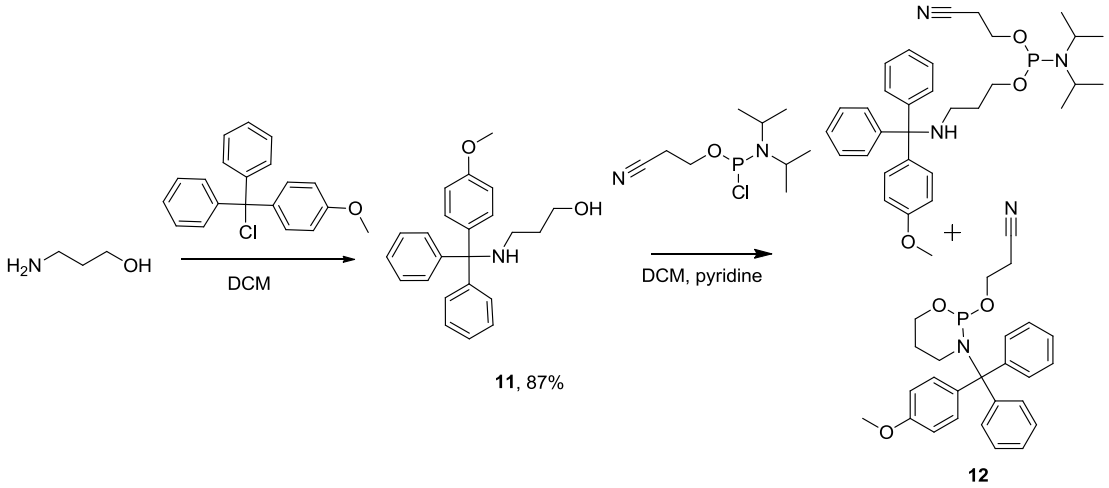
3.2.1.2 5' Terminal linkers

The C_2 (**1**) and C_3 (**2**) 5' terminal modifiers were also first protected with Fmoc (9-Fluorenylmethyloxycarbonyl)[7] and then the protecting group was changed to TFA for **1** and finally to MMT-Cl (monomethoxytrityl chloride).[5] An advantage of the latter is that during DNA synthesis the coupling efficiency can be calculated spectrophotometrically by measuring the trityl release. For the same reason DMT is used as the protecting group for the secondary hydroxyl group in the central modifier. The synthesis of amine linkers **1**, **2** and **3** is depicted in Scheme 2 and 3.[5] Surprisingly, the phosphoramidite coupling reaction with 3-aminopropanol resulted in two compounds. Analysis performed by mono and multinuclear two-dimensional NMR analysis (^{1}H-^{1}H, ^{1}H-^{13}C, ^{1}H-^{31}P correlations) showed that the isopropylamino group of the phosphoramidite is substituted by the amino group of the 3-aminopropanol resulting in a more stable cyclic product (compound **12**) (Scheme 3). This cyclization can also be caused by the use of pyridine during the reaction. It is

possible that the pyridinium salt formed cleaves the isopropylamino group facilitating the attack by the terminal amine as also seen for compound **4**. Therefore, a two carbon bridge (ethanolamine) and a five carbon bridge (pentanolamine) were used for the synthesis of the terminal modifier assuming that this would reduce the chance of cyclisation.



Scheme 2. Synthesis of C_2 and C_5 linkers.[5]



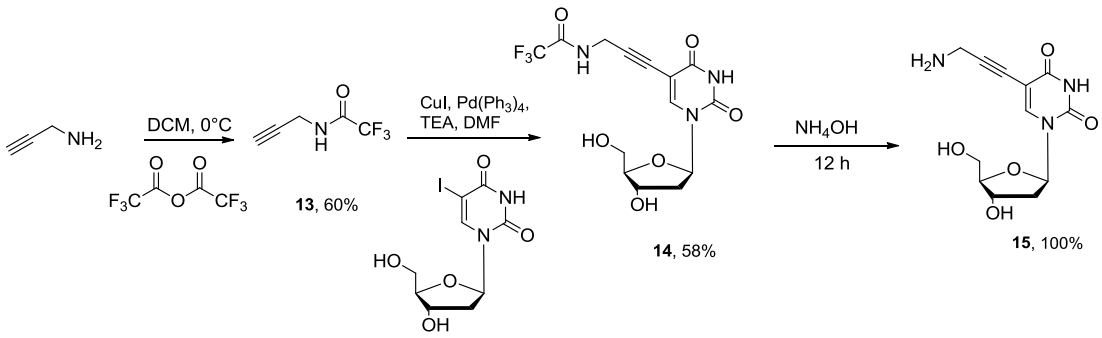
Scheme 3. Synthesis of C_3 amine linker resulting in desired product and cyclized compound.

3.2.2 Synthesis of monomers

As explained later in this chapter the central aliphatic linker resulted in DNA degradation and therefore, a nucleoside was modified with an amine functionality. Several of such modified nucleotides have been described in the literature, in particular, several groups have reported the synthesis of thymidine containing a propargylamino group.[8-14] This base modification was chosen because of its straightforward synthesis. The amine functionality was introduced by a propargylamine precursor (**13**). Linkers with longer bridges can be used for this purpose,[15, 16] although this specific linker was considered ideal for two main reasons.

Firstly, it can be synthesized in one step from the commercially available propargylamine and trifluoroacetic anhydride (Scheme 4), and secondly, linkers with longer chains would place the phosphine moiety further away from the DNA probably reducing the chance of efficient chiral induction. Compound **13** was first synthesized using ethyl trifluoroacetate in methanol resulting in a low yield of crude product (42%). Trifluoroacetic anhydride gave better conversion with a 60% yield of pure product after vacuum distillation.[17]

Pd-catalyzed Sonogashira coupling of **13** to 5'-iodo-2-9deoxyuridine (IdU) affords the modified nucleobase **14** (58% yield).[12] During this reaction triethylamine (TEA) neutralizes the hydrogen iodide formed giving the $Et_3N{\cdot}HI$. This salt co-elutes along with the desired product during purification with column chromatography. Although, it could be easily eliminated by stirring the purified product with K_2CO_3 in tetrahydrofuran to neutralize the ammonium salt back to triethylamine. Higher yields were obtained by lowering the quantities of the Pd catalyst and CuI.



Scheme 4. Synthesis of propargyl amine modified thymidine.[12]

Modified thymidine can be deprotected by using concentrated aqueous ammonia. The deprotected base contains trifluoroacetate salt according to ^{19}F NMR. This salt could be removed in the case of compound **15** by column chromatography yielding crystals of the pure product, whose X-ray structure is depicted in Figure 4. The crystal structure shows that the amine functionality points away from the chiral centre and that the absolute configuration is retained. The purification of compound **15** however was not routinely carried out because it was found that the presence of TFA salt did not affect the coupling with phosphinocarboxylic acids.

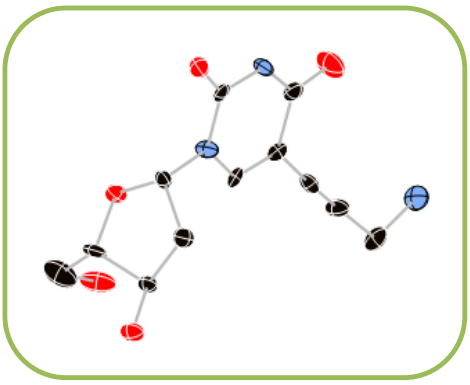
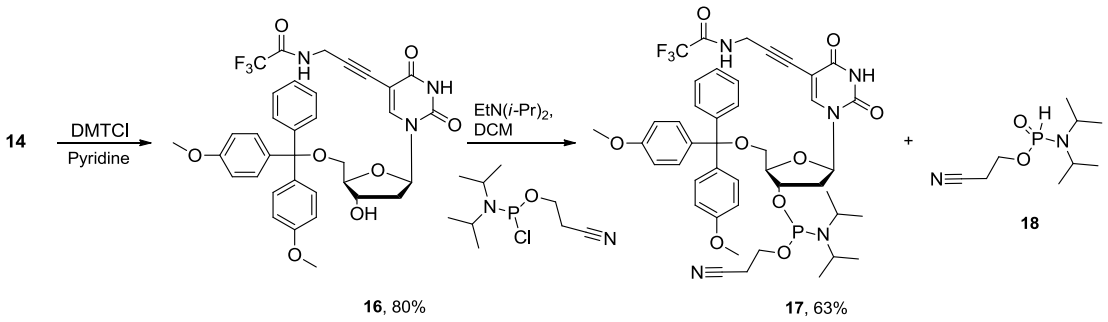


Figure 4. Ortep representation for **15**. Thermal ellipsoids drawn at 50% probability.

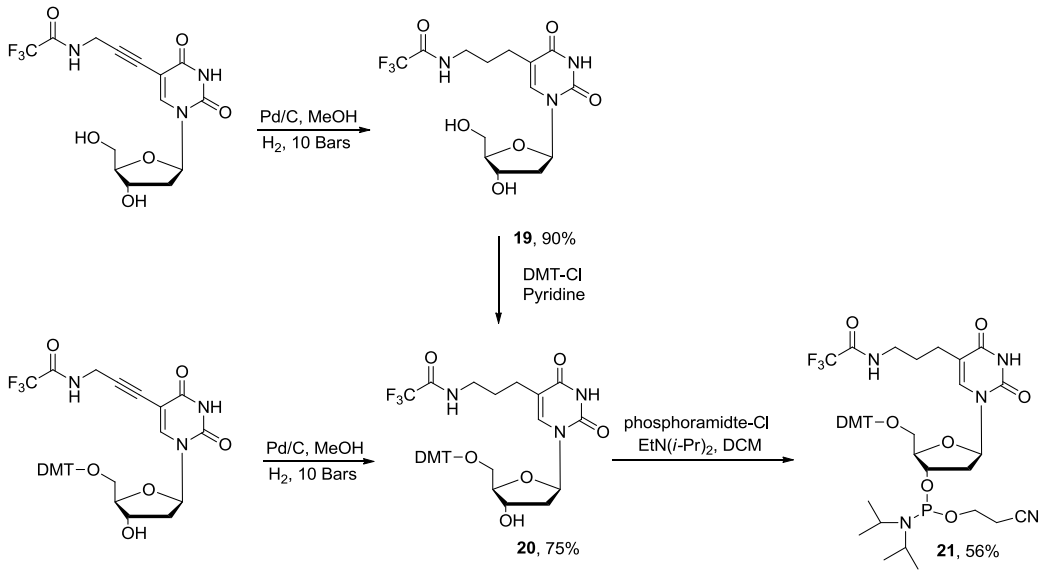
For introduction of the monobase into the DNA it is necessary that the amine function remains protected. Continuing from compound **14** the next two steps are protection of the primary hydroxyl group with 4,4'-dimethoxytrityl chloride (DMT-Cl) to give compound **16** (80% yield)[12] and phosphoramidite introduction to yield compound **17**,[12] which was contaminated with 40% of hydrolyzed phosphoramidite reagent (**18**) after purification by column chromatography (Scheme 5). This contamination was also reported in literature.[12]



Scheme 5. Synthesis of amine modified thymidine phosphoramidite.[12]

The triple bond of monobase **14** was hydrogenated to increase the flexibility and reactivity of the amino group (compound **19**). Both $NaBH_4/NiCl_2$ and 5% PtO_2[8] gave mixtures of saturated and unsaturated products along with starting material. 20% PtO_2 and 10% Pd/C instead both afforded the hydrogenated product with less than 4% impurities. As Pd/C is considerably less expensive than PtO_2, the former was chosen as the preferred catalyst. Filtration of the reaction mixture resulted in the pure desired product without any further purification. Protection of the primary hydroxyl group

with DMT-Cl gave the tritylated nucleoside (**20**) in 75% yield. The protected nucleoside (**16**) was also hydrogenated with Pd/C and afforded the desired product in 95% yield. Phosphoramidite introduction produced the expected product without any hydrolysis in 56% yield (**21**) (Scheme 6).

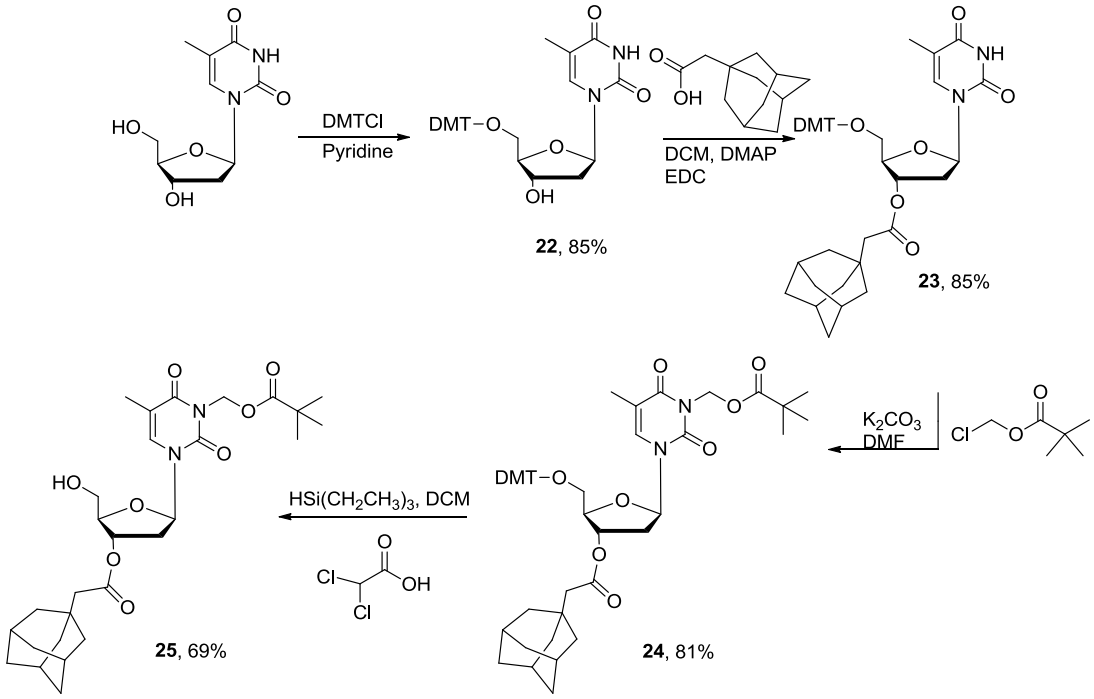


Scheme 6. Synthesis of propylamino modified thymidine phosphoramidite.[11]

3.2.3 Synthesis of trimer

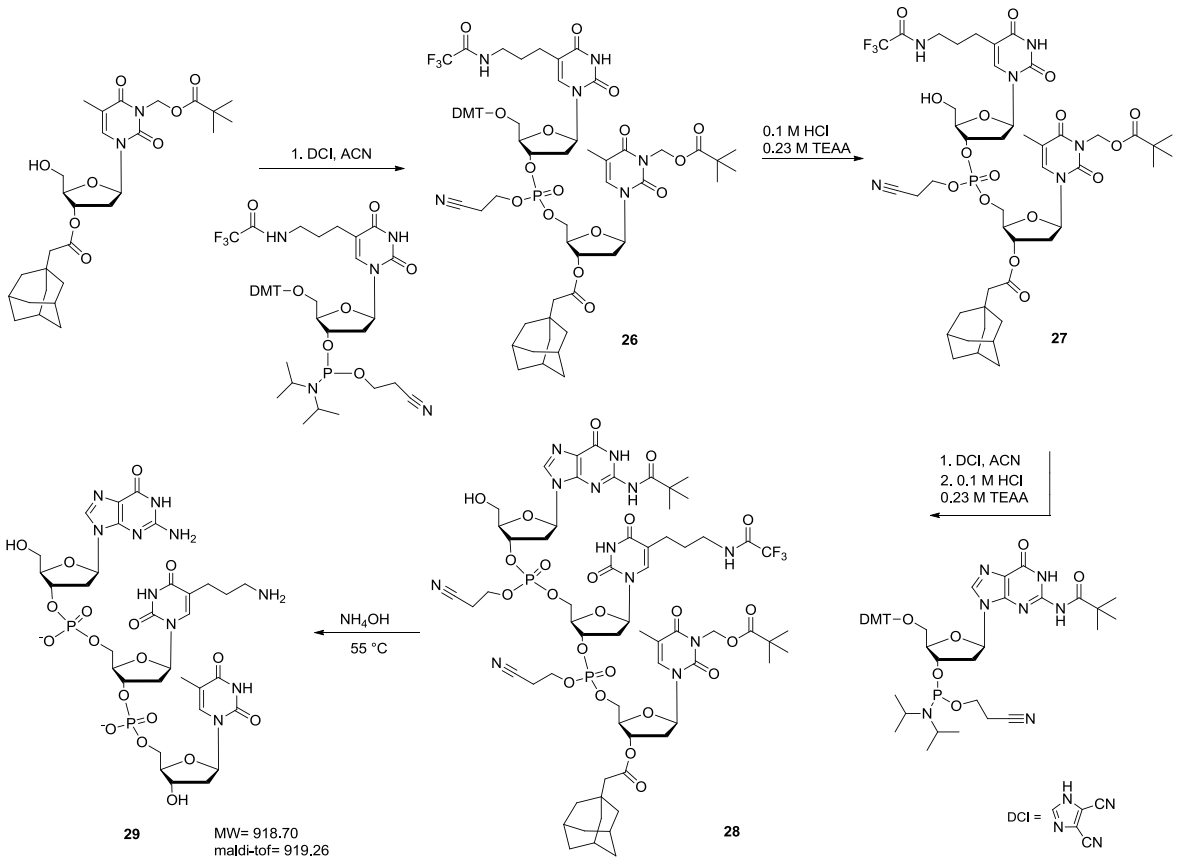
A DNA trimer is a good model for the study of longer oligonucleotides, with the advantage that they can be prepared in solution in much larger quantities. De Koning et al[18] reported an efficient and fast synthesis of short DNA strands in solution. Following their procedure a trimer was prepared with an amine modified monomer in the central position. For the synthesis of DNA in solution the first base requires a protecting group for the secondary alcohol at the 3' position compatible with DNA synthesis conditions while amenable to be deprotected during ammonia treatment at the end of the synthesis. The protecting group reported is the adamantylacetyl ester and the chosen nucleoside for the 3' position was thymidine. The primary hydroxyl group was protected with DMT affording compound **22** in 85% yield, then the secondary hydroxyl group could be protected with adamantane acetic acid (AdaOH) leading to compound **23** in 85% yield. The amino group present in thymidine was then protected with chloromethylpivalate (Pom) to increase the solubility in organic

solvents producing compound **24** in 81% yield. The last step was DMT removal under acidic conditions to afford **25**. The yield of this last step was 69% (Scheme 7).



Scheme 7. Synthesis of protected thymidine.[18]

The trimer is synthesized starting from compound **25**, by coupling with modified nucleoside phosphoramidite (**21**) and guanine phosphoramidite respectively (dTdXdG) (Scheme 8). Aqueous ammonia at 55 ˚C for 3 days is used for the deprotection and as explained in chapter 2 purification is performed with FPLC using different concentrations of TEAA (triethylamine acetic acid) buffer as eluent. MALDI-TOF analysis shows the molecular weight peak corresponding to the desired product.



Scheme 8. Synthesis of trimers in solution with the amine modified base at the central position.[18]

3.2.4 Synthesis of 15-mers

3.2.4.1 Introduction of aliphatic linkers into DNA

A complete turn of a double stranded DNA requires at least 10 base pairs. 15-mers were considered to have the optimal length for a modified oligonucleotide because they can form a duplex with a complementary strand and in optimal conditions its synthesis gives relatively high yields (20-40%). Longer strands in our findings would inevitably be obtained in considerably lower yields and shorter strands might not be able to give duplex formation due to structural distortions caused by the modification. DNA modifications can be introduced at different locations of the strand at will. Phosphine moieties in a central position of a DNA strand are more likely to be influenced by the chiral environment especially when a double helix is formed by introducing a complementary strand. On the other hand, phosphine modification at the terminal positions together with a complementary strand allows the templated formation of a bidentate. (Figure 1)

Compound **4** (Figure 5) was introduced into several 15-mers.

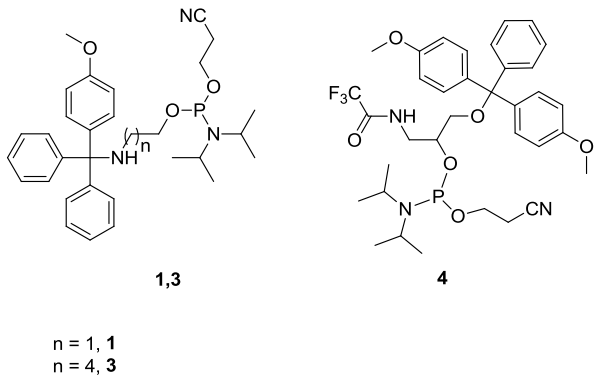


Figure 5. Terminal aliphatic linkers and central aliphatic linker.[5, 6]

The purification of DNA was initially performed with an FPLC ion exchange column followed by a desalting step. HPLC purification is most commonly used for the purification of DNA strands so it was decided to change our method of purification to preparative HPLC with a C18 clarity column. There are two major advantages of HPLC purification. Firstly, it uses a volatile buffer, therefore the desalting step is no longer required and additionally it drastically reduces the amount of sodium salts present in the DNA. Secondly, DNA strands of several lengths can be purified. The only drawback of HPLC is that only small amounts of DNA can be purified in each run. The maximum column load for a C18 Clarity column is 5 μmol while in contrast, for an HP 16/10 QXL Sepharose FPLC column the maximum load can be up to 70 μmol depending on the sample purity and the length of the strand.

When the 15-mers were purified by preparative HPLC several peaks appeared and the main peak did not give the expected molecular weight in MALDI-TOF analysis. This suggests that the DNA decomposes into smaller fragments. The deprotection procedure was then changed from 1:1 concentrated aqueous ammonia/40% methylamine, to concentrated aqueous ammonia 80 ˚C for 8 h and finally to aqueous concentrated ammonia at 50˚C for 17 h but no major change in chromatograms were observed. Despite numerous attempts, only on a few occasions fractions containing the desired product (according to MALDI-TOF analysis) could be collected. The quantity of the 15-mer collected was much less than 1 nmol. Therefore, the use of linker was abandoned because of its instability and difficulty of purification.

It is important to emphasize that sample preparation is important for MALDI-TOF analysis. Salts are known to disrupt the analysis of DNA therefore several methods were tested to reduce the amount of salts present in the samples. The best results were

obtained with a combination of two different desalting methods. The DNA samples are first desalted using a desalting column, followed by Dowex 50X, which is an ion exchange resin and finally prior to analysis desalting tips are used.

Linkers **1** and **3** were also introduced into a DNA 15-mer although the desired strand could not be synthesized. The final coupling during the automated synthesis of the 15mer with C_2 (**1**) as terminal linker gave 78% yield although MALDI-TOF analysis after FPLC purification resulted in 73 mass units less than the expected molecular weight. When the C_5 linker **3** was introduced into the terminal position of the 15mer, again by standard DNA synthesis, the coupling efficiency was 0% indicating that the coupling had failed. To analyze the coupling efficiency of compound **3** a test was employed with methanol and tetrazole in solution. After the addition of tetrazole the phosphoramidite peak in ^{31}P NMR successfully moved from 146.8 ppm to 139.5 ppm showing that coupling to methanol was successful. Coupling of compound **3** to a DNA strand was attempted several times but the same results were obtained. Because of those unsuccessful results it was decided to buy a commercial 15mer with a C_3 linker at the 3' position.

3.2.5.2 Introduction of amino modified thymidine into 15-mers

Compound **16** was introduced in the central and terminal position of DNA strands of 15 bases long (15-mers). These syntheses are typically performed automatically in a DNA synthesizer and the steps have been explained in the previous chapter. Modified base **16** was successfully introduced in the central position and 5' position of several 15-mers (Table 1).

Strand #	Strands Synthesized	Molecular Weight	Mass Found
S1	5'-TAAGCCAMCATCCGC-3'	4535.9	4534.3
S2	5'-ATTCGGTMGTAGGCG-3'	4678.0	4676.4
S3	5'-GCGGATGMTGGCTTA-3'	4678.0	4676.9
S4	5'-MATTCGGTAGTAGGCG-3'	4991.3	4989.4

Table 1. Mass spec analysis (MALDI-TOF) of the synthesized 15-mers M= amine modified base **16**.

Once the desired DNA strand had been synthesized it was cleaved off from the solid support and all the protecting groups were deprotected simultaneously using basic conditions. The first method of deprotection used was the most commonly

encountered in the literature: concentrated aqueous ammonia at 80 ˚C for 8 h or at 55 ˚C overnight. After purification using a preparative HPLC column, MALDI-TOF analysis for 15-mers **S2** and **S3** showed an extra peak next to the desired peak at 53 mass units higher (Figure 6). A likely explanation for this peak is that the acrylonitrile released from the phosphate is attacked by the free amine of the linker before being quenched by the ammonia (Scheme 9).

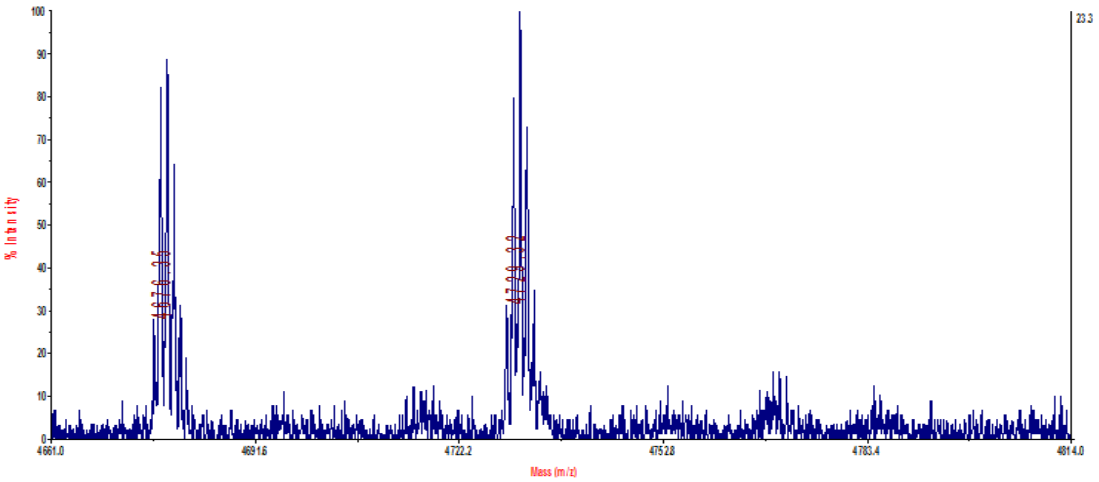
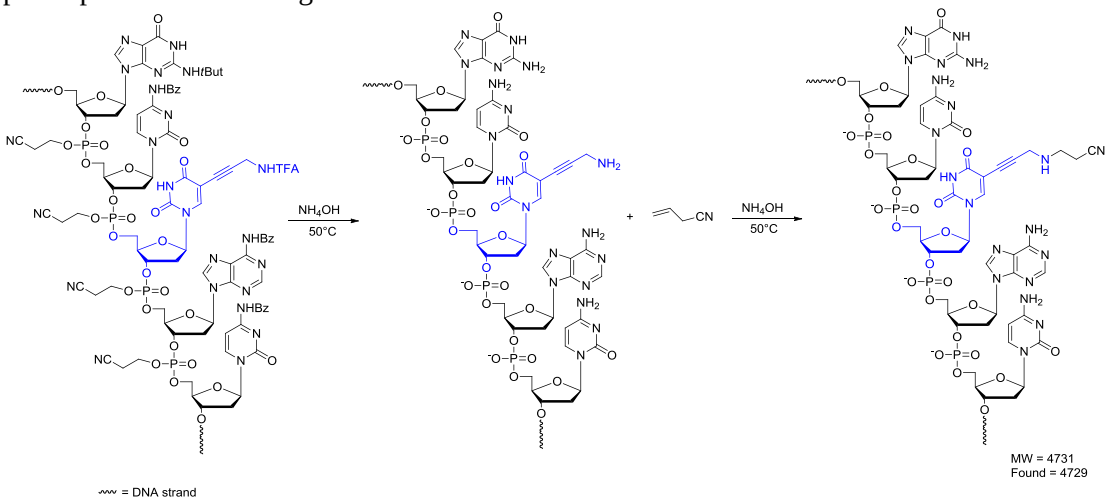


Figure 6. MALDI-TOF analysis of 15-mer **S2** showing the peak of the expected compound plus a peak of 53 units higher. ˚



Scheme 9. Attack of the amine linker by the acrylonitrile during oligonucleotide cleavage from solid support with ammonia.

