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Designed thiazole orange nucleotides for the synthesis of single labelled oligonucleotides that fluoresce upon matched hybridization†

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Probe molecules that enable the detection of specific DNA sequences are used in diagnostic and basic research. Most methods rely on the specificity of hybridization reactions, which complicates the detection of single base mutations at low temperature. Significant efforts have been devoted to the development of oligonucleotides that allow discrimination of single base mutations at temperatures where both the match and the mismatch probe–target complexes coexist. Oligonucleotides that contain environmentally sensitive fluorescence dyes such as thiazole orange (TO) provide single nucleotide specific fluorescence. However, most previously reported dye–DNA conjugates showed only little if any difference between the fluorescence of the single and the double stranded state. Here, we introduce a TO-containing acyclic nucleotide, which is coupled during automated oligonucleotide synthesis and provides for the desired fluorescence-up properties. The study reveals the conjugation mode as the most important issue. We show a design that leads to low fluorescence of the unbound probe (background) yet permits TO to adopt fluorescent binding modes after the probe–target complex has formed. In these probes, TO replaces a canonical nucleobase. Of note, the fluorescence of the "TO–base" remains low when a base mismatch is positioned in immediate vicinity.

Introduction

Fluorescent nucleotides are invaluable tools for studies of DNA–DNA and DNA–protein recognition processes.[1,2] For example, environmentally sensitive fluorochromes can be used to report changes of local DNA structure.[3,4] This property is of interest in the field of nucleic acid diagnostics, in particular for the analysis of single base mutations. Considerable efforts have been devoted to the development of fluorogenic oligonucleotide probes that allow real-time detection of nucleic acids.[2,5] Most DNA-detection methods rely on the interaction between two, terminally appended dyes.[6] The sequence specificity of these methods is typically determined by the selectivity of the hybridization. Oligonucleotide probes that contain a single fluorescent nucleotide can offer an additional level of sequence discrimination that is provided by the environmental sensitivity of the fluorophore.[7,8] In order to provide for high sensitivity and specificity of DNA detection, useful probes should have (a) low fluorescence intensity in the single stranded state, (b) high fluorescence intensity after hybridization with matched targets and, ideally, (c) weak fluorescence after hybridization with (single) mismatched targets.

The contact of dyes with nucleobases usually leads to quenching of fluorescence. Phenanthridinium dyes and some cyanines are notable exceptions.[9] Thiazole orange (TO), oxazole yellow and their dimeric forms are perhaps the most powerful DNA stains. Up to 3200-fold enhancement of fluorescence upon binding of soluble dyes with DNA has been reported.[10] In attempts to develop fluorescence-up oligonucleotides, TO, oxazole yellow and ethidium dyes have been attached to oligonucleotides (Fig. 1).[11–14] However, most of the reported single fluorophore-labelled DNA conjugates showed only little if any difference between the fluorescence of the single and the double stranded state.[15] Here, we introduce a new TO nucleotide building block, which provides for the desired enhancements of fluorescence upon hybridization. The TO building block can be used in automated synthesis of oligonucleotides. Our study reveals the conjugation mode as the most important issue in the design of single labelled fluorescence-up probes. This issue has received little attention in previous studies. We show a design that confers a significant reduction of the (background) fluorescence of single stranded state while maintaining the high fluorescence of the double stranded state. Thus, the resulting oligonucleotide probes provide enhancements of fluorescence upon hybridization. We demonstrate that the fluorescence of the thiazole orange nucleotide responded to the introduction of mismatched base pairs. This enabled the discrimination of single base mutations even at temperatures where both the match and the mismatch probe–target complexes coexist.

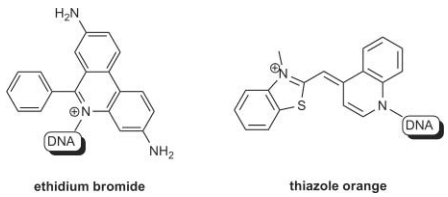

Fig. 1 DNA conjugates of ethidium bromide and thiazole orange.

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Results and discussion

Design

DNA stains such as thiazole orange experience dramatic fluorescence enhancements upon binding to nucleic acids.[9,10] Intercalation and minor groove binding of double helical DNA are preferred binding motives and nucleic acid recognition is predominantly driven by rather unselective stacking and ionic interactions. Thus, single stranded DNA also provides opportunities for binding of thiazole orange. Such interactions are probably facilitated in oligonucleotide conjugates due to proximity effects. The accompanying increase of fluorescence of the unbound state explains why it has been so difficult to develop single labelled fluorescence-up probes.[12–14] The challenge is to design a linkage that prevents stacking of TO within the single stranded probe conjugate yet permits intercalation (or minor groove binding) and, hence, fluorescence after the probe–target complex has formed. According to this analysis, tethering *via* flexible linkers shall be avoided. We assumed that the steric hindrance provided by the glycoside in the spacer-free TO nucleotide **A** (Fig. 2) would confer the desired properties. Stacking in the single stranded state would be hindered. In the double helix, TO should occupy the position of a canonical nucleobase. This may enforce stacking and fluorescence may occur.

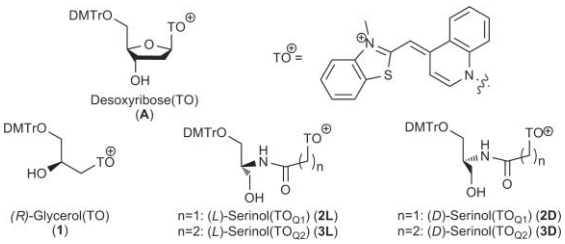
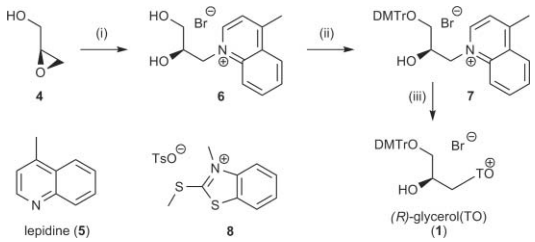


Fig. 2 Structures of a theoretical desoxyribose–TO monomer and of the desired acyclic TO-labelled DNA monomers.

The probe design is reminiscent of the recently disclosed FIT probes (FIT = forced intercalation), in which TO is used as an artificial base in peptide nucleic acids (PNA).[7,16,17] However, synthesis of ribose-linked TO–DNA conjugates is virtually impossible because the glycosidic bond in the hypothetical nucleotide **A** would not be strong enough to sustain the conditions of DNA synthesis. We therefore decided to explore open chain analogues. We considered the use of sterically confined (*R*)-glycidol backbone in **1**, which is known from glycerol nucleic acid (GNA) and probably the smallest backbone capable of Watson–Crick base pairing.[18,19] The serinol backbone in **2** and **3** is known from amino acid nucleic acids (AANA)[20] and provides bioisosteric internucleotide linkage while the flexibility of the chromophore–backbone linkage is restricted by means of the peptide bond.

Synthesis of building blocks and TO-DNA-conjugates

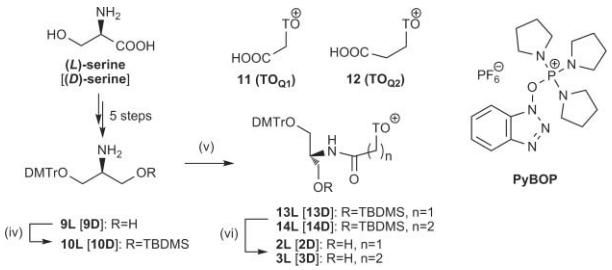
For the synthesis of the glycerol–TO nucleoside **1** (Scheme 1), (*S*)-glycidol (**4**) was treated with $MgBr_2$ and lepidine (**5**). The Lewis acid-meditated ring opening furnished lepidinium salt **6**, which was subsequently converted in the presence of imidazole into the DMTr-ether **7**. The TO chromophore in **1** was established



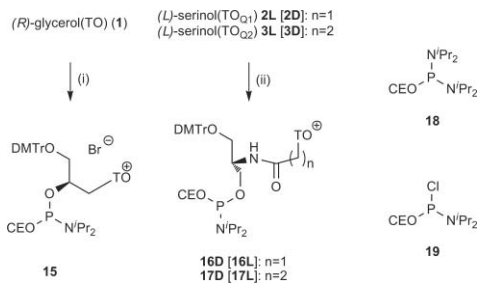
Scheme 1 Synthesis of (*R*)-glycidol(TO) (**1**). (i) 1. $MgBr_2$, CH_2Cl_2, 0 °C, 2. lepidine (**5**), RT, 2 d, 77%; (ii) DMTrCl, imidazole, DMF, RT, 12 h, 95%; (iii) **8**, NEt_3, CH_2Cl_2, RT, 24 h, 98%.

in the base-promoted reaction of **7** with the benzothiazolium compound **8**.

