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# Surprising duplex stabilisation upon mismatch introduction within triply modified duplexes

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ABSTRACT

Three different modified phosphoramidite nucleoside building blocks equipped with additional protected imidazole, masked alcohol and masked carboxylate functionality are synthesized and incorporated into oligonucleotides. Based on the serine-protease active site model, doubly and triply modified duplexes are created and tested for stability. Analysis of different spatial distributions of the extra functionalities shows that careful positioning can even overcome duplex destabilisation caused by the introduction of mismatches.



## 1. Introduction

Nature typically uses protein based catalysts for a range of chemical transformations and a whole series of synthetic mimics of these catalysts has been developed and investigated throughout the past decade. In comparison to the wealth of research dealing with the design of protein or peptide based catalysts, considerably less effort has been invested in generating synthetic nucleic acid based catalytic systems. Although various studies have been reported on the creation of so-called DNAzymes for cleavage of recruited RNA sequences and in some cases even DNA sequences, no studies have been reported concerning synthetic nucleic acid based systems designed for cleavage of amide bonds. In view of the negatively charged backbone that could severely compromise recognition of an anionic transition state analog this is not surprising. This further underscores the difficulty in designing suitable systems and illustrates the need for introduction of additional functionality into the DNA. In general, the application of DNA or oligonucleotides as real catalysts is still only an emerging field of research.

However, the DNA double helical structure represents a completely untapped potential framework for precise positioning of functional groups. A particular attractive feature of using DNA as scaffold resides in the fact that once a specific sequence is incubated with a complementary sequence, both will recognize each other and inevitably form a duplex, in an inherently predictable and programmed manner. This illustrates a great advantage of oligonucleotides over proteins, namely the precise control over secondary structure, resulting in the possibility to design specific 3-dimensionally defined assemblies.

Whereas this ability has been exploited by various people in the context of using DNA as a template to facilitate programmed reaction or polymerization between associated and adequately functionalized building blocks [1–6], fewer efforts have been directed towards the use of DNA as a catalytic system. Moreover the scope of DNA catalysis has until now been mostly limited to oligonucleotide substrates [7]. Examples include RNA cleavage [8,9], oxidative DNA cleavage [10] and RNA [11] and DNA ligation [12]. Only few examples of DNA catalysis on non-oligonucleotide substrates have been reported. Next to the use of nucleoside triphosphates (NTPs) as substrates [13], porphyrin metalation has been reported [14], both based on the use of DNA made up of natural building blocks. One specific case of organic reaction catalysis reports on a Diels–Alder deoxyribozyme as the first example of a DNA catalyst for C–C bond formation [15,16].

One way to optimize the catalytic performance of DNA is to incorporate functional groups that are able to participate directly in chemical catalysis. An elegant approach by Feringa and co-workers reports on the use of intercalating Cu(II) complexing ligands to create an enantioselective DNA based catalyst for asymmetric Diels–Alder reactions [17,18]. The exact position of the complexing ligand within the DNA is however undefined, making it difficult to assess the exact role of the DNA. Furthermore functionalisation can be achieved making use of random incorporation of unnatural nucleotides via polymerase reactions [19]. Site-specific inclusion of extra chemical functionality in DNA is a promising approach towards improving DNA catalysis, but apart from a few isolated examples [20–22], this potential is largely unexplored [7]. The current paper describes our first and preliminary efforts towards the design and synthesis of functionalized DNA duplexes inspired by the active site of hydrolytic enzymes. Design of catalytic systems being a tremendous task where various aspects of binding,

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orientation of functional moieties and transition state stabilisation come into play, therefore a stepwise approach is recommended where optimization of each parameter can be important for final success. In view of the importance of the relative positioning of functional groups within an enzyme's active site, we have decided to carefully explore different possible spatial orientations and combinations of modified residues in order to gain insight into the delicate balance between the orientation of the extra functional groups and the resulting duplex stability.