Studies with the nucleoside (**14**) and acrylonitrile in ammonia showed that the amine linker is very reactive towards acrylonitrile. Reaction of one equivalent of the protected nucleoside in ammonia with 14 equivalents of acrylonitrile resulted in complete addition of the amino group to acrylonitrile, whereas around 25% of the

modified base is found to be coupled to acrylonitrile when the ratio is one to one. Surprisingly, this is not observed in the trimer and not in all the 15-mers, indicating that the exact sequence of the strand has a large influence on its reactivity. Hydrogenated amine-modified monomer (**20**) was introduced into three DNA strands (table 2).

Strand #	Strands Synthesized	Molecular Weight	Mass Found
5H	5'-ATTCGGTHGTAGGCG-3'	4682	4683
6H	5'-CGCCTACHACCGAAT-3'	4540	4540
7H	5'-HTTCGGTAGTAGGCG-3'	4682	4680

Table 2. Mass spec analysis (MALDI-TOF) of the synthesized 15-mers H= amine modified base **20**.

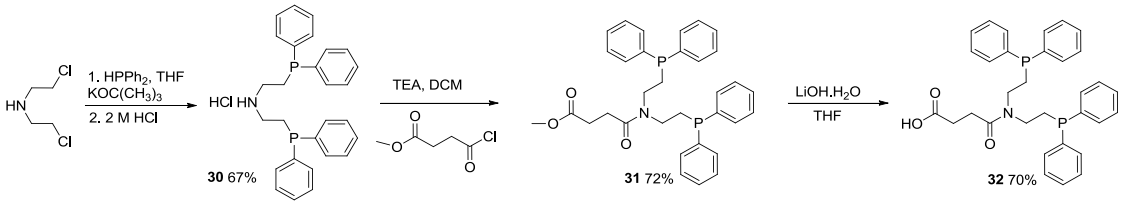
Strand **5H** was used to test two different methods of deprotection: a 1:1 mixture solution of ammonia/aqueous 40% methylamine for 17 h at 55 ˚C, and concentrated aqueous methyl amine (40%) for 8 h at 55 ˚C. The strand was cleaved off with both methods as shown by two main overlapping peaks in the HPLC chromatograms. MALDI-TOF analysis of those two peaks showed the desired product although the last peak contained a signal about 53 units higher indicating that a small amount of oligonucleotide is still attacked by acrylonitrile. This indicates that a small amount of DNA still reacts with acrylonitrile even when a strongly nucleophilic base such as methylamine is used for the deprotection.

3.2.5 Coupling of phosphine moieties to monomers, trimers and 15-mers

3.2.5.1 Coupling of phosphines to monomers

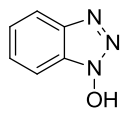
Before coupling phosphine moieties to DNA, phosphine modified monomers were synthesized to serve as models for longer DNA chains. *Ortho*-, *meta*- and *para*-diphenylphosphinobenzoic acids and 4-(*bis*(2-diphenylphosphino)ethyl)amino)-4-oxobutanoic acid (Whitesides carboxylic acid) were successfully coupled to the amine modified thymidine. *Ortho*- and *para*-diphenylphosphinobenzoic acids are commercially available, whereas *meta*-diphenylphosphinobenzoic acid and the Whitesides carboxylic acid were synthesized following literature procedures.[19] Compound **30** was synthesized from *bis*(2-chloroethyl) amine hydrochloride and diphenylphosphine in 67% yield. This compound is commonly referred to as "Whitesides ligand" after George M. Whitesides.[20] Methyl-4-chloro-4-oxobutyrate

was added to compound **30** to give the protected compound **31**[19] in 72% yield. (Scheme 10). Purification of this compound proved to be troublesome because part of the desired product co-eluted with an unidentified brown impurity during column chromatography purification. Later it was found in the group that by replacing methanol by acetone as eluent the desired product could be obtained in pure form in 81% yield. Deprotection with LiOH afforded the desired product **32** in 70 % yield.

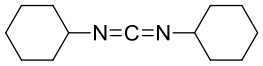


Scheme 10. Synthesis of 4-(*bis*(2-diphenylphosphino)ethyl)amino)-4-oxobutanoic acid.

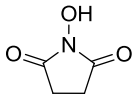
The coupling of phosphine moieties to the nucleosides was performed under the same conditions as those to be employed with DNA strands (Scheme 11). The phosphinocarboxylic acids were activated with EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)/NHS (*N*-Hydroxysuccinimide) in dry DMF and added to the modified bases in a 0.1 M aqueous solution of $NaHCO_3$. The amine functionality of the modified thymidine is deprotected prior to the coupling by using concentrated aqueous ammonia. Purification by column chromatography gave pure product with less than 5% oxidation in good yields up to 82%. Preparation of monomer **40** modified with compound **32** gave yields lower than 10% and its synthesis was not further pursued. The three phosphinocarboxylic acids mentioned before were coupled to both propargyl and propylamino modified monomers. The coupling reaches about 80% conversion as monitored by ^{31}P-NMR spectroscopy when the propylamino base is used. In order to improve the yields, other coupling reagents were tested such as DCC (Dicyclohexylcarbodiimide)/HOBT (Hydroxybenzotriazole)/TEA,[21] CDI (1,1'-Carbonyldiimidazole), NHS/EDC/TEA[22] and pentafluorophenol/DCC/IPEA[23] (Figure 7) but none of the reactions provided the desired product.



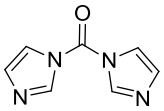
Hydroxybenzotriazole (HOBT)



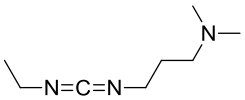
Dicyclohexylcarbodiimide (DCC)



N-Hydroxysuccinimide (NHS)

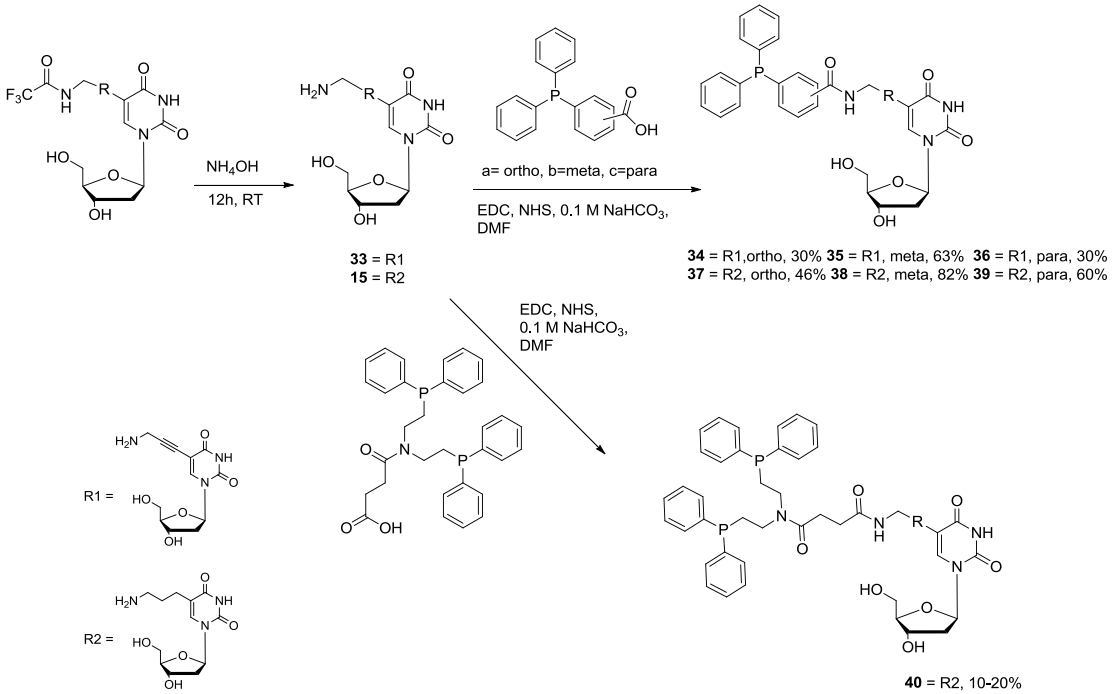


1,1'-Carbonyldiimidazole (CDI)



1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)

Figure 7. Common reagents used for amide bond formation.



Scheme 11. Synthesis of phosphine modified monomers with *ortho*-, *meta*-, *para*-diphenylphopshinobenzoic acids and 4-(*bis*(2-diphenylphosphino)ethyl)amino)-4-oxobutanoic acid.

Crystals suitable for X-ray crystallography of compounds **37** (Figure 8) and **38** (Figure 9) were obtained by slow evaporation of acetonitrile solutions at room temperature.

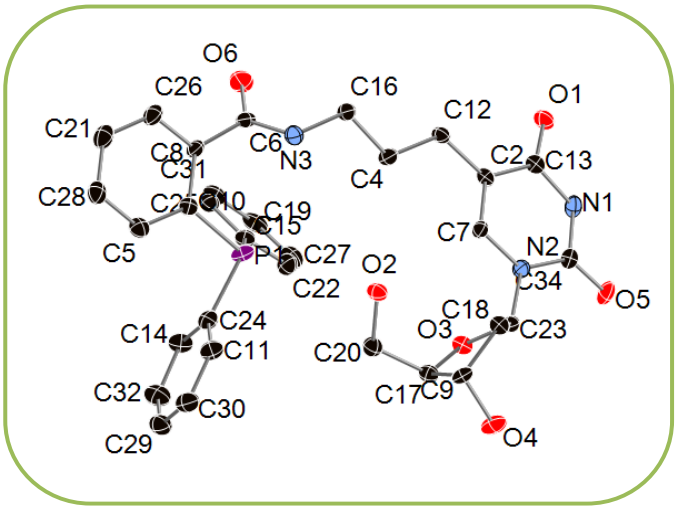


Figure 8. Ortep representation for **37**. Thermal ellipsoids are drawn at 50% probability.

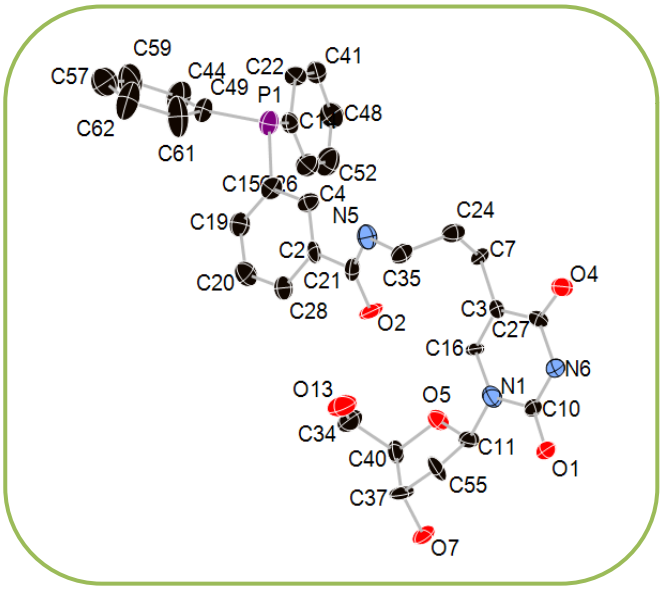


Figure 9. Ortep representation for **38**. Thermal ellipsoids are drawn at 50% probability.

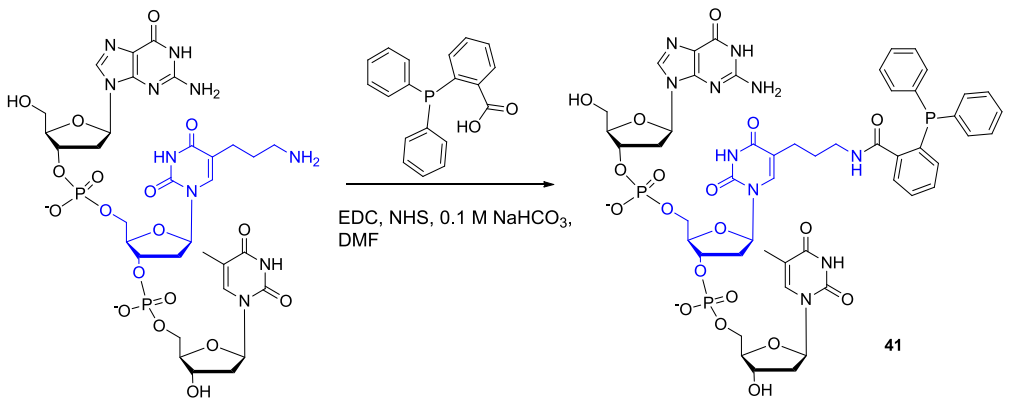
The crystal structures proved the identity of compounds **37** and **38** and confirmed the expected absolute configurations of the stereogenic carbons of the the ribose ring. Bonding distances and angles are similar to those found in other compounds.[24] In spite of that, both structures look very different. **38** presents an open structure with the diphenylphosphino unit far away from the ribose ring and the electron pair of the phosphorus atom pointing outwards of the molecule. This conformation is probably forced by an intramolecular hydrogen bond between O(13)H and the O(2) of the

carbonyl of the amido group. In contrast, **37** has a much more closed structure, with the PPh_2 group pointing inwards, in the direction of the 5'-OH of the ribose ring and without the hydrogen bond found for **38**.

3.2.5.2 Coupling of phosphine moieties to DNA strands

Similar coupling conditions[1] were used to introduce the phosphine moiety onto the trimer and several 15-mers. The differences between the coupling of the phosphine moiety to the monomer and the oligonucleotides were the quantities of DNA employed and the equivalents of phosphinocarboxylic acid used. The amounts of oligonucleotides used for those syntheses are in the micro and nanomolar range and therefore, the purification and analysis of those compounds can not be achieved with the standard organic synthesis methods. For the trimer, 4 equivalents of activated phosphinobenzoic acid were used while for the 15-mers 250-500 equivalents were employed. Trimer modified with *ortho*-diphenylphosphinobenzoic acid (Scheme 11), was purified by FPLC using an ion exchange column. $^{31}P\{^1H\}$ NMR before purification showed the phosphate peaks at around 0 ppm, a peak at −6.94 ppm which is the expected region for the coupled phosphine, a peak at −8.58 ppm which corresponds to the phosphine starting material and some other minor peaks. After purification, $^{31}P\{^1H\}$ NMR shows the phosphate peaks at 0 ppm and a peak at 36 ppm which is most likely the oxidized coupled phosphine with an integral ratio of 2 to 1. The coupling reaction was repeated and the crude product was split in two fractions which were purified by HPLC and FPLC, in parallel. For both methods, the contact with air of the reaction mixture was minimized and all solvents were degassed. $^{31}P\{^1H\}$ NMR of product after FPLC showed the phosphate peak and the peak of the phosphine oxide. HPLC purification gave no peaks corresponding to the trimer. It is most likely that the trimer is not soluble in the eluting medium and it precipitates on the column. Because of the sensitivity of the phosphine to oxygen it was decided that it was necessary to protect the phosphorus atom before the purification step. BH_3 was chosen as protective group because the DNA is compatible with its deprotection conditions. However, previous experience in the group on the deprotection of phosphine compounds showed that BH_3 removal could be troublesome. For the trimer it is necessary to find a deprotection method that does not require any further purification. Van Overschelde *et al*[25] successfully deprotected phosphine-boranes by simply stirring them in refluxing methanol or ethanol. This

method was tested for the deboronation of the trimer. After coupling of *ortho*-diphenylphosphinobenzoic acid to the trimer the crude mixture was treated with $BH_3 \cdot SMe_2$ to protect the phosphine ligand and perform purification under air. Purification by HPLC was repeated and MALDI-TOF analysis of the purified trimer showed a peak corresponding to the expected mass of 1221.6. The sample was then deboronated by refluxing in methanol for 1 h. After being concentrated *in vacuo* a $^{31}P\{^{1}H\}$ NMR spectrum was taken which showed the phosphates signal, a peak at −6 ppm, which corresponds to the deprotected phosphine coupled to the DNA and several other peaks around the +36 ppm region which probably correspond to the oxidized phosphine. Metal complexation with $[Rh(acac)(CO)_2]$ was attempted and the reaction was followed by MALDI-TOF but no peak corresponding to the metal complex formation could be observed. However, MALDI-TOF may not be an adequate technique of the analysis of DNA-metal complexes.



Scheme 12. Coupling of *ortho*-diphenylphosphinobenzoic acid to the trimer.[1]

15-mers with central and terminal modifiers were also functionalized with phosphine moieties using the same coupling conditions as with the trimers and monomers. Because of the higher molecular weight 15-mers can be purified using other methods. They were initially purified by using a membrane filter with a cut-off of molecular weight at 3000 daltons. The filter consists of a cellulose membrane that separates molecules by their molecular weight using centrifugal force (Figure 10).

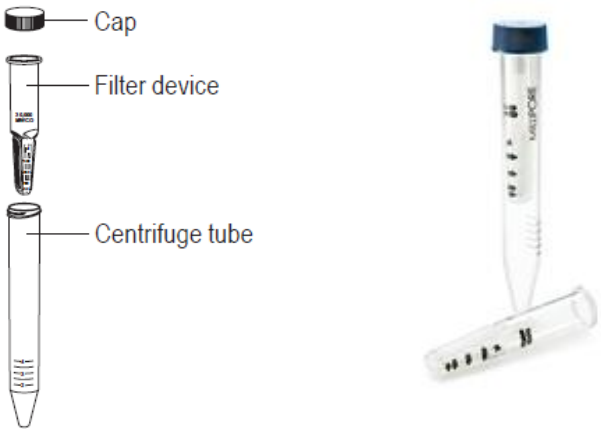


Figure 10. Illustration of a membrane filter from Millipore.

After DCM or $CHCl_3$ washing the aqueous layer contains a white precipitate which was removed by centrifugation. The clear aqueous layer was placed in the membrane filter and it was centrifuged several times with degassed water to separate all the low molecular weight compounds from the desired strand. The contact with air was minimized as much as possible. $^{31}P\{^1H\}$ NMR spectrum of the water phase solution remaining in the membrane filter still showed the peak corresponding to the excess of phosphine. MALDI-TOF analysis showed the molecular weight of the phosphine oxide coupled to DNA. To exclude a DNA catalyzed oxidation of the phosphine with water, $^{31}P\{^1H\}$ NMR spectrum of a DNA strand in presence of 240 equivalents of *p*-diphenylphosphinobenzoic acid was recorded. 50 measurements were taken every 30 minutes (64 scans) to monitor the appearance of oxidized phosphine. After 25 h no oxidation was observed suggesting that the DNA does not favour the oxidation of the phosphine moiety. Another result indicating that the phosphine unit is not oxidized by the DNA or during purification was mass spectrometric analysis with electrospray ionisation. In this case, the molecular weight corresponds to the non oxidized phosphine (15-mer **S4** with *o*-dppba).

It has to be mentioned that many attempts were also carried out to couple strands **S1**, **S3** and **S4** to *o*-, *p*-diphenylphosphinobenzoic acid (dppba) and **5** but unfortunately, the coupling reactions with the propargylic amines exhibited bad reproducibility. The only reproducible coupling was obtained with a commercial strand (**7CS**) of sequence 5'-GCTGATGACTATCGAM-3' which has a C_3 3' flexible terminal linker. The Propargylic amines are considerably less basic and nucleophilic than those bearing

saturated groups. Moreover, the rigidity of the carbon bridge might render the coupling more difficult. For these reasons we discontinued our efforts with propargylic amine modified strands **S1**, **S2**, **S3** and **S4** and the triple bond of monobase **14** was hydrogenated to increase both the flexibility of the bridge and the nucleophilicity of the nitrogen atom, potentially improving the coupling efficiency. In addition, to simplify the method and to obtain purer strands, the purification of the coupled DNA 15-mers was changed to ethanol precipitation. Precipitation of 217 nmol crude product resulted in 155 nmol of DNA, therefore about 30% of the DNA is lost during this procedure. Due to the unreliability of MALDI-TOF analysis, a more robust and complementary analytical technique was required. Therefore, ^{31}P-NMR analysis was attempted although it was anticipated to be problematic due to the extremely small amounts of phosphine modified DNA available. Strand **5H** was coupled to *p*-(diphenylphosphino) benzoic acid and after washing and ethanol precipitation a ^{31}P{^{1}H} NMR sample was prepared under inert atmosphere. The ^{31}P{^{1}H} NMR spectrum (Figure 11) showed the peaks of the phosphates at 0 ppm a small peak at −5.5 ppm corresponding to the coupled phosphine moiety, small peaks at 35 ppm corresponding to partial oxidation of the coupled phosphine and an unidentified extra peak at 24 ppm. No excess of free phosphine was present. The integration of the two phosphorus peaks does not correspond to 1 to 14. The different relaxation times of the phosphine and the phosphates makes integration unreliable. In spite of that, the NMR strongly suggests the success of the coupling and to the best of our knowledge constitutes the first NMR evidence of a phosphine moiety coupled to a relatively long DNA strand. Figure 14 shows a model of the strand with the phosphine moiety in yellow drawn with the modelling program pymol. MALDI-TOF analysis of the same strand used for NMR analysis gave the molecular weight expected for the oxidized coupled compound (Figure 12), indicating that the oxidation takes place during the MALDI-TOF measurements. No peak corresponding to the uncoupled 15-mer could be observed. Analysis by MALDI-TOF of a mixture of phosphorylated and uncoupled 15-mer was attempted to obtain a better indication of the coupling efficiency but no signal above the mass of 1200 could be observed. Due to the large amount of salts present with the DNA, the 15mer was filtered through a desalting NAP-10 column. This procedure was performed under inert conditions. ^{31}P{^{1}H}NMR after desalting showed complete oxidation of the phosphine moiety as indicated by peaks at 35 ppm. The coupling was repeated with strand **6H** and in this case

$^{31}P\{^{1}H\}$NMR showed about 50% oxidation of the coupled phosphine moiety and again a small peak at 24 ppm. This strand was complexed with dimer [PdCl(η^3-allyl)]$_2$. $^{31}P\{^{1}H\}$NMR of the Pd allyl chloride complexed 15-mer shows the appearance of a broad peak at 27 ppm and the disappearance of the peak at −5.5 ppm suggesting the successful formation of the Pd complex with the phosphine moiety coupled to the DNA 15-mer (Figure 13). MALDI-TOF analysis was attempted but the peak corresponding to the expected molecular weight was not detected. Strand **7H** was coupled to the Whitesides carboxylic acid, MALDI-TOF analysis shows the molecular weight of the coupled oxidized phosphine ligand proving a successful coupling although $^{31}P\{^{1}H\}$NMR shows complete oxidation of the coupled phosphine ligand. The Whitesides ligand is more oxygen sensitive compared to diphenylphosphino benzoic acid because the third electron withdrawing phenyl ring is replaced by an aliphatic chain therefore increasing the electron density on the phosphorous atom making it more prone to oxidation.

The oxidized phosphine strands were analyzed by HPLC to identify the coupling efficiency although only two overlapping peaks were visible with a retention time of 6 minutes. Using the same gradient for purification of the modified DNA the 15-mer was eluted after 26 minutes. This suggests that the phosphine moiety drastically changes the interaction between the 15-mer and the C18 reversed phase material of the column.

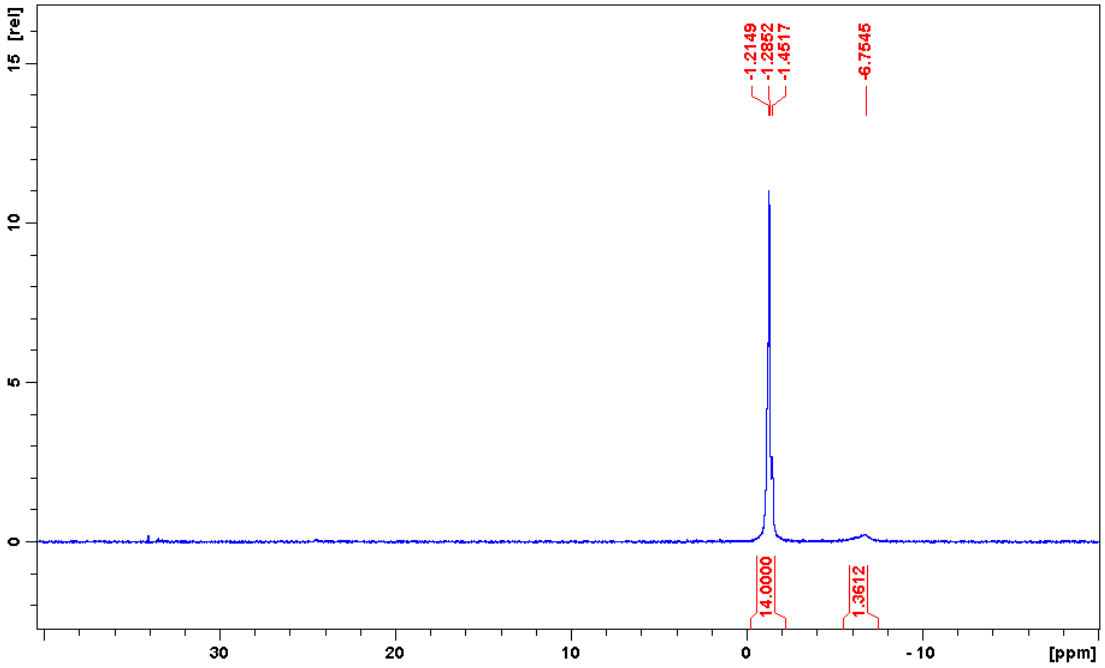


Figure 11. $^{31}P\{^{1}H\}$NMR spectrum after 16 h of acquisition time with a 500 MHz NMR machine. The peak at −1.2 ppm is the phosphate peak and the peak at −6.7 ppm is the peak of the phosphine coupled to the 15-mer.

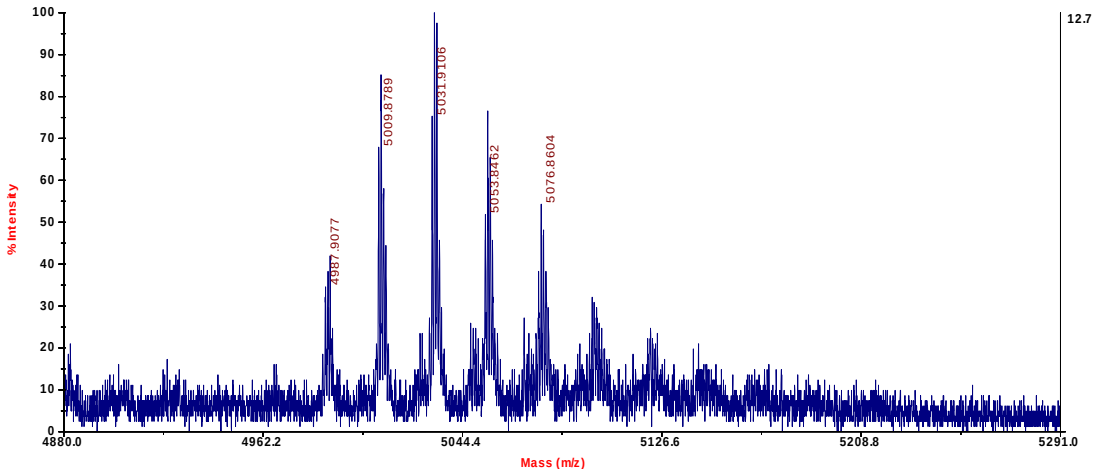


Figure 12. MALDI-TOF analysis of the phosphine modified 15-mer. Calculated mass is of 4970, mass found is 4987 which represents the coupled oxidized compound, peaks at 5009.9, 5031.9, 5053.6, and 5076.8 are the coupled compound plus Na.