The synthesis of (*D*)- and (*L*)-serinol-based nucleosides was commenced from (*D*)- and (*L*)-serine, which were converted to the DMTr-protected (*D*)- or (*L*)-serinol building blocks **9D** and **9L** by applying procedures described in the literature (Scheme 2).[20] The coupling of the carboxyalkylated TO dyes such as **11** was surprisingly difficult. The coupling proceeded smoothly when the alcohol in **9D/9L** was protected as TBDMS ether in **10D/10L**. Subsequent TBAF treatment furnished the TO-nucleosides **2D**, **2L**, **3D**, **3L**, which were converted to the phosphoramidites **15–17** (Scheme 3) and used without further purification in automated DNA synthesis.



Scheme 2 Synthesis of TO-labelled serinol monomers **2L**, **2D**, **3L** and **3D**. The scheme only depicts the synthesis of the enantiomerically pure monomers derived from (*L*)-serine. Monomers derived from (*D*)-serine were synthesised in the same procedure. (iv) TBDMSCl, NEt_3, DMAP, CH_2Cl_2, 0 °C, 16 h, 86%; (v) **11** or **12**, PyBOP, NMM, PPTS, DMF, RT, 16 h, 56%, (vi) TBAF, THF, 2 h, RT, 22–33%.



Scheme 3 Synthesis of phosphoramidites **15–17**. (i) Dicyanoimidazole, **18**, MeCN, 2 h, 65%; (ii) DIPEA, **19**, CH_2Cl_2, full conversion.

The synthesis of TO-nucleotide-containing oligonucleotides was performed by applying known protocols.[21] The TO-labelled phosphoramidites were coupled at 0.2 M (CH_3CN) concentration over a period of 30 minutes. Their coupling efficiency was equal

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Table 1 Melting temperatures (T_M)[a] of duplexes of TO–DNA conjugates **15–19** or unmodified reference DNA **20** with complementary DNA **21–24**

5′-G C C G T A A A T A G C C G-3′	(**20**)
5′-G C C G T X **Z** X T A G C C G-3′	(**21X-25X**, X = A, T, C, G)
3′-C G G C A Y T Y A T C G G C-5′	(Y = T, **26**; A, **27**; G, **28**; C, **29**)

Monomer **Z**	XY = AT	XY = TA	XY = CG	XY = GC
dA	**20·26** 55 °C			
1	**21A·26** 54 °C	**21T·27** 56 °C	**21C·28** 62 °C	**21G·29** 62 °C
2L	**22A·26** 55 °C	**22T·27** 57 °C	**22C·28** 65 °C	**22G·29** 61 °C
2D	**23A·26** 51 °C	**23T·27** 53 °C	**23C·28** 61 °C	**23G·29** 59 °C
3L	**24A·26** 53 °C	**24T·27** 49 °C	**24C·28** 59 °C	**24G·29** 59 °C
3D	**25A·26** 52 °C	**25T·27** 52 °C	**25C·28** 62 °C	**25G·29** 59 °C

[a] Recorded as denaturation curves. **20**, **21X–25X**, **26–29** 1 μM in 10 mM NaH_2PO_4 buffer, 100 mM NaCl, pH = 7, 1 h degassed.

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to commercially available canonical phosphoramidites. For the release of 5′-DMTr-oligonucleotides, the solid support was treated with aqueous concentrated ammonia solution for 4 h at 55 °C. The oligonucleotides were purified by HPLC prior to, as well as after, acidolytic DMTr-cleavage and desalted by size exclusion filtration.

Thermal stability

The TO–DNA conjugates **21X–25X** were hybridized with complementary DNA **26–29** (Table 1). The thermal stability was assessed by means of UV-monitored melt analyses. The sigmoid shape of melting curves suggested cooperative base-pairing. The replacement of the central adenine in **20** by TO removes a Watson–Crick base pair. Of note, TO was found to compensate not only for the loss of hydrogen bonding, but even for the perturbations caused by the open chain ribose analogues. For example, the (*S*)-glycerol(TO) containing duplex **21A·26** revealed almost unchanged thermal stability compared with the unmodified complex **20·26**, although canonical (*S*)-glycerol nucleotides destabilize duplex DNA by $\Delta T_M = 8$ °C.[18] Moreover, it has been reported that (*D*)- or (*L*)-serinol(TO) nucleotides reduce duplex stability by $\Delta T_M = 9$ °C or 12 °C.[20] In contrast, the TO nucleotides in **22A·26** and **23A·26** showed little if any destabilization. The extension of the linker that connects the TO with the (*L*)-serinol backbone by one carbon atom resulted in decreases of duplex stability (**22T·27** *vs.* **24T·27**, $\Delta T_M = 8$ °C or **22C·28** *vs.* **24C·28**, $\Delta T_M = 6$ °C). By contrast, the (*D*)-backbone better tolerated the linker extension. This may indicate that the (*D*)-serinol linkage in duplex DNA provides TO with a higher degree of flexibility than the (*L*)-form, which is in line with the lower stability of duplexes that contained the TO-nucleotide **2D** rather than **2L**.

Fluorescence measurements

The responsiveness of TO fluorescence was studied by measurements of emission spectra prior to and after hybridization with fully complementary DNA. Fig. 3 shows fluorescence spectra of probes, which differ only in the backbone. A comparison of the probes in the single stranded form revealed major differences. The glycerol–TO in **21T** and the (*L*)-serinol–TO nucleotide in **22T** had low emission intensity. The (*D*)-serinol in single strands **23T** and **25T** as well as the (*L*)-serinol that carried the C3-linked chromophore in **24T** conferred significantly higher intensities of TO fluorescence. The hybridization of probes **23T**, **24T** and **25T**, all of which showed high single strand fluorescence, with complementary DNA **27** was accompanied by decreases of TO emission. Such decreases of fluorescence have previously been observed for oligonucleotides in which TO was attached *via* a flexible tether.[12,22] From the 5 modifications studied, only the (*L*)-serinol (**22A–22G**) conferred enhancements of fluorescence

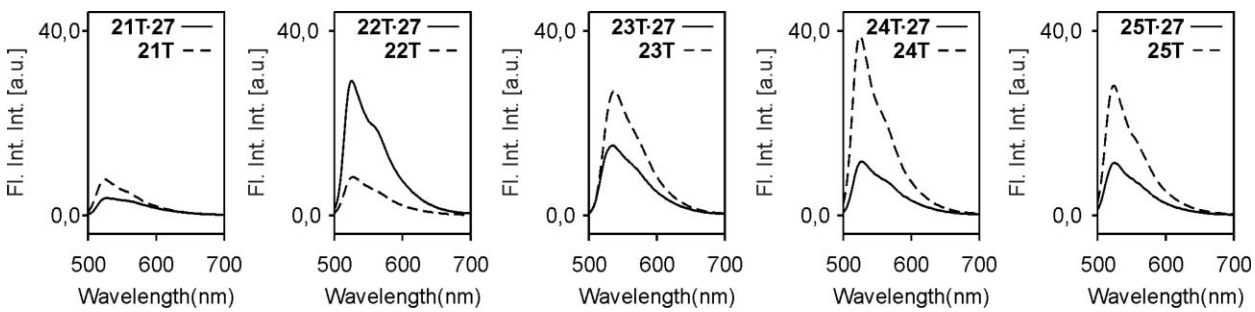

Fig. 3 Fluorescence emission spectra of TO–DNA conjugates **21T–25T** before (dashed line) and after (solid line) addition of fully complementary DNA **27** at 25 °C, $\lambda_{ex} = 495$ nm, $Slit_{Ex} = 5$, $Slit_{Em} = 2.5$, **21T–25T**, **27** 1 μM in phosphate buffer (see conditions in Table 1).

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upon matched hybridization. The general trend of the fluorescence properties was retained in probes that contained different next neighbours of TO (ESI, Tables S1 and S2†). Again, the (*S*)-glycidol and the (*D*)-serinol failed in providing the enhancement of TO emission upon matched hybridization.

The stacking of TO with nucleobases closes fluorescence decay channels because torsions around the central methine bridge in the excited state are restricted.[17,23] Recent investigations have shown that a tight TO–nucleobase contact provides options for depletion of the TO excited state.[4] This complicates the analysis of the fluorescence of "TO–bases" in DNA. High hybridization-induced fluorescence enhancements probably call for a subtle balance between linker constraints required to prevent TO–nucleobase stacking in the single stranded state, and linker flexibility to avoid strong electronic coupling between TO and nucleobases in the double stranded state. The sterically confined glycerol in **21T** efficiently reduced single strand fluorescence, but forced TO to adopt non-fluorescent modes in the double strand. On the contrary, the linkers **3D** in **24T** and **3L** in **25T** were probably too flexible to limit single strand fluorescence. High fluorescence was also observed for single stranded **23T**. This is in line with the melt analyses which suggested that **23T** confers more degrees of freedom than **22T**. Only the (*L*)-serinol with the short carboxymethyl spacer in **2L** provided the desired properties. The nucleotide apparently is sufficiently constrained to reduce background fluorescence yet allows TO to adopt fluorescent binding modes when in the double strand.