## 2. Material and methods

### 2.1. Chemicals and reagents

All reagents were obtained from Aldrich, ACROS Organics or Fluka and were used without prior purification. Dichloromethane and $Et_3N$ were distilled from calciumhydride and MeOH was dried on $Mg/I_2$. Reactions were performed under argon atmosphere. Analytical TLC was carried out on glass plates precoated with silica gel (Merck, 60F-254, 0.25 mm) and compounds were visualized using phosphomolybdic acid (PMA) and $KMnO_4$. Flash chromatography was performed on silica gel (KieselgelMerck, 230–400 mesh, Typ 9385, 60 Angström). $^{31}P$ NMR spectra were recorded on a Bruker 300 MHz spectrometer at 121 MHz. The deuterated solvent $CDCl_3$ was obtained from Aldrich. Chemical shifts (δ units) are expressed in parts per million (ppm) relative to TMS and the internal solvent peak was used for calibration. Mass spectra (ES-MS) were recorded on a quadrupole ion trap LC mass spectrometer (Thermo Finnigan MAT LCQ mass spectrometer) equipped with electrospray ionization and EI-MS spectra were acquired on a Hewlett–Packard 5998 A (MS, EI) spectrometer.

### 2.2. Oligonucleotide synthesis

Oligonucleotide synthesis was carried out using an Applied Biosystems DNA synthesizer under standard synthetic procedures based on phosphoramidite chemistry. Modified building blocks are coupled manually. Therefore the DNA synthesis cycle is stopped and the activator (1 M 4,5-dicyanoimidazole in acetonitrile) and the phosphoramidite (a 0.05 M solution in acetonitrile) are successively added in different portions onto the solid support. For each 2 parts of phosphoramidite, 3 parts of activator are used. Before use, both were dried on 3 Å molecular sieves for 15 min. Coupling times are extended to 15 min. The oligonucleotides are synthesized using the DMTr-ON method. The last DMTr protecting group will be cleaved manually using a Sep-pak® purification (vide infra) under acidic conditions. Oligonucleotides are deprotected and cleaved from the solid support by treatment with concentrated ammonia (28%) at 55 °C for 15 h. During this procedure, the acetyl protecting group of $\mathbf{U^o}$ and the Boc-protection group of $\mathbf{U^I}$ are also cleaved. For the deprotection of the allylgroup of $\mathbf{U^C}$, a different procedure, which is performed before the cleavage of the solid phase, is needed. Therefore, the solid support is suspended in dichloromethane and morpholine. Tetrakis(triphenylphosphine)-Pd(0) and triphenylphosphine are added. This mixture is shaken in the dark for 40 min. The dichloromethane solution is removed with a syringe and the solid support is rinsed with dichloromethane, ethanol and water. After this procedure, the solid support is treated with a concentrated ammonia solution to cleave and deprotect as described before.

### 2.3. Sep-Pak® purification

The cleaved and deprotected oligonucleotide (DMTr-ON) is purified using a Sep-Pak® procedure. The Sep-Pak® cartridge is activated using successively 10 mL $CH_3CN$ and 10 mL 5 mM triethyl ammonium acetate solution (TEAA solution). The oligonucleotide solution is applied on the cartridge and the resulting solution is reapplied three times. The cartridge is now washed with 15 mL 2.8% $NH_4OH$ solution followed by 10 mL $H_2O$ to remove the capped sequences. The desired oligonucleotide can then be removed from the cartridge after cleavage of the DMTr group. For this deprotection, 2 × 5 mL 1.5% TFA is added and the cartridge is washed with 10 mL milli-Q water. The deprotected oligonucleotide is collected by washing with 3 × 1 mL 20% acetonitrile in water. These three fractions are separately collected, lyophilized using the speedvac and analyzed with RP-HPLC.

### 2.4. RP-HPLC purification

Purification was carried out by reversed phase HPLC using a Phenomenex Clarity 5 μm Oligo-RP column with a gradient of 0–100% A over 30 min (at 0.8 mL/min; A: methanol, B: 0.1 M TEAA buffer (pH 7), 250 mL TEAA buffer 1 M, 2125 mL milli-Q water and 125 mL acetonitrile).

### 2.5. Determination of $T_m$

To determine the melting temperatures, the oligonucleotides are dissolved in 1 M NaCl solution, 0.1 M sodiumphosphate buffer (pH 7) and water. 10% of the 1 M NaCl solution and 10% of the 0.1 M sodiumphosphate buffer of the total volume is added and the mixture is diluted with milli-Q water to the desired volume. The concentration of the oligonucleotide in the total volume is 1 μM. The melting temperatures are defined using a CARY 300 BIO UV/VIS spectrophotometer. The oligonucleotides are annealed by heating the samples quickly until 95 °C and then allowing them to cool down until 18 °C. The program Thermal, in which the samples are heated from 18 °C to 95 °C at a rate of 0.3 °C/min, is used to determine the melting temperatures.