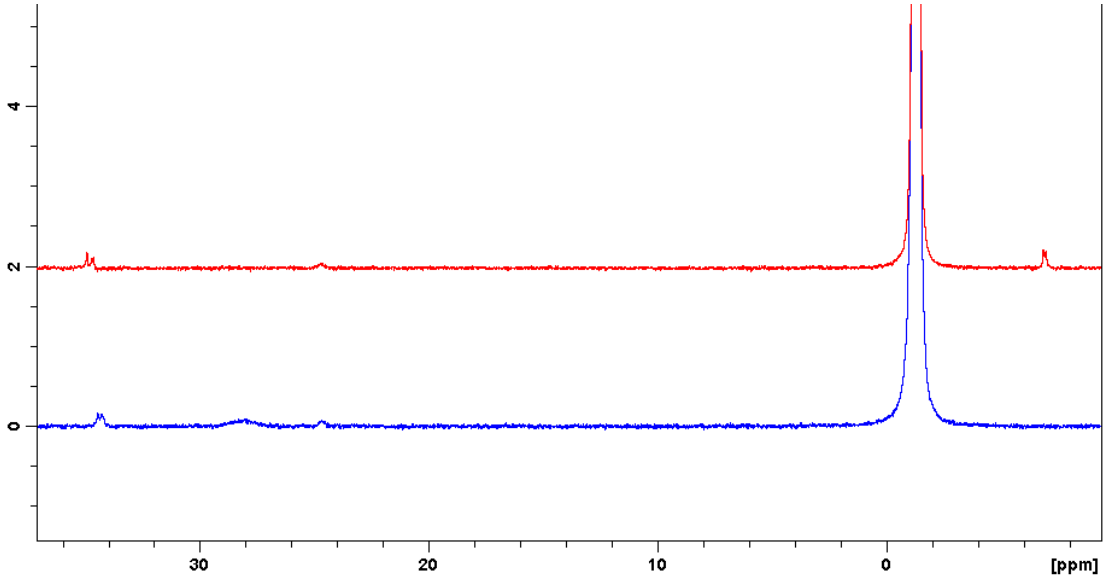


Figure 13. Overlapping ^{31}P NMR spectra of phosphine modified 15-mer. Red spectrum shows a peak at −5.5 ppm representing the coupled phosphine. Blue spectrum shows the Pd complexed phosphine with a peak at 27 ppm.

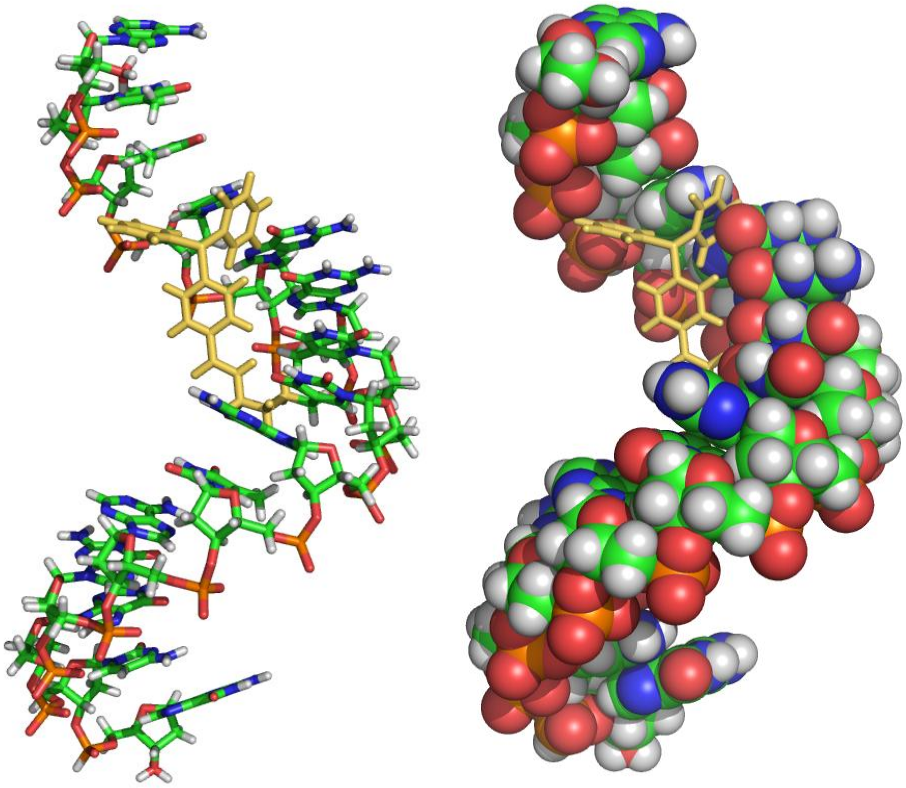
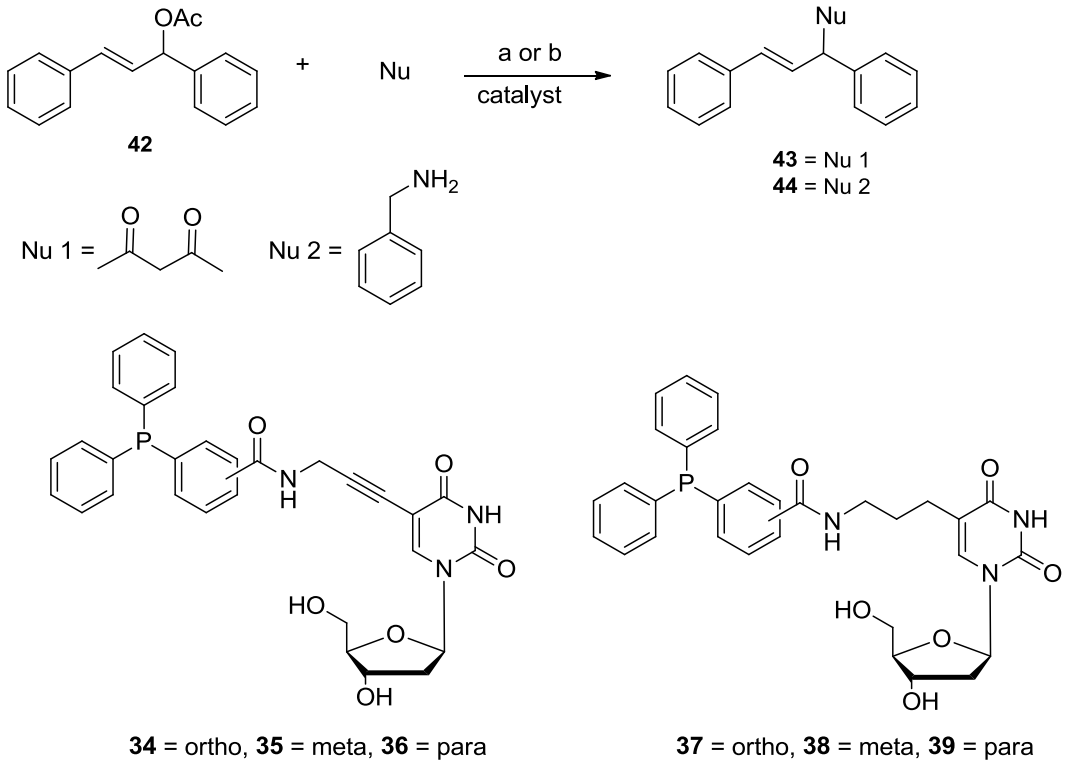


Figure 14. Stick and sphere representation of phosphine (in yellow) modified 15-mer using Pymol.

3.2.6 Catalysis with modified bases

As explained in the introduction, phosphine modified monobases have been used as ligands for Pd-catalyzed allylic substitution resulting in enantoselectivities up to

80%.[24] Pd-catalyzed allylic substitution is a model reaction for Pd catalyzed C-C bond formation and it is a very well established reaction.[26, 27] For those reasons allylic substitution of 1,3-diphenylallyl acetate with dimethyl malonate as nucleophile was chosen to study the catalytic properties of the six phosphine modified bases (Scheme 13). The reactions were performed in CH_2Cl_2 and THF.



Scheme 13. Allylic alkylation (a) BSA (*N*,*O*-Bis(trimethylsilyl)acetamide), $[Pd(allyl)Cl]_2$, KOAc) and allylic amination (b) $[Pd(allyl)Cl]_2$) reactions using the modified nucleobases as ligands.

Allylic alkylation in CH_2Cl_2 resulted in 100% conversion for compounds **35**, **36**, **38**, **39** and less than 10% conversion for compounds **37** and **34** (Table 3). Enantioselectivities were lower than 4%. The same reaction repeated in THF resulted in much lower conversion and ee lower than 5%. The much stronger coordination properties of THF probably slow down the reaction.

Compounds **34**, **35**, **36** were also tested in allylic alkylation at 0 ˚C to observe any change in enantioselectivity. No change in ee was observed although the conversions dropped to lower than 20% (Table 3).

Ligand	Solvent	Conv. %
34	DCM	1.6, 0*
34	THF	0
35	DCM	100, 15*
35	THF	14.5
36	DCM	100, 19*
36	THF	3.4
37	DCM	9.2
37	THF	3.4
38	DCM	100
38	THF	9.8
39	DCM	100
39	THF	4.4

Table 3. 0.5 μmol of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$, 1,3-diphenylallyl acetate:dimethyl malonate: BSA:Pd = 50:150:150:1, 400 μl of solvent at 25 ˚C, for 24 h. * reactions performed at 0 ˚C for 24 h.

Allylic amination was performed with the same substrate and benzylamine acting both as base and nucleophile. Allylic amination was more problematic, in CH_2Cl_2 the conversions for compounds **35**, **36**, **38**, **39** were 99-100%. Conversion for compound **37** was lower than 10% and for **34** the results were not consistent (Table 4). In THF the results were non reproducible and the reactions were not tried again.

Ligand	Solvent	Conv. %
35	DCM	100
36	DCM	100
37	DCM	8.2
38	DCM	99.5
39	DCM	100

Table 4. 0.5 μmol of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$, 1,3-diphenylallyl acetate:benzylamine:Pd = 50:150:1 400 μl of solvent at 25 ˚C, for 24 h.

The Pd complex of the 15-mer described above was tested as ligand for Pd-catalyzed allylic alkylation of the benchmark substrate 1,3-diphenylallyl acetate with dimethyl malonate but unfortunately no conversion was observed.

The same 15-mer was synthesized again and analyzed by $^{31}P\{^1H\}$NMR spectroscopy, which showed the unoxidized phosphine peak at −5.5 ppm. The strand was complexed in situ with $[Rh(acac)(CO)_2]$ and used directly for hydroformylation of styrene, which did not give any conversion either.

3.3 Conclusion

This chapter details the successful synthesis of two modified thymidines with amino groups and central and terminal aliphatic linkers. Those modifications were successfully introduced into oligonucleotides using standard solid phase synthesis and amine modified thymidine was also introduced in the central position of a trimer using solution phase synthesis. *Ortho*-, *meta*-, and *para*-diphenylphosphinobenzoic acids were coupled to amine modified thymidine and used in Pd-catalysed allylic substitution reactions. Although the reactions were not selective towards any specific enantiomer, four ligands gave complete conversions in allylic alkylation. A trimer was successfully synthesized in solution phase containing thymidine modified with an alkene amine linker in the central position. *Ortho*- diphenylphosphinobenzoic acid was coupled to it resulting in 50% oxidation after purification by preparative HPLC and BH_3 deprotection in refluxing methanol. 15-mers were modified with thymidine containing an alkyne amine linker, thymidine containing an alkane modified linker, a central aliphatic amine linker, a 3' C_3 linker and a 5' C_3, C_5 terminal aliphatic amine linkers. Some of those amine modified oligonucleotides could be coupled to phosphine moieties. Oxidation of the phosphine moiety was prevented after several attempts and after the use of several different purification methods. Methods were developed for the purification of oligonucleotides prior and after phosphine modification and an appropriate method was found for the MALDI-TOF analysis of oligonucleotides. A Pd metal complex was obtained as indicated by NMR analysis and a few attempts were made in using the phosphine modified 15-mer ligands in catalysis.

Having optimized the coupling and purification procedures, future work should be focused on the use of DNA phosphine modified oligonucleotides as ligands for asymmetric catalytic reactions. A bidentate system could be generated from single stranded oligonucleotides with phosphine modification at the 5' and 3' position and different complementary strands could be used to change the local environment of the substrate. Moreover, several different phosphines could be synthesized bearing carboxylic acid moieties and then introduced on the DNA increasing the variety of ligands that could be synthesized in a relatively short period of time.

Part of the work shown in this chapter was done in collaboration with Dr. Arnald Grabulosa. Part of this work was also published with cover in the European Journal of Organic Chemistry

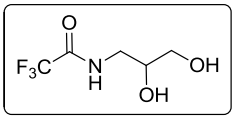
3.4 Experimental

General Procedures

All reactions were performed under argon using standard Schlenk techniques. Chemicals were purchased from Aldrich Chemical Company, Acros, Fluka, Link Technologies and Glen Research. CH_2Cl_2 and acetonitrile were distilled from CaH_2; ethanol and methanol were distilled from Mg/I_2; toluene was distilled from Na, THF and Et_2O were distilled from sodium /benzophenone and all the distillations were performed under nitrogen atmosphere. DMF was purchased "Extra dry" and stored over molecular sieves (4 Å) under argon. Aqueous solutions were degassed by three freeze/thaw cycles under vacuum and kept under argon. Thin Layer Chromatography (TLC) was performed on silica plates (Polygram 0.2 mm silica gel with fluorescent indicator UV_{254}). Ninhydrin spray was used for the detection of primary and secondary amines on TLC silica plates. Silica gel 60 particle size 0.063-0.2 mm from Fluka was used for flash chromatography. NMR spectra were recorded at room temperature on Bruker Avance spectrometers (300, 400, and 500 MHz). Positive chemical shifts (δ) are given (in ppm) for high-frequency shifts relative to a TMS reference (^1H and ^{13}C) or an 85% H_3PO_4 reference (^{31}P). ^{13}C, ^{31}P, and ^{19}F spectra were measured with ^1H decoupling. The following abbreviations have been used in the description of the spectra: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were recorded on a Perkin-Elmer Spectrum GX spectrometer in KBr pellets, with a window between 4000 and 400 cm^{-1}. Mass spectrometry: MALDI-TOF mass spectra were recorded on a 4800 Plus MALDI TOF/TOF™ Analyzer using a 9:1 3-hydroxypicolinic acid (HPA): ammonium citrate matrix. The matrix is made with 50 mg/ml of HPA in 50:50 MeCN:H_2O and 50 mg/ml of ammonium citrate in H_2O. The desalting column used for the preparation of MALDI-TOF samples was a GE Healthcare Illustra® micron G-25 and the desalting tips were Millipore® zip-tip C18, the Dowex used for desalting was a 50WX4 200-400 mash resin. Oligonucleotides prepared at our laboratories were synthesized by an Applied Biosystems 392 DNA/RNA synthesizer using protocols obtained from Applied Biosystems. The reagents used for the synthesis of oligonucleotides were bought from Glen research and were: oxidizing solution 0.10 M I_2 in THF/pyridine/water; cap mix B (10% 1-methylimidazole in THF/pyridine); cap mix A (THF/pyridine/acetic anhydride); deblocking mix (3% trichloro acetic acid in DCM); activator (sublimed 1H-tetrazole in anhydrous acetonitrile); dA, dC, dG, dT phosphoramidites. Coupling yields for DNA synthesis are based on measurements of DMT release which is determined by conductivity measurements. Purification by ion exchange, size exclusion and desalting were carried out with a FPLC AktaBasic 100 (P-901), monitor UV-900. The column used for purification of the modified oligonucleotides was a HiLoad 16/10 Q Sepharose HP with the following buffers: buffer A = 1 M NaCl, 10 mM NaOH and buffer B = 10 mM NaOH. The column for the desalting was a HiPrep 26/10 Desalting with a linear gradient of 0.15 M NH_4HCO_3 buffer. HPLC purification of oligonucleotides was carried out in a Waters machine equipped of a Waters 2700 sample manager, Waters 600 controller and Waters 2487 dual absorption detector. The reverse phase column used was a Phenomenex, Clarity® C18 5 μ oligo-RP, 250 x 21.20 mm. Oligonucleotides synthesis at the University of Leiden was performed

with a AKTA-oligopilot DNA synthesizer. Chiral HPLC analyses were carried out in an Agilent Technologies 1200 Series apparatus equipped with a UV-Vis photodiode array detector. The measurements were performed at 254 nm, with an injection volume of 1 µl.

_N_-(2,3-dihydroxypropyl)trifluoroacetamide (5)[6]



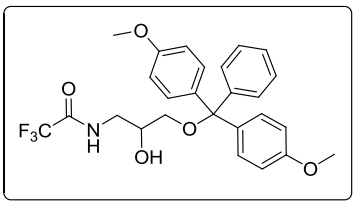
Compound **5** was synthesized according to literature procedure. The desired compound is a white solid. Yield: 1.29 g, 92%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, CD_3OD): δ = 3.80-3.72 (m, 1H, H-4), 3.52-3.41 (m, 3H, CH, CH_2), 3.33-3.26 (m, 1H, CH)

^{19}F NMR (300 MHz, CD_3OD): δ = −73.86

_N_-(_2_,3-dihydroxy-3-_O_-dimethoxytritylpropyl)trifluoroacetamide (6)[6]



Compound **6** was synthesized according to literature procedure. The desired compound is a white foam. Yield: 3.0 g, 47%

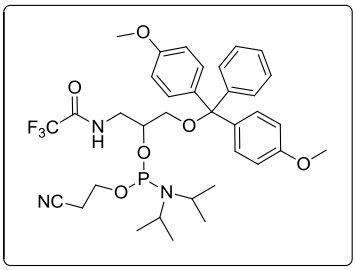
Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34-7.31 (m, 2H, Ar), 7.21-7.17 (m, 7H, Ar), 6.78-6.75 (m, 4H, Ar), 6.60 (bs, 1H), 3.87-3.81 (m, 1H), 3.71 (s, 6H), 3.57-3.48 (m, 1H), 3.27-3.18 (m, 2H), 3.09-3.04 (m, 1H), 3.36 (d, 1H, J = 4.6 Hz)

^{13}C NMR (300 MHz, CD_3OD): δ = 160.1, 159.2 (q), 146.4 (Ar), 137.3 (Ar), 131.3 (Ar), 129.3 (Ar), 129.8 (Ar), 127.8 (Ar), 117.5 (q), 114.1, 87.52, 70.1, 66.8, 55.7, 44.5

^{19}F NMR (300 MHz, $CDCl_3$): δ = −76.30

_N_-(2,3-dihydroxypropyl-2-_O_-cyanoethyl-_N_,_N_-diisopropylphosphoramidite-3-_O_-dimethoxytritylpropyl)trifluoroacetamide (4)[6]



Compound **4** was synthesized according to literature procedure. The desired compound was obtained as a white powder. Yield: 420 mg, 64%

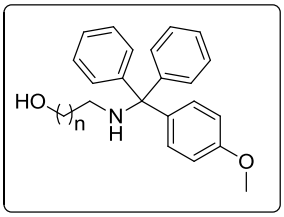
Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, CD_3OD): δ = 7.32-7.10 (m, 9H, Ar), 6.7 (m, 4H, Ar), 6.68 (br. s, 1H), 3.98 (m, 2H), 3.84 (m, 1H), 3.72 (s, 6H), 3.58 (m, 2H) 3.30 (m, 1 H), 3.19 (d, 1H), 3.14 (dd, 1H), 3.05 (dd, 1H), 2.57, 2.38 (t, 2H), 1.10 (m, 12H)

^{31}P NMR (300 MHz, CD_3OD): 150.6 (s)

MS: 712.22 ($M+Na^+$)

***N*-[(methoxyphenyl)diphenylmethyl]-2-aminoethanol (9)**[28]



Compound **9** was synthesized according to literature procedure. The desired product was obtained as a white solid. Yield: 1.6 g, 73%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41-7.08 (m, 12H, Ar), 7.76-7.73 (m, 2H Ar), 3.71 (s, 3H), 3.61 (t, 2H, J = 5.2 Hz), 2.28 (t, 2H, J = 5.3 Hz), 1.88 (bs, 2H)

***N*-[(methoxyphenyl)diphenylmethyl]-3-aminopropanol (11)**[5]

Compound **11** was synthesized according to literature procedure. The desired product was obtained as a white solid. Yield: 2.0 g, 87%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46-7.42 (m, 4H, Ar), 7.36-7.27 (m, 6H, Ar), 7.23-7.18 (m, 2H, Ar), 6.84-6.80 (m, 2H, Ar), 3.89 (t, 2H, J = 5.4 Hz), 3.79 (s, 3H), 2.41 (t, 2H, J = 5.9 Hz), 1.71 (q, 2H, J = 5.8 Hz)

^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 156.9, 144.6, 136.4, 128.7, 127.4, 126.90, 125.4, 112.2, 69.9, 62.6, 54.2, 42.3, 31.3

***N*-[(methoxyphenyl)diphenylmethyl]-5-aminopentanol (10)**[5]

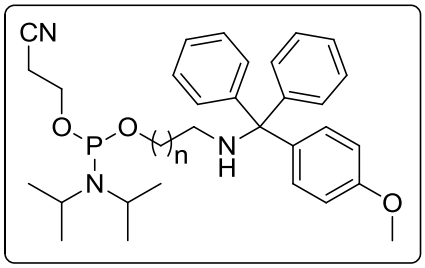
The name method as for **11** was employed. Yiled 3.9 g, 79%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40-7.37 (m, 4H, Ar), 7.30-7.27 (m, 2H, Ar), 7.20-7.16 (m, 4H, Ar), 7.11-7.07 (m, 2H, Ar), 6.74-6.70 (m, 2H, Ar), 4.70 (s, 3H), 3.52 (t, 2H, J = 6.6 Hz), 2.05 (t, 2H, J = 6.6 Hz), 1.47-1.39 (m, 6H), 1.33-1.37 (m, 2H)

^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 156.7, 145.5, 137.4, 128.7, 127.5, 126.7, 125.1, 112.0, 69.3, 61.7, 54.1, 42.5, 31.6, 29.6, 22.5

***N*-[(methoxyphenyl)diphenylmethyl]-2-aminoethanol-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (1)**



Compound **9** (1.6 g, 4.8 mmol, 1eq), was dissolved in 15 ml of THF, and cooled to −70˚C. Diisopropylethyl amine (1.5 ml, 9.6 mmol, 3 eq) and 2-cyanoethyl diisopropylphosphoramidite (7 ml of a 1M solution in ACN, 7 mmol, 1.5 eq) were added to the reaction mixture and stirred at room temperature for 1.5 h. The reaction mixture was concentrated to driness and the resulting oil was purified by column chromatography (SiO_2, petroleoum ether: acetone 80:20→70:30) The desired product resulted as a solid. Yield: 1.2 g, 47%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.07 (m, 12H, Ar), 6.75-6.71 (m, 2H, Ar), 3.76-3.64 (m, 7H), 3.56-3.44 (m, 2H), 2.49 (t, 2H, J = 6.7 Hz), 2.30-2.28 (m, 2H), 1.9 (bs, 1H), 1.09 (t, 12H, J = 6.7 Hz)

_N_-[(methoxyphenyl)diphenylmethyl]-5-aminopentanol-_O_-Cyanoethyl-_N_,_N_-diisopropylphosphoramidite (3)

The same method as for **1** was employed. Yield: 1.0 g, 63%

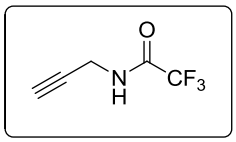
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73-7.70 (m, 4H, Ar), 7.59-7.56 (m, 2H, Ar), 7.30-7.10 (6H, Ar), 6.87-6.84 (m, 2H, Ar), 3.80-3.59 (m, 4H), 3.51-3.37 (m, 5H), 2.34 (t, 2H, J = 6.4 Hz), 1.85-1.79 (m, 2H), 1.66-1.41 (m, 7H), 1.26-1.22 (m, 12H)

^{31}P NMR (101 MHz, $CDCl_3$): δ = 147.8

_N_-(2-propynyl)trifluoroacetamide (13)[17]

Compound **13** was synthesized according to literature procedure resulting in a clear oil. Yield: 7.9 g, 57%



Characterization of the product fully agreed with data from literature.

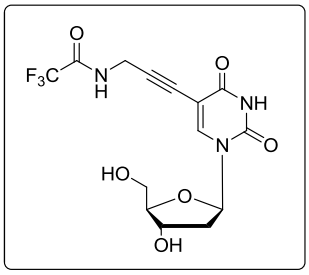
^{1}H NMR(400 MHz, $CDCl_3$): δ = 6.72 (bs, 1H), 4.15 (ddd, 2H, J = 2.60 Hz, J = 5.4 Hz, J = 0.5 Hz), 2.33(1H, J = 2.6 Hz)

^{19}F NMR (376.3 MHz, $CDCl_3$): δ = −76.45

^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 157.1 (q, $COCF_3$, J = 37.9 Hz), 115.5 (q, CF_3, J = 287.7 Hz), 73.0, 29.6

5-[1-(_N_-trifluoroacetyl-3-amino-1-propynyl)]deoxyuridine (14)[12]

This compound was prepared with a slightly modified procedure than those previously published.



5-Iodo-2'-deoxyuridine (2.00 g, 5.65 mmol, 1 eq) and CuI (108.6 mg, 0.57 mmol, 0.1 eq) were suspended in 30 ml of anhydrous DMF. The mixture was stirred for 30 minutes protected from light. To this mixture triethylamine (1.60 ml, 11.50 mmol, 2 eq), *N*-propynyltrifluoroacetamide (2.60 g, 17.20 mmol, 3 eq) and *tetrakis*(triphenylphosphine)palladium(0) (330 mg, 0.28 mmol, 0.05 eq) were added in this order. The yellow clear solution was stirred for 18 h protected from light giving an orange solution. The solvents were then removed *in vacuo* and the residue was dissolved in 70 ml of THF. Solid potassium carbonate (approximately 4 g) was added and the suspension was stirred in an open flask for 2 hours. The salts were filteringed and the liquid was concentrated to dryness. The gummy residue was treated with 30 ml of methanol causing the precipitation of a yellow solid. This solid was filtered and discarded. The remaining liquid was concentrated to dryness and the methanol treatment was repeated until no solid precipitates (usually two or three times). The filtrate was concentrated in vacuum again and the residue was recrystallized from 30 ml of acetonitrile. After several hours in the freezer, the desired product was obtained as a yellow powder. Yield: 1.6 g, 58%

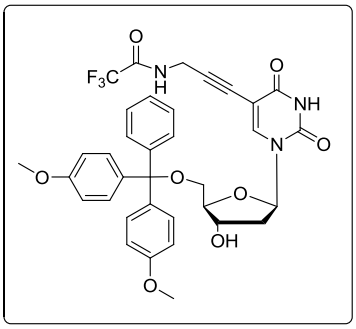
Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, MeOD): δ = 8.22 (s, 1H), 6.13 (t, 1H, J = 6.6 Hz), 4.30-4.27 (m, 1H), 4.17 (s, 2H), 3.85-3.82 (m, 1H), 3.66 (ddd, 2H, J = 3.0 Hz, J = 12.1 Hz, J = 16.1 Hz), 2.24-2.04 (m, 2H)

^{13}C NMR (100.6 MHz, MeOD): δ = 163.8, 157.7 (q, $COCF_3$, J = 37.3 Hz), 150.3, 144.9, 116.6 (q, CF_3, J = 286.7 Hz), 98.7, 88.3, 87.53, 86.2, 75.3, 71.1, 61.7, 40.9, 29.9

5-[1-(N-trifluoroacetyl-3-amino-1-propynyl)]-5'-*O*-dimethoxytrityldeoxyuridine (16)[12]

Compound **14** (800 mg, 2.1 mmol, 1 eq), was azeotropically dried 3 times with 2 ml of toluene and then dissolved in 10 ml of pyridine. DMT-Cl (862 mg, 2.54 mmol, 1.2 eq), dissolved in 10 ml of pyridine, was slowly added to the solution of compound 4. After 4h, 4 ml of MeOH was added and the reaction mixture was concentrated to dryness. The resulting oil was purified by column chromatography (SiO_2, MeOH:CH_2Cl_2, 2:98→3:97). The desired product was obtained as a white powder. Yield: 1.3 g, 80%



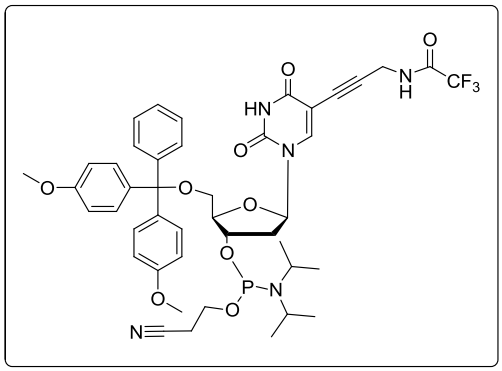
Characterization of the product fully agreed with data from literature.

