Synthesis and Biophysical Properties of Oligodeoxynucleotides Containing 2′-Deoxy-5-(4-nitro-1*H*-imidazol-1-yl)-*β*-D-uridine and 2′-Deoxy-5-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-*β*-D-uridine Monomers

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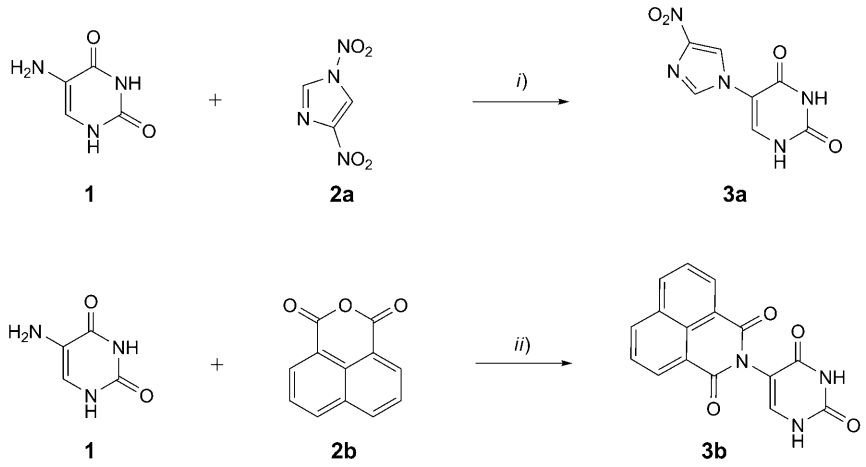
Detection of single-nucleotide polymorphisms (SNPs) of biologically relevant DNA and RNA samples remain a scientific and practical challenge. We have synthesized phosphoramidite building blocks and oligodeoxynucleotide probes containing novel 2′-deoxyuridine monomers modified by 5-(4-nitro-1*H*-imidazol-1-yl; (monomer **X**) or 2′-deoxy-5-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl; monomer **Y**) substituents. The effects of monomers **X** and **Y** on duplex thermal stability, and their capability towards discrimination of single-base mismatches were furthermore studied. Encouraging results were obtained with respect to thermal mismatch discrimination using oligodeoxynucleotides containing monomer **X** and fluorescence-based discrimination using oligodeoxynucleotides containing monomer **Y**.

Introduction. – Detection of single-nucleotide polymorphisms (SNPs) has in the last decade attracted much interest in the field of oligonucleotide chemistry [1]. Related hereto are base-pair mutations of gene sequences which may result in modulation of gene expression and eventually disease [2]. Analysis of mutations and SNPs may be performed by the use of oligonucleotide probes. The majority of existing methods embark on differences in hybridization efficiency of matched and mismatched oligonucleotide probes and the target sequence, and the use of a fluorescent reporter group [3]. The reporter group can be attached directly to the sugar or the nucleobase, or it can be a planar fluorophore which is able to structurally mimic the nucleobase [4]. Ideally, SNPs and mutations are detected by monitoring increased fluorescence of the probe as read-out, either in case of fully matched hybridization between probe and target [5], or if a mismatched base pair is present in the duplex [6]. Alternatively, if hybridization, *i.e.*, duplex formation, is dependent of a full match between probe and target, the use of probes which fluoresce only when hybridized is an option [7]. Good candidates for improved SNP probes are nucleotide monomers bearing modified nucleobases which are directly conjugated to reporter groups [8]. Herein, we describe synthesis, thermal denaturation, and fluorescence properties of novel oligodeoxynucleotide (ODN) probes bearing a 4-nitro-1*H*-imidazol-1-yl or 1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl [9] substituent attached at C(5) of uracil base moieties.