The conjugation mode enforces TO to respond to changes of local architecture. Mismatched base-pairs "soften" the duplex structure. We assumed that the increase of available space introduced by mismatched base-pairs would attenuate the fluorescence of the "TO–nucleobase". Indeed, the enhancements of TO fluorescence upon mismatched hybridization of **22T** are significantly lower than the fluorescence enhancements upon matched hybridization (Fig. 4A). The background-corrected specificity factor D is a measure for the power of discrimination between matched and mismatched targets (Fig. 4B). Most fluorescence-up hybridization probes provide for $D \approx 1$ at temperatures below the T_m of the mismatched probe–target complex. In contrast, the background-corrected fluorescence of the matched duplex **22T**·**27A** was 9-fold higher than the fluorescence of TT-mismatched duplex **22T**·**27T** (Fig. 4B). The TG- and the TC-mismatch were discriminated with specificity factors of $D = 6.5$ and $D = 5.6$, respectively. This specificity was achieved at 25 °C, a temperature where both matched and single mismatched probe–target complexes exist. The discrimination was even higher when the hybridization was performed at 50 °C. Under these conditions the presence of the matched target **27A** resulted in a 30-fold higher background-corrected fluorescence than the presence of the mismatched target **27C**.

Detection of G12V mutation of human Ras

The findings discussed above are not restricted to **22T**. The (*L*)-serinol(TO_{Q2})-monomer was also incorporated into a probe designed to recognize a segment of the human *ras* gene, carrying a carcinogenic *G12V* mutation, caused by a single base exchange of a G (wild type) against T (mutant). Hybridization of **30** with fully complementary DNA **31Mu** at 25 °C is signalled by enhancement of the TO-fluorescence. Even at this temperature, which is far below the T_m of the mismatched probe–target-complex, (T_m = 59 °C) the presence of the matched complex can be distinguished from the presence of the single mismatched complex (Fig. 5A). A notable feature of probes such as **30**, in which TO serves as base surrogate, is the ability to signal the presence of complementary target and to discriminate single mismatched targets over a broad temperature range. Fig. 5B shows the temperature dependence of the hybridization-induced enhancements of TO fluorescence, as well as the background corrected discrimination of single mismatched target **31WT**. The fluorescence responsiveness was highest at a temperature close to the T_m = 64 °C of the matched probe–target duplex. This is in the range of typical annealing temperatures used in quantitative PCR analysis. Moreover, the fluorescence intensity of matched probe–target duplex **30**·**31Mu** is always higher than the intensity provided of the single mismatched probe–target duplex **30**·**31WT**. Thus, the mismatched target can be discriminated at any temperature below 64 °C. This behaviour stands in stark contrast to detection methods that solely draw upon the specificity of probe-target recognition.

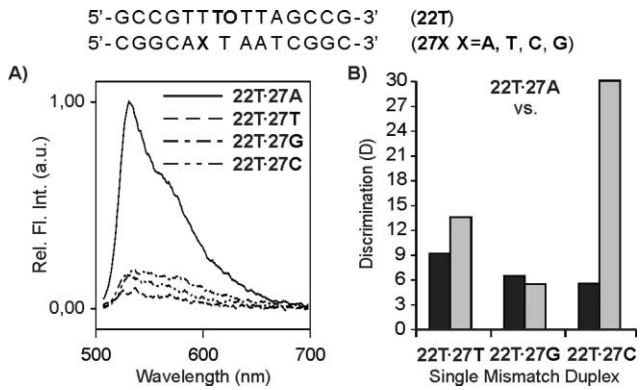

Fig. 4 Background-corrected (A) relative fluorescence at 25 °C and (B) discrimination of single mismatched *vs.* fully complementary targets. **22T**·**27A** (match) *vs.* **22T**·**27T**, **22T**·**27G** and **22T**·**27C** (mismatch) at 25 °C (black bars) and 50 °C (grey bars). *Rel. Fl. Int.* = $(F - F_0)/(F_{ma} - F_0)$, D = $(F_{ma} - F_0)/(F_{mi} - F_0)$, ($F_0$, fluorescence intensity of single strands; F_{ma}, F_{mi}, fluorescence intensity of matched and mismatched duplexes).

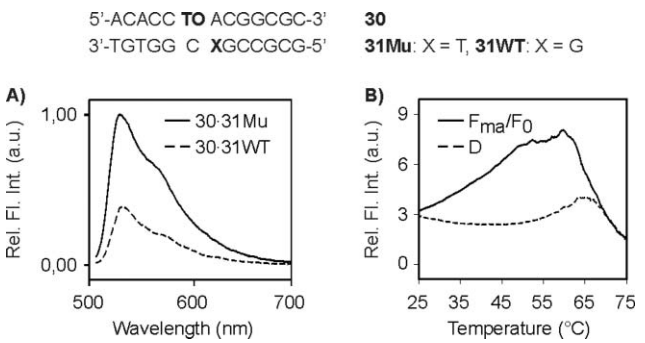

Fig. 5 Background-corrected fluorescence of probe targeting G12V mutation of human Ras gene (A) **30**·**31Mu** (match, solid line) *vs.* **30**·**31WT** (mismatch, dashed line) at 25 °C. (B) Temperature dependent fluorescence enhancement (solid line) of **30** upon hybridization with **31Mu** and background corrected discrimination (dashed line) of **30**·**31Mu** (match) *vs.* **30**·**31WT** (mismatch). λ_{ex} = 495 nm, λ_{em} 525= 5 nm $Slit_{Ex}$ = 5, $Slit_{Em}$ = 5, **30**, **31WT**, **31WT** 1 μM in phosphate buffer (see condition in Table 1).

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Conclusions

It was the aim of this study to develop single labelled oligonucleotides that experience strong fluorescence enhancements only upon matched hybridization. We and others introduced thiazole orange as an artificial base in DNA.[14] The presented data highlight the central importance of the backbone and linker structure that anchors the "thiazole orange base". There are, to our surprise, only few reports about the influence of linkers on the fluorescence properties of dye–nucleotide conjugates.[24] An analysis of the linker properties required to achieve the desired fluorescence behaviour of single labelled dye–DNA conjugates has not been reported. Our results clearly indicate that T_m optimization[25] is a poor guideline for the design of single labelled fluorescence-up probes (exemplified by the high T_m of weakly fluorescent duplexes that contain the glycidol-TO-DNA **21T**).

The careful linker optimization presented in this study may enable a generic design of TO labelled DNA oligonucleotides that gain fluorescence upon hybridization. Such properties have until recently only been provided by TO-labelled peptide nucleic acids (PNA) such as the Light-Up probes[26] and FIT-PNA.[4,7,17] Both probes capitalize on the low affinity of TO for PNA, which helps reduce the fluorescence of the unbound state (= background). Despite the complications that arise from the intrinsically higher single strand fluorescence, DNA-based TO probes offer opportunities difficult to access by the PNA backbone. The DNA backbone is amenable to cellular delivery by means of lipofection, which may facilitate the detection of DNA and RNA in live cells. DNA is easier to synthesize and enzymatic methods allow further modification.

In summary, we synthesised the first DNA conjugates, in which thiazole orange served as an artificial base that signals hybridization by enhancements of fluorescence. The measured data shows that our TO–DNA conjugates proved responsive to adjacent base mismatches. This suggests applications in the analysis of single base mutations. The study revealed the critical influence of the backbone and the linker that connects the "thiazole orange base" with the oligonucleotide. However, we wish to note that changes of the anchor position may allow for further improvements. This investigation was focused on N-linked TO conjugates. Attachment of TO through one of the adjacent quinoline carbon atoms would change the angle in which TO penetrates the base stack, which may have an impact on the fluorescence intensity achievable through matched hybridization. Our analysis suggests that hybridization-induced fluorescence enhancements can probably only be obtained when the backbone/linker structure prevent intramolecular thiazole orange–nucleobase stacking in the single stranded state yet confers sufficient flexibility to allow thiazole orange to adopt fluorescent binding modes in the double stranded state. Ongoing work is directed to the homogeneous detection of RNA in biological samples.

Experimental section

Solvents (CH_2Cl_2, THF) were dried by using the solvent purification system *SPS 800* of *MBraun*. Dry DMF (H_2O < 0.01%) was purchased from Fluka. Aqueous solutions were made of water of Milli-Q-Pore purity. Other used compounds were commercially available and not further purified prior to usage. Column chromatography was performed with *SDS 60 ACC* silica gel using a *Büchi Sepacore*™ flash chromatographer and TLC with *E. Merck Silica Gel 60 F254* plates. Optical rotations were measured at the sodium D-line with an *Perkin Elmer 241 polarimeter* using a 100 mm glass cuvette. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded with *Bruker DPX 300* spectrometer. The signals of the residual protonated solvent ($CDCl_3$, CD_3OD or CD_3CN) were used as reference signals. Coupling constants are given in Hz. High resolution mass spectra were measured with a *Hewlett-Packard GCMS 5995-A* (*ESI+*) spectrometer. Semi preparative HPLC was carried out on a *1105 HPLC System* from *Gilson*, for analytical RP-HPLC a *1105 HPLC System* of *Gilson* and a *Acquity UPLC* System of *Waters* were used. A UV-detector at a wavelength $\lambda = 260$ nm and $\lambda = 520$ nm was used for the detection. Semi preparative separations were carried out by using a *Polaris C18 A 5μ* (*PN A 2000-250 × 100*)-column of *Varian* (pore size 220 Å) at a flow rate of 4 mL min^{-1} at 55 °C. Analytical HPLC was carried out by using a *XBridge C18 5 μ* (*250 × 046*)-column of *Waters* (pore size 130 Å) at a flow rate of 1 mL min^{-1} at 55 °C or a *BEH 130 C18 1.7 μm* (*2.1 × 50*)-column of *Waters* (pore size 130 Å) at a flow rate of 1 mL min^{-1} at 55 °C. As mobile phase a binary mixture of A (0.1 M TEAA buffer, pH = 7, aq.) and B (acetonitrile) was used. MALDI-TOF mass spectra were measured on a *Voyager-DE*™ Pro Biospectrometry Workstation of *PerSeptive Biosystems*. As matrix was used a solution of 1 part of a solution of 20 mg 2′,4′,6′-trihydroxyacetophenone in 1 mL MeCN and 1 part of a solution of 50 mg diammonium citrate in 1 mL water was. The sample concentration was calculated by using oligo calculation at www.proligo.com and $\varepsilon(TO) = 6600$ L mol^{-1}. UV-Vis measurements were performed with a *Varian Cary 100*, fluorescence emission spectra were recorded on a *Varian Cary Eclipse* using 10 mm UV quartz cuvettes.