^{1}H NMR(400 MHz, $CDCl_3$): δ = 8.23 (s, 1H), 7.43-7.40(m, 2H, Ar), 7.33-7.20 (m, 7H, Ar), 6.94 (bs, 1H, NH), 6.86-6.82 (m, 4H, Ar), 6.36-6.33 (m, 1H), 4.61-4.58 (m, 1H), 4.13-4.10 (m, 1H), 3.98-3.86 (m, 2H), 3.78 (s, 6H), 3.42-3.32 (m, 2H), 2.56-2.50 (m, 1H), 2.36-2.29 (m, 1H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 161.6, 157.6 (Ar), 157.7 (Ar), 155.5 (q, $COCF_3$, J = 37.6 Hz) 148.7, 143.5 (Ar), 142.5, 134.4 (Ar), 129.0 (Ar), 128.9 (Ar), 127.0 (Ar), 126.8 (Ar), 126.0 (Ar), 114.5 (q, CF_3, J = 289.4 Hz), 112.3 (Ar), 98.0, 86.4, 86.1, 86.0, 84.0, 74.2, 71.4, 62.5, 54.2, 40.6, 29.4

5-[1-(N-trifluoroacetyl-3-amino-1-propynyl)]-3'-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-5'-*O*-dimethoxytrityldeoxyuridine (17)[12]

Compound **16** (1.1 g, 1.6 mmol, 1eq) was azeotropically dried 3 times with 2 ml of toluene. It was dissolved in 20 ml of CH_2Cl_2 and cooled to 0 ˚C. Diisopropylethylamine (0.68 ml, 4.8 mmol, 3 eq) was added followed by slow addition of 2-cyanoethyl diisopropylphosphoramidite (2.8 ml of a 1 M solution in acetonitrile, 1.3 eq). After 3 h the reaction mixture was diluted with 100 ml of ethyl acetate and washed with 100 ml of 5% aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under vacuum at 35 ˚C. The resulting yellow oil was purified by column chromatography (SiO_2, DCM:MeOH:TEA, 96:3:1). The desired product resulted in a transparent solid, containing 40% of hydrolyzed 2-cyanoethyl diisopropylphosphoramidite was present with the product. Yield: 880 mg, 63%

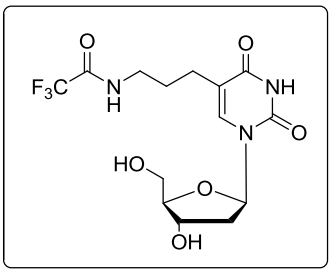


Characterization of the product fully agreed with data from literature.

^{1}H NMR(400 MHz, $CDCl_3$): δ = 8.17, 8.13 (2s, 1H), 7.39-7.36 (m ,2H), 7.28-7.13 (m, 7H), 6.80-7.76 (m, 4H), 6.29-6.24 (m, 1H), 4.59-5.53 (m, 1H), 4.14-4.08 (m, 1H), 3.86-3.32 (m, 13H), 3.27-3.22 (m, 1H), 2.56-2.46 (m, 2H), 2.35 (t, 1H, J = 6.3 Hz), 2.31-2.24 (m, 1H), 1.22-1.19 (m, 12H)

^{31}P NMR (162 Hz, $CDCl_3$): δ = 148.9, 148.6

5-[1-(N-trifluoroacetyl-3-aminopropyl)]deoxyuridine (19)



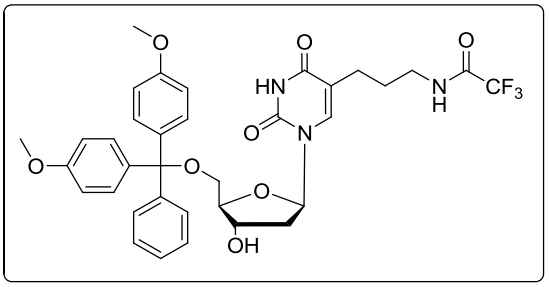
The mononucleoside **14** (2.0 g, 5.3 mmol) and 10% Pd/C (564 mg, 0.53 mmol of Pd) were suspended in 30 ml of dry methanol and hydrogenated under 10 bar of H_2 for 26 hours. The resulting suspension was filtered through a pad of celite and concentrated *in vacuo*. The crude was purified via a short column (SiO_2, EtOAc:MeOH, 90:10) to yield, after removal of the solvent, the title product as a pale yellow solid. Yield: 1.78 g, 90%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.28 (bs, NH), 9.40 (bs, NH), 7.67 (s, 1H), 6.15 (t, 1H, J = 6.9 Hz), 5.24 (bs, OH), 5.02 (bs, OH) 4.24-4.21 (m, 1H), 3.76-3.74 (m, 1H), 3.60-3.50 (m, 2H), 3.16 (t, J = 6.8 Hz), 2.25-2.02 (m, 4H), 1.67-1.60 (m, 2H)

^{13}C NMR (100.6 MHz, MeOD): δ = 165.7, 158.7 (q, $COCF_3$, J = 39.7 Hz) 151.4, 137.93, 116.0 (q, CF_3, J = 285.57 Hz), 113.9, 86.5, 85.1, 70.3, 60.9, 38.7, 38.6, 26.2, 23.6

5-[1-(N-trifluoroacetyl-3-aminopropyl)]-5'-*O*-dimethoxytrityldeoxyuridine (20)[12]



The mononucleoside **16** (2.0 g, 2.94 mmol) and 10% Pd/C (313 mg, 0.294 mmol of Pd) were suspended in 30 ml of dry methanol and hydrogenated under 10 bar of H_2 for 24 hours. The resulting suspension was filtered through a pad of celite and concentrated *in vacuo*. The crude product was purified via a short column (SiO_2, CH_2Cl_2:MeOH:NEt_3, 89:10:1) to yield, after removal of the solvent, the title product as a pale yellow solid. Yield: 1.91 g, 95%

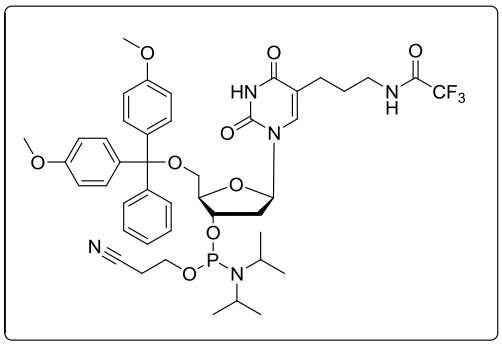
Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.67 (s, 1H), 7.56 (bt, 1H), 7.38-7.35 (m, 2H, Ar), 7.32-7.23 (m, 7H, Ar), 6.84-6.81 (m, 4H, Ar), 6.49-6.44 (m, 1H), 4.63-4.59 (m, 1H), 4.11-4.08 (m, 1H), 3.78 (s, 6H), 3.37 (ddd, 2H, J = 3.0 Hz, 10.8 Hz, J = 38.8 Hz), 3.12-2.98 (m, 2H), 2.48-2.41 (m, 1H), 2.38-2.29 (m, 1H) 1.87-1.79 (m, 1H), 1.69-1.59 (m, 1H) 1.38-1.29 (m, 2H)

^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 165.1, 159.2 (Ar), 157.6 (q), 150.9, 144.5 (Ar), 137.9, 135.6 (Ar), 130.6 (Ar), 130.6 (Ar), 128.7 (Ar), 128.4 (Ar), 127.7 (Ar), 116.5 (q), 114.3, 113.7 (Ar), 87.3, 86.9, 85.3, 72.8, 63.9, 55.7, 41.5, 38.8, 28.6, 23.5

^{19}F NMR (282 MHz, $CDCl_3$): δ = −77.78

5-[1-(*N*-trifluoroacetyl-3-aminopropyl)]-3'-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-5'-*O*-dimethoxytrityldeoxyuridine (21)[12]



Compound **20** (900 mg, 1.32 mmol, 1 eq) was azeotropically dried once with acetonitrile and 3 times with toluene. It was dissolved in 20 ml of DCM and diisopropylethylamine (0.9 ml, 5.3 mmol, 4 eq) was added. After cooling to 0 ˚C 2-cyanoethyl diisopropylphosphoramidite (2.6 ml of a 1 M solution in acetonitrile, 2 eq) was added dropwise. After this addition the reaction was slowly warmed to room temperature and stirred for 3 h. The reaction mixture was then diluted with 100 ml of ethyl acetate and washed with 50 ml of 5% $NaHCO_3$ and 50 ml of brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* at 35 ˚C. The resulting yellow oil was purified by silica column chromatography using DCM:EtOAc:TEA 60:39:1 mixture. Yield: 600 mg, 67%

Characterization of the product fully agreed with data from literature.

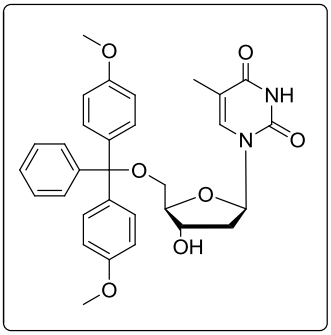
^{1}H NMR: (400 HMz, $CDCl_3$): δ = 7.62, 7.58 (2s, 1H), 7.31-7.17 (m, 9H), 6.79-6.75 (m, 4H), 6.38-6.33 (m, 1H), 4.64-4.60 (m, 1H), 4.12-4.05 (2m, 1H), 3.80-3.45 (m, 12H), 3.25 (dt, 1H, J = 10.1 Hz, 2.5 Hz), 3.06-2.89 (m, 2H), 2.56 (t, 1H, J = 6.0 Hz), 2.53-2.41 (m, 1H), 2.34 (t, 1H, J = 6.0 Hz), 2.33-2.56 (m, 1H), 1.83-1.76 (m, 1H), 1.65-1.56 (m, 1H), 1.26-1.20 (m, 2H), 1.11-1.08 (m, 10H), 0.97 (d, 2H, J = 6.6 Hz)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 162.6, 156.9, 155.2, 148.3, 148.2, 142.2, 135.6, 135.5, 133.3, 128.4, 126.5, 126.4, 126.1, 125.5, 125.4, 115.8, 115.5, 115.5, 112.6, 112.1, 113.0, 111.1, 85.0, 83.9, 83.5, 82.9, 82.7, 71.8, 71.7, 71.5, 71.4, 61.0, 61.0, 56.4, 56.3, 56.2, 56.1, 53.4, 41.5, 41.4, 41.4, 41.3, 38.2, 38.1, 36.4, 26.4, 22.7, 21.0, 18.5, 18.3

^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 140.9, 148.7

^{19}F NMR (376.3 MHz, $CDCl_3$): δ = −76.50

5'-*O*-dimethoxytrityl-deoxythymidine (22)[18]



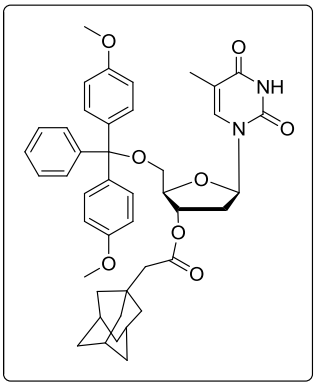
Compound **22** was synthesized according to literature procedure. The desired product was isolated as a slightly yellow powder. Yield: 13.8 g, 85%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (s,1H), 7.33-7.31 (m, 2H, Ar), 7.23-7.13 (m, 7H, Ar), 6.72 (d, 4H, Ar, J = 8.9 Hz), 6.36 (t, 1H, J = 6.9 Hz), 4.53-4.50 (m, 1H), 4.02-4.00 (m, 1H), 3.65 (s, 6H), 3.36-3.24 (ddd, 2H, J = 3.1 Hz, J = 10.5 Hz, J = 20.8 Hz), 2.38-2.34 (m, 1H), 2.23-2.20 (m, 1H), 1.33 (s, 3H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 163.3, 157.6, 149.8, 143.4, 134.9, 134.4, 134.4, 129.1, 127.1, 126.9, 126.0, 112.2, 110.2, 85.8, 85.4, 83.8, 71.4, 62.7, 54.2, 44.7, 39.9, 10.8

3'-*O*-Adamantaneacetyl-5'-*O*-dimethoxytrityl-deoxythymidine (23)[18]

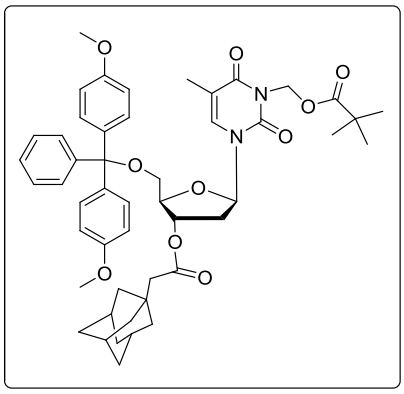


Compound **23** was synthesized according to literature procedure. The desired compound was obtained as white foam. Yield: 12.8 g, 88%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.47 (bs, 1H), 7.53 (s, 1H), 7.33-7.31 (m, 2H), 7.23-7.13 (m, 7H), 6.75 (d, 4H, J = 8.7 Hz), 6.37 (t, 1H, J = 7.1 Hz), 5.38-5.37 (m, 1H), 4.06-4.04 (m, 1H), 3.70 (s, 6H), 3.40 (m, 1H), 2.40-2.34 (m, 2H), 2.01 (bs, 2H), 1.88 (bs, 3H), 1.63-1.52 (m, 12H), 1.30 (s, 3H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.2, 162.9, 157.7, 149.7, 143.2, 134.8, 134.7, 134.2, 129.1, 129.1, 127.1, 127.0, 126.2, 112.3 , 110.6, 86.1, 83.4, 83.2, 73.7, 62.7, 54.2, 47.6, 41.4, 37.0, 35.6, 32.0, 27.5, 10.6

3'-*O*-Adamantaneacetyl-5'-*O*-dimethoxytrityl-*N*-pivaloyloxymethyl-deoxythymidine (24)[18]



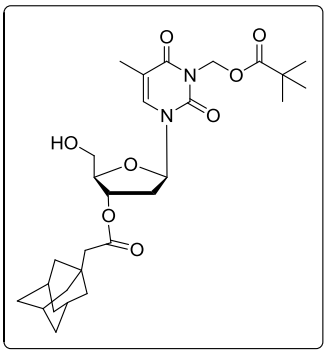
Compound **24** was synthesized according to literature procedure. The desired compound was obtained as a white foam. Yield: 12.0 g, 81%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (s, 1H), 7.33-7.30 (m, 2H), 7.22-7.19 (m, 6H), 7.16-7.12 (m,1H), 7.76-6.74 (d, 4H, J = 8.9 Hz), 6.38 (t, 1H, J = 7.1 Hz), 5.89-5.84 (m, 2H), 5.40-5.37 (m, 1H), 4.07-4.05 (m, 1H), 3.68 (s, 6H), 3.40 (m, 2H), 2.40-2.37 (m, 2H), 1.99 (s, 2H), 1.87 (bs, 3H), 1.62-1.50 (m, 12H), 1.33 (s, 3H), 1.10(s, 9H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 175.7, 165.4, 160.7, 157.0, 148.6, 142.5, 133.5, 133.4, 132.7, 128.3, 126.4, 125.4, 111.5, 108.9, 85.4, 83.3, 72.8, 63.4, 61.8, 53.4, 46.8, 40.7, 37.0, 36.3, 34.9, 31.2, 26.7, 25.3, 10.5

3'-*O*-Adamantaneacetyl-*N*-pivaloyloxymethyl-deoxythymidine (25)[18]



Compound **25** was synthesized according to literature procedure. The desired compound is a white foam. Yield: 2.0 g, 69%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.61 (s, 1H), 6.25 (t, 1H, J = 7.3 Hz), 5.91-5.85 (m, 2H), 5.30-5.26 (m, 1H), 4.05-4.03 (m, 1H), 3.87 (m, 2H), 2.35-2.31 (m, 2H), 2.03 (s, 2H), 1.90 (bs, 3H), 1.66-1.53 (m, 12H), 1.11 (s, 9H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 177.7, 171.6, 162.6, 150.4, 135.3, 110.5, 86.2, 85.3, 74.2, 65.1, 62.5, 48.6, 42.4, 38.8, 37.5, 36.6, 33.0, 28.5, 27.0, 13.2

General procedure for one synthesis cycle toward trimer (29) synthesis[18]

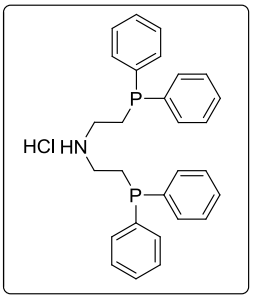
Compound **25** (266 mg, 0.5 mmol, 1 eq) and compound **21** (663 mg, 0.75 mmol, 1.5 eq) were coevaporated with acetonitrile three times and dissolved in 3.3 ml of acetonitrile. DCI (266 mg, 2.25 mmol, 4.5 eq) was added and the reaction mixture was stirred for 1 h. 200 µl of water was added and the reaction mixture was stirred for 2 minutes, 7.2 ml of 0.2 M I_2 in THF:pyridine 4:1 was added and the reaction mixture was stirred for 5 minutes. The reaction mixture was diluted with 25 ml of ethyl acetate and 5 ml of THF and washed with a saturated solution of $Na_2S_2O_3$ (20 ml), 10% aqueous $KHSO_4$ (2 x 20 ml), 10% aqueous $NaHCO_3$ (20 ml), and 1:1 brine:water (20 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness.

The protected nucleoside was dissolved in 14 ml of 0.1 M HCl solution made from AcCl in MeCN:MeOH 1:6. After 30 min, 6 ml of 0.23 M TEAA was added and the reaction changed colour from red to clear. It was diluted with 10 ml of acetonitrile and washed with heptane:diethylether 2:1 (4 x 30 ml). Ethyl acetate:THF, 5:2 was added to the water layer and it was washed with water (2 x 20 ml), a saturated solution of $NaHCO_3$ (2 x 20 ml), brine:water 1:1 (20 ml). The final organic layer was dried over $MgSO_4$, filtered and concentrated to dryness.

General procedure for deprotection and purification of trimer

Oligonucleotides were deprotected in a solution of 40% methylamine in ammonia for 3 days at 55 ˚C. The reaction mixture was concentrated to dryness at 60 ˚C, dissolved in 6 ml of water and centrifuged**.** The supernatant was purified using an ion exchange sepharose column with buffer A: 20 mM TEAA, buffer B: 2 M TEAA.

***N,N-Bis*(2-(diphenylphosphino)ethyl)ammonium chloride (30)**[29]



Compound **30** was synthesized according to literature procedure. The desired product resulted as white crystals. Yield: 27.0 g, 67%

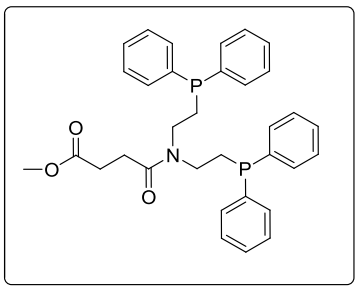
Characterization of the product fully agreed with data from literature.

^{1}H NMR: (400 HMz, $CDCl_3$): δ = 7.40-7.37 (m, 8H), 7.32-7.30 (m, 12H), 2.95-2.94 (m, 4H), 2.58-2.53 (m, 4H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 136.1 (d, J_{CP} = 12.1 Hz), 132.7 (d, J_{CP} = 19.1 Hz), 129.2, 128.8 (d, J_{CP} = 6.6 Hz), 44.2 (d, J_{CP} = 24.1 Hz), 23.8 (d, J_{CP} = 16.3 Hz)

^{31}P NMR (161.9 MHz, $CDCl_3$): δ = −21.0

Methyl-4-(*N,N-bis*(2-(diphenylphosphino)ethyl)amino)-4-oxobutanoate (31)[29]



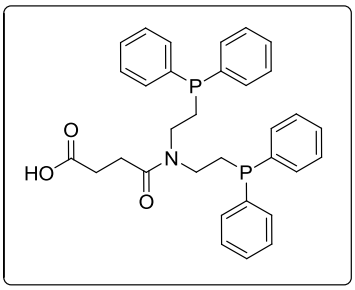
Compound **31** was synthesized according to literature procedure resulting in a slight yellow solid. Yield: 1.8 g, 72%

Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34-7.22 (m, 20H, Ar), 3.60 (s, 3H), 3.36-3.30 (m, 2H), 3.22-3.16 (m, 2H, C*H*$_2$), 2.47 (t, J = 6.6 Hz, 2H), 2.23-2.14 (m,6H)

^{31}P NMR (162 MHz, $CDCl_3$): δ = −20.9, −22.1

4-(*N,N-bis*(2-(diphenylphosphino)ethyl)amino)-4-oxobutanoic acid (32)[29]



Compound **32** was prepared according to literature procedure resulting in a white powder. Yield: 1.0 g, 70%

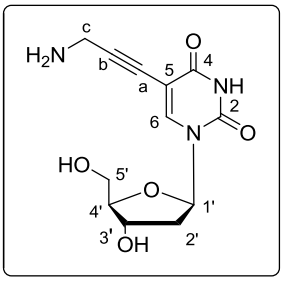
Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24-7.14 (m, 20H), 3.21-3.19 (m, 2H), 3.0 (m, 2H), 2.23-1.98 (m, 8H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 178.4, 171.9, 136.9 (d, J_{CP} = 13.0 Hz), 136.2 (d, J_{CP} = 12.4 Hz), 131.6 (d, J_{CP} = 19.0 Hz), 128.0, 127.7, 127.6, 127.5, 127.4 (d, J_{CP} = 6.6 Hz), 44.2 (d, J_{CP} = 26.2 Hz), 43.6 (d, J_{CP} = 24.0 Hz), 30.9, 28.1, 26.4 (d, J_{CP} = 15.5 Hz), 25.1 (d, J_{CP} = 14.7 Hz)

^{31}P NMR (121.47 MHz, $CDCl_3$): δ = −20.4, −21.6

5-(3-amino-1-propynyl)-2'-deoxyuridine (15)[11]



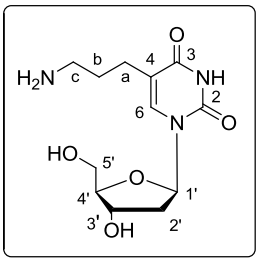
The protected base **14** (100 mg, 0.27 mmol) was dissolved in 10 ml of concentrated ammonia and stirred for 12 h. Removal of the liquid furnished the desired deprotected base **15** in quantitative yield as a yellowish oil. This product was used without any purification for the couplings with the phosphinocarboxylic acids. However ^{19}F NMR spectroscopy showed the presence of the trifluoroacetate anion at –75 ppm. This impurity was removed by column chromatography (SiO_2, CH_2Cl_2/MeOH, 70:30), to quantitatively yield the pure product as a yellow solid.

Characterization of the product fully agreed with data from literature.

^{1}H NMR(400 MHz, MeOD): δ 7.88 (s, 1H, H-6), 5.90 (t, 1H, J = 6.4 Hz, H-1'), 4.13-4.09 (m, 1H, H-3'), 3.74-3.71 (m, 1H, H-4'), 3.68 (s, 2H, H-c), 3.54-3.41 (m, 2H, H-5'), 2.15-2.09 (m, 1H, H-2'), 2.05-1.98 (m, 1H, H-2')

X-ray diffraction Crystals suitable for X-ray diffraction were grown from evaporation of dichloromethane/methanol solution at room temperature.

5-(3-aminopropyl)-2'-deoxyuridine (33)[11]

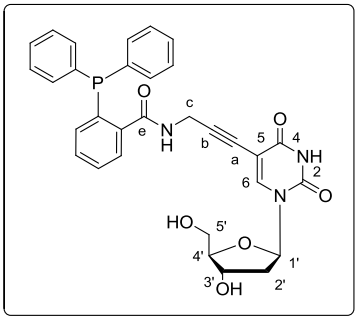


The protected base **19** (114.6 mg, 0.3 mmol) was dissolved in 6 ml of concentrated ammonia and stirred for 12 hours. Removal of the liquid furnished the desired deprotected base **33** in quantitative yield as a yellowish oil. This product was used without any purification for the couplings with the phosphinocarboxylic acids.

Characterization of the product fully agreed with data from literature.

^{1}H NMR (300 MHz, D_2O): δ = 7.57 (s, 1H, H-6), 6.12 (dd, 1H, J = 6.8, H-1'), 4.33-4.29 (m, 1H, H-3'), 3.89-3.86 (m, 1H, H-4'), 3.71-3.67 (m, 1H, H5'), 3.63- 3.59 (m, 1H, H-5'), 2.87-2.83 (m, 2H, H-c), 2.28-2.18 (m, 4H, H-2', H-a), 1.70 (tt, 2H, J = 7.8 Hz, 7.5 Hz, H-b).

(ortho) (34)



o-Diphenylphosphinobenzoic acid (306 mg, 1.2 mmol, 1.2 eq), EDC·HCl (230 mg, 1.2 mmol, 1.2 eq) and NHS (173 mg, 1.5 mmol, 1.5 eq) were dissolved in 20 ml of dry degassed DMF and stirred for 2 h.