Results and Discussion. – 5-Aminouracil (**1**) was used for synthesis of *C(5)*-functionalized uracil derivatives **3a** and **3b** (*Scheme 1*). 5-(4-Nitro-1*H*-imidazol-1-

yl)uracil (**3a**) was obtained in an *Anrorc*-type reaction of **1** with 1,4-dinitro-1*H*-imidazole (**2a**) in DMSO as described in [10]. 5-(1,3-Dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)uracil (**3b**) was synthesized in 71% yield by reaction of **1** with naphthalene-1,8-dicarboxylic anhydride **2b** (*Scheme 1*). This reaction was performed in a mixture of EtOH and DMSO in the presence of Et_3N and DMAP. The use of the typical solvents for such a transformation like glacial AcOH [11] or pyridine [12] were not efficient because of poor solubility of **1** in these solvents.

Scheme 1



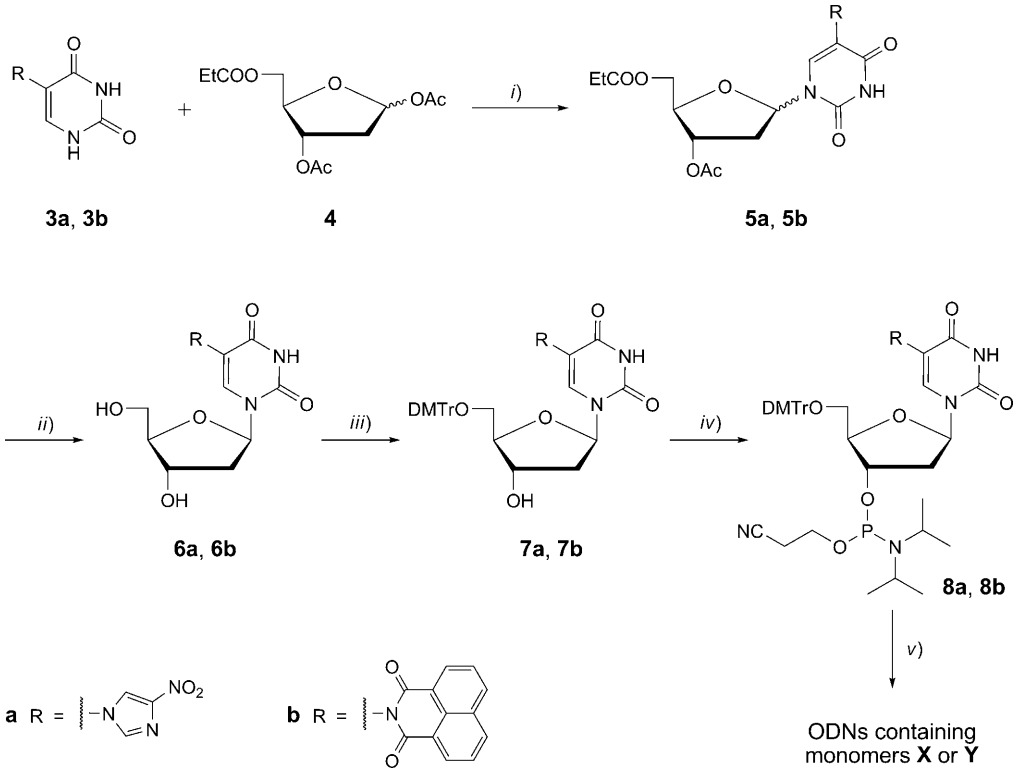
i) DMSO, r.t. *ii*) Et_2N (TEA), 4-(dimethylamino)pyridine (DMAP), DMSO/EtOH 1:1 (*v*/*v*); 71%.