### 2.6. General synthesis procedure for 3′-O-P-β-cyanoethyl-N,N′-diisopropylphosphoramidites $U^I$, $U^O$, $U^C$ from the corresponding 5′-dimethoxytrityl compounds

The starting dimethoxytritylated compound was dried in vacuo overnight before being dissolved in anhydrous dichloromethane. DIPEA (3 eq.) and 2-cyano-*N,N′*-diisopropylchlorophosphoramidite (1.5 eq.) were added dropwise to the solution at 0 °C. The reaction mixture was slowly allowed to warm to room temperature and stirred for 4–6 h under argon. The mixture was cooled down to 0 °C and quenched with a 5% $NaHCO_3$ solution ($\mathbf{U^I}$, $\mathbf{U^C}$) or methanol ($\mathbf{U^o}$) and stirred for 10 min. The solution was diluted by the addition of dichloromethane and washed with 5% $NaHCO_3$ solution (2×) and brine (2×). The organic phase was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with a mixture of hexane, acetone and $Et_3N$ (49:49:2). The desired fractions were combined and concentrated under vacuum yielding a white foam.

*2′-(3-Acetoxypropanoyl)amido-5′-O-(4,4′-dimethoxytrityl)-3′-O-(P-β-cyanoethyl-N,N′-diisopropylaminophosphinyl)-2′-deoxyuridine* ($\mathbf{U^o}$): Rf (hexane:acetone:$Et_3N$ 49:49:2): 0.36; $^{31}P$ NMR ($CDCl_3$, 121 MHz) δ 151.27, 149.16 ppm; MS ESI$^+$ *m/z* 303.2 $[DMTr]^+$, 876.5 $[M+NH_4]^+$.

*2′-((4-Allyloxy-4-oxo)butanoyl)amido-5′-O-(4,4′-dimethoxytrityl)-3′-O-(P-β-cyanoethyl-N,N′-diisopropylaminophosphinyl)-2′-deoxyuridine* ($\mathbf{U^C}$): Rf (hexane:acetone:$Et_3N$ 49:49:2): 0.28; $^{31}P$ NMR ($CDCl_3$, 121 MHz) δ 151.30, 149.50 ppm; MS ESI$^+$ *m/z* 303.3 $[DMTr]^+$, 885.6 $[M]^+$, 902.6 $[M+NH_4]^+$.

*2′-(3-(1-tert-Butoxycarbonyl)imidazole-4-yl-acryloyl)amido-5′-O-(4,4′-dimethoxytrityl)-3′-O-(P-β-cyanoethyl-N,N′-diisopropylamino-*

*phosphinyl)-2′-deoxyuridine (**U$^{I}$**)*: Rf (hexane:acetone:$Et_3N$ 49:49:2): 0.30; $^{31}P$ NMR ($CDCl_3$, 121 MHz) δ 150.90, 150.80 ppm; MS ESI$^+$ *m*/*z* 303.3 $[DMTr]^+$, 965.4 $[M]^+$, 966.3 $[M+H]^+$.

## 3. Results and discussion

### 3.1. Design of a DNA-based serine protease mimic

The most intensively studied natural system for amide bond hydrolysis is probably the class of serine protease enzymes. Equipped with an active site containing a so-called catalytic triad of Aspartic acid, Histidine and Serine, these enzymes are responsible for cleavage of peptide bonds in protein rich food [23]. The unique cooperation between the side-chain functionalities in the active site allows serine proteases to perform under very mild conditions [24]. A vast array of serine protease mimics has been described [25] based on cyclodextrins [26–28], polymer backbones [29] or peptidic structures [30–37] to name the most frequently applied strategies. In a totally different strategy we decided to explore the potential of the DNA double helix to act as a scaffold for the correct positioning of functional groups in an active site mimic. In view of the intrinsic programmability of DNA sequences and the state-of-the-art technology available for facile and automated synthesis of oligonucleotides, these modified building blocks can then be incorporated at will into any desired position, whereby the design and synthesis of a suitable active site with specific three dimensional arrangement of catalytic groups can be reduced to the design and synthesis of suitably functionalized individual modified nucleoside building blocks.