The nucleoside **15** (281 mg, 1.0 mmol, 1 eq) was dissolved in 10 ml of previously degassed 0.1 M $NaHCO_3$ aqueous solution and the activated phosphine was added by syringe. A white precipitate formed but redissolved after a few minutes, giving a clear orange mixture, which was stirred for 12 h. Water (50 ml), $CHCl_3$ (50 ml) and brine (10 ml) were added to the reaction mixture giving a biphasic system. The organic phase was separated and the aqueous layer extracted twice with $CHCl_3$ (50 ml). The combined organic layers were washed with water (100 ml) and dried over Na_2SO_4. After filtration, the solvent was removed under vacuum (heating to 60 ºC was necessary to remove the last traces of DMF) giving a gummy white residue. This crude was purified by column chromatography (SiO_2, $CHCl_3$:MeOH, 90:10) to give the title product as a white foam after solvent removal. Yield: 171 mg, 30%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3411, 3054, 1700 ν(C=O), 1653 ν(C=O), 1584, 1465, 1434, 1282, 1093, 747, 697

^{1}H NMR (500 MHz, CD_3OD): δ = 8.34 (s, 1H, H-6), 7.60 (ddd, 1H, J = 7.6 Hz, 3.6 Hz, 1.0 Hz, Ar), 7.43 (td, 1H, J = 7.5 Hz, 1.1 Hz, Ar), 7.27-7.37 (m, 11 H, Ar), 7.01(ddd, 1H, J = 7.6 Hz, 4.1 Hz, 0.7 Hz), 6.27 (t, 1H, H-1', J = 6.6 Hz), 4.43-4.41 (m, 1H, H-3'), 4.13 (s, 2H, H-c), 3.97 (dd, 1H, H-4', J = 6.5 Hz, 3.2 Hz), 3.83 (dd, 1H, H-5', J = 12.0 Hz, 3.1 Hz), 3.75 (dd, 1H, H-5', J = 12.0 Hz, 3.5 Hz), 2.36-2.31 (m, 1H, H-2'), 2.26-2.21 (m, 1H, H-2')

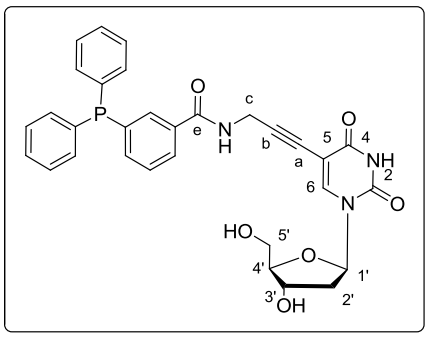
^{13}C NMR (126 MHz, CD_3OD): δ = 171.4 (e), 164.8 (4), 151.2 (2), 145.5 (6), 142.0 (d, C, J_{CP} = 24.5 Hz); 138.9 (d, J_{CP} = 8.6 Hz), 138.8 (d, J_{CP} = 13.6 Hz), 135.2-128.6 (Ar), 100.0 (5), 89.8 (a); 89.2 (4'), 87.1 (1'), 75.6 (b), 72.1 (3'), 62.7 (5'), 41.8 (2'), 31.0 (c)

^{31}P NMR (162 MHz, CD_3OD): δ = –9.5

MS (ES+): 592.24 (M+Na$^+$), HRMS calculated for $C_{31}H_{28}N_3NaO_6P$, 592.1613, found 592.1615

MP**:** Decomposes over 135 ºC

(meta) (35)



The same method as for **34** was employed using *m*-diphenylphosphinobenzoic acid. Yield: 107 mg, 63%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3423, 1686 ν(C=O), 1542, 1459, 1434, 1283, 1093, 746, 696

^{1}H NMR (400 MHz, CD_3OD): δ = 8.31 (s, 1H, H-6), 7.90 (s, 1H), 7.86 (dt, 1H, J = 8.1 Hz, 1.5 Hz), 7.82 (dt, 1H, J = 7.7 Hz, 1.3 Hz), 7.45 (tdd, 1H, J = 7.7 Hz, 1.3 Hz, 0.6 Hz), 7.39-7.26 (m, 10 H), 6.23 (t, 1H, H1', J = 6.6 Hz), 4.39-4.37 (m, 1H, H3'), 4.31 (s, 2H, H-c), 3.93 (dd, 1H, H-4', J = 6.6 Hz, 3.3 Hz), 3.80 (dd, 1H, H-5', J = 12.0 Hz, 3.1 Hz), 3.72 (dd, 1H, H-5', J = 12.0 Hz, 3.5 Hz), 2.33-2.27 (m, 1H, H-2'), 2.24-2.219 (m, 1H, H-2')

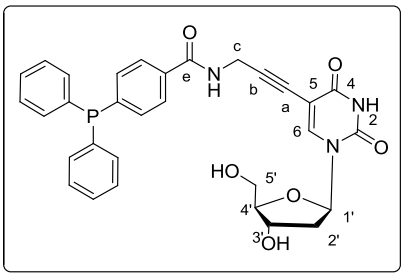
^{13}C NMR (101 MHz, CD_3OD): δ = 169.3 (C, e), 164.7 (4), 151.1 (2), 145.5 (6), 140.0 (d, J_{CP} = 13.5 Hz), 138.0 (d, J_{CP} = 10.9 Hz), 137.7 (d, J_{CP} = 15.5 Hz), 135.4 (d, J_{CP} = 7.5 Hz), 134.9-128.7 (Ar), 99.9 (5), 90.0 (a), 89.1 (4'), 87.0 (1'), 75.2 (b), 72.0 (3'), 62.6 (5'), 41.7 (2'), 31.0 (c)

^{31}P NMR (162 MHz, CD_3OD): δ = –5.5

MS (ES+): 592.03 (M+Na^{+}), HRMS calculated for $C_{31}H_{28}N_3NaO_6P$, 592.1613, found 592.1605

MP: Decomposes over 140 ºC

(para) (36)



The same method as for **34** was employed using *p*-diphenylphosphinobenzoic acid. 171 mg, 30%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3394; 3054; 2963; 1691 ν(C=O), 1535, 1465, 1434, 1263, 1094, 802, 746, 696

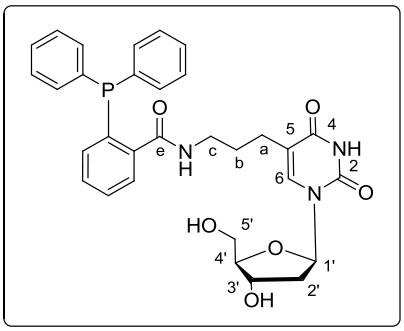
^{1}H NMR (300 MHz, CD_3OD): δ = 8.32 (s, 1H, H-6), 7.81-7.78 (m, 2H), 7.39-7.26 (m, 12 H), 6.23 (t, 1H, H-1', J = 6.6 Hz), 4.40-4.35 (m, 3H, H-3', H_c), 3.93 (dd, 1H, H-4', J = 6.6 Hz, 3.3 Hz), 3.80 (dd, 1H, H-5', J = 12.0 Hz, 3.1 Hz), 3.72 (dd, 1H, H-5', J = 12.0 Hz, 3.5 Hz), 2.34-2.20 (m, 2H, H-2')

^{13}C NMR (75 MHz, CD_3OD): δ = 169.3 (e), 164.7 (4), 151.1 (2), 145.5 (6), 144.0 (d, J_{CP} = 8.9 Hz), 137.8 (d, J_{CP} = 6.0 Hz), 135.2-128.3 (Ar), 99.9 (5), 90.0 (a), 89.2 (4'), 87.0 (1'), 75.2 (b); 72.0 (3'), 62.6 (5'), 41.7 (2'), 31.0 (c)

^{31}P NMR (121 MHz, CD_3OD): δ = –5.5

MS (ES+): 592.02 (M+Na^{+}), HRMS calculated for $C_{31}H_{28}N_3NaO_6P$, 592.1613, found 592.1617

(ortho) (37)



The same method as for **34** was employed starting from 0.3 mmol (85.84 mg) of mononucleoside **33**. Yield: 80 mg, 46%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3403, 3053, 2930, 1671 ν(C=O), 1475, 1434, 1275, 1055, 746, 698

^{1}H NMR (300 MHz, CD_3OD): δ = 7.85 (s, 1H, H-6), 7.52 (ddd, 1H, J = 7.6 Hz, 3.6 Hz, 1.3 Hz), 7.42 (dt, 1H, J = 7.8 Hz, 13 Hz), 7.36-7.23 (m, 11 H), 6.96 (ddd, 1H, J = 4.1 Hz, 3.6 Hz, 0.9 Hz), 6.27 (t, 1H, H-1', J = 6.8 Hz), 4.41-4.36 (m, 1H, H-3'), 3.90 (dd, 1H, H-4', J = 6.9 Hz, 3.5 Hz), 3.77 (dd, 1H, H-5', J = 12.0 Hz, 3.1 Hz), 3.68 (dd, 1H, H-5', J = 12.0 Hz, 3.8 Hz), 3.18 (t, 2H, H-c, J = 6.7 Hz), 2.28-2.21 (m, 4H, H-2', H_a), 1.63-1.55 (m, 2H, H-b)

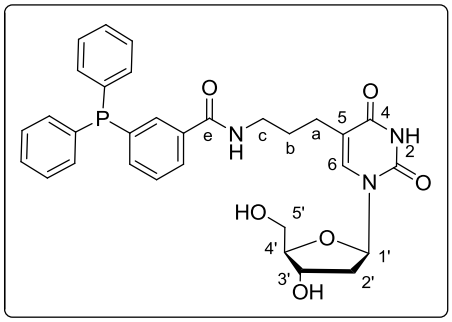
^{13}C NMR (75 MHz, CD_3OD): δ = 172.2 (e), 166.0 (4), 152.3 (2), 138.9 (6), 138.6-128.4, 114.8 (5), 88.9 (4'), 86.4 (1'), 72.2 (3'), 62.9 (5'), 41.2 (2'), 39.7 (c), 28.9 (b), 25.0 (a)

^{31}P NMR (121 MHz, CD_3OD): δ = –10.1

MS (ES+): 572.19 (M–H), 573.20 (M), HRMS calculated for $C_{31}H_{31}N_3O_6P$, 573.1950; found 572.1942

X-ray diffraction Crystals suitable for X-ray diffraction were grown from evaporation of an acetonitrile solution at room temperature.

(meta) (38)



The same method as for **34** was employed starting from 0.3 mmol (85.84 mg) of mononucleoside **33**. Yield: 142 mg, 82%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3421, 3054, 2926, 1675 ν(C=O),_1539, 1465, 1434, 1275, 1093, 744, 696

^{1}H NMR (300 MHz, CD_3OD): δ = 7.87 (s, 1H, H-6), 7.85-7.77 (m, 2H), 7. 44 (td, 1H, J = 7.6 Hz, 0.7 Hz), 7.37-7.26 (m, 11 H), 6.27 (t, 1H, H-1', J = 6.7 Hz), 4.41-4.37 (m, 1H, H-3'), 3.90 (dd, 1H, H-4', J = 6.7 Hz, 3.4 Hz), 3.78 (dd, 1H, H-5', J = 12.1 Hz, 3.1 Hz), 3.69 (dd, 1H, H-5', J = 12.1 Hz, 3.7 Hz), 3.35 (t, 2H, H-c, J = 6.8 Hz), 2.37 (t, 2H, H-a, J = 7.2 Hz), 2.25-2.21 (m, 2H, H-2'), 1.83-1.74 (m, 2H, H-b)

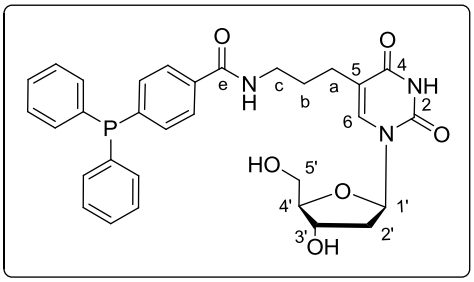
^{13}C NMR (75 MHz, CD_3OD): δ = 169.9 (e), 166.1 (4), 152.2 (2), 138.8 (6), 139.9-128.6, 114.8 (5), 88.9 (4'), 86.4 (1'), 72.2 (3'), 62.8 (5'), 41.3 (2'), 49.1 (c), 29.2 (b), 25.1 (a)

^{31}P NMR (121 MHz, CD_3OD): δ = –5.4

MS (ES+): 596.11 (M+Na$^+$), 612.11 (M+O+Na$^+$), HRMS calculated for $C_{31}H_{32}N_3NaO_6P$, 596.1926; found 596.1908

X-ray diffraction Crystals suitable for X-ray diffraction were grown by evaporation of an acetonitrile solution at room temperature

(para) (39)



The same method as for **34** was employed using *p*-diphenylphosphinobenzoic acid. Yield: 103 mg, 60%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3423, 3054, 2927, 1686 ν(C=O), 1475, 1434, 1276, 1093, 746, 697

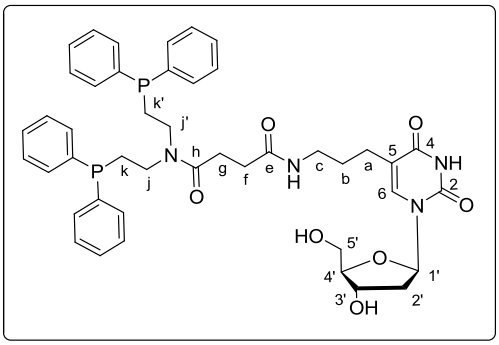
^{1}H NMR (300 MHz, CD_3OD): δ = 7.89 (s, 1H, H-6), 7.79 (d, 1H, J = 1.4 Hz), 7.76 (d, 1H, J = 1.4 Hz), 7.39-7.26 (m, 12 H), 6.27 (t, 1H, H-1', J = 6.8 Hz), 4.42-4.38 (m, 1H, H-3'), 3.90 (dd, 1H, H-4', J = 6.7 Hz, 3.4 Hz), 3.80 (dd, 1H, H-5', J = 12.1 Hz, 3.1 Hz), 3.71 (dd, 1H, H-5', J = 12.1 Hz, 3.7 Hz), 3.40 (t, 2H, H-c, J = 6.7 Hz), 2.39 (t, 2H, H-a, J = 7.2 Hz), 2.25-2.22 (m, 2H, H-2'), 1.82 (quint, 1H, H-b, J = 6.9 Hz)

^{13}C NMR (75 MHz, CD_3OD): δ = 170.0 (e), 166.1 (4), 152.2 (2), 138.8 (6), 135.1-128.2, 114.8 (5), 88.9 (4'), 86.3 (1'), 72.1 (3'), 62.8 (5'), 41.3 (2'), 40.0 (c), 29.2 (b), 25.2 (a)

^{31}P NMR (121 MHz, CD_3OD): δ = –5.6

MS (ES+): 596.02 (M+Na$^+$), HRMS calculated for $C_{31}H_{32}N_3NaO_6P$, 596.1926; found 596.1915

(Whitesides) (40)



The same method as for **34** was employed using *Whitesides*. Yields 10-20%

IR (KBr), $\bar{\nu}$(cm^{-1}): 3423, 3054, 2923; 1671 (C=O); ν1475; 1434; 1276; 1096; 742; 698

^{1}H NMR (500 MHz, CD_3OD): δ = 7.85 (s, 1H, H-6), 7.39-7.29 (m, 20 H), 6.25 (t, 1H, H-1', J = 6.8 Hz), 4.39-4.37 (m, 1H, H-3'), 3.89 (dd, 1H, H-4', J = 6.3 Hz, 3.2 Hz), 3.79 (dd, 1H, H-5', J = 12.1 Hz, 3.0 Hz), 3.69 (dd, 1H, H-5', J = 12.1 Hz, 3.5 Hz) 3.39-3.31 (m, 4H, H-j, H-j'), 3.20-3.12 (t, 2H, H-c), 2.37 (bs, 4H, H-f, H-g), 2.30 (m, 4H), 2.25 (t, 2H, H-k or k' J = 7.8 Hz), 2.19-2.16 (m, 2H, H-2'), 1.71-1.564 (m, 2H, H-b)

^{13}C NMR (125 MHz, CD_3OD): δ = 174.8 (e), 173.6 (h), 165.9 (4), 152.2 (2), 139.4 (d, J_{CP} = 12.6 Hz), 139.0 (d, J_{CP} = 12.2 Hz), 138.8 (6), 133.9-133.6, 130.3-129.6, 114.8 (5), 88.9 (4'), 86.4 (1'), 72.2 (3'), 62.9 (5'), 46.6 (d, j or j', J_{CP} = 24.7 Hz), 45.0 (d, j or j', J_{CP} = 24.1 Hz), 41.4 (C-2'), 39.2 (c), 31.8 (f), 29.5 (g), 29.2 (b), 28.5 (d, k or k', J_{CP} = 14.6 Hz), 27.3 (d, j or j', J_{CP} = 13.8 Hz), 24.9 (a)

^{31}P NMR (121 MHz, CD_3OD): δ = –21.0; –21.7

MS (ES+): 831.02 (M+Na$^+$), 847.02 (M+O+Na$^+$), HRMS calculated for $C_{44}H_{50}N_4NaO_7P_2$, 831.3052; found 831.3040

General procedure for DNA phosphorylation[1]

A DNA 15-mer (15 nmol) was dissolved in a 300 µl solution of 0.1 M aqueous $NaHCO_3$. Diphenylphosphinobenzoic acid (2.3 mg, 7.5 µmol, 500 eq), EDC·HCl (1 mg, 9 µmol, 600 eq), and NHS (1.4 mg, 7.5 µmol, 500 eq) were dissolved in 600 µl of DMF and stirred for 2 h. The activated phosphine was added to the DNA solution and stirred for 12 h. 600 µl of water was added to the reaction mixture and the reaction mixture was washed with dichloromethane (3 x 2 ml). The water layer was concentrated *in vacuo* and redissolved in 1 ml of water. 200 µl of this solution was placed in an eppendorf. 20 µl of a 3 M NaAc aqueous solution and 700 µl of cold EtOH were added to the DNA solution and placed at 0 ˚C for 1 h. It was centrifuged for 30 minutes at 4 ˚C at 13000 rpm. The supernatant was removed and a cold 70% aqueous EtOH solution was added and the sample was centrifuged for another 30 minutes.

General procedure for allylic alkylation of 1,3-diphenylallyl acetate with dimethyl malonate

A mixture of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (0.5 µmol) and ligand (2 µmol) were dissolved in THF or DCM, and they were stirred for 30 minutes. A trace of KOAc, 1,3-diphenylallyl acetate (100 µmol), diphenyl ether (100 µmol), dimethyl malonate (300 µmol) and BSA (300 µmol) were added to the metal complex solution in this order. The reaction mixture was stirred for 24 h. Each solution was filtered through SiO_2 and left to evaporate overnight. A CHIRACEL OD-H chiral column (Daicel Chemical Industry Ltd.) was employed using a 99:1 (hexane:*i*PrOH) isochratic mixture with a flow of 1 ml/min. t_R (S) 23.6 min, t_R (R) 24.3 min.

General procedure for allylic amination of 1,3-diphenylallyl acetate with benzylamine

A mixture of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (0.5 µmol) and ligand (2 µmol) were dissolved in THF or DCM, and they were stirred for 30 minutes. 1,3-diphenylallyl acetate (100 µmol), diphenyl ether (100 µmol) and benzylamine (300 µmol) were added to the metal complex solution in this order. The reaction mixture was stirred for 24 h. Each solution was filtered through SiO_2 and left to evaporate overnight. A CHIRACEL OD-H chiral column (Daicel Chemical Industry Ltd.) was employed using a 99.5:0.5 ((hexane:*i*PrOH) isochratic mixture with a flow of 0.5 ml/min. t_R (R) 28.4 min, t_R (S) 30.3 min.

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Chapter 4: Modified PNA in Catalysis

4.1 Introduction

Peptide nucleic acids (PNA), a mimic of DNA, were first synthesized by Nielsen and coworkers in 1991.[1] The PNA backbone is based on *N*-(2-aminoethyl)glycine units, which contain purine and pyrimidine bases, linked through amide bonds as in peptide chains (Figure 1).

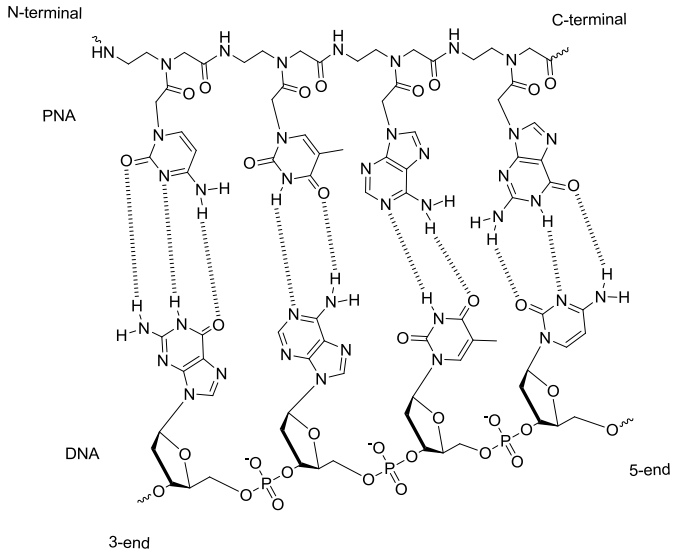


Figure 1. Example of a PNA DNA duplex.[2] Reprinted from L. Bialy *et al*. *Tetrahedron* 2005, **61**, 8295-8305. Copyright 2005, with permission from Elsevier.

PNA forms duplexes with single stranded DNA and triplexes with double stranded DNA. In both cases the interstrand interactions are stronger than in natural DNA-DNA double strands. This is explained by the neutral backbone of PNA which replaces the anionic phosphate backbone of DNA, thereby avoiding electrostatic repulsion.[3] DNA has been replaced by PNA in many occasions because of its increased stability and compatibility with organic synthesis. PNA has been used for biosensors such as microarrays,[4,5] in diagnostic devices[6] and as antisense oligonucleotides.[7]

Transition metal complexes have been introduced in PNA for several reasons. The most common ligands used contain nitrogen atoms for metal coordination. 6-(2-pyridyl)-4-pyrimidine (**A**), 5-(2-pyridyl)-1*H*-3-pyrazole (**B**) and bis(2-picolyl)-amine (**C**) complexes with Zn^{II}, Cu^{II} and Ni^{II} have been studied as tools for suppression of

gene expression.[8] A hybrid between a metallosalen and a metallopeptide where the metal is $Ni^{(II)}$ (**D**) has been used for selective modification of DNA with an aldehyde functionality that can be used for further functionalization such as labelling and binding to proteins.[9] A ferrocene and a tris(bipyridine)ruthenium^{II} complex served as markers for binding between PNA and DNA for future use in genetic diagnostics.[10] Bipyridines have also replaced nucleic bases in specific locations of PNA for the introduction of Ni^{II}, Co^{II}, and Cu^{II} in the double helix[11] (Figure 2). The majority of those ligands have been introduced on the PNA through amide bond formation at the terminal position of a PNA strand or as a replacement of a base.

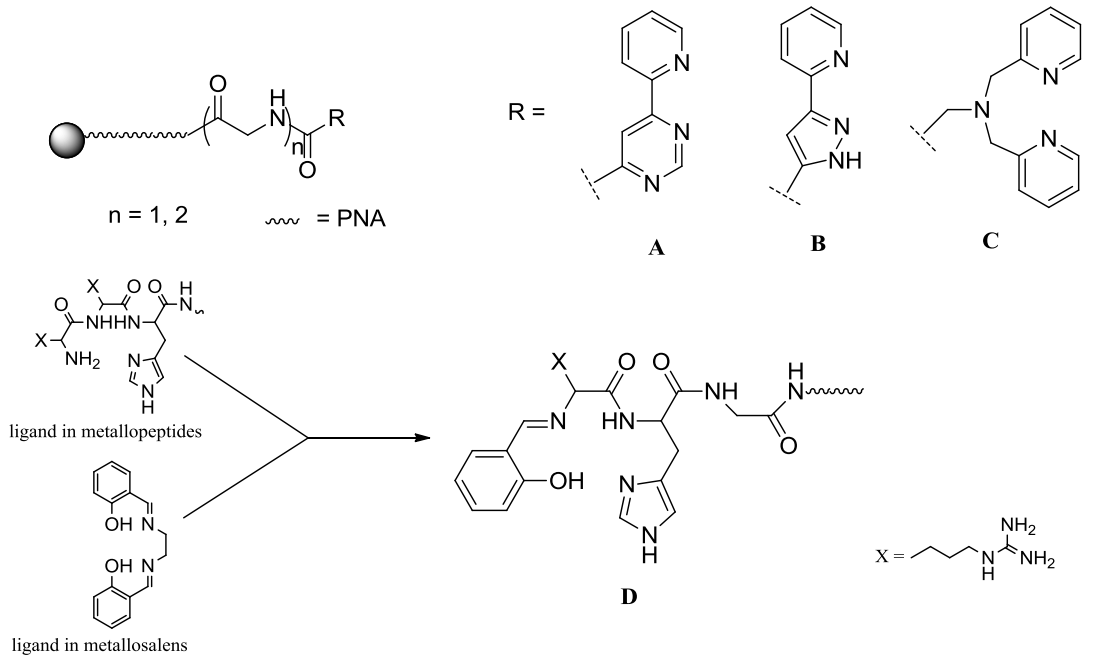
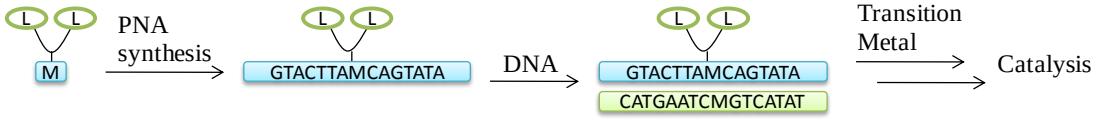


Figure 2. A selection of ligands coupled to PNA strands.[8-11]

As previously explained in chapters 2 and 3, DNA's high selectivity and specificity towards small molecules makes it a good candidate as a ligand for catalytic asymmetric reactions. Despite its appealing qualities DNA is only soluble in water and only minute quantities can be produced per synthesis. In contrast, PNA is soluble in organic solvents and can be synthesized at larger scale. Even though PNA is not a chiral molecule, the use of chiral amino acids[12] or duplex formation with DNA strands[13,14] can result in strand chirality.

Our objective is to synthesize a PNA strand containing a bidentate ligand which could be complexed to transition metals and used in asymmetric catalysis (Scheme 1). Two different strategies were employed; the first strategy is based on the introduction of phosphines on PNA strands. The backbone of PNA is resistant to the conditions of phosphine deprotection therefore a phosphine functionality can be protected and introduced directly on the PNA monomer prior to strand synthesis. This method facilitates the purification of the phosphine modified strand and guarantees complete coupling of the phosphine moiety to the PNA strand. As explained in chapter 3, phosphines are very sensitive towards oxidation. Therefore the second strategy presented in this chapter is exploiting the synthesis of a bipyridine monomer, which can also be introduced on the PNA during strand synthesis and it is not air sensitive. Bipyridine ligands are known to be compatible with DNA and have been used for Diels-Alder catalyzed reactions.[15] Our aim is to build chiral ligands to use with transition metals as catalysts in asymmetric synthesis. Our method is to use these novel PNA-based ligands in combination with DNA and to test them in transition metal catalyzed asymmetric reactions (Scheme 1). This chapter details the synthesis of a diphosphine modified and bipyridine modified PNA monomer and the successful synthesis of a short modified PNA strand.



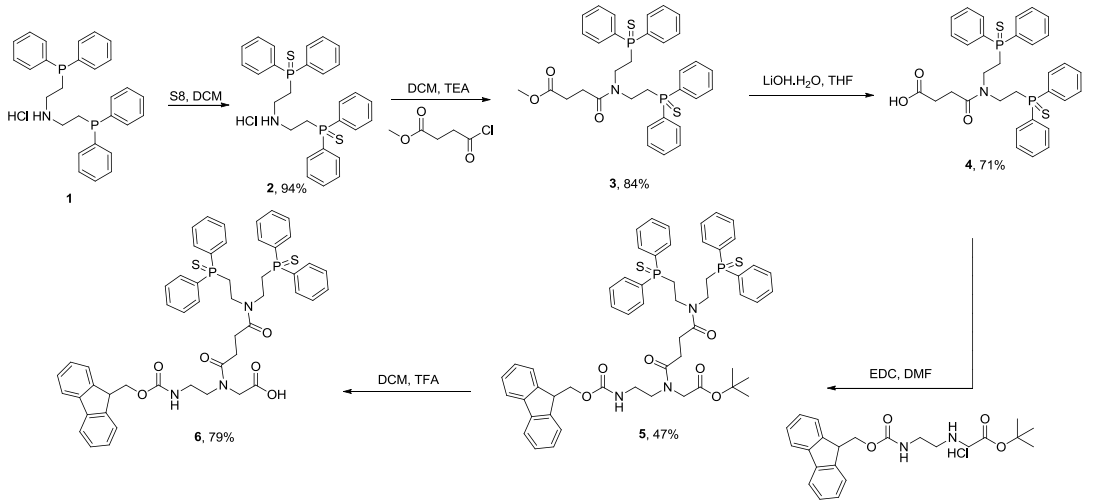
Scheme 1. Introduction of modified monomer into a PNA strand and its use in catalysis.