Coupling of *C(5)*-modified uracil **3a** or **3b** with 2-deoxy-1,3-di-*O*-acetyl-5-*O*-propanoyl-D-ribofuranose (**4**) [13] was carried out in MeCN according to the *Vorbrüggen* method [14] using TMSOTf as a catalyst (*Scheme 2*). The products were isolated as inseparable anomeric mixtures of nucleoside derivatives **5a** and **5b** in 93 and 86% yield, respectively. Subsequent deacylation with MeONa in anhydrous MeOH, followed by fractional crystallization from MeOH, afforded pure β-anomers **6a** and **6b** in yields of 36 and 33%, respectively. The 5′-OH group of nucleosides **6a** and **6b** was regioselectively protected using 4,4′-dimethoxytrityl chloride (DMTrCl) in anhydrous pyridine. The appropriate nucleoside derivatives **7a** and **7b** were isolated in yields of 80 and 86%, respectively. Finally, *O(3′)*-phosphitylation using 2′-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite in the presence of *Hünig*'s base furnished the phosphoramidite building blocks **8a** (83%) and **8b** (73%) suitable for oligonucleotide synthesis.

Synthesis of ODNs containing monomers **X** and **Y** (see *Fig. 1* for structures, and *Tables 1*, *2*, and *3* for ODN sequences) was performed in 0.2 μmol scale using an automated DNA synthesizer[1]). Standard reagents and procedures were used except for extended coupling times (0.05M in MeCN, 15 min; 1*H*-tetrazole as a catalyst) for phosphoramidites **8a** and **8b**. The stepwise coupling yields were >99% for unmodified

[1]) See *Exper. Part.*

Scheme 2



i) 1,1,1,3,3,3-Hexamethyldisilazane (HMDS), $(NH_4)_2SO_4$, then MeCN, trimethylsilyl triflate (Tf = trifluoromethylsulfonyl; TMSOTf), −15 to 0°; **5a**: 93%, **5b**: 86%. *ii*) *a*) MeONa/MeOH, 90 min, r.t.; *b*) *Amberlist IRC 120*; **6a**: 36%, **6b**: 33%). *iii*) Bis(4-methoxyphenyl)(phenyl)methyl chloride (DMTrCl), pyridine, r.t.; **7a**: 80%, **7b**: 86%. *iv*) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, MeCN, $EtN(i\text{-}Pr)_2$, r.t.; **8a**: 83%, **8b**: 73%. *v*) DNA Synthesizer.

DNA phosphoramidites, and *ca.* 99% for **8a** and **8b**. Removal of nucleobase-protecting groups and cleavage from the solid support for ODNs containing monomer **X** (**ON2**, **ON3**, and **ON11**) were achieved using standard conditions (32% aq. NH_3 for 12 h at 55°). In the case of ODNs containing monomer **Y** (**ON4** and **ON12**), application of the standard deprotection conditions resulted in substantial cleavage of the 1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl moiety (up to 40% cleavage)[2]), resulting in formation of by-products containing *C(5)*-amino-2′-deoxyuridine monomers. When a shorter deprotection time was employed (up to maximum 6 h at 55°), the undesired cleavage product was still isolated (in up to a yield of 30%)[2]). The best results were obtained when the deprotection was carried out at room temperature for 24 h leading to benzo-isoquinolin-2-yl cleavage to an extent of only 5–10%. Previously, when 1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl-protected dA, dG, and dC monomers were used for

[2]) Reported percentages are based on ion-exchange chromatography.

synthesis of nucleotide dimers, the benzoisoquinolinyl moieties were cleaved completely using 40% aq. NH_3 (50° for 4 h) [12]. Our results showed that the 1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl moiety formed on 5-aminouracil is relatively stable and can be successfully installed on ODN derivatives when using mild deprotection conditions. The crude *O5′*-DMTr protected ODNs were purified by RP-HPLC, followed by detritylation using 80% aq. AcOH for 20 min at room temperature and EtOH precipitation, to afford the pure ODNs. The composition and purity of the ODNs was confirmed by HR-MALDI-MS and ion-exchange HPLC, respectively[1]).