(*R*)-2-Lepidinium-propane-1,3-diol bromide (6)

To a cooled (0 °C) solution of (*S*)-glycidol (**4**), (1.00 g,13.5 mmol, 0.9 mL) in 23 mL CH_2Cl_2–THF (1 : 2, v/v) under argon was added $MgBr_2$ (4.20 g, 23.0 mmol). After 15 min TLC indicated full conversion of the starting material. The reaction was stopped by addition of 3 mL of aqueous NH_4Cl solution (10%). The resulting mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and filtered. The volatiles were removed under reduced pressure yielding 1.51 g (9.74 mmol) (*S*)-3-bromopropane-1,2-diol (72%). The crude material was used without further purification. To the bromodiol was added lepidine (**5**) (2.81 g, 19.6 mmol) and the mixture was stirred for 2 days at 70 °C. The reaction product precipitated upon addition of CH_2Cl_2. The residue was washed with cyclohexane and ether and was dried at reduced pressure. Yield: 2.23 g (7.48 mmol, 77%), brown solid, $C_{13}H_{16}BrNO_2$ (298.18 g mol^{-1}). R_f 0.75 (CH_2Cl_2–MeOH, 75 : 25, v/v). $[\alpha]_D = 8.9$ ($c = 0.10$, MeOH). HRMS (*m/z*) calculated: 218.1176 $[C_{13}H_{16}NO_2]^+$, found: 218.1174. ^{1}H-NMR (CD_3OD): $\delta =$ 9.19 (d, $J = 6.0$ Hz, 1H), 8.62-8.58 (m, 2H), 8.30-8.24 (m, 1H), 8.10-8.00 (m, 2H), 5.50 (dd, $J = 14.1$ Hz and 2.7 Hz, 1H), 4.84 (dd, $J = 14.2$ Hz and 9.4 Hz, 1H), 4.20-4.10 (m, 1H), 3.84 (dd, $J =$ 11.2 Hz and 4.7 Hz, 1H), 3.74 (dd, $J = 11.2$ Hz and 6.4 Hz, 1H),

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3.33-3.32 (m, 1H), 3.099 (s, 1H); ^{13}C-NMR (CD_3OD): $\delta = 160.9$, 150.3, 136.4, 130.5, 128.3, 123.4, 120.3, 71.0, 64.7, 61.5, 20.4.

(*R*)-1-DMT-2-Lepidinium-propane-3-ol bromide (7)

To a solution of (*R*)-3-lepidinium-propane-1,3-diol bromide (**6**) (900 mg, 3.02 mmol) in 30 mL DMF was added dimethoxytritylchloride (1.80 g, 5.13 mmol) and imidazole (665 mg, 9.66 mmol). After 12 h, 2 mL methanol was added. The volatiles were removed under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2–MeOH–NEt_3, 92.5 : 7 : 0.5, v/v/v). Yield: 1.14 g (1.90 mmol, 63%) brown solid, $C_{34}H_{34}BrNO_4$ (599.17 g mol^{-1}). R_f 0.30 (CH_2Cl_2–MeOH, 90 : 10, v/v). ^{1}H-NMR (CD_3OD): $\delta = 9.31$ (d, $J = 6$ Hz, 1H), 8.33-8.23 (m, 2H), 7.98-7.84 (m, 2H), 7.63-7.59 (m, 2H), 7.38-7.35 (m, 2H), 7.28-7.14 (m, 6H), 6.77 (d, $J = 8.8$ Hz, 4H), 5.30 (dd, $J = 13.8$ Hz and 2.5 Hz, 1H), 4.99-4.92 (m, 1H), 4.19-4.16 (m, 1H), 3.69 (s, 6H), 3.51-3.46 (m, 1H), 3.26-3.20 (m, 1H), 2.86 (s, 3H). ^{13}C-NMR (CD_3OD): δ = 158.6, 148.8, 144.2, 137.4, 135.4, 135.3, 134.7, 129.8, 129.3, 128.0, 127.9, 127.6, 126.3, 122.4, 121.2, 118.8, 113.4, 86.9, 68.4, 64.7, 60.4, 55.2, 46.1, 46.1, 20.4.

(*R*)-1-DMT-Glycerol(TO) (1)

To a solution of compound (**7**) (1.14 g, 1.90 mmol) and 2-methyl-methylthiobenzothiazolium tosylate (**8**) in CH_2Cl_2 was added triethylamine (486 mg, 670 µL, 4.80 mmol). After 24 h the volatiles were removed under reduced pressure and the residue was purified by flash column chromatography (CH_2Cl_2–MeOH–NEt_3, 97.5 : 2 : 0.5, v/v/v). Yield: 1.39 g (1.86 mmol, 98%), red solid, $C_{42}H_{39}BrN_2O_4S$ (747.74 g mol^{-1}). $[\alpha]_D = -1.5$ ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 667.2625 $[C_{42}H_{39}N_2O_4S]^+$, found: 667.2625. ^{1}H-NMR ($CDCl_3$/NEt_3): $\delta = 8.45$ (d, $J = 7.2$ Hz, 1H), 8.39 (d, $J = 7.5$ Hz, 1H), 7.83 (d, $J = 8.4$ Hz, 2H), 7.53-7.11 (m, 15H), 6.93 (d, $J = 7.1$ Hz, 1H), 6.74 (dd, $J = 8.9$ Hz and 2.0 Hz, 4H), 6.48 (s, 1H), 5.14 (d, $J = 7.4$ Hz, 1H), 4.79-4.73 (m, 1H), 4.61-4.54 (m, 1H), 4.13 (s, 1H), 3.79 (s, 3H), 3.69 (s, 6H), 3.43-3.39 (m, 1H), 3.28-3.22 (m, 1H); ^{13}C-NMR ($CDCl_3$): δ = 159.4, 158.4. 148.9, 144.9, 144.6, 140.0, 137.4, 135.6, 135.5, 132.8, 129.9, 128.1, 127.9, 126.9, 126.8, 125.5, 124.4, 123.9, 122.2, 117.6, 112.1, 108.1, 87.9, 86.6, 68.6, 64.7, 57.2, 55.2, 46.2, 34.4, 8.8.

(*R*)-1-DMT-3-TBDMS-Serinol (10D)

To a solution of (*S*)-3-DMT-serinol (**9D**) (2.64 g, 6.71 mmol) in 50 mL CH_2Cl_2 was added at 0 °C triethylamine (781 mg, 1.08 mL, 7.72 mmol), dimethylaminopyridine (94.3 mg, 0.772 mmol) and *tert*-butyldimethylsilyl chloride (1.11 g, 7.38 mmol). After 16 h the reaction mixture was washed with 50 mL saturated aqueous $NaHCO_3$ solution, 2 times with 50 mL water and once with 50 mL saturated aqueous NaCl solution. The organic phase was dried over $MgSO_4$. The solids were removed by filtration and the volatiles removed under reduced pressure. The residue was further purified by flash column chromatography. Yield: 2.92 g (5.75 mmol, 86%), colorless syrup, $C_{30}H_{41}NO_4Si$ (507.74 g mol^{-1}). R_f 0.75 (CH_2Cl_2–MeOH–NEt_3, 100 : 10 : 0.1, v/v/v). $[\alpha]_D = 1.9$ ($c = 1.00$, CH_3CN). ^{1}H-NMR ($CDCl_3$): $\delta = 0.05$ (6H, m, $SiMe_2$), 0.89 (9H, s, Si^{t}Bu), 3.06 (2H, m, CH, CH_2), 3.17 (1H, m, CH_2), 3.59 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 9.8$ Hz, CH_2), 3.68 (1H, dd, $J_1 = 4.4$ Hz, $J_2 = 9.9$ Hz, CH_2), 3.80 (6H, s, DMT-OCH_3), 6.85 (4H, m, DMT), 7.20-7.38 (7H, m, DMT), 7.48 (4H, m, DMT). ^{13}C-NMR ($CDCl_3$): $\delta = -5.5$ ($SiMe_2$), 18.1 (Si^{t}Bu), 25.8 (Si^{t}Bu), 53.1 (CH), 55.1 (DMT-OCH_3), 65.2 (CH_2), 65.2 (CH_2), 85.7 (DMT-C_q), 112.9 (4DMT-ArCH), 126.6 (1DMT-ArCH), 127.7 (2DMT-ArCH), 128.1 (2DMT-ArCH), 130.0 (4DMT-ArCH), 136.2 (2DMT-ArC_q), 145.1 (DMT-ArC_q), 158.3 (2DMT-ArC_q).