4.2 Results and Discussion

4.2.1 Synthesis of phosphine modified PNA monomer

The Whitesides ligand described in chapter 3 (**1**) was used for the synthesis of the PNA monomer. This ligand was protected with sulfur to give (**2**) in 94% yield. Reaction with methyl-4-chloro-4-oxobutyrate and subsequent deprotection gave compound (**4**) in 71% yield. The PNA backbone is commercially available as the HCl salt and, therefore, coupling of this compound to the PNA backbone was preceded by preliminary neutralization by washing with a saturated solution of $NaHCO_3$.[16] This method afforded the desired product (**5**) in 47% yield. Partial deprotection of the

Fmoc group during basic washing accounts for the low yields. ^{1}H NMR of the PNA backbone after washing with $NaHCO_3$ showed partial cleavage of the Fmoc group. For this reason neutralization of HCl was attempted with the addition of TEA but this procedure was not successful and the previous method was used to synthesize the desired product. The ^{1}H NMR of the desired compound was very complex. Mass spectroscopy analysis showed the desired product and the integrations in the ^{1}H NMR spectra corresponded to the expected number of protons. High temperature $^{31}P\{^{1}H\}$ NMR studies were performed to test if the desired compound exists as different conformers. At high temperature the peaks become sharper and merged into one peak at 393 °K which suggests that the desired product is a conformer. Deprotection of the Boc group with trifluoroacetic acid gave the desired product after filtration without the need of any further purification (**6**) (79% yield) (Scheme 2).

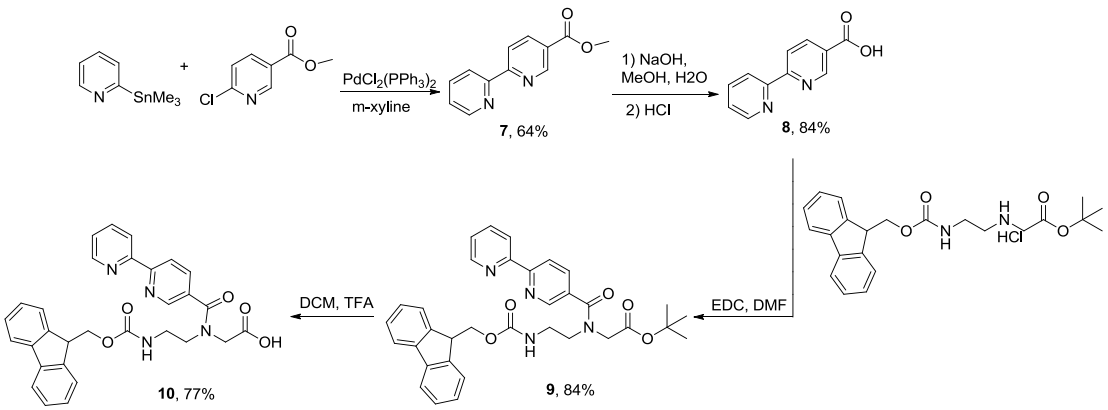


Scheme 2. Synthesis of phosphine modified PNA monomer.

4.2.2 Synthesis of bipyridine modified PNA monomer

A second monomer with a bipyridine ligand was also synthesized. 2,2-Bipyridine-5-carboxylic acid **8** was prepared from trimethyl-(2-pyridyl)tin and methyl-6-chloropyridine-3-carboxylate to give compound **7** in 64% yield. Deprotection under basic conditions gave the desired product **8** in 84% yield.[17] Compound **8** was coupled to the Fmoc protected PNA backbone using the same procedure as for compound **6** (84% yield) (Scheme 3). The ^{1}H NMR spectrum of this compound after purification was very complex as in compound **5**. High temperature ^{1}H NMR studies were

performed to test if the compound exists as different conformers. As shown in the ^{1}H NMR spectra taken from 313 to 393 K (Figure 3) the peaks become sharper and merge into one peak belonging to the desired product. This behaviour at high temperatures suggests that compound (**9**) indeed exists as different conformers. This behaviour has also been reported for a similar compound by Williams *et al*.[18] Deprotection with trifluoroacetic acid gave compound (**10**) in 77% yield.



Scheme 3. Synthesis of bipyridine modified PNA monomer.[17]

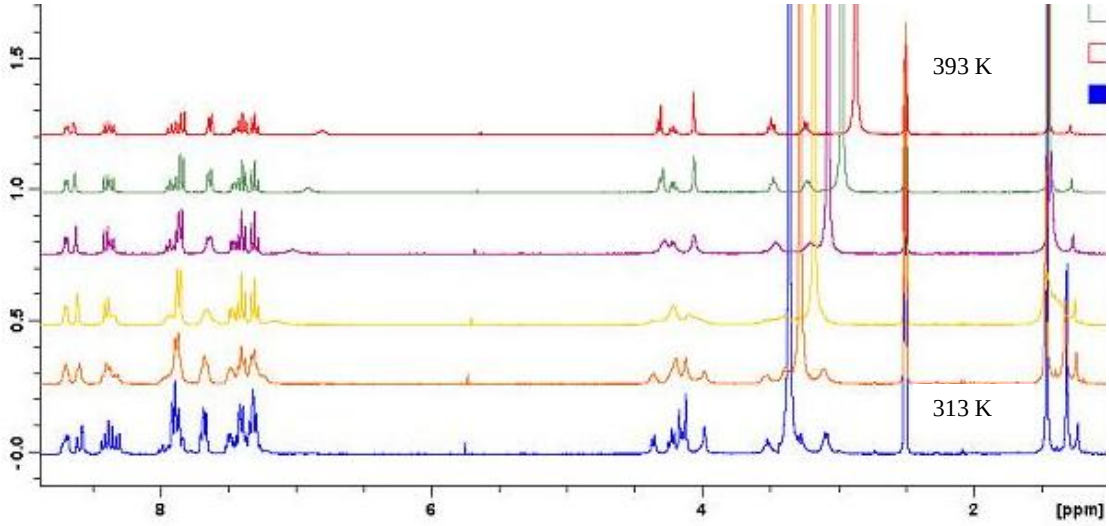


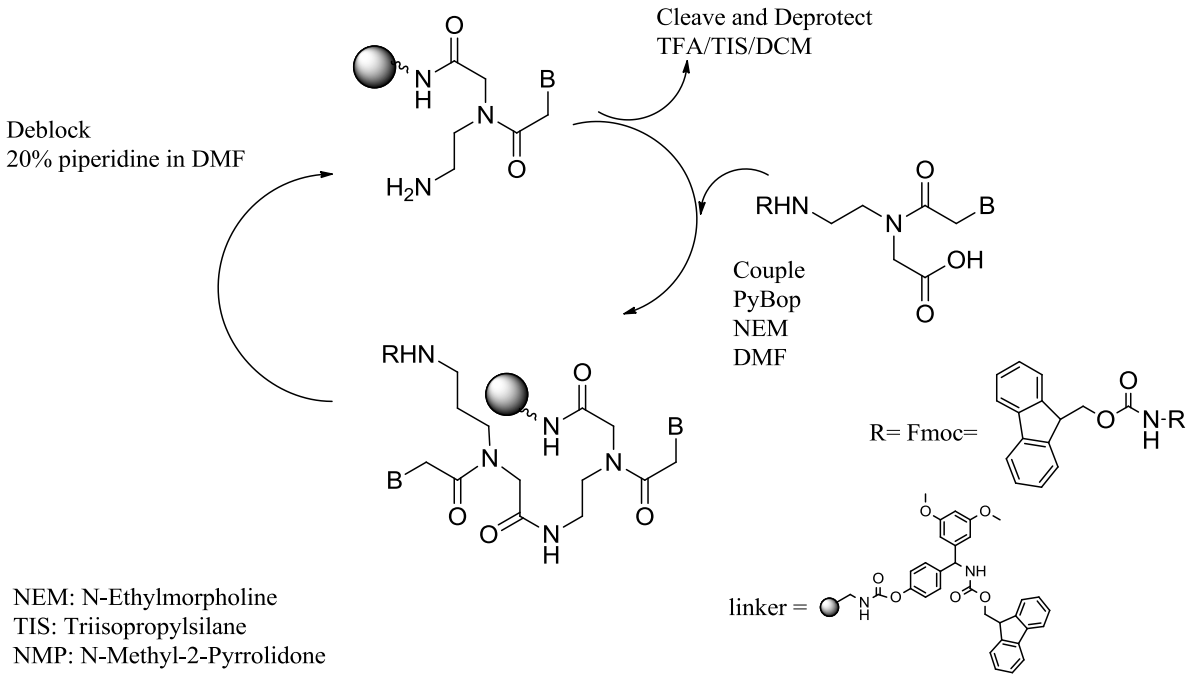
Figure 3. Overlap of six 300 MHz ^{1}H NMR spectra taken at different temperatures. The spectra coloured in blue represents compound (**9**) taken at 313 K, the spectra above it were taken at an increase interval of temperature of 16 K.

4.2.3 Synthesis of PNA strands

Introduction of **10** into a PNA strand was attempted following the protocol of Bradley *et al* using PyBop (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) and NEM (*N*-ethylmorpholine).[19] A linker was placed on a

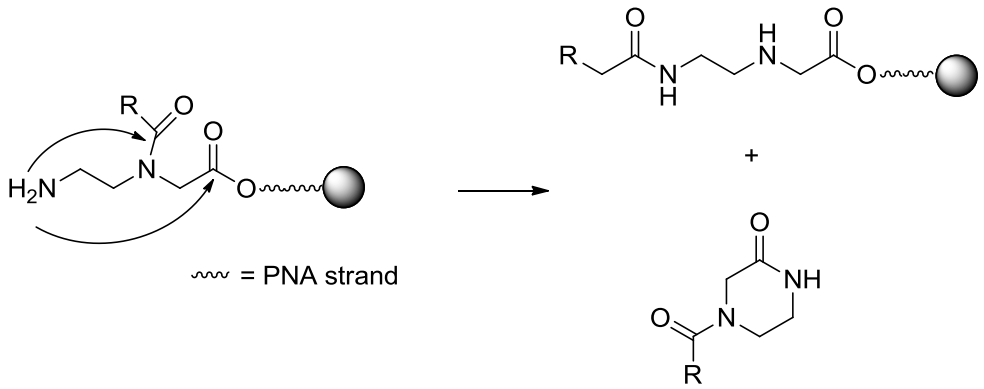
polystyrene (PS) solid support. This linker contained an amine group protected by Fmoc which was deprotected by using 20% piperidine in DMF. PNA monomers are most commonly synthesized with Fmoc (9-Fluorenylmethyloxycarbonyl)/Bhoc (benzhydryloxycarbonyl)[20] or Boc (*tert*-Butyloxycarbonyl)/Cbz (Carbobenzyloxy)[21] protecting groups. Monomers A, C, G and T are commercially available containing Fmoc as protecting group. Coupling and deblocking steps are repeated until the desired length is synthesized. Each step is analyzed using the Kaiser test.[22] The Kaiser test can give an indication of the coupling success although at a certain strand length the test loses its validity.

TFA/TIS(triisopropylsilane)/DCM is used to cleave the strand from the solid support (Scheme 4). Reverse phase HPLC is the common method of purification.



Scheme 4. Synthesis of PNA strands on solid support.

During the synthesis of the 15 mer, a small sample, after the introduction of the 8th monomer, was taken for HPLC analysis to monitor the process of the synthesis. This chromatrogram showed several peaks and MALDI-TOF analysis also showed several peaks; none of them corresponding to the desired product. Therefore, the synthesis was not further continued.



Scheme 5. Cyclization of deprotected PNA monomer.[23]

As shown in Scheme 4 during PNA synthesis basic conditions were applied to deprotect the primary amine from the Fmoc group. It is possible that during this deprotection step a small percentage of product cyclizes. When terminal amines are not protected two undesirable side reactions can take place under basic conditions (Scheme 5). The free amine can attack the acetyl group of the heterocyclic base transferring it from the central secondary amine to the terminal primary amine.[24] The other possible side reaction is the attack of the acetyl group of the PNA backbone by the primary terminal amine.[25,23] Both side reactions result in termination of strand synthesis.

Another point in the synthesis where this reaction can occur is during the coupling step because it is performed under basic conditions (NMM as base). Some of the couplings were left overnight therefore increasing the possibility of Fmoc deprotection by NMM. This can not only lead to cyclization but also to multiple coupling. The synthesis failure can be related to partial product cyclization preventing the continuation of the strand and multiple coupling therefore giving a various range of different strands as seen by HPLC analysis.

The second strategy used was to synthesize phosphine modified compound (**6**) and introduce it into PNA. The synthesis of the PNA strands was performed with A, T, and C monomers containing Dde (1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl) protecting groups kindly provided by Professor Bradley's group. The same group[2] has developed the synthesis of PNA nucleosides with Dde/MMT protecting groups. This method was initially developed for the synthesis of PNA-peptide conjugates although it also gives a more robust protection to the terminal amine functionality than the

more common Fmoc method. The Dde protecting group is more resistant to basic conditions then Fmoc therefore it is less affected by the basic conditions used during the coupling step. A 5-mer, 9-mer and 15-mer were the target strands (Table 1). The 5-mer was the only successful synthesis (Figure 4) because in the syntheses of the 9-mer and 15-mers mixtures of shorter strands were obtained.

Strand #	Strands Synthesized	Molecular Weight	Mass Found
S1	5'-TAMCA-3'	1773	1793
S2	5'-ACTTMAATC-3'	2832	2603, 2336
S3	5'-TCAACCAMCATCCTC-3'	4360	3314, 3049,2799

Table 1. Molecular weight of PNA strands synthesized containing compound **6** (M) and their mass found by MALDI-TOF analysis.

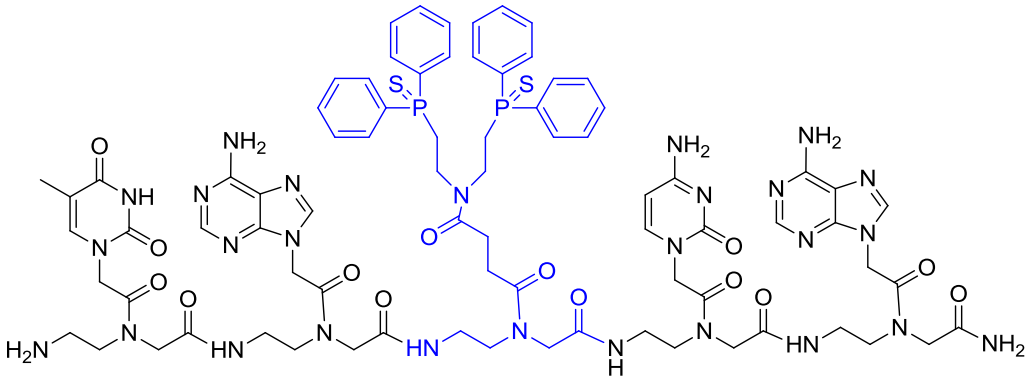


Figure 4. PNA 5-mer (TAMCA, M = compound **6**) synthesized using Dde as the protecting group for the PNA monomers.

Because PNA is a lipophilic compound, it can aggregate on the solid support during the synthesis, decreasing the coupling efficiency as the strand grows longer.[26] The synthesis of Dde protected PNA monomers consists of several steps and therefore those monomers were not readily available to test the synthesis of bipyridine modified PNA.

4.2.4 Phosphine deprotection

Gilbertson *et al* have been introducing sulfur protected phosphine moieties into peptide chains for their use in catalytic reactions.[27] The two methods used for phosphine deprotection have been Raney-Ni after purification of the peptide strands and MeOTf / HMPT on solid supported peptides. The 15-mer strand which resulted in

a shorter strand was tested for sulfur deprotection using Raney-Ni. MALDI-TOF analysis showed the same pattern of peaks for the strand before deprotection suggesting that the deprotection was not successful. The presence of modified phosphine monomer in the failed 15-mer strand was confirmed by calculating the possible combinations in the sequence of the monomers in the 15-mer. The molecular weights present in the MALDI-TOF can only correspond to shorter strands (9-mer, 10-mer 11-mer) containing the phosphine moiety. Phosphine deprotection tests were made with sulfur protected Whitesides ligand as a model for PNA strands. Following the procedure of Gilbertson *et al*[28] for the deprotection of phosphine modified peptides, Raney nickel was employed. The main advantage of using this deprotection method is that Raney nickel can be removed from the desired compound by filtration with no need of any further purification. The deprotection was attempted several times with different batches of Raney nickel but was unsuccessful. The HMPT method resulted in successful deprotection but gave rise to unidentified phosphorus impurities. A clean method of deprotection is necessary to avoid any further purification and possible oxidation of the phosphine ligand. The HMPT method was tested with compound (**6**) coupled on solid support and $^{31}P\{^{1}H\}$ NMR showed complete deprotection. The disadvantage of this method is that after phosphine deprotection with HMPT on solid support the PNA can not be purified because that would probably result in oxidation of the phosphine during HPLC purification.

4.3 Conclusion

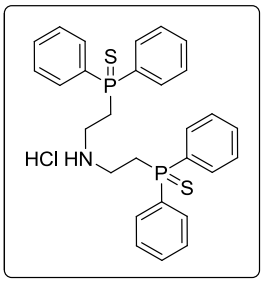
In this chapter PNA monomers containing a diphosphine sulfide and a 2,2'-bipyridine moiety were synthesized. The phosphine modified monomer was successfully introduced into a 5-mer but the sulfide deprotection was troublesome. Synthesis failure of PNA strands could be prevented by using Dde instead of Fmoc protecting group. Also, the synthesis of shorter strands would give higher yields and reduce the risk of cyclization. Two short strands with terminal monodentates could be combined with a DNA complementary strand to receive chirality and place the ligands in the central position of a helix.

4.4 Experimental

General procedure

All reactions were performed under argon using standard Schlenk techniques. Chemicals were purchased from Aldrich Chemical Company, Acros, Fluka, Link Technologies and Glen Research. CH_2Cl_2 and acetonitrile were distilled over CaH_2; ethanol and methanol were distilled over Mg/I_2; toluene was distilled over Na, THF and Et_2O were distilled over sodium /benzophenone and all the distillations were performed under nitrogen atmosphere. DMF was purchased "Extra dry" and stored over molecular sieves (4 Å) under argon. Aqueous solutions were degassed by three freeze/thaw cycles under vacuum and kept under argon. Thin Layer Chromatography (TLC) was performed on silica plates (Polygram 0.2 mm silica gel with fluorescent indicator UV_{254}). Ninhydrin spray was used for the detection of primary and secondary amines on TLC silica plates. Silica gel 60 particle size 0.063-0.2 mm from Fluka was used for flash chromatography. NMR spectra were recorded at room temperature with Bruker Avance spectrometers (300, 400, and 500 MHz). Positive chemical shifts (δ) are given (in ppm) for high-frequency shifts relative to a TMS reference (^1H and ^{13}C) or an 85% H_3PO_4 reference (^{31}P). ^{13}C, ^{31}P, and ^{19}F spectra were measured with ^1H decoupling. The following abbreviations have been used in the description of the spectra: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were recorded in a Perkin-Elmer Spectrum GX spectrometer in KBr pellets, with a window between 4000 and 400 cm^{-1}. Mass spectrometry: electrospray mass spectra were recorded on waters, micromass LCT time of flight mass spectrometer coupled to a Waters 2975 HPLC. MALDI spectra were acquired on a Voyager-De™ STR MALDI-TOF MS(Applied Biosystems). The matrix was sinapinic acid (10 mg/ mL) in 50 % MeCN in water with 0.1 % TFA. HPLC used for the purification of PNA was an HPLC/Prep Agilent 1100 series. The column used was a Phenomenex Prodigy 5 µ ODS (3) 100 A size 250 x 10 mm. The eluents used were ACN with 0.1 % TFA and H_2O with 0.1 % TFA.

Bis(2-(diphenylphosphorothioyl)ethyl)amine (2)



Compound **1** (1 g, 2.1 mmol, 1 eq) was added to a solution of sulfur (0.80 g, 2.5 mmol, 1.2 eq) in 20 ml of dichloromethane. After a few minutes a white precipitate was formed which was filtered and recrystallized with dichloromethane. Yield: 900 mg, 90%

^1H NMR (400 MHz, $CDCl_3$): δ = 10.20 (bs, 2H), 7.79-7.73 (m, 8H), 7.46-7.36 (m, 12H), 3.18 (m, 4H), 3.08 (m, 4H)

^{31}P NMR: 38.7 (s, 2P)

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 132.3, 131.1, 131.04, 131.0, 130.2, 129.2, 129.0, 42.9, 28.7, 28.2

MS (ES+): 505.78 ($M+Na^+$), HRMS calculated for $C_{28}H_{30}NNaP_2S_2$, 506.1295 found 506.1299

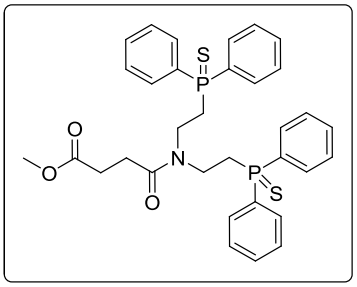
Elemental analysis calculated for $C_{28}H_{30}ClNP_2S_2$: C 62.04, H 5.58, N 2.58:

Found: C 62.12, H 5.43, N 2.46

IR: 3437, 2910, 2702, 2436, 1587, 1436, 1104

mp: 232-234 ˚C

Methyl 4-(bis(2-(diphenylphosphorothioyl)ethyl)amino)-4-oxobutanoate (3)[29]



This compound was prepared using the same method as for compound **26** of chapter 3. Yield: 1.0, 84%

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87-7.72 (m, 8H, Ar), 7.50-7.34 (m, 12H, Ar), 3.64 (s, 3H, CH3), 3.62-6.56 (m, 2H), 3.49-3.42 (m, 2H), 2.78-2.66 (m, 4H), 2.53-2.49 (m, 2H), 2.38-2.34 (m, 2H)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 173.4, 171.8, 132.9, 132.3, 131.9, 131.7, 131.1, 131.0, 130.8, 129.0, 128.9, 128.8, 128.6, 51.8, 43.3, 41.5, 32.2, 31.5, 31.0, 30.3, 28.8, 28.0

P NMR (121.5 MHz, $CDCl_3$): δ = 40.3, 38.4

MS (ES+): 641.73 ($M+Na^+$), HRMS calculated for $C_{33}H_{35}NNaO_3P_2S_2$, 642.1431 found 642.1429

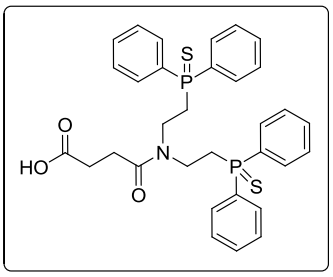
Elemental analysis calculated for $C_{33}H_{35}NO_3P_2S_2$: C 63.96, H 5.69, N 2.26:

Found: C 63.89, H 5.79, N 2.15

IR: 3447, 3057, 2943, 1735, 1646, 1480, 1437, 1366, 1161, 1104

mp: 133-135 ˚C

4-(bis(2-(diphenylphosphorothioyl)ethyl)amino)-4-oxobutanoic acid (4)[29]



This compound was synthesized using the same method as for compound **27** of chapter 3 Yield: 1.65 g, 88%

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90-7.78 (m, 8H), 7.55-7.40 (m, 12H), 3.67 (m, 2H), 3.52 (m, 2H), 2.80 (m, 4H), 2.58 (m, 2H), 2.43 (m, 2H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 179.2, 173.8, 132.7, 132.2, 131.9, 131.8, 131.6, 131.4, 131.1, 131.0, 130.9, 129.0, 128.8, 128.7, 128.6, 43.0, 32.3, 31.7, 31.2, 30.5, 29.9, 29.6

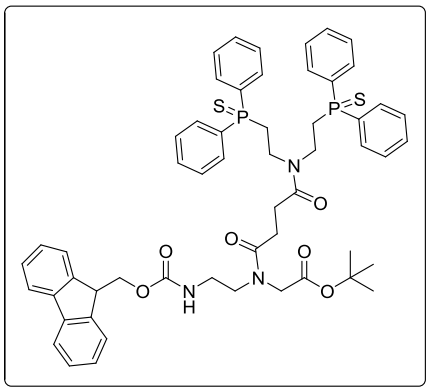
^{31}P NMR (121.4 MHz, $CDCl_3$):δ = 40.0, 38.3

MS (ES+): 627.76 ($M+Na^+$), HRMS calculated for $C_{32}H_{33}NNaO_3P_2S_2$, 628.1275 found 628.1274

IR: 3431, 3047, 2928, 2364, 1701, 1644, 1437, 1169, 1104

mp: 189-190 ˚C

***N*-[2-(Fmoc-amino)-ethyl]-gly-O-*tert*-Butyl-*N*-(bis(2-(diphenylphosphorothioyl)ethyl)amino)-4-yl (5)**



N-[2-(Fmoc-amino)-ethyl]-gly-*O*-tBu·HCl (0.6 g, 1.4 mmol, 1 eq) was dissolved in 40 ml of chloroform and washed with a saturated solution of $NaHCO_3$ (2 x 20 ml). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The resulting oil was dissolved in 12 ml of DMF and compound **3** (0.95 g, 1.6 mmol, 1.1 eq) was added. When all the compounds were completely dissolved EDC HCl (0.3 g, 1.6 mmol, 1.1 eq) was added and the reaction mixture was left stirring overnight. The reaction mixture was concentrated to dryness, redissolved in DCM and washed with 5 ml of water. The organic layer was dried over Na_2SO_4 and purified by column chromatography (SiO_2, MeOH:DCM, 1:99). The desired product resulted as a white powder. Yield: 650 mg, 47%.