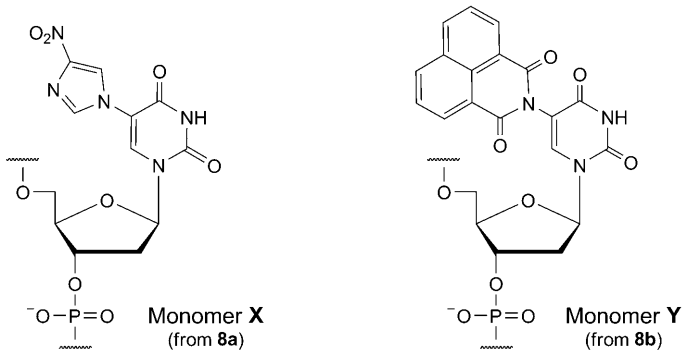


Fig. 1. *Structures of monomers* **X** *and* **Y**

Table 1. *Thermal Denaturation Studies*[a]) *of Nonamer ODNs Containing Monomer* **X**[b]) *or* **Y**[b]) *towards DNA and RNA Complements, and Singly Mismatched DNA Targets*[c]). T_m Values[c]) in ° (ΔT_m values in parentheses).

		DNA: 3′-CAC T**B**T ACG				**RNA**
		ON5	**ON6**	**ON7**	**ON8**	**ON9**
	B:	**A**	**C**	**G**	**T**	**A**
ON1	5′-GTG ATA TGC	28.5	12.0 (– 16.5)[d])	19.0 (– 9.5)[d])	11.5 (– 17.0)[d])	26.5
ON2	5′-GTG A**X**A TGC	23.0 (– 5.5)[d])	n.t.[e])	n.t.[e])	n.t.[e])	19.0 (– 7.5)
ON3	5′-G**X**G A**X**A **X**GC	n.t.[e])	–[f])	–[f])	–[f])	n.t.[e])
ON4	5′-GTG A**Y**A TGC	14.0 (– 14.5)[f])	–[f])	–[f])	–[f])	n.t.[e])

[a]) Thermal denaturation temperatures were measured as the maximum of the first derivative of the melting curve (A_{260} *vs.* temperature) recorded in medium salt buffer ([Na^+] = 110 mM, [Cl^-] = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 μM concentrations of the two complementary strands. [b]) See *Fig. 1* for structures of monomer **X** and **Y**. [c]) T_m Values are average values of at least two measurements. [d]) ΔT_m Value calculated relative to the DNA:DNA (**ON1**:**ON5**) or DNA:RNA (**ON1**:**ON9**) reference duplexes. [e]) No clear transition observed above 10°. [f]) Denaturation experiment not performed.

The effect of monomers **X** or **Y** on duplex stabilities under medium salt conditions was evaluated by UV thermal denaturation experiments in nonamer (**ON2**–**ON4**, *Table 1*) and 21-mer sequence contexts (**ON11** and **ON12**; *Tables 2* and *3*). The thermal denaturation curves of all modified duplexes displaying a thermal denaturation temperature (T_m value; *Tables 1–3*) exhibited sigmoidal monophasic transition curves

with shapes similar to those obtained for the unmodified reference duplexes. A single incorporation of monomer **X** into a nonamer ON (**ON2**; *Table 1*) resulted in a substantial decrease in thermal stability of duplexes with complementary DNA or RNA (-5.5 and $-7.5°$, respectively, when compared to the unmodified reference duplexes formed with **ON1**). Incorporation of monomer **X** induces a less pronounced stability-decreasing effect when incorporated into a 21-mer ODN (*i.e.*, **ON11**; *Tables 2* and *3*). The affinity-decreasing effect of monomer **X** was further confirmed in the nonamer context, as three incorporations of monomer **X** (*i.e.*, **ON3**; *Table 1*) prevented duplex formation towards both complementary DNA and RNA. Single incorporation of monomer **Y** induced an even stronger affinity-decreasing effect for a nonamer duplex with the DNA complement ($-14.5°$ compared to the unmodified DNA duplex), whereas no duplex was formed with complementary RNA (*Table 1*). A single incorporation of monomer **Y** into the 21-mer ON, **ON12**, accordingly induced substantial decreases in the thermal stabilities of the duplexes formed ($-5.5°$ against DNA and $-6.0°$ against RNA; *Tables 2* and *3*).