(*S*)-1-DMT-3-TBDMS-Serinol (10L)

As described for **10D**, (*S*)-3-DMT-serinol (**9L**) (2.64 g, 6.71 mmol) in 50 mL CH_2Cl_2 was reacted with triethylamine (781 mg, 1.08 mL, 7.72 mmol), dimethylaminopyridine (94.3 mg, 0.772 mmol) and *tert*-butyldimethylsilyl chloride (1.11 g, 7.38 mmol). Yield: 2.11 g (4.16 mmol, 43% d. Th.), colorless syrup, $C_{30}H_{41}NO_4Si$ (507.74 g mol^{-1}). R_f 0.75 (CH_2Cl_2–MeOH–NEt_3, 100 : 10 : 0.1, v/v/v). $[\alpha]_D = -1.7$ ($c = 1.00$, CH_3CN). ^{1}H-NMR ($CDCl_3$): $\delta = 0.05$ (6H, m, $SiMe_2$), 0.88 (9H, s, Si^{t}Bu), 3.06 (2H, m, CH, CH_2), 3.17 (1H, m, CH_2), 3.57 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 9.8$ Hz, CH_2), 3.68 (1H, dd, $J_1 = 4.4$ Hz, $J_2 = 9.9$ Hz, CH_2), 3.81 (6H, s, DMT-OCH_3), 6.85 (4H, m, DMT), 7.20-7.37 (7H, m, DMT), 7.47 (4H, m, DMT). ^{13}C-NMR ($CDCl_3$): $\delta = -5.5$ ($SiMe_2$), 18.2 (Si^{t}Bu), 25.8 (Si^{t}Bu), 53.1 (CH), 55.1 (DMT-OCH_3), 65.3 (CH_2), 65.3 (CH_2), 85.7 (DMT-C_q), 113.0 (4DMT-ArCH), 126.6 (1DMT-ArCH), 127.7 (2DMT-ArCH), 128.1 (2DMT-ArCH), 130.0 (4DMT-ArCH), 136.2 (2DMT-ArC_q), 145.1 (DMT-ArC_q), 158.3 (2DMT-ArC_q).

(*R*)-1-DMT-3-TBDMS-Serinol(TO_{Q1}) (13D)

In a volume of 30 mL DMF, thiazole orange (TO_{Q1}, **11**) (1.30 g, 3.02 mmol) was suspended by applying ultrasound over a period of 2 min. Subsequently, PyBOP (1.56 g, 3.02 mmol) and *N*-methylmorpholine (305 mg, 332 µL, 101.15 mmol) were added. The mixture was stirred for 2 min, whereupon the red suspension considerably cleared up. Subsequently, PPTS (759 mg, 3.02 mmol) was added. The mixture was again stirred for 2 min. To the clear solution was added 1.02 g (2.01 mmol) (*R*)-1-DMT-3-TBDMS-serinol (**10D**) in 10 mL DMF. The mixture was stirred for 16 h. The volatiles were removed under reduced pressure. The residue was suspended in 100 mL CH_2Cl_2. After filtration, the residue was discarded and the filtrate was washed with 100 mL saturated aqueous $NaHCO_3$ solution, twice with 100 mL water and dried over $MgSO_4$. The solids were removed by filtration and the volatiles were removed under reduced pressure. The residue was further purified by flash column chromatography. Yield: 1.03 g (1.13 mmol, 56%), red solid, $C_{50}H_{56}BrN_3O_5SSi$ (919.05 g mol^{-1}). R_f 0.75 (CH_2Cl_2–MeOH00NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D$ = –2.7 ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 838.3704 $[C_{50}H_{56}N_3O_5SSi]^+$, found: 838.3697. ^{1}H-NMR (CD_3CN): $\delta = 0.01$ (3H, s, $SiMe_2$), 0.02 (3H, s, $SiMe_2$) 0.83 (9H, s, Si^{t}Bu), 3.10 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 9.1$ Hz, CHH′), 3.21 (1H, dd, $J_1 = 5.7$ Hz, $J_2 = 9.0$ Hz, CHH′), 3.62 (3H, s, TO-CH_3), 3.73 (8H, m, 2DMT-OCH_3, CH_2), 4.10 (1H, m, CH), 4.90 (2H, s, TO-CH_2), 6.33 (1H, s, TO-CH), 6.83 (4H, m, 4DMT), 6.89 (1H, m, TO), 7.03 (1H, m, TO), 7.10-7.50 (14H, m, 9DMT, 5TO), 7.57 (1H, m, TO), 7.86 (1H, m, TO), 8.17 (1H, m, TO). ^{13}C-NMR (CD_3CN): δ = –5.3 ($SiMe_2$), –5.3 ($SiMe_2$), 18.7 (Si^{t}Bu), 26.1 (Si^{t}Bu), 34.6 (TO-CH_3), 52.6 (CH), 55.8 (DMT-OCH_3), 57.2 (TO-CH_2), 62.7 ($2CH_2$), 86.8 (DMT-C_q), 89.5 (TO-CH), 108.5 (TO-Ar-CH), 113.7

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(TO-Ar-CH), 113.9 (4DMT-ArCH), 118.0 (TO-Ar-CH), 123.3 (TO-Ar-CH), 124.7 (TO-Ar-CH), 125.3 (TO-Ar–C_q), 125.7 (TO-Ar–C_q), 126.0 (TO-Ar-CH), 127.5 (TO-Ar-CH), 127.7 (1DMT-ArCH), 128.7 (2DMT-ArCH), 128.9 (2DMT-ArCH), 129.1 (TO-Ar-CH), 130.9 (2DMT-ArCH), 130.9 (2DMT-ArCH), 134.0 (TO-Ar-CH), 136.8 (DMT-ArC_q), 137.0 (DMT-ArC_q), 138.6 (TO-Ar–C_q), 141.1 (TO-Ar–C_q), 145.5 (TO-Ar-CH), 146.1 (DMT-Ar–C_q), 149.6 (TO-Ar–C_q), 159.5 (2DMT-ArC_q), 161.9 (TO-Ar–C_q), 166.1 (TO-C_q).

(*S*)-1-DMT-3-TBDMS-Serinol(TO_{Q1}) (13L)

As described for **13D**, thiazole orange (**TO_{Q1}**, **11**) (1.30 g, 3.02 mmol) was reacted with PyBOP (1.56 g, 3.02 mmol), *N*-methylmorpholine (305 mg, 332 µL, 101.15 mmol), PPTS (759 mg, 3.02 mmol) and 1.02 g (2.01 mmol) (*R*)-1-DMT-3-TBDMS-serinol (**10L**). Yield: 1.22 g (1.33 mmol, 66% d. Th.), red solid, $C_{50}H_{56}BrN_3O_5SSi$ (919.05 g mol^{-1}). R_f 0.75 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D = 2.5$ ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 838.3704 $[C_{50}H_{56}N_3O_5SSi]^+$, found: 838.3705. ^{1}H-NMR (CD_3CN): $\delta = 0.00$ (3H, s, $SiMe_2$), 0.00 (3H, s, $SiMe_2$) 0.81 (9H, s, Si^{t}Bu), 3.11 (1H, dd, $J_1 = 5.9$ Hz, $J_2 = 9.1$ Hz, CHH′), 3.21 (1H, dd, $J_1 = 5.3$ Hz, $J_2 = 9.1$ Hz, CHH′), 3.66-3.77 (11H, m, TO-CH_3, 2DMT-OCH_3, CH_2), 4.08 (1H, m, CH), 5.06 (2H, s, TO-CH_2), 6.51 (1H, s, TO-CH), 6.81 (4H, m, 4DMT), 7.04 (1H, m, TO), 7.20-7.30 (8H, m, 7DMT, TO), 7.38-7.55 (7H, m, 2DMT, 5TO), 7.70 (1H, m, TO), 7.99 (1H, m, TO), 8.31 (1H, m, TO). ^{13}C-NMR (CD_3CN): $\delta = -5.3$ ($SiMe_2$), −5.2 ($SiMe_2$), 18.7 (Si^{t}Bu), 26.2 (Si^{t}Bu), 34.4 (TO-CH_3), 52.5 (CH), 55.8 (DMT-OCH_3), 57.1 (TO-CH_2), 62.6 (CH_2), 62.7 (CH_2), 86.8 (DMT-C_q), 89.3 (TO-CH), 108.3 (TO-Ar-CH), 113.6 (TO-Ar-CH), 113.9 (4DMT-ArCH), 117.6 (TO-Ar-CH), 123.2 (TO-Ar-CH), 124.5 (TO-Ar-CH), 125.2 (TO-Ar–C_q), 125.6 (TO-Ar–C_q), 125.8 (TO-Ar-CH), 127.5(TO-Ar-CH), 127.7 (1DMT-ArCH), 128.8 (2DMT-ArCH), 128.8 (2DMT-ArCH), 129.1 (TO-Ar-CH), 130.9 (4DMT-ArCH), 133.9 (TO-Ar-CH), 136.8 (DMT-ArC_q), 136.9 (DMT-ArC_q), 138.4 (TO-Ar–C_q), 140.9 (TO-Ar–C_q), 145.3 (TO-Ar-CH), 146.0 (DMT-Ar–C_q), 149.2 (TO-Ar–C_q), 158.3 (2DMT-ArC_q), 161.5 (TO-Ar–C_q), 166.0 (TO-C_q).