Depending on the position of an extra functional group introduced within the nucleoside building block and the specific assembled sequence, it should be possible to obtain duplexes structures with the functional groups converging in either major or minor groove (see Fig. 1 for a generalistic picture of the envisaged strategy). In order to introduce the necessary functionalities, we have initially focused on the modification of 2′-amino-2′-deoxyuridine [38].

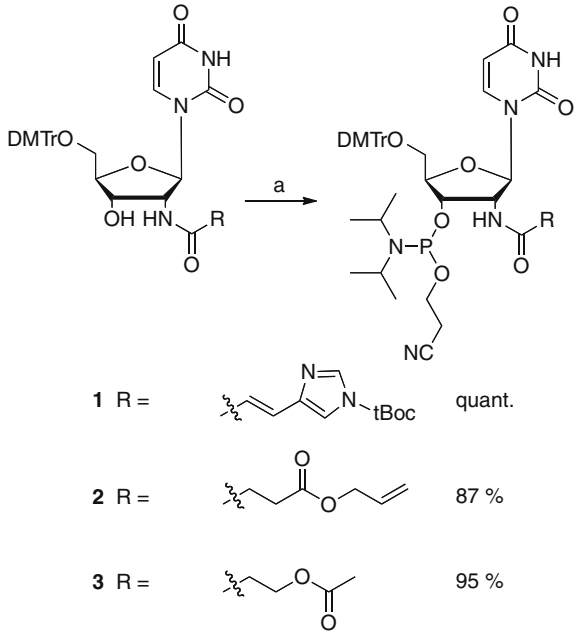
### 3.2. Synthesis of functionalized phosphoramidite building blocks

We have earlier reported on the design and synthesis of a series of suitable 2′-modified nucleosides [39]. As shown in Scheme 1, three modified nucleoside building blocks which were synthesized starting from 2′-amino-2′-deoxyuridine, were converted into the necessary phosphoramidites for incorporation into suitable DNA sequences. All three reactions occurred smoothly using 2-cyano-*N*′,*N*′-diisopropylchlorophosphoramidite and building blocks **1**, **2** and **3** could be isolated in good yield and purity.

### 3.3. Synthesis of modified oligonucleotides

In view of the *tert*-butyloxycarbonyl (*t*Boc)-protection on **U$^{I}$** (**1**) and acetyl protecting group on **U$^{o}$** (**3**), which can both be removed under classical conditions for cleavage and deprotection of oligonucleotides involving ammonium hydroxide [40,41], it was decided to incorporate these two modified uridines in one chain. Cleavage of the allyl protecting group on the carboxylate function of the **U$^{C}$** (**2**) building block requires a separate treatment with $Pd(PPh_3)_4/PPh_3$/morpholine before detachment from the solid support [42]. Therefore this building block was incorporated into a singly modified complementary chain. DNA sequences were chosen as to avoid self-complementarity and ending in three G–C pairs in order to avoid end-fraying. Oligonucleotides were synthesized using standard protocols with 1H-tetrazole activation [43] for natural building blocks while 4,5-dicyanoimidazole [44] was used for coupling of modified phosphoramidites. Automated synthesis of the 3′-end of the chain was interrupted for manual coupling of **U$^{I}$**, **U$^{o}$** or **U$^{C}$** at the desired position. Stock solutions of **1**, **2** and **3** were prepared and manually injected onto the solid support. It was shown to be important not to mix nucleoside and activator beforehand in order to achieve good coupling yields.

Table 1 gives an overview of the synthesized oligodeoxynucleotide 20mers. Two different **U$^{C}$** containing oligonucleotides **ODN1** and **ODN2** were synthesized in order to obtain different positioning of the functional carboxylate moiety within the duplex. Furthermore two different doubly modified sequences **ODN3** and **ODN4** were constructed where both the extra imidazole and extra alcohol moiety are represented. In literature, examples of serine proteases with




a) 2-cyano-*N,N*′-diisopropylchlorophosphoramidite, DIPEA, DCM, 0 °C-rT, 4-6 h.

**Scheme 1.** Synthesis of phosphoramidites for DNA incorporation. Symbols **U$^{I}$**, **U$^{C}$** and **U$^{o}$** will be used throughout the text to represent incorporated building blocks **1**, **2** and **3** respectively.