Table 2. *Thermal Denaturation Studies*[a]*) of 21-mer ODNs Containing Monomers* **X** *or* **Y** *towards Complementary DNA and Singly Mismatched DNA Targets.* T_m Values[c]) in °C (ΔT_m values in parentheses).

		DNA: 3′-ACG TGA CAT **B**CA GAC ATG GTA			
		ON13	**ON14**	**ON15**	**ON16**
	B:	**A**	**C**	**G**	**T**
ON10	5′-TGC ACT GTA **T**GT CTG TAC CAT	61.0	53.0 (-8.0)[b])	57.5 (-3.5)[b])	55.5 (-5.5)[b])
ON11	5′-TGC ACT GTA **X**GT CTG TAC CAT	58.5 (-2.5)[b])	52.5 (-6.0)[c])	55.0 (-3.5)[c])	54.0 (-4.5)[c])
ON12	5′-TGC ACT GTA **Y**GT CTG TAC CAT	55.5 (-5.5)[b])	54.0 (-1.5)[d])	55.5 (0.0)[d])	54.5 (-1.0)[d])

[a]) For conditions of thermal denaturation experiments, see legend of *Table 1*. [b]) ΔT_m Values calculated relative to duplex **ON10**:**ON13**. [c]) ΔT_m Values calculated relative to duplex **ON11**:**ON13**. [d]) ΔT_m Values calculated relative to that of duplex **ON12**:**ON13**.

Watson–Crick base-pairing selectivity of ONs containing a single incorporation of monomers **X** or **Y** was studied by evaluating duplex formation with DNA and RNA targets containing a singly mismatched nucleotide positioned directly opposite to the centrally positioned **X** and **Y** monomers. It is noteworthy that **ON2** containing monomer **X** did not form duplexes with singly mismatched DNA target strands (*Table 1*), and that the 21-mer ON with a single monomer **X** (**ON11**) displayed satisfactory base-pairing selectivity approaching that of the unmodified **ON10** against both the DNA and RNA complements (*Table 2* and *3*). Incorporation of a single monomer **Y** into the nonamer or 21-mer sequences induced poor base-pairing selectivity based on the thermal denaturation effects observed (*Tables 1–3*). The ability to discriminate a single base-mismatch target strands is one of the desirable characteristics of nucleic acid probes for potential applications in therapeutics and diagnostics [15]. The results obtained for monomer **X** suggest that ODNs containing monomer **X** are promising probe candidates, and confirm the suitability of the 5-position of uracil bases as a site of attachment of aromatic moieties.

Table 3. *Thermal Denaturation Studies*[a]) *of 21-mer ODNs Containing Monomers* **X** *or* **Y** *towards Complementary RNA and Singly Mismatched RNA Targets.* T_m Values[c]) in °C (ΔT_m values in parentheses).

		RNA: 3′-ACG UGA CAU **B**CA GAC AUG GUA			
		ON17	**ON18**	**ON19**	**ON20**
	B:	**A**	**C**	**G**	**U**
ON10	5′-TGC ACT GTA **T**GT CTG TAC CAT	63.0	54.5 (– 8.5)[b])	60.5 (– 2.5)[b])	54.0 (– 9.0)[b])
ON11	5′-TGC ACT GTA **X**GT CTG TAC CAT	59.0 (– 4.0)[b])	53.0 (– 6.0)[c])	57.0 (– 2.0)[c])	54.0 (– 5.0)[c])
ON12	5′-TGC ACT GTA **Y**GT CTG TAC CAT	57.0 (– 6.0)[b])	54.0 (– 3.0)[d])	55.5 (– 1.5)[d])	54.5 (– 2.5)[d])

[a]) For conditions of thermal denaturation experiments, see legend of *Table 1*. [b]) ΔT_m Values calculated relative to duplex **ON10**:**ON17**. [c]) ΔT_m Values calculated relative to duplex **ON11**:**ON17**. [d]) ΔT_m Values calculated relative to that of duplex **ON12**:**ON17**.