(*R*)-1-DMT-Serinol(TO_{Q1}) (2D)

To a solution of 800 mg (0.872 mmol) (*R*)-1-DMT-3-TBDMS-serinol(TO_{Q1}) (**13D**) in 15 mL THF under an argon atmosphere was added 1.74 mL of a 1 M solution of TBAF in THF (1.74 mmol). After 1 h 80 mL saturated aqueous $NaHCO_3$ solution was added. The resulting red precipitate was collected, washed 4 times with ethyl acetate and dried under reduced pressure. Yield: 533 g (0.663 mmol, 76%), red solid, $C_{44}H_{42}BrN_3O_5S$ (804.79 g mol^{-1}). R_f 0.62 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D = -5.1$ ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 724.2840 $[C_{44}H_{42}N_3O_5S]^+$, found: 724.2842. ^{1}H-NMR (CD_3CN): $\delta = 3.11$ (2H, m, CH_2), 3.67 (2H, m, CH_2), 3.73 (6H, m, 2DMT-OCH_3), 3.77 (3H, s, TO-CH_3), 4.09 (1H, m, CH), 5.03 (2H, s, TO-CH_2), 6.55 (1H, s, TO-CH), 6.81 (4H, m, 4DMT), 7.09 (1H, d, $J = 7.0$, TO), 7.20-7.60 (17H, m, 9DMT, 7TO, 1NH), 7.75 (1H, d, $J = 7.8$, TO), 8.01 (1H, d, $J = 7.8$, TO), 8.35 (1H, d, $J = 8.0$, TO). ^{13}C-NMR (CD_3CN): $\delta = 34.6$ (TO-CH_3), 52.9 (CH), 55.9 (2DMT-OCH_3), 57.3 (TO-CH_2), 62.2 (CH_2), 63.2 (CH_2), 86.8 (DMT-C_q), 89.5 (TO-CH), 108.6 (TO-Ar-CH), 113.7 (TO-Ar-CH), 113.9 (4DMT-ArCH), 123.5 (TO-Ar-CH), 124.8 (TO-Ar–C_q), 125.4 (TO-Ar–C_q), 125.8 (TO-Ar-CH), 126.1 (TO-Ar-CH), 127.6 (TO-Ar-CH), 127.7 (1DMT-ArCH), 128.8 (2DMT-ArCH), 128.9 (2DMT-ArCH), 129.2 (TO-Ar-CH), 130.9 (4DMT-ArCH), 134.1 (TO-Ar-CH), 136.9 (DMT-ArC_q), 137.0 (DMT-ArC_q), 138.8 (TO-Ar–C_q), 141.3 (TO-Ar–C_q), 145.6 (TO-Ar-CH), 146.0 (DMT-Ar–C_q), 149.8 (TO-Ar–C_q), 159.5 (2DMT-ArC_q), 162.0 (TO-Ar–C_q), 166.3 (TO-C_q).

(*S*)-1-DMT-Serinol(TO_{Q1}) (2L)

As described for **2D**, (*R*)-1-DMT-3-TBDMS-serinol(TO_{Q1}) (**13D**) (276 mg, 0.300 mmol) was reacted with 600 µL of a 1 M solution of TBAF in THF (0.600 mmol). Yield: 181 g (0.225 mmol, 75%), red solid, $C_{44}H_{42}BrN_3O_5S$ (804.79 g mol^{-1}). R_f 0.62 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D = 4.9$ ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 724.2840 $[C_{44}H_{42}N_3O_5S]^+$, found: 724.2847. ^{1}H-NMR (CD_3CN): $\delta = 3.12$ (2H, m, CH_2), 3.67 (2H, m, CH_2), 3.73 (6H, m, 2DMT-OCH_3), 3.77 (3H, s, TO-CH_3), 4.09 (1H, m, CH), 5.03 (2H, s, TO-CH_2), 6.56 (1H, s, TO-CH), 6.81 (4H, m, 4DMT), 7.10 (1H, d, $J = 7.0$, TO), 7.20-7.60 (17H, m, 9DMT, 7TO, 1NH), 7.75 (1H, d, $J = 7.8$, TO), 8.01 (1H, d, $J = 7.8$, TO), 8.35 (1H, d, $J = 8.1$, TO). ^{13}C-NMR (CD_3CN): $\delta = 34.6$ (TO-CH_3), 52.9 (CH), 55.8 (2DMT-OCH_3), 57.3 (TO-CH_2), 62.2 (CH_2), 63.2 (CH_2), 86.8 (DMT-C_q), 89.5 (TO-CH), 108.5 (TO-Ar-CH), 113.7 (TO-Ar-CH), 113.9 (4DMT-ArCH), 123.4 (TO-Ar-CH), 124.8 (TO-Ar–C_q), 125.3 (TO-Ar–C_q), 125.7 (TO-Ar-CH), 126.0 (TO-Ar-CH), 127.5 (TO-Ar-CH), 127.7 (1DMT-ArCH), 128.7 (2DMT-ArCH), 128.9 (2DMT-ArCH), 129.2 (TO-Ar-CH), 130.9 (4DMT-ArCH), 134.1 (TO-Ar-CH), 136.8 (DMT-ArC_q), 136.9 (DMT-ArC_q), 138.7 (TO-Ar–C_q), 141.2 (TO-Ar–C_q), 145.6 (TO-Ar-CH), 146.0 (DMT-Ar–C_q), 149.7 (TO-Ar–C_q), 159.5 (2DMT-ArC_q), 161.9 (TO-Ar–C_q), 166.3 (TO-C_q).

(*R*)-1-DMT-3-TBDMS-Serinol(TO_{Q2}) (14D)

In a volume of 15 mL DMF thiazole orange (**TO_{Q2}**, **12**) (844 mg, 1.77 mmol) was suspended by applying ultrasound over a period of 2 min. Subsequently, PyBOP (921 mg, 1.77 mmol) and *N*-methylmorpholine (716 mg, 659 µL, 7.08 mmol) were added. To the clear solution was added (*R*)-1-DMT-3-TBDMS-serinol (**10D**) (450 mg, 0.886 mmol) in 5 mL DMF. The mixture was stirred for 16 h. The volatiles were removed under reduced pressure. The residue was suspended in 100 mL CH_2Cl_2. After filtration, the residue was discarded and the filtrate was washed with 100 mL saturated aqueous $NaHCO_3$ solution, twice with 100 mL water and dried over $MgSO_4$. The solids were removed by filtration and the volatiles were removed under reduced pressure. The residue was further purified by flash column chromatography. Yield: 273 mg (0.274 mmol, 31%), red solid, $C_{51}H_{58}F_6N_3O_5PSSi$ (998.14 g mol^{-1}). R_f 0.73 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D = -2.1$ ($c = 0.05$, CH_3CN). HRMS (*m*/*z*) calculated: 852.3861 $[C_{51}H_{58}N_3O_5SSi]^+$, found: 852.3852.^{1}H-NMR (CD_3CN): $\delta = -0.11$ (6H, m, $SiMe_2$), 0.71 (9H, s, Si^{t}Bu), 2.81 (2H, t, $J = 5.9$, CH_2-CO-), 2.90 (1H, dd, $J_1 = 5.4$, $J_2 = 9.1$, CHH′), 2.99 (1H, dd, $J_1 = 6.1$ Hz, $J_2 = 9.1$ Hz, CHH′), 3.47 (1H, dd, $J_1 = 5.8$, $J_2 = 10.2$, CHH′), 3.58 (1H, dd, $J_1 = 4.5$ Hz, $J_2 = 10.2$, CHH′), 3.70 (6H, m, 2DMT-OCH_3), 3.82 (3H, s, TO-CH_3), 4.06