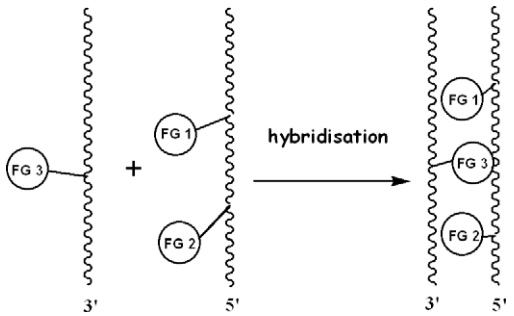



**Fig. 1.** Creation of an active site via hybridisation of functionalized complementary oligonucleotides.

**Table 1**
Preparation and MS analysis (ESI) of modified oligodeoxynucleotides **ODN1**–**6**.

| | Isolated sequences | Calcd | Obsd |
|---|---|---|---|
| **ODN1** | d(5′-GGCGTGCAAAU$^{C}$TCACAGGCG-3′) | 6267.1 | 6267.7 |
| **ODN2** | d(5′-GGCGTGCAAU$^{C}$TTCACAGGCG-3′) | 6258.0 | 6257.1 |
| **ODN3** | d(5′-CGCCTGTGAAU$^{I}$U$^{O}$TGCACGCC-3′) | 6260.1 | 6261.9 |
| **ODN4** | d(5′-CGCCTGTGAAU$^{I}$TU$^{O}$GCACGCC-3′) | 6260.1 | 6261.9 |
| **ODN5** | d(5′-GGCGTGCAGAU$^{O}$TCACAGGCG-3′) | 6253.1 | 6253.1/6277.0 (+Na) |
| **ODN6** | d(5′-CGCCTGTGAAU$^{I}$CTGCACGCC-3′) | 6172.1 | 6174.3 |

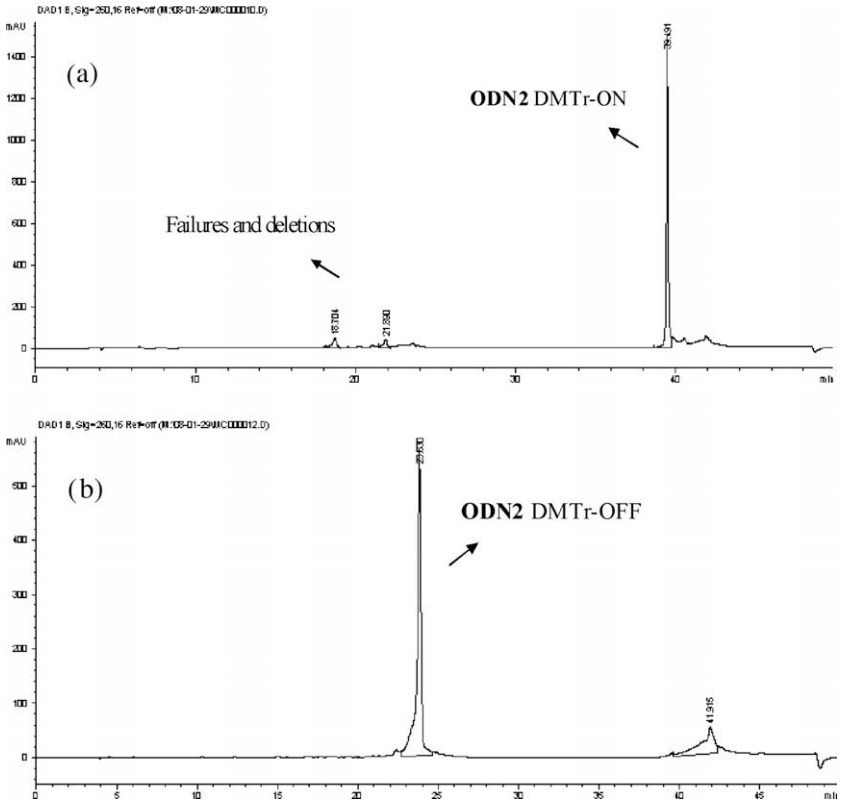



**Fig. 2.** HPLC chromatograms of the singly modified oligodeoxynucleotide **ODN2**. (a) Crude product after cleavage from solid support. (b) Sep-Pak® purified product.