Monomer **Y** was selected for this study because of its fluorescence properties [9]. Steady-state fluorescence emission spectra[1]) of **ON12** and the corresponding 21-mer duplexes with matched and mismatched DNA or RNA targets were recorded in the T_m buffer at 10°[3]) using 1.0 μM of each ODN strand and an excitation wavelength of 343 nm (*Fig. 2*). The fluorescence-emission spectrum of single-stranded **ON12** is characterized by a broad unstructured band (λ_{max} *ca.* 415 nm). Interestingly, increases in the fluorescence emission intensity were observed upon hybridization with complementary DNA and RNA. In addition, the duplex with DNA, *i.e.*, **ON12**:**ON13**, displayed a significant red shift (*ca.* 15 nm) of its fluorescence emission maxima compared to that of the duplex formed with complementary RNA, *i.e.*, **ON12**:**ON17**.

Fluorescence emission spectra of duplexes formed between the probe **ON12**, and singly mismatched DNA and RNA targets are also included in *Fig. 2*. Duplexes with **Y**:C and **Y**:T/U mismatches display moderately increased fluorescence intensity relative to that of the single stranded **ON12**, but decreased fluorescence intensities relative to the matched duplexes. Interestingly, **Y**:G mismatches constitute a special case, as these duplexes displayed significant increases in the fluorescence intensities (3.5- and 7.5-fold, resp.) compared to the single stranded **ON12**. In addition, it is noteworthy that these **Y**:G mismatched duplexes, *i.e.*, **ON12**:**ON15** and **ON12**:**ON19**, are characterized by having fluorescence maxima at 393 nm. Such a distinctive fluorescence behavior reveals that monomer **Y** may render such ODN probes useful for nucleic acid-based applications in line with other base-discriminating fluorescent (BDF) probes [4].

Conclusions. – We have successfully synthesized 2′-deoxy-5-(nitroimidazolyl)- and 2′-deoxy-5-benzoisoquinolinyl)uridine phosphoramidite building blocks in four steps starting from 1,3-*O*-diacetyl-2-deoxy-5-*O*-propanoyl-D-ribofuranose and 5-functionalized uracil derivatives. The obtained monomers **X** and **Y** were incorporated into oligodeoxynucleotides according to standard procedures. Incorporation of these monomers into nonamer and 21-mer oligodeoxynucleotides resulted in considerable decreases of the thermal stability of duplexes with DNA or RNA complements. Oligodeoxynucleotides containing the *C(5)*-(nitroimidazolyl)-ribofuranosyl monomer

[3]) Similar results were observed at 20°.