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(1H, m, CH), 4.73 (2H, s, TO-CH_2-), 6.41 (1H, d, J = 8.8, TO), 6.62 (1H, s,–CH=), 6.74 (4H, m, 4DMT), 7.03 (1H, d, J = 7.3, TO), 7.19 (8H, m, 7DMT, TO), 7.35 (3H, m, 2DTM, 1NH), 7.54 (2H, m. 2TO), 7.67 (1H, m, TO), 7.77 (1H, m, TO), 7.90 (2H, m, 2TO), 8.22 (1H, d, J = 7.3, TO), 8.49 (1H, d, J = 8.6, TO). ^{13}C-NMR (CD_3CN): δ = −5.4 ($SiMe_2$), −5.4 ($SiMe_2$), 18.6 (Si^tBu), 26.0 (Si^tBu), 34.5 (TO-CH_3), 35.4 (CH_2-CO-), 51.7 (TO-CH_2-), 52.0 (CH), 55.8 (2DMT-OCH_3), 62.9 (CH_2), 63.1 (CH_2), 86.6 (DMT-C_q), 89.0 (–CH=), 108.8 (TO-Ar-CH), 113.5 (TO-Ar-CH), 113.8 (4DMT-ArCH), 118.6 (TO-Ar-CH), 123.4 (TO-Ar-CH), 125.2 (TO-Ar–C_q), 125.4 (TO-Ar–C_q), 125.6 (TO-Ar-CH), 126.4 (TO-Ar-CH), 127.6 (1DMT-ArCH), 128.6 (DMT-ArCH), 128.9 (DMT-ArCH), 129.1 (TO-Ar-CH), 130.8 (2DMT-ArCH), 130.9 (2DMT-ArCH), 134.1 (TO-Ar-CH), 136.9 (DMT-ArC_q), 137.0 (DMT-ArC_q), 138.1 (TO-Ar–C_q), 141.4 (TO-Ar–C_q), 145.5 (TO-Ar-CH), 146.1 (DMT-Ar–C_q), 150.1 (TO-Ar–C_q), 159.5 (DMT-ArC_q), 159.5 (DMT-ArC_q), 161.7 (TO-Ar–C_q), 169.8 (TO-Ar–C_q).

(*S*)-1-DMT-3-TBDMS-Serinol(TO_{Q2}) (14L)

As described for **14D**, thiazole orange (**TO_{Q2}**, **12**) (844 mg, 1.77 mmol) was reacted with PyBOP (921 mg, 1.77 mmol), *N*-methylmorpholine (716 mg, 659 µL, 7.08 mmol) and (*R*)-1-DMT-3-TBDMS-serinol (**10L**) (450 mg, 0.886 mmol). Yield: 280 mg (0.281 mmol, 32%), red solid, $C_{51}H_{58}F_6N_3O_5PSSi$ (998.14 g mol^{-1}). R_f 0.73 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D$ = 2.0 (c = 0.05, CH_3CN). HRMS (m/z) calculated: 852.3861 $[C_{51}H_{58}N_3O_5SSi]^+$, found: 852.3851. ^{1}H-NMR (CD_3CN): δ = −0.12 (6H, m, $SiMe_2$), 0.70 (9H, s, Si^tBu), 2.80 (2H, t, J = 5.8, CH_2-CO-), 2.89 (1H, dd, J_1 = 5.4, J_2 = 9.1, CHH′), 2.98 (1H, dd, J_1 = 6.1 Hz, J_2 = 9.0 Hz, CHH′), 3.47 (1H, dd, J_1 = 5.8, J_2 = 10.2, CHH′), 3.58 (1H, dd, J_1 = 4.4 Hz, J_2 = 10.1, CHH′), 3.69 (6H, m, 2DMT-OCH_3), 3.79 (3H, s, TO-CH_3), 4.06 (1H, m, CH), 4.71 (2H, s, TO-CH_2-), 6.42 (1H, d, J = 8.9, TO), 6.59 (1H, s,–CH=), 6.73 (4H, m, 4DMT), 6.98 (1H, d, J = 7.3, TO), 7.16 (8H, m, 7DMT, TO), 7.33 (3H, m, 2DTM, 1NH), 7.46 (1H, m. TO), 7.53 (1H, m. TO), 7.65 (1H, m, TO), 7.74 (1H, m, TO), 7.87 (2H, m, 2TO), 8.20 (1H, d, J = 7.3, TO), 8.46 (1H, d, J = 8.6, TO). ^{13}C-NMR (CD_3CN): δ = −5.4 ($SiMe_2$), −5.4 ($SiMe_2$), 18.6 (Si^tBu), 26.0 (Si^tBu), 34.5 (TO-CH_3), 35.4 (CH_2-CO-), 51.7 (TO-CH_2-), 52.0 (CH), 55.8 (2DMT-OCH_3), 62.9 (CH_2), 63.1 (CH_2), 86.6 (DMT-C_q), 89.0 (–CH=), 108.8 (TO-Ar-CH), 113.5 (TO-Ar-CH), 113.8 (4DMT-ArCH), 118.6 (TO-Ar-CH), 123.4 (TO-Ar-CH), 125.2 (TO-Ar–C_q), 125.4 (TO-Ar–C_q), 125.6 (TO-Ar-CH), 126.4 (TO-Ar-CH), 127.6 (1DMT-ArCH), 128.6 (DMT-ArCH), 128.9 (DMT-ArCH), 129.1 (TO-Ar-CH), 130.8 (2DMT-ArCH), 130.9 (2DMT-ArCH), 134.1 (TO-Ar-CH), 136.9 (DMT-ArC_q), 137.0 (DMT-ArC_q), 138.0 (TO-Ar–C_q), 141.3 (TO-Ar–C_q), 145.4 (TO-Ar-CH), 146.1 (DMT-Ar–C_q), 150.0 (TO-Ar–C_q), 159.4 (DMT-ArC_q), 159.5 (DMT-ArC_q), 161.6 (TO-Ar–C_q), 169.8 (TO-Ar–C_q).

(*R*)-1-DMT-Serinol(TO_{Q2}) (3D)

To a solution of (*R*)-1-DMT-3-TBDMS-Serinol(TO_{Q2}) (**14D**) (273 mg, 0.274 mmol) in 15 mL THF under an argon atmosphere was added TBAF·$3H_2O$ (179 mg, 0.566 mmol). After 1 h 80 mL saturated aqueous $NaHCO_3$ solution was added. The resulting red precipitate was collected, washed 4 times with ethyl acetate and dried under reduced pressure. Yield: 162 g (0.213 mmol, 78%), red solid, $C_{45}H_{44}FN_3O_5S$ (757.91 g mol^{-1}). R_f 0.44 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D$ = −4.5 (c = 0.05, CH_3CN). HRMS (m/z) calculated: 738.2996 $[C_{45}H_{44}N_3O_5S]^+$, found: 738.2983. ^{1}H-NMR (CD_3CN): δ = 2.80 (2H, m, CH_2-CO-), 2.88 (1H, dd, J_1 = 5.2, J_2 = 9.3, CHH′), 2.97 (1H, dd, J_1 = 5.9 Hz, J_2 = 9.2 Hz, CHH′), 3.45 (2H, m, CH), 3.67 (6H, m, 2DMT-OCH_3), 3.73 (3H, s, TO-CH_3), 4.06 (1H, m, CH), 4.66 (2H, s, TO-CH_2-), 6.48 (1H, s,–CH=), 6.56 (1H, d, J = 8.9, TO), 6.70 (4H, m, 4DMT), 6.83 (1H, d, J = 7.3, TO), 7.15 (7H, m, 7DMT), 7.30 (4H, m, 2DMT, 1NH, 1TO), 7.38 (1H, m. TO), 7.47 (1H, m. TO), 7.58 (1H, m, TO), 7.66 (1H, m, TO), 7.79 (2H, m, 2TO), 8.13 (1H, d, J = 7.3, TO), 8.34 (1H, d, J = 8.7, TO). ^{13}C-NMR (CD_3CN): δ = 34.4 (TO-CH_3), 35.5 (CH_2-CO-), 51.6 (TO-CH_2-), 52.4 (CH), 55.9 (2DMT-OCH_3), 62.5 (CH_2), 63.6 (CH_2), 86.6 (DMT-C_q), 88.9 (–CH=), 108.8 (TO-Ar-CH), 113.5 (TO-Ar-CH), 113.9 (4DMT-ArCH), 118.5 (TO-Ar-CH), 123.3 (TO-Ar-CH), 125.2 (TO-Ar–C_q), 125.3 (TO-Ar–C_q), 125.6 (TO-Ar-CH), 126.3 (TO-Ar-CH), 127.7 (DMT-ArCH), 127.7 (TO-ArCH), 128.7 (2DMT-ArCH), 128.9 (2DMT-ArCH), 129.1 (TO-Ar-CH), 130.8 (2DMT-ArCH), 130.9 (2DMT-ArCH), 134.1 (TO-Ar-CH), 136.9 (2DMT-ArC_q), 137.9 (TO-Ar–C_q), 141.2 (TO-Ar–C_q), 145.2 (TO-Ar-CH), 146.0 (DMT-Ar–C_q), 149.8 (TO-Ar–C_q), 159.5 (DMT-ArC_q), 159.5 (DMT-ArC_q), 161.4 (TO-Ar–C_q), 170.6 (TO-Ar–C_q).