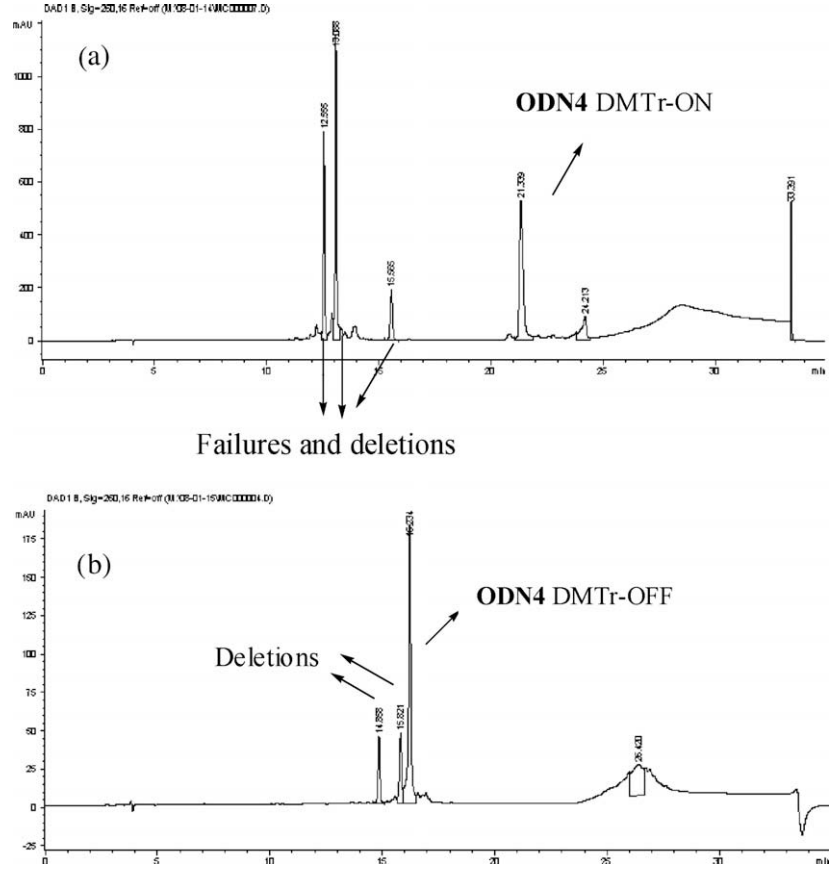



**Fig. 3.** HPLC chromatograms of the doubly modified oligodeoxynucleotide **ODN4**. (a) Crude product after cleavage from solid support. (b) Sep-Pak® purified product.

catalytic dyads have been reported functioning on the basis of a histidine and serine catalytic residue only [45,46]. In order to evaluate the importance of the role of the carboxylate and possible creation of catalytic dyads next to triads, **ODN5** and **ODN6** were constructed containing **U$^{o}$** and **U$^{I}$** as single modifications.

Coupling of modified building blocks **2** (**U$^{C}$**) and **3** (**U$^{o}$**) proceeded smoothly. In order to efficiently purify these sequences, oligonucleotides were synthesized leaving the final 5′-OH group protected (DMTr-ON method). This allows for an initial simple purification over Sep-Pak® cartridges (see Experimental Section for details). As for **ODN1** and **ODN2**, this simple Sep-Pak® treatment proved to be sufficient to obtain pure products as illustrated in Fig. 2 for **ODN2**.

Introduction of modified nucleoside **1** (**U$^{I}$**) suffered from somewhat lower yields. More specifically the simultaneous introduction of two modified building blocks in one chain such as in **ODN3** and **ODN4** proved to be more problematic and led to the occurrence of failure and deletion sequences as shown in Fig. 3a. The poor quality of crude doubly modified oligonucleotides can be ascribed to the lower coupling yield for introduction of **1** (**U$^{I}$**). Moreover since the modified residues are introduced via manual coupling, double modification implies interruption of the automated synthesis cycle twice probably giving rise to more impure synthesis products. Next to an initial purification over Sep-Pak® cartridges, which removes most of the undesired side-products with concurrent 5′-deprotection (see Fig. 3b), a final HPLC purification was performed to remove the remaining deletion sequences and allowed to obtain **ODN4** in good purity (Supporting information). An identical procedure was followed for purification of the crude **ODN3** that was contaminated by comparable failure and deletion sequences.