^{1}H NMR (300 MHz, DMSO-d_6, 393 K): δ = 7.89-7.78 (m, 12H), 7.54-7.46 (m, 12H), 7.41 (t, 2H, J = 7.3 Hz), 7.34 (t, 2H, J = 7.3 Hz), 3.98 (s, 2H), 3.56-3.48 (m, 5H), 3.37 (t, 2H, J = 6.5 Hz), 2.90-2.75 (m, 10H), 2.39 (t, 2H, 6.5 Hz), 1.43 (s, 9H)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.5, 172.3, 156.8, 156.6, 141.3, 132.9, 132.4, 131.8, 131.5, 131.3, 131.2, 131.1, 130.8, 129.0, 128.8, 128.7, 128.6, 127.8, 127.7, 127.1, 125.2, 120.0, 82.1, 66.8, 49.7, 49.5, 47.2, 43.3, 41.6, 39.7, 28.1, 27.4

^{31}P NMR (121.4 MHz, $CDCl_3$): δ = 40.0, 39.9, 38.2, 38.1

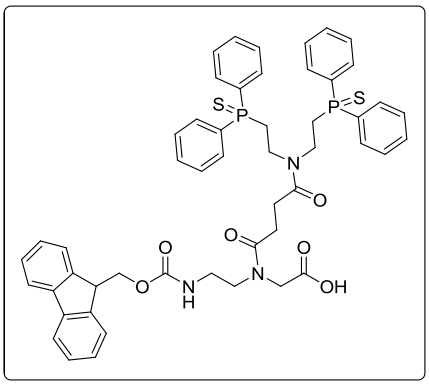
IR: 3421, 3054, 2925, 2847, 2357, 1966, 1912, 1720, 1645, 1517, 1478, 1450, 1436, 1309, 1242, 1155, 1103

Elemental Analysis Calculated for $C_{55}H_{59}N_3O_6P_2S_2$: 67.12, H 6.04, N 4.27, found: C 67.24, H 5.69, N 4.10

MS (ES+): 1005.72 ($M+Na^+$)

mp: 90-92 ˚C

***N*-[2-(Fmoc-amino)-ethyl]-gly-*N*-(bis(2-(diphenylphosphorothioyl)ethyl)amino)-4-yl(6)**



Compound **5** (0.6 g, 0.6 mmol, 1 eq) was dissolved in 3 ml of DCM and 4 ml of TFA (5 mmol, 8 eq) were added at 0 ˚C. The reaction mixture was stirred for 2 h and concentrated to dryness. The resulting dry yellow powder was dissolved and washed with a saturated solution of $NaHCO_3$ (2 x 20 ml). The organic layer was dried over Na_2SO_4 and concentrated to dryness resulting in a slightly yellow powder, with no further purification required. Yield: 440 mg, 79%

^{1}H NMR (300 MHz, DMSO-d_6, 363 K): δ = 7.88-7.75 (m, 12H, Ar), 7.50-7.42 (m, 12H, Ar), 7.36 (dt, 2H, J = 7.3 Hz, 1.2 Hz), 7.30 (dt, 2H, J = 7.4 Hz, 1.4 Hz), 3.70 (s, 1H), 3.61-3.59 (m, 1H), 3.54-3.42 (m, 5H), 3.14-2.85 (m, 6H), 2.52-2.40 (m, 2H), 2.31 (m, 2H)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 175.2, 174.6, 172.3, 172.0, 156.2, 155.8, 142.9, 140.1, 131.8, 131.3, 131.2, 130.7, 130.5, 130.2, 130.1, 129.8, 127.9, 127.8, 127.7, 127.6, 127.5, 126.6, 126.1, 124.3, 124.0, 120.0, 118.8, 65.7, 46.0, 42.1, 40.0, 38.8, 38.5, 38.2, 35.4, 30.5, 29.8, 29.4, 28.7, 27.1, 26.6

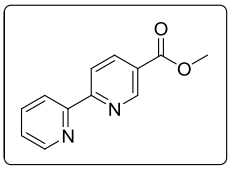
^{31}P NMR (121.4 MHz, $CDCl_3$): δ = 40.2, 40.1, 38.5

IR: 3421, 3055, 2944, 1969, 1912, 1912, 1715, 1636, 1521, 1437, 1478, 1451, 1369, 1311, 1246, 1168, 1104

MS (ES+): 949.79 ($M+Na^+$)

mp: 125-130 ˚C

Methyl 2,2'-bipyridine-5-carboxylate (7)[17]

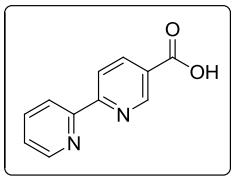


This compound was prepared following literature procedure. Yield: 1.45 g, 64%

Characterization of the product fully agreed with data from literature.

^1H NMR (400 MHz, $CDCl_3$): δ = 9.20-9.19 (m, 1H), 8.65-8.63 (m, 1H), 8.45-8.39 (m, 2H), 8.35-8.32 (m, 1H), 7.78 (t, 1H, J = 7.8 Hz, 1.7 Hz), 7.29 (m, 1H), 3.91 (s, 3H)

2,2'-bipyridine-5-carboxylic acid (8)[17]



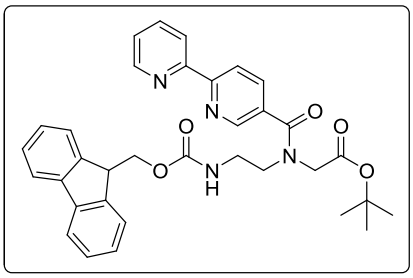
This compound was prepared following literature procedure. Yield: 590 mg, 84%

Characterization of the product fully agreed with data from literature.

^1H NMR (400 MHz, MeOD): δ = 9.15-9.14 (m, 1H), 8.73-7.72 (m, 1H), 8.51-8.38 (m, 3H), 7.98 (dt, 1H, J = 7.9 Hz, 1.54 Hz), 7.52-7.49 (m, 1H)

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 166.1, 158.3, 154.1, 150.1, 149.5, 138.2, 137.5, 126.5, 124.9, 121.3, 120.2

***N*-[2-(Fmoc-amino)-ethyl]-gly-*O*-*tert*-butyl-*N*-2,2'-bipyridine-5-carboxamido)acetate (9)**



N-[2-(Fmoc-amino)-ethyl]-gly-*O*-tBu·HCl (350 mg, 0.81 mmol, 1 eq) was dissolved in 20 ml of chloroform and washed with 2 x 40 ml of an aqueous saturated solution of $NaHCO_3$ The water layer was extracted with 40 ml of chloroform and the combined organic layers were dried over Na_2SO_4 and concentrated to dryness. The resulting oil was coevaporated with toluene and compound **8** (194 mg, 0.97 mmol, 1.2 eq), EDC·HCl (217 mg, 1.13 mmol, 1.4 eq) and 20 ml of DCM were added. The reaction was stirred for 12 h and washed with 10 ml of water. The water layer was back extracted with 20 ml of DCM and the combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The residue product was purified by column chromatography (SiO_2, DCM:MeOH, 96:4, SiO_2). The desired compound resulted as a white powder. Yield: 390 mg, 84%

^{1}H NMR (300 MHz, DMSO-d_6 at 393 K): δ = 8.70-8.63 (m, 2H), 8.41-8.34 (m, 2H), 7.94-7.82 (m, 4H), 7.62 (d, 2H, J = 7.5 Hz), 7.46-7.37 (m, 3H), 7.30 (t, 3H, J = 7.5 Hz, 1.1 Hz), 6.80 (bs, 1H), 4.31 (d, 2H, J = 6.7 Hz), 4.21 (t, 1H, J = 6.4 Hz), 4.06 (s, 2H), 3.50 (t, 2H, J = 6.4 Hz), 3.23 (q, 2H, J = 5.8 Hz, 6.2 Hz), 1.44 (s, 9H)

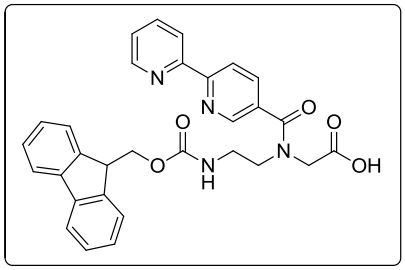
^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.1, 169.0, 168.7, 156.8, 156.2, 155.1, 149.4, 147.3, 143.9, 141.3, 137.1, 135.7, 135.4, 131.1, 127.7, 127.1, 125.2, 124.3, 124.2, 121.6, 120.6, 112.0, 83.2, 82.7, 67.0, 66.9, 52.7, 50.7, 49.2, 47.7, 47.2, 39.2, 28.1, 28.0

IR: 3357, 2978, 2361, 1720, 1589, 1518, 1434, 1369, 1246, 1152, 1091, 1018

MS (ES+): 601.02 ($M+Na^+$), HRMS calculated for $C_{34}H_{34}N_4NaO_5$, 601.2427; found, 601.2427

mp: 75-76 ˚C

_N_-[2-(Fmoc-amino)-ethyl]-gly-_N_-2,2'-bipyridine-5-carboxamido)acetate (10)



Compound **9** (50 mg, 0.865 mmol) was dissolved in TFA:water (5:1 mixture) and stirred for 2 h. Cold Et_2O was added and a precipitate was formed. The solution was filtered and the solid washed with Et_2O. The slightly yellow solid was dried *in vacuo*. Yield: 35 mg, 77%

^{1}H NMR (300 MHz, DMSO-d_6 at 363 K): δ = 8.68-8.63 (m, 2H), 8.37 (d, 2H, J = 8.1 Hz), 7.98 (t, 1H, J = 7.6 Hz), 7.89 (dd, 1H, J = 8.2 Hz, 2.1 Hz), 7.80 (d, 2H, J = 7.5 Hz), 7.60 (bd, 2H, J = 7.4 Hz), 7.51-7.47 (m, 1H), 7.35 (t, 2H, J = 7.4 Hz), 7.26 (dt, 2H, J = 7.5 Hz, 1.2 Hz), 6.95 (bs, 1H), 4.27-4.15 (m, 3H), 4.08 (bs, 2H), 3.46 (bs, 2H), 3.20 (bs, 2H)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 171.1, 170.9, 169.7, 169.4, 159.5 (q, CF_3, J = 38.46 Hz), 157.7, 157.4, 157.1, 148.6, 148.5, 147.7, 147.4, 145.9, 145.6, 145.3, 143.8, 143.7, 143.5, 143.4, 142.7, 141.1, 140.6, 137.2, 136.6, 134.5, 134.3, 133.9, 127.6, 127.4, 126.9, 126.86, 126.78, 124.8, 124.79, 124.5, 124.0, 123.6, 122.0, 121.9, 121.8, 119.6, 119.5, 116.8, 114.8 (q, CF_3, 283.26 Hz), 114.6, 67.2, 66.5, 66.4, 51.2, 50.4, 49.6, 46.8, 46.4, 38.5, 37.9

IR: 3369, 3065, 1719, 1638, 1531, 1452, 1192, 1020

MS (ES+): 522.94 ($M+Na^+$), HRMS calculated for $C_{30}H_{27}N_4O_5$, 523.1981; found, 523.1972

mp: 105-109 ˚C

General procedure for PNA synthesis[19]

A PEGA resin with a rink amide linker (10 µm, 1 eq) was placed in a SP extractor and shaken with DMF for 30 min. The resin was washed with DCM, MeOH, and DMF 3 times. 20% piperidine in DMF was added and it was shaken for 10 min. This procedure was repeated twice. The resin was washed again and the monomer A (41 mg, 56.1 µmol, 5.5 eq) and PyBOP (25.5 mg, 49 µmol, 4.8 eq), in *N*-ethylmorpholine (11.7 mg, 102 µmol, 10 eq) and 550 µl of DMF were added. The mixture was placed in a microwave for 20 min at 60˚C. The resin was washed and the deprotection mix in DMF (700 µl:140 µl, 5:1) was added and shacked for 1 hour (deprotection mix: $NH_2OH{\cdot}HCl$ (2.5 g, 3.6 mmol), imidazole (1.836 g, 2.7 mmol) in 10 ml of 1-methyl-

2-pyrrolidone). The resin was washed again with DCM, MeOH, and DMF 3 times and another monomer was added to it. This procedure was repeated until the desired PNA length was obtained. Cleavage of PNA strand from resin was done by using TFA:TIS:DCM, 90:5:5 and shaking for one hour. The resulting mixture was collected in an eppendorf and the resin was washed with 0.6 ml of DCM and it was placed together with the other fraction. The mixture was dried by a flow of N_2 and a yellow solid was formed. Diethylether was added and a white precipitate was formed. It was sonicated and centrifuged for 5 min. Diethylether was removed and this procedure was repeated a second time. The white solid was dried by a flow of N_2 and purified by preparative HPLC.

When monomers containing Fmoc protecting group were used for PNA synthesis the coupling was performed by shaking for 2 h at room temperature and a PS resin was used.

4.5 References

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Chapter 5: Preliminary Studies of the Use of Aptamers in Catalysis

5.1 Introduction

Aptamers are RNA and DNA strands which are capable of selectively binding small molecules.[1-4] They are selected for a specific compound using systematic evolution of ligands by exponential enrichment (SELEX).[5] A library of oligonucleotides is synthesized and multiplied by the Polymerase Chain Reaction (PCR). This library is then used for screening binding affinity towards target substrates. Two main screening methods are used; the first screening method consists of incubating the random polynucleotide mixture and the target molecule in a buffer. The aptamers that bind to the target are filtered by using a nitrocellulose filter partitioning. The aptamers that are not selective to the target elute first, the aptamers bound to the target elute last. The second method involves immobilization of the target molecule on a solid support. All the sequences that do not bind to the target are washed away. This selection is repeated several times until the desired strongest binding aptamer is found.[4,5,7-10] This method has been applied to discover aptamers for several molecules.[2,4,11,12]

The use of chiral molecules that non-covalently bind to transition metal coordinating ligands has been increasing over the past 30 years. After the pioneering work of Whitesides[13] other groups have used proteins[14-16] and DNA[17,18] as a tool to transfer chirality to asymmetric catalytic reactions.

Aptamers are chiral molecules that could potentially transfer their chirality to asymmetric reactions. Both DNA and RNA aptamers have been produced for selective binding of arginine as target substrate.[19,20-22] This molecule is a commercially available amino acid containing an amine moiety that can be functionalized through amide bond formation. Our project aim is to use the high selectivity of DNA aptamers and their chirality to produce ligands for asymmetric reactions. Arginine will act as a non-covalent binding target, which will be modified with a bipyridine ligand via amide bond formation and its aptamer will be used to transfer its chirality to the Cu bipyridine catalyst for Diels-Alder reactions (Figure 1).

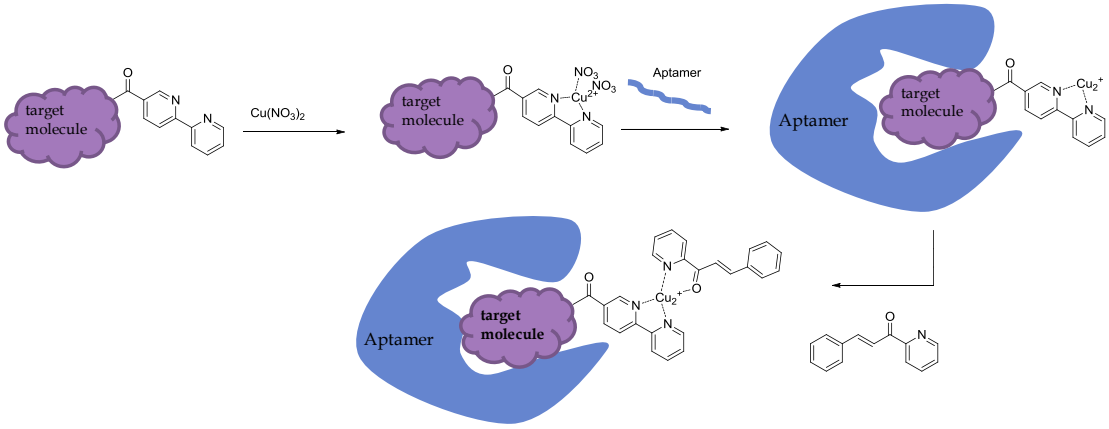
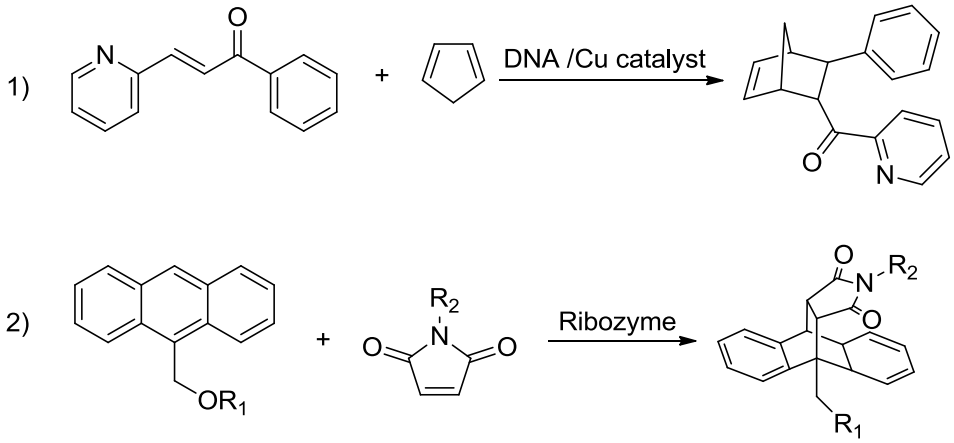


Figure 1. Example of a Diels-Alder reaction using the high recognition of aptamers towards a bipyridine ligand. Target molecule in purple represents arginine and the aptamer is represented in blue.

Bipyridines are compatible with DNA as ligands for Diels-Alder reactions resulting in enantioselectivities up to 99% for reaction 1 represented in scheme 1.[17,23,24] Moreover, biomolecules such as ribozymes (RNA capabe to catalyze reactions) have also been known to catalyze Diels-Alder reactions.[25,26] Jäschke *et al*[27,28] were the first to synthesize a Diels-Alderase ribozyme which enantioselectively catalyzed a carbon-carbon bond formation between anthracene and a maleimide achieving over 95% ee for (reaction 2 represented in scheme 1). This catalytic reaction was chosen as a model because it is well known and is compatible with biomolecules. This chapter ldescribes the synthesis of a bipyridine modified arginine.



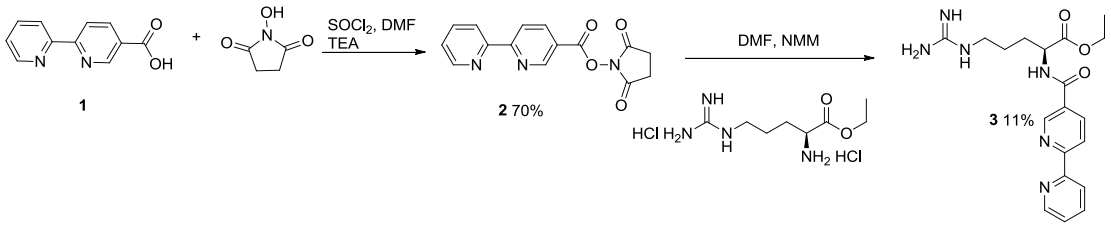
R_1 = H or $(C_2H_4O)_6H$
R_2 = spacer containing biotin, hydrazide or carboxylic acid at the terminal postion

Scheme 1. Diels-Alder reactions using a DNA catalyst (1)[17,23,24] and a ribozyme catalyst (2)[27,28]

5.2 Results and Discussion

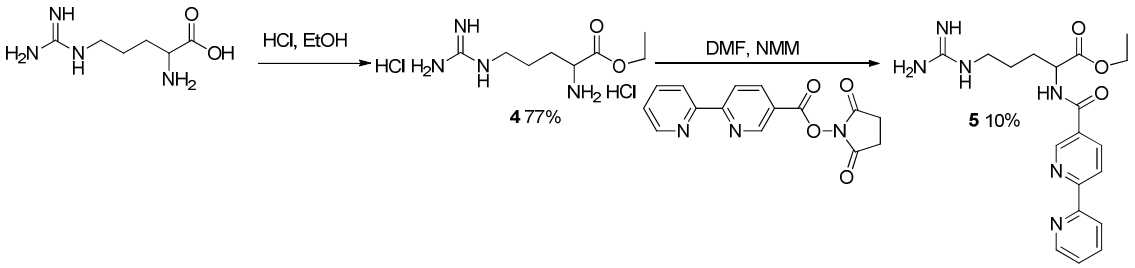
5.2.1 Synthesis of modified arginine

Bipyridine modified arginine was synthesized in three steps. The preparation of bipyridine carboxylic acid was previously represented in chapter 4 **1**. To couple the bipyridine moiety to arginine, bipyridine carboxylic acid **1** was activated with *N*-hydroxysuccinimide (NHS) to afford **2** in 70% yield. Coupling of **2** to L-arginine ethyl ester resulted in 98% conversion according to ^{1}H NMR. Purification of this compound proved troublesome. Because of its high polarity it could not be purified by silica or alumina column chromatography and precipitation with a variety of solvent mixtures was unsuccessful. Finally, compound **3** was purified by using reverse phase HPLC on a C18 phenomenex column, although the final yields were poor (11%). The buffer used for the purification of this compound was triethylamine and acetic acid which proved difficult to remove from the desired compound after purification. By co-evaporation with acetonitrile and heating to 80 ˚C *in vacuo* most of the buffer was removed although one equivalent of acetic acid was still present possibly because of hydrogen bonding with the guanidinium group of the arginine.



Scheme 2. Synthesis of bipyridine modified arginine.[29]

During the addition of bipyridine carboxylic acid racemization of the desired compound is conceivable. Therefore, a racemic version of compound **3** was synthesized. Racemic ethylester arginine is not commercially available so it was synthesized from the commercial racemic arginine using HCl gas in ethanol to yield 77% of compound **4**.[30] The same steps as in scheme 1 were performed to achieve the desired racemic bipyridine ligand **5,** again in poor yield.



Scheme 3. Synthesis of racemic arginine bipyridine ligand.[30]

Analysis of compound **5** by chiral reverse phase chromatography showed two peaks of similar ratio (57:43), (**A** in Figure 2). The same analysis performed with compound **3** results in one single peak (**B** in Figure 2) with very similar retention times as the second peak shown in Figure 1. This proves that compound **3** does not racemize during its synthesis.

A

B

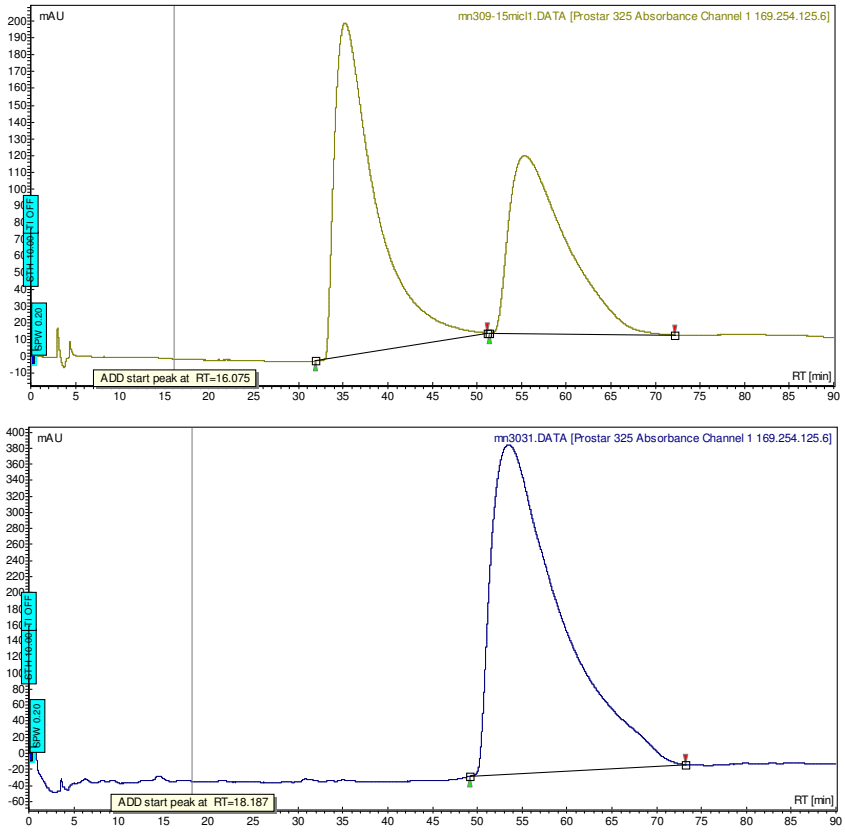
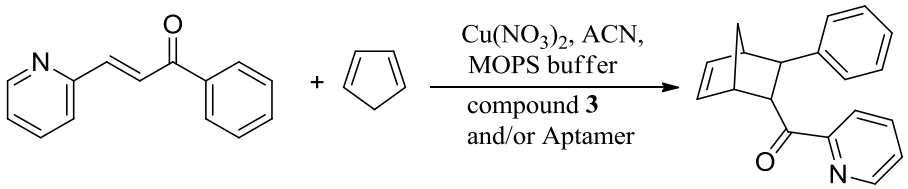


Figure 2. Compound **5** (**A**) and **3** (**B**) analyzed using a CHIRACEL OD-H column using hexane:*i*propanol 90:10 plus 1% TFA.

Frankel and Harada[19] have identified an aptamer (5'-GATCGAAACGTAGCGCCTTCGATC-3') which binds to Arginine and specifically

to the guanidinium part of the molecule. They have also shown that the negative charge of the carboxylate present in arginine reduces the binding affinity for the aptamer. For this reason it was decided to use protected arginine bipyridine ligands **3** for preliminary catalytic studies. Preliminary studies were performed for copper catalyzed Diels-Alder reaction of azachalcone with cyclopentadiene using $Cu(NO_3)_2 \cdot 3H_2O$ as precursor (Scheme 4).



Scheme4. Diels-Alder of azachalcone with cyclopentadiene using compound 3 and/or aptamer.

The copper complex of the aptamer adduct with compound **3** was used as catalyst for the Diels Alder reaction between azachalcone and cyclopentadiene resulting in 13% ee of the product. Under the same reaction conditions the copper catalyst derived from compound **3** as ligand only resulted in a racemic mixture. This preliminary study suggests that the DNA has a positive effect on the enantioselectivity of the reaction. It could be possible that the DNA binds to arginine transferring the chirality to the reaction or that the aptamer interacts with the copper precursor forming a metal complex which is active in this reaction.

5.3 Conclusion

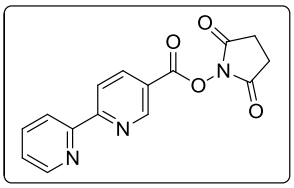
A bipyridine modified L-arginine was synthesized and analyzed by chiral HPLC. This ligand was tested with and without an aptamer in copper catalyzed Diels-Alder reaction resulting in 13% ee when the aptamer is used. This suggests that DNA induces enantioselectivity of the reaction. Further studies could be focused on the optimization of the catalytic reaction by changing the concentration of the aptamer used and also by partially changing the structure of the aptamer to improve binding affinity. Also, the binding affinity of the aptamer toward the bipyridine modified arginine could be analyzed by CD spectroscopy.

5.4 Experimental

General procedure

All reactions were performed under argon using standard Schlenk techniques. Chemicals were purchased from Aldrich Chemical Company and Acros and used as received. CH_2Cl_2 and acetonitrile were distilled over CaH_2; ethanol and methanol were distilled over Mg/I_2; THF and Et_2O were distilled over sodium /benzophenone and all the distillations were performed under nitrogen atmosphere, *N*-methylmorpholine was distilled over sodium. DMF was purchased "Extra dry" and stored over molecular sieves (4 Å) under argon. Thin Layer Chromatography (TLC) was performed on silica plates (Polygram 0.2 mm silica gel with fluorescent indicator UV_{254}). Silica gel 60 particle size 0.063-0.2 mm from Fluka was used for flash chromatography. NMR spectra were recorded at room temperature with Bruker Avance spectrometers (300, 400, and 500 MHz). Positive chemical shifts (δ) are given (in ppm) for high-frequency shifts relative to a TMS reference (^1H and ^{13}C) or an 85% H_3PO_4 reference (^{31}P). ^{13}C and ^{31}P spectra were measured with ^1H decoupling. The following abbreviations have been used in the description of the spectra: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectrometry: electrospray mass spectra were recorded on waters, micromass LCT time of flight mass spectrometer coupled to a Waters 2975 HPLC. HPLC purification of Arginine bipyridine was carried out in a Waters machine equipped of a Waters 2700 sample manager, Waters 600 controller and Waters 2487 dual absorption detector. The reverse phase column used was a Phenomenex, Clarity® C18 5 µ oligo-RP, 250 x 21.20. Chiral HPLC analyses of the Diels-Alder product was carried out on an Agilent Technologies 1200 Series apparatus equipped with a UV-Vis photodiode array detector. The measurements were performed at 215 nm, with an injection volume of 1 µl. Chiral HPLC analysis of Arginine bipyridine was carried on a Varian ProStar with an auto sampler Model 410.

N-Hydroxysuccinimide-2,2'-bipyridine-5-carboxylate (2)[29]



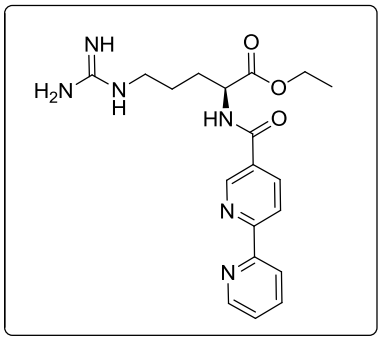
Compound **2** was prepared following literature procedure. Yield: 210 mg, 70 %

Characterization of the product fully agreed with data from literature.