(*S*)-1-DMT-Serinol(TO_{Q2}) (3L)

As described for **3D**, (*R*)-1-DMT-3-TBDMS-serinol(TO_{Q2}) (**14L**) (280 mg, 0.281 mmol) was reacted with TBAF·$3H_2O$(183 mg, 0.580 mmol). Yield: 187 g (0.247 mmol, 88%), red solid, $C_{45}H_{44}FN_3O_5S$ (757.91 g mol^{-1}). R_f 0.44 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v). $[\alpha]_D$ = 3.8 (c = 0.05, CH_3CN). HRMS (m/z) calculated: 738.2996 $[C_{45}H_{44}N_3O_5S]^+$, found: 738.2986. ^{1}H-NMR (CD_3CN): δ = 2.80 (2H, m, CH_2-CO-), 2.87 (1H, dd, J_1 = 5.2, J_2 = 9.3, CHH′), 2.97 (1H, dd, J_1 = 6.0 Hz, J_2 = 9.3 Hz, CHH′), 3.46 (2H, m, CH), 3.66 (6H, m, 2DMT-OCH_3), 3.73 (3H, s, TO-CH_3), 4.05 (1H, m, CH), 4.66 (2H, s, TO-CH_2-), 6.48 (1H, s,–CH=), 6.56 (1H, d, J = 8.8, TO), 6.70 (4H, m, 4DMT), 6.83 (1H, d, J = 7.3, TO), 7.15 (7H, m, 7DMT), 7.30 (4H, m, 2DMT, 1NH, 1TO), 7.39 (1H, m. TO), 7.47 (1H, m. TO), 7.58 (1H, m, TO), 7.66 (1H, m, TO), 7.79 (2H, m, 2TO), 8.13 (1H, d, J = 7.3, TO), 8.34 (1H, d, J = 8.7, TO). ^{13}C-NMR (CD_3CN): δ = 34.4 (TO-CH_3), 35.5 (CH_2-CO-), 51.6 (TO-CH_2-), 52.4 (CH), 55.8 (2DMT-OCH_3), 62.5 (CH_2), 63.6 (CH_2), 86.6 (DMT-C_q), 88.9 (–CH=), 108.8 (TO-Ar-CH), 113.4 (TO-Ar-CH), 113.9 (4DMT-ArCH), 118.5 (TO-Ar-CH), 123.3 (TO-Ar-CH), 125.2 (TO-Ar–C_q), 125.3 (TO-Ar–C_q), 125.6 (TO-Ar-CH), 126.3 (TO-Ar-CH), 127.7 (DMT-ArCH), 127.7 (TO-ArCH), 128.7 (2DMT-ArCH), 128.9 (2DMT-ArCH), 129.1 (TO-Ar-CH), 130.8 (2DMT-ArCH), 130.9 (2DMT-ArCH), 134.1 (TO-Ar-CH), 136.9 (2DMT-ArC_q), 137.9 (TO-Ar–C_q), 141.2 (TO-Ar–C_q), 145.2 (TO-Ar-CH), 146.0 (DMT-Ar–C_q), 149.8 (TO-Ar–C_q), 159.4 (DMT-ArC_q), 159.5 (DMT-ArC_q), 161.4 (TO-Ar–C_q), 170.6 (TO-Ar–C_q).

(*S*)-1-DMT-Glycerol(TO)-2-(*O*-cyanoethyl-*N*,*N*-di*iso*propyl)-phosphoamidite (15)

In an argon atmosphere, (*R*)-glycerol(TO) (666 mg, 0.891 mmol) (**1**) was dissolved in dicyanoimidazole (0.25 M, 10.2 ml

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solution in acetonitrile) and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (714 µl, 2.10 mmol) was added. After 2 h the reaction was stopped by the addition of 1 ml MeOH. Subsequently, 50 ml CH_2Cl_2 was added and the organic phase was three times washed with 20 ml saturated aqueous $NaHCO_3$ solution. The solvent was removed under reduced pressure and the crude product was further purified by flash column chromatography (CH_2Cl_2–MeOH–NEt_3, 97.5 : 2 : 0.5, v/v/v). Yield: 546 mg (0.576 mmol, 65%), red solid, $C_{51}H_{56}BrN_4O_5PS$ (947.96 g mol^{-1}).

(*D*)-1-DMT-Serinol(TO$_{Q1}$)-3-(*O*-cyanoethyl-*N*,*N*-di*iso*propyl)-phosphoamidite (16D)

In an argon atmosphere, (*D*)-serinol(TO$_{Q1}$) (**2D**) (295 mg, 0.366 mmol) was dissolved in 10 mL dry CH_2Cl_2 and DIPEA (283 mg, 383 µL, 2.20 mmol) was added, followed by 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphorodiamidite (390 mg, 1.65 mmol, 368 µL). After 1 h, the reaction was stopped by the addition of 10 mL saturated aqueous $NaHCO_3$ solution. The organic phase was separated, washed twice with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. The crude product was dissolved in 1.8 mL dry acetonitrile, filtered and used without further purification for automated DNA synthesis. Yield: quantitative, red solid, $C_{53}H_{59}BrN_5O_6PS$ (1005.0 g mol^{-1}). R_f 0.65 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v).

(*L*)-1-DMT-Serinol(TO$_{Q1}$)-3-(*O*-cyanoethyl-*N*,*N*-di*iso*propyl)-phosphoamidite (16L)

A solution of (*L*)-serinol(TO$_{Q1}$) (**2L**) (345 mg, 0.427 mmol) in 15 mL dry CH_2Cl_2 was allowed to react with DIPEA (331 mg, 447 µL, 2.56 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphorodiamidite (454 mg, 1.92 mmol, 428 µL) as described for **16D**. The crude product was dissolved in 2.1 mL dry acetonitrile, filtered and used without further purification for automated DNA synthesis. Yield: quantitative, red solid, $C_{53}H_{59}BrN_5O_6PS$ (1005.0 g mol^{-1}). R_f 0.64 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v).

(*D*)-1-DMT-Serinol(TO$_{Q2}$)-3-(*O*-cyanoethyl-*N*,*N*-di*iso*propyl)-phosphoamidite (17D)

A solution of (*D*)-serinol(TO$_{Q2}$) (**3D**) (180 mg, 0.237 mmol) in 10 mL dry CH_2Cl_2 was allowed to react with DIPEA (153.6 mg, 207 µL, 1.19 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphorodiamidite (141 mg, 0.594 mmol, 134 µL) as described for **16D**. The crude product was dissolved in 1.2 mL dry acetonitrile, filtered and used without further purification for automated DNA synthesis. Yield: quantitative, red solid, $C_{53}H_{59}BrN_5O_6PS$ (1005.0 g mol^{-1}). R_f 0.53 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v).

(*L*)-1-DMT-Serinol(TO$_{Q2}$)-3-(*O*-cyanoethyl-*N*,*N*-di*iso*propyl)-phosphoamidite (17L)

A solution of (*L*)-serinol(TO$_{Q2}$) (**3L**) (150 mg, 0.198 mmol) in 10 mL dry CH_2Cl_2 was allowed to react with DIPEA (153.6 mg, 207 µL, 1.19 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphorodiamidite (141 mg, 0.594 mmol, 134 µL) as described for **16D**. The crude product was dissolved in 1.2 mL dry acetonitrile, filtered and used without further purification for automated DNA synthesis. Yield: quantitative, red solid, $C_{53}H_{59}BrN_5O_6PS$ (1005.0 g mol^{-1}). R_f 0.53 (CH_2Cl_2–MeOH–NEt_3, 89.5 : 10 : 0.5, v/v/v).

DNA synthesis, workup, purification and characterization

The oligodeoxynucleotides were assembled by using an *AB Applied Biosystems Synthesyzer Model 3400* and phosphoramidite methodology. CPGs were purchased from *Applied Biosystems* and *Link Technologies* (1 µmol, pore size 500 Å) and DNA syntheses reagents from *Applied Biosystems* and *Roth* (dry acetonitrile, 2% dichloroacetic acid in CH_2Cl_2, 4% tetrazole in acetonitrile, acetic anhydride in 2,6-lutidin–THF (1 : 1 : 8), 16% 1-methylimidazole in THF, Iod in water–pyridine–THF (3 : 2 : 20 : 75)). The phosphoramidites dT-, dA^{Pac}-, dC^{Ac}-, $dG^{iPr\text{-}Pac}$, dA^{Bz}-, dC^{Bz} and dG^{DMF} were used following the manufacturers instructions (0.1 M dry acetonitrile). The phosphoramidites **15**, **16D**, **16L**, **17D** and **17L** were used in 0.2 M solution. The synthesizer was programmed to yield oligomers carrying the terminal DMT protective group ("trityl-on"). After synthesis the resulting CPGs were treated with 1 mL of saturated aqueous NH_4OH was for 4 h at RT, the supernatant was collected and the volatiles were evaporated by using a *Uniequip Speed-vac Unijet II*. The crude product was further purified by RP-HPLC. Afterwards, DMT removal was induced through the addition of 50% AcOH aqueous solution over 30 min. The crude product was again purified by RP-HPLC. The resulting oligomers were concentrated and desalted using *NAP-5 Sephadex* columns of *GE Healthcare* or *Amersham Biosciences*. Finally, the oligomers were freeze dried with a *Christ LDC 1m* lyophilizer. The residues were dissolved in water (Milli-Q-Pore) to reach a final concentration of 0.1 mM. Identity and purity was determined by using analytical RP-HPLC or UPLC and MALDI-TOF mass spectroscopy (see the ESI†).

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