**Table 2**
Melting temperatures for modified and non-modified duplexes. Melting temperatures are recorded in 0.01 M sodium phosphate buffer at pH 7.0 containing 0.1 M NaCl. Oligonucleotide concentrations were 1 μM. $\Delta T_m$ was determined compared to the unmodified duplexes.

| | | Duplexes | $T_m$ | $\Delta T_m$ |
|---|---|---|---|---|
| Entry 1 | **ODN1**:**ODN3** | d(5′-GGC GTG CAA A**U$^{C}$**T CAC AGG CG-3′)<br>d(3′-CCG CAC GT**U$^{o}$ U$^{i}$**AA GTG TCC GC-5′) | 53.8 °C | −16.4 °C |
| Entry 2 | **ODN2**:**ODN3** | d(5′-GGC GTG CAA **U$^{C}$**TT CAC AGG CG-3′)<br>d(3′-CCG CAC GT**U$^{o}$ U$^{i}$**AA GTG TCC GC-5′) | 56.3 °C | −7.4 °C |
| Entry 3 | **ODN1**:**ODN4** | d(5′-GGC GTG CA A A**U$^{C}$**T CAC AGG CG-3′)<br>d(3′-CCG CAC G**U$^{o}$**T **U$^{i}$**AA GTG TCC GC-5′) | 54.8 °C | −15.4 °C |
| Entry 4 | **ODN2**:**ODN4** | d(5′-GGC GTG CA A **U$^{C}$**TT CAC AGG CG-3′)<br>d(3′-CCG CAC G**U$^{o}$**T **U$^{i}$**AA GTG TCC GC-5′) | 58.4 °C | −5.3 °C |
| Entry 5 | **ODN5**:**ODN6** | d(5′-GGC GTG CAG A**U$^{o}$**T CAC AGG CG-3′)<br>d(3′-CCG CAC GTC **U$^{i}$**AA GTG TCC GC-5′) | 61.9 °C | −9.4 °C |
| Entry 6 | **ODN7**:**ODN4** | d(5′-GGC GTG CAA ATT CAC AGG CG-3′)d<br>(3′-CCG CAC G**U$^{o}$**T **U$^{i}$**AA GTG TCC GC-5′) | 60.9 °C | −9.3 °C |
| Entry 7 | **ODN7**:**ODN8**[a] | d(5′-GGC GTG CAA ATT CAC AGG CG-3′)<br>d(3′-CCG CAC GTT TAA GTG TCC GC-5′) | 70.2 °C | / |
| Entry 8 | **ODN9**:**ODN10**[b] | d(5′-GGC GTG CAG ATT CAC AGG CG-3′)<br>d(3′-CCG CAC GTC TAA GTG TCC GC-5′) | 71.3 °C | / |
| Entry 9 | **ODN11**:**ODN8**[c] | d(5′-GGC GTG CAA TTT CAC AGG CG-3′)<br>d(3′-CCG CAC GTT TAA GTG TCC GC-5′) | 63.7 °C | / |

[a] Unmodified reference for **ODN1**:**ODN3**, **ODN1**:**ODN4** and **ODN7**:**ODN4**.
[b] Unmodified reference for **ODN5**:**ODN6**.
[c] Unmodified reference for **ODN2**:**ODN3** and **ODN2**:**ODN4**.

### 3.4. Formation and evaluation of doubly and triply modified duplexes

With the six different modified sequences in hand, six different doubly and triply modified duplex combinations can be considered. In order to evaluate the influence of the different modifications and the relative positioning of the functional groups on the duplex stability, melting temperatures were recorded. Table 2 summarizes the obtained results for all combinations. Commercial oligodeoxynucleotides **ODN7–11** were further used to construct the reference non-modified duplexes and the corresponding melting temperatures are included for comparison.

As can be noticed from Table 2, two types of modified duplexes are considered. Triply modified duplexes for creation of potential catalytic triads include **ODN1**:**ODN3, ODN2**:**ODN3, ODN1**:**ODN4** and **ODN2**:**ODN4**. Next to potential triads, two potential catalytic diads were considered **ODN5:ODN6** and **ODN7:ODN4**.