^1H NMR (400 MHz, $CDCl_3$): δ = 9.30-9.29 (m, 1H), 8.68-8.66 (m, 1H), 8.56-8.53 (m, 1H), 8.47-8.42 (m, 2H), 7.81 (dt, 1H, J = 1.8 Hz, 7.7 Hz), 7.35-7.32 (m, 1H), 2.88 (bs, 4H)

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 169.0, 161.0, 160.9, 154.4, 151.1, 149.4, 138.8, 137.3, 125.1, 122.3, 121.1, 120.8, 25.7

Ethyl 2-([2,2-bipyridine]-5-carboxamido)-5-guanidinopentanoate (3)



Compound **2** (100 mg, 0.336 mmol, 1 eq), *L*-arginine ethyl ester·HCl (92 mg, 0.336 mmol, 1 eq), and *N*-methylmorpholine (81 µl, 0.740 mmol, 2.2 eq), were mixed together in DMF (5 ml) and stirred overnight. Afterwards the reaction mixture was concentrated to dryness, dissolved in 50:50 solution of water:methanol, filtered again and purified by Preparative HPLC using A: 0.1 mM TEAA (triethylamine acetic acid) buffer, B: methanol. Gradient 20 →55% B in 40 min. t_R 25.40 min. Yield: 14.6 mg, 11%

^{1}H NMR (400 MHz, D_2O): δ = 8.73 (m, 1H), 8.44 (m, 1H), 8.09-8.06 (m, 1H), 7.91-7.80 (m, 3H), 7.36 (m, 1H), 4.49-4.45 (m, 1H), 4.15 (q, 2H, J = 7.1 Hz), 3.13 (t, 2H, J = 6.7 Hz), 1.95-1.89 (m, 1H), 1.85-1.74 (m, 1H), 1.66-1.60 (m, 2H), 1.17 (t, 3H, J = 7.4 Hz)

^{13}C NMR (75.5 MHz, MeOD): δ = 168.3, 159.7, 158.8, 156.2, 150.5, 149.6, 138.8, 137.6, 130.9, 125.9, 123.1, 121.9, 62.6, 54.0, 41.9, 29.5, 26.6, 14.5

MS (ES+): 384.91 (M+), HRMS calculated for $C_{19}H_{25}N_6O_3$ (acetic acid salt); 385.1988, found 385.1987

mp: 42-45

[α] = +13.5 (c = 1, methanol)

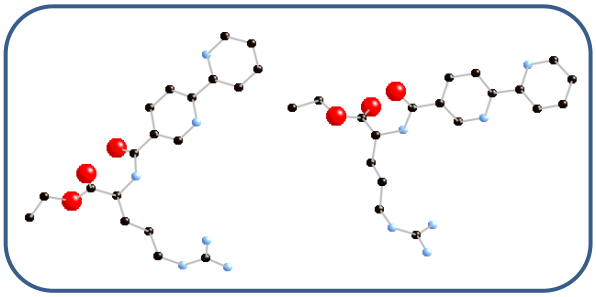
Chiral HPLC analysis using a CHIRACEL OD-H column, hexane:*i*propanol 90:10 plus 1% TFA, eluted the desired compound at t_R 53.55min.

Compound **5** was synthesized following the procedure of compound **3** using racemic arginine ethylester. Yield: 3.6 mg, 10%.

^{1}H NMR (400 MHz, MeOD): δ = 9.1 (m, 1H), 8.70 (m, 1H), 8.47-8.43 (m, 2H), 8.38-8.35 (m, 1H), 7.98 (dt, 1H, J = 7.8 Hz, 1.8 Hz), 7.51-7.48 (m, 1H) 4.70-4.66 (m, 1H), 4.26 (q, 2H, J = 7.1 Hz), 3.29-3.25 (m, 2H), 2.10-2.04 (m, 1H), 2.00-1.88 (m, 1H), 1.80-1.71 (m, 2H), 1.31 (t, 3H, J = 7.4 Hz)

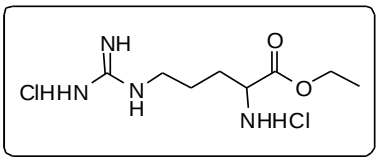
Chiral HPLC analysis using a CHIRACEL OD-H column, hexane:*i*propanol 90:10 plus 1% TFA, eluted the desired compound at t_R (D)33.38 min, t_R (L) 50.78 min.

Crystals suitable for X-ray crystal structure analysis were obtained by slow evaporation with acetonitrile. Both enantiomers were found in the crystal structure.



DL-Arninine ethyl ester(4)[30]

Compound **5** was synthesized according to literature procedure resulting in a white powder. Yield: 1.2 g, 77%



Characterization of the product fully agreed with data from literature.

^{1}H NMR (400 MHz, MeOD): δ = 4.32 (q, 2H, J = 7.0 Hz), 4.10 (t, 1H, J = 6.5 Hz), 3.29 (t, 2H, J = 6.9 Hz), 2.08-1.94 (m, 2H), 1.85-1.68 (m, 2H), 1.34 (t, 3H, 7.1 Hz)

General procedure for Diels-Alder reactions[29]

Aptamer 5'-GATCGAAACGTAGCGCCTTCGATC-3' (30 µl, 30 nmol, 2.6 eq), compound **3** (10 µl, 15 nmol, 1.3 eq), and $Cu(NO_3)_2 \cdot 3H_2O$ (120 µl, 12 nmol) in MOPS buffer (20 mM, pH 6.5) were added together and cooled for 1.5 h at 0˚C. Azachalcone (3 µl, 296 nmol, 25 eq) and freshly distilled cyclopentadiene (1 µl, 10.9 µmol, 363 eq), were added and the reaction mixture was shaken for 3 days at 4˚C. Then the reaction mixture was extracted with Et_2O which was evaporated by a flow of air. The products were analyzed by chiral HPLC using an OD-H column and hexane:*i*PrOH 99:1 with a flow rate of 0.5ml/min. 15.4, 18.6 (*exo* isomer), 22.0, 30.1 (*endo* isomer)

5.5 References

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Conclusion and Future Work

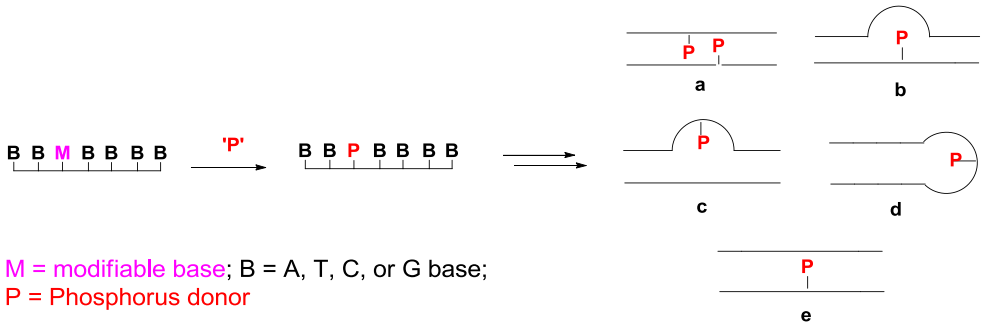
This thesis focuses on the development of metalloDNAzymes for the application in asymmetric catalytic reactions. Two methods are presented; the covalent and noncovalent approach. The covalent approach consists of introducing modified nucleosides with thiol or amine functionalities into oligonucleotides. This thesis reports the synthesis of several modified nucleosides, their introduction into oligonucleotides and their coupling to phosphine moieties. Thiol functionalized oligonucleotides were the initial target with the aim to form thioesters with phosphine moieties. Thioester bonds can be cleaved easily and therefore have high potential for the recycling of DNA ligands. The functionalization of thiol modified oligonucleotides proved troublesome due to unwanted reactions of the free thiol group during the deprotection step. These difficulties prompted us to change the strategy and use a less reactive linker for the introduction of phosphine moieties into DNA strands. Two amine modified nucleosides were successfully introduced into oligonucleotides. *Ortho*-, *meta*-, and *para*-diphenylphosphinobenzoic acids were successfully coupled to amine modified nucleosides and the resulting ligands were used as models for DNA strands in Pd-catalysed allylic substitution reactions. Although no enantioselectivity was observed, four of the ligands gave complete conversions in this reaction. Longer amine modified oligonucleotides could also be functionalized with phosphine moieties and a ^{31}P NMR spectra of a 15-mer modified with *para*-diphenylphosphinobenzoic acid and its metal complex with a Pd allylic moiety was successfully obtained. The method developed in this thesis enables the synthesis of several phosphine modified oligonucleotides as ligands for asymmetric catalytic reactions.

To circumvent the problem of phosphine oxidation another covalent method is presented in this thesis, which consists of the synthesis of a sulfur protected phosphine moiety coupled to PNA strands. PNA is a stable achiral mimic of DNA. Chirality in PNA can be easily introduced by placing DNA as complementary strand. PNA strands can be synthesized with modified monomers already containing the ligand functionality. Since the PNA backbone is resistant to sulfur deprotection conditions, a protected phosphine moiety can be introduced in a PNA strand during its synthesis. Unfortunately, the preparation of long modified PNA strands was found troublesome

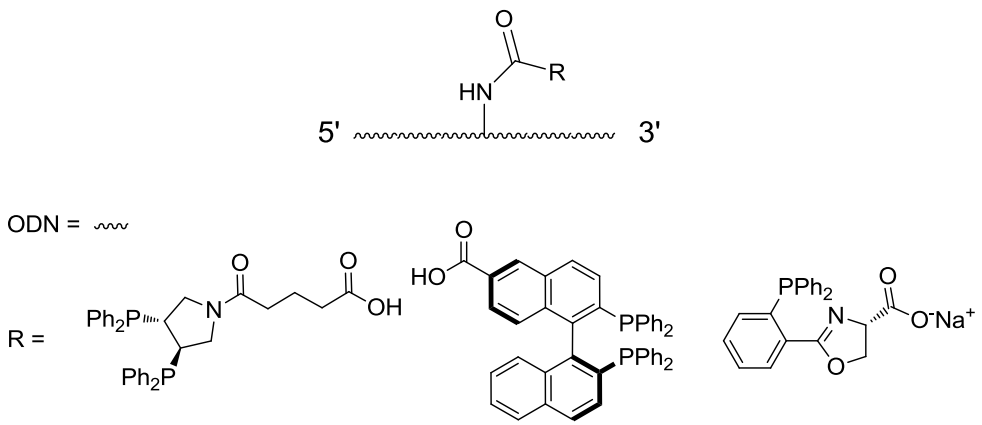
although a PNA 5-mer containing a bidentate sulfur protected phosphine could be successfully prepared. Further work on this method will allow the introduction of protected phosphine moieties into PNA strands during its synthesis and therefore easing ligand purification.

As an alternative to the covalent approach, a preliminary study in using aptamers as chiral scaffold has also been presented in this thesis. Several aptamers have been reported to bind to L-arginine, which has been chosen as the target molecule to couple to a transition metal coordinating ligand. A 2,2'-bipyridine modified L-arginine was successfully synthesized and tested with and without an aptamer in a copper catalyzed Diels-Alder reaction, resulting in 13% ee when the aptamer was used. This suggests that the DNA aptamer induces enantioselectivity in the reaction.

The successful preparation of phosphine modified oligonucleotides developed in this thesis is a promising starting point for using phosphine modified "DNAzymes" as ligands for enantioselective transition-metal catalyzed reactions. This thesis contains the ground work to obtain DNA modified phosphines, which in the future can be tested in several different catalytic reactions. In addition, different complementary strands can be used to change the local environment of the substrate (Scheme 1); different transition metals can be used such as Rh, Pd, and Ir; and different types of phosphines bearing a carboxylic acid moiety can be coupled to the DNA, as shown by Jäschke[1] (Scheme 2), increasing the variety of ligands that can be synthesized in a relatively short period of time.

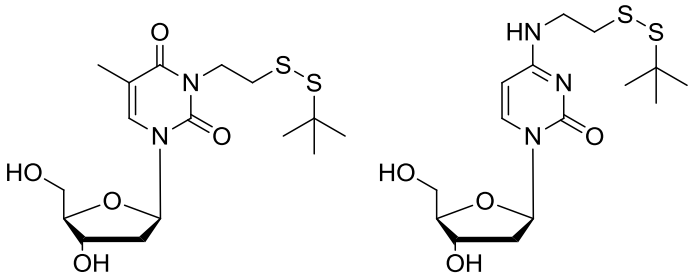


Scheme 1. Coupling of a phosphine moiety to a DNA strand and introduction of complementary strands. Introduction of a complementary strand also containing a phosphine moiety results in a bidentate (**a**), mismatches in the complementary strand sequence can give rise to loops in the complementary strands (**b**) in the modified strand (**c**), or in a self complementary strand (**d**), addition of a fully complementary strand (**e**) produces yet another environment.



Scheme 2. DNA strands containing different phosphine ligands.[1]

In future work the use of thiol functionalities, which provide a convenient reversible thioester linkage between the DNA and the phosphine functionality, can be carried out by synthesizing nucleosides which do not contain a reactive alkynyl functionality. A few examples of such monomers can be found in the work of Glick[2] and Eritja[3] (Scheme 3).



Scheme 3. Nucleosides containing a thiol linker.[2, 3]

PNA is a biomolecule with high potential as ligand in catalytic reactions. This thesis shows the successful introduction of protected phosphine moieties in short PNA strands. In future work Dde instead of Fmoc protecting group can be used to avoid problems during strand synthesis. PNA binds more strongly to DNA than DNA does with itself and therefore shorter strands can be synthesized to simplify the syntheses. Phosphine deprotection was unsuccessful therefore different phosphine moieties that might be more prone to sulfur deprotection or the use of a phosphorous scavenger such as tributylphosphine[4] can also be tested. As with DNA, PNA ligands could be modified by changing the complementary DNA strands, using different types of ligand functionalities and different transition metals.

Finally, the preliminary study presented in this thesis demonstrates that the arginine aptamer plays a role in the enantioselectivity of a Diels-Alder reaction. This method will allow the easy synthesis of ligands without the need to apply modifications to the DNA structure and without the need of anaerobic conditions. Further studies can be focused on the optimization of the catalytic reaction by changing the concentration of the aptamer used and by partially changing the structure of the aptamer or arginine to improve binding affinity. In addition, the binding affinity of the aptamer toward the 2,2'-bipyridine modified arginine can be analyzed by CD spectroscopy. For a better insight on the catalytic reaction, the racemic arginine bipyridine ligand can be used in the catalytic reactions to understand the importance of the chirality of the arginine ligand for binding to the aptamer. In addition, catalytic runs with only DNA and copper are necessary to understand whether the enantioselectivity observed is generated from the aptamer/arginine ligand or from the aptamer alone interacting with the copper precursor.

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Appendix: Crystallographic Data

The complete crystallographic data (.cif file) for the compounds bellow can be found in the CD accompanying this thesis.

Table 1. Crystal data and structure refinement for compound **5** (Chapter 2).

Identification code	10	
Empirical formula	C15 H17 N3 O5 S	
Formula weight	351.38	
Temperature	93(2) K	
Wavelength	0.71075 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 5.2132(11) Å	α = 90°.
	b = 9.1903(18) Å	β = 90°.
	c = 32.342(7) Å	γ = 90°.
Volume	1549.5(6) $Å^3$	
Z	4	
Density (calculated)	1.506 Mg/m^3	
Absorption coefficient	0.242 mm^{-1}	
F(000)	736	
Crystal size	0.15 x 0.10 x 0.01 mm^3	
Theta range for data collection	2.30 to 25.33°.	
Index ranges	-6<=h<=5, -11<=k<=10, -38<=l<=38	
Reflections collected	15517	
Independent reflections	2831 [R(int) = 0.0252]	
Completeness to theta = 25.00°	99.9 %	
Absorption correction	multiscan	
Max. and min. transmission	0.9976 and 0.9646	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2831 / 0 / 217	
Goodness-of-fit on F^2	1.119	
Final R indices [I>2sigma(I)]	R1 = 0.0567, wR2 = 0.1607	
R indices (all data)	R1 = 0.0576, wR2 = 0.1618	
Absolute structure parameter	-0.01(15)	
Largest diff. peak and hole	0.691 and -0.296 $e.Å^{-3}$	

Table 2. Crystal data and structure refinement for compound **11** (Chapter 2).

Identification code	2	
Empirical formula	C20 H20 N2 O6 S	
Formula weight	416.44	
Temperature	173(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 4.8756(4) Å	α = 90°.
	b = 16.2429(13) Å	β = 90°.
	c = 23.7663(19) Å	γ = 90°.
Volume	1882.1(3) $Å^3$	
Z	4	
Density (calculated)	1.470 Mg/m^3	
Absorption coefficient	1.902 mm^{-1}	
F(000)	872	
Crystal size	0.20 x 0.20 x 0.03 mm^3	
Theta range for data collection	3.30 to 68.00°.	
Index ranges	-5<=h<=5, -19<=k<=19, -28<=l<=28	
Reflections collected	20497	
Independent reflections	3104 [R(int) = 0.0521]	
Completeness to theta = 68.00°	93.9 %	
Absorption correction	multiscan	
Max. and min. transmission	0.9451 and 0.7022	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3104 / 0 / 263	
Goodness-of-fit on F^2	1.003	
Final R indices [I>2sigma(I)]	R1 = 0.0651, wR2 = 0.1734	
R indices (all data)	R1 = 0.0686, wR2 = 0.1766	
Absolute structure parameter	0.05(4)	
Largest diff. peak and hole	0.567 and -0.303 $e.Å^{-3}$	

Table 3. Crystal data and structure refinement for compound **19** (Chapter 2).

Identification code	1	
Empirical formula	C13 H16 N2 O5 S	
Formula weight	312.34	
Temperature	93(2) K	
Wavelength	0.71070 Å	
Crystal system	Monoclinic	
Space group	C2	
Unit cell dimensions	a = 20.69(5) Å	α = 90°.
	b = 6.819(16) Å	β = 111.36(6)°.
	c = 11.35(4) Å	γ = 90°.
Volume	1491(7) $Å^3$	
Z	4	
Density (calculated)	1.391 Mg/m^3	
Absorption coefficient	0.240 mm^{-1}	
F(000)	656	
Crystal size	0.35 x 0.11 x 0.01 mm^3	
Theta range for data collection	2.11 to 25.44°.	
Index ranges	-24<=h<=24, -8<=k<=8, -13<=l<=13	
Reflections collected	7338	
Independent reflections	2715 [R(int) = 0.3862]	
Completeness to theta = 25.44°	98.9 %	
Absorption correction	multiscan	
Max. and min. transmission	0.9976 and 0.9208	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2715 / 1 / 92	
Goodness-of-fit on F^2	1.495	
Final R indices [I>2sigma(I)]	R1 = 0.2236, wR2 = 0.4824	
R indices (all data)	R1 = 0.2379, wR2 = 0.5160	
Absolute structure parameter	-1.0(4)	
Largest diff. peak and hole	1.080 and -1.200 $e.Å^{-3}$	

Table 4. Crystal data and structure refinement for compound **15** (Chapter 3).

Identification code	4	
Empirical formula	C12 H15 N3 O5	
Formula weight	281.27	
Temperature	93(2) K	
Wavelength	0.71069 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.633(3) Å	α = 90°.
	b = 10.934(5) Å	β = 90°.
	c = 17.203(8) Å	γ = 90°.
Volume	1247.7(10) $Å^3$	
Z	4	
Density (calculated)	1.497 Mg/m^3	
Absorption coefficient	0.118 mm^{-1}	
F(000)	592	
Crystal size	0.30 x 0.10 x 0.03 mm^3	
Theta range for data collection	3.29 to 28.14°.	
Index ranges	-6<=h<=8, -13<=k<=14, -16<=l<=22	
Reflections collected	7432	
Independent reflections	2606 [R(int) = 0.1290]	
Completeness to theta = 28.14°	90.7 %	
Absorption correction	multiscan	
Max. and min. transmission	0.9965 and 0.9654	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2606 / 0 / 182	
Goodness-of-fit on F^2	0.991	
Final R indices [I>2sigma(I)]	R1 = 0.1225, wR2 = 0.2564	
R indices (all data)	R1 = 0.1904, wR2 = 0.2910	
Absolute structure parameter	2(4)	
Largest diff. peak and hole	0.518 and -0.436 $e.Å^{-3}$	

Table 5. Crystal data and structure refinement for compound **37** (Chapter 3)

Identification code	agpk5	
Empirical formula	C31 H32 N3 O6 P	
Formula weight	573.57	
Temperature	93(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 8.2680(15) Å	α = 90°.
	b = 12.254(2) Å	β = 92.063(5)°.
	c = 13.839(3) Å	γ = 90°.
Volume	1401.2(4) $Å^3$	
Z	2	
Density (calculated)	1.359 Mg/m^3	
Absorption coefficient	0.148 mm^{-1}	
F(000)	604	
Crystal size	0.1000 x 0.1000 x 0.1000 mm^3	
Theta range for data collection	2.22 to 25.37°.	
Index ranges	-8<=h<=9, -12<=k<=14, -12<=l<=16	
Reflections collected	8867	
Independent reflections	4839 [R(int) = 0.0272]	
Completeness to theta = 25.00°	99.9 %	
Absorption correction	Multiscan	
Max. and min. transmission	1.0000 and 0.9836	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4839 / 5 / 387	
Goodness-of-fit on F^2	0.715	
Final R indices [I>2sigma(I)]	R1 = 0.0479, wR2 = 0.1285	
R indices (all data)	R1 = 0.0500, wR2 = 0.1326	
Absolute structure parameter	0.19(11)	
Largest diff. peak and hole	1.388 and -0.358 $e.Å^{-3}$	

Table 6. Crystal data and structure refinement for compound **38** (Chapter 3)

Identification code	agpk3	
Empirical formula	C31 H32 N3 O6 P	
Formula weight	573.57	
Temperature	93(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P1	
Unit cell dimensions	a = 8.321(2) Å	α = 75.93(2)°.
	b = 10.6140(19) Å	β = 88.69(3)°.
	c = 18.263(6) Å	γ = 68.52(3)°.
Volume	1451.9(6) $Å^3$	
Z	2	
Density (calculated)	1.312 Mg/m^3	
Absorption coefficient	0.143 mm^{-1}	
F(000)	604	
Crystal size	0.1500 x 0.0800 x 0.0500 mm^3	
Theta range for data collection	2.13 to 25.37°.	
Index ranges	-10<=h<=9, -10<=k<=12, -21<=l<=21	
Reflections collected	9047	
Independent reflections	6748 [R(int) = 0.0774]	
Completeness to theta = 25.00°	96.7 %	
Absorption correction	Multiscan	
Max. and min. transmission	1.0000 and 0.9748	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6748 / 11 / 764	
Goodness-of-fit on F^2	0.981	
Final R indices [I>2sigma(I)]	R1 = 0.0792, wR2 = 0.1737	
R indices (all data)	R1 = 0.1114, wR2 = 0.2032	
Absolute structure parameter	0.1(2)	
Largest diff. peak and hole	1.029 and -0.376 $e.Å^{-3}$	

Table 7. Crystal data and structure refinement for compound **5** (Chapter 5).

Identification code	9	
Empirical formula	C19 H24 N6 O3	
Formula weight	384.44	
Temperature	173(2) K	
Wavelength	1.54187 Å	
Crystal system	Orthorhombis	
Space group	Pbca	
Unit cell dimensions	a = 30.217(6) Å	α = 90°.
	b = 16.724(3) Å	β = 90°.
	c = 9.365(2) Å	γ = 90°.
Volume	4732.8(17) $Å^3$	
Z	8	
Density (calculated)	1.079 Mg/m^3	
Absorption coefficient	0.621 mm^{-1}	
F(000)	1632	
Crystal size	0.30 x 0.01 x 0.01 mm^3	
Theta range for data collection	2.92 to 68.07°.	
Index ranges	-36<=h<=36, -19<=k<=20, -11<=l<=11	
Reflections collected	57858	
Independent reflections	4289 [R(int) = 0.2367]	
Completeness to theta = 67.00°	99.9 %	
Absorption correction	multiscan	
Max. and min. transmission	0.9938 and 0.8356	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4289 / 0 / 130	
Goodness-of-fit on F^2	1.942	
Final R indices [I>2sigma(I)]	R1 = 0.2212, wR2 = 0.5181	
R indices (all data)	R1 = 0.2631, wR2 = 0.5468	
Largest diff. peak and hole	2.612 and -1.010 e.$Å^{-3}$	

Table of Abbreviations

AdaOH	adamantane acetic acid
CAST	combinational active-site saturation test
Cbz	carbobenzyloxy
CDI	1,1′-carbonyldiimidazole
CS	commercial strand
Bhoc	benzhydryloxy carbonyl
Boc	*tert*-butyloxycarbonyl
BSA	(*N*,*O*-bis(trimethylsilyl)acetamide)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DCM	dichloromethane
Dde	1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl
DMF	dimethylformamide
DMT-Cl	4,4'-dimethoxytrityl chloride
DNA	deoxyribonucleic acids
dppba	diphenylphosphine benzoic acid
EDC	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide
EPA	environmental protection agency
FMN	flavin mononucleotide
Fmoc	9-Fluorenylmethyloxycarbonyl
FPLC	fast protein liquid chromatography
15-mer	oligonucleotide 15 bases long
Trimer	oligonucleotide 3 bases long
HMBC	heteronuclear multiple bond correlation
HOBT	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
IPEA	diisopropylethylamine
IU	5-iodo-2’-deoxyuridine
MW	molecular weight
MMT-Cl	4-Methoxytriphenylmethyl chloride
NHS	*N*-hydroxysuccinimide
NMR	nuclear magnetic resonance
nt	nucleotides
PCR	polymerase chain reaction

SELEX	systematic evolution of ligands by exponential enrichment
TCEP	*tris*(2-carboxyethyl)phosphine hydrochloride
TEA	tryethylamine
TEAA	triethylamine acetic acid
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIS	triisopropylsilane
VEGF	endothelial growth factor

Acknowledgments

This thesis would not be completed without thanking the people that help me with this project and mostly helped me grow to be the person that I'm now. Fist of all I would like to thank Professor Paul Kamer to give me the opportunity to participate in this challenging project. Special thanks go to Dr. Bert Swennenhuis mostly because of his company in the lab and his encouragement. I would like to thank Dr. Loic Ropartz for training me during my first year, Jason for the help with organic chemistry and for the nice conversations, Dr. Wouter Laan for the help with the biochemistry side of my project, Dr. Debby Dodds for the nice cakes, and the rest of the group for the help and for keeping the lab organized and tidy. I would like to express my deep gratitude to Dr. Arnald Grabulosa for correcting my thesis and being like a brother to me; also for never needing a pencil again. I would also like to thank Mrs Melanja Smith and Dr. Tomas Lebl for their outstanding NMR service, Dr. Catherine Botting, Dr. Sally Shirran and Mr Alex Houston for the help with MALDI-TOF analysis, Mr. Bobby Cathcart, Mr. Colin Smith, Mrs Marjory Parker, Dr. Qingzhi Zhang, Mrs Caroline Horsbursh, for all their help during those 5 years. A million thanks go to Professor Alex Slawin for all the amazing support during the dark times of my PhD. Moreover, thanks go to Professor Mark Bradley, Dr. Juan Jose Diaz-Mochon and Dr. Eftychia Koini for the help with the PNA project. Many thanks go to Karen and Federica for all the nice evenings spent together. Special thanks go to Aga, Marcin and Dominik for their friendship and for always being there for me. Thanks to Jacorien and William for the amazing dinners and the nice wedding dresses trips. Thanks go to Irina, Andrea and Trudy for the nice moments spent at the university cottage. Last but not least I would like to thank my Family, Peter and my distant friends Lesli, Jeevanthie, Soo-Hyun (Irene), and Barbara for the support. Finally, thanks go to Micio, for all the purring and to Frodo, Andor and Mafros for the nice walks on the beach.