As expected, the two duplexes containing only two modified building blocks have a higher stability than the triply modified duplexes (entries 5–6 versus entries 1–4). In comparison with the unmodified references, a destabilization of ∼9.5 °C can be noticed. When considering the triply modified combinations **ODN1:ODN3** and **ODN1:ODN4**, a destabilization of 15–16 °C can be observed, confirming the trend of an average destabilization of ∼5 °C per modified building block introduced (entries 1 and 3). This value

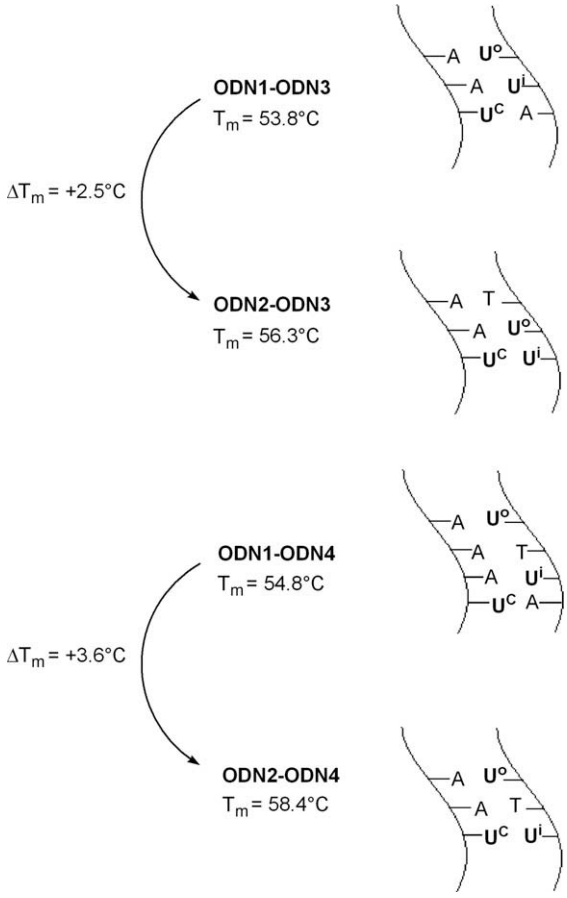



**Fig. 4.** Visual representation of formed triads and their respective stabilities. Modified residues are depicted in bold.

corresponds to what can be expected based upon previous examples of incorporation of 2′-modified building blocks into oligonucleotides [47]. For the two remaining combinations containing three modifications in a singly mismatched sequence **ODN2:ODN3** (entry 2) and **ODN2:ODN4** (entry 4), one would expect an even lower duplex stability. Fig. 4 visually represents the difference in stability between matched and mismatched sequences. It is surprising to notice that these duplexes are at least 10 °C more stable than expected (on the basis of ~5 °C destabilization per modification plus an additional mismatch). Not only is the destabilization less than expected, moreover these triply modified mismatched duplexes are more stable than the fully matched triply modified analogs **ODN1:ODN3** and **ODN1:ODN4**.

Inspection of both mismatched sequences **ODN2:ODN3** and **ODN2:ODN4** in Fig. 4 shows that the complementary modified residues $\mathbf{U^C}$ and $\mathbf{U^I}$ are facing each other. It is tempting to ascribe the observed stabilization to a combined hydrogen bonding/electrostatic interaction between the negatively charged carboxylate modified residue and the partially positively charged imidazole moiety on the other side of the duplex, counteracting the loss in stability due to the occurrence of a mismatch.

Future experiments with a variety of model substrates will show whether the presumed interaction and concurrent increased duplex stability exert a beneficial influence on eventual hydrolytic activity.

## 4. Conclusions

We have successfully constructed duplex systems containing a diad or triad of functional groups by incorporation of suitably modified nucleoside building blocks into a series of oligonucleotide sequences. Through careful analysis of different combinations and positioning of extra functionalities in a DNA duplex, it has been shown that duplex stability can be carefully tuned. When positioned in a correct way, interactions between complementary functionalities are able to counteract and overcome mismatch destabilization. These considerations pave the way for design of a suitable active site within the DNA, where correct positioning of and interaction between catalytic functionalities are of paramount importance for catalytic activity.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2010.01.002.

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