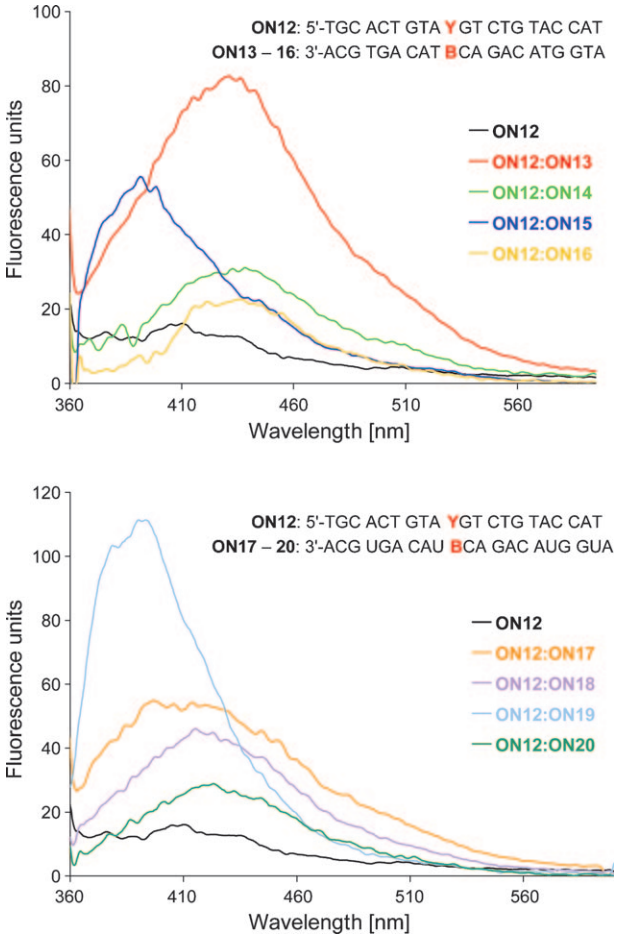


Fig. 2. *Steady-state fluorescence emission spectra of* **ON12** *and its duplexes with complementary and single-base-mismatched DNA* (left) *and RNA* (right) *targets*

X show satisfactory thermal mismatch discrimination against single base-mismatched DNA or RNA targets. Oligonucleotides containing the *C(5)*-(benzoisoquinolinyl)-ribofuranosyl monomer **Y** display interesting mismatch discrimination based on fluorescence emission, in particular against guanine mismatches in both DNA and RNA targets. The straightforward synthesis and interesting single base mismatch-discriminative power make oligodeoxynucleotides containing monomers **X** and **Y** promising candidates for *in vitro* and *in vivo* applications.

Experimental Part

General. All reagents and solvents were of anal. grade, obtained from commercial suppliers and used without further purification except for CH_2Cl_2, which was distilled prior to use. Anh. pyridine was used as obtained from commercial suppliers. MeCN was dried through storage over activated 3-Å molecular

sieves. Anh. CH_2Cl_2, $ClCHCH_2Cl$, $EtN(i\text{-}Pr)_2$, 1,4-dioxane, and Et_3N were dried through storage over activated 4-Å molecular sieves. Reactions were conducted under Ar, whenever anh. solvents were used. All reactions were monitored by TLC using silica-gel-coated plates with a fluorescence indicator (SiO_2 *60*, *F-254*), which were visualized *a*) by UV light and *b*) by dipping in 5% conc. H_2SO_4 in abs. EtOH (*v*/*v*), followed by heating. Column chromatography (CC) was performed using silica gel *60*-packed columns (particle size 0.040–0.063 mm, *Merck*) and moderate pressure (pressure ball). Evaporation of solvents was carried out under reduced pressure at a temp. below 50°. After CC, appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 12 h. ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were recorded at 300, 75.5, and 121.5 MHz, resp., chemical shifts δ are reported in ppm relative to Me_4Si or deuterated solvent as internal standard (δ(H) $CDCl_3$ 7.26 ppm, (D_6)DMSO 2.50 ppm; δ(C) $CDCl_3$ 77.16 ppm, (D_6)DMSO 39.52 ppm; 85% δ(P) H_3PO_4 0 ppm); coupling constants *J* are given in Hz; traces of solvents in NMR spectra were identified by reference to published data [16]. HR-ESI-Mass spectra were recorded in positive-ion mode on an *IonSpec Fourier* transform mass spectrometer with 2,5-dihydroxybenzoic acid as a matrix; in *m*/*z*.

1-(3′-O-*Acetyl-2′-deoxy-5′*-O-*propanoyl*-D-*ribofuranos-1′-yl)-5-(4″-nitro-1*H-*imidazol-1″-yl)-pyrimidine-2,4-(1*H,*3*H*)-dione* (**5a**). A mixture of *5-(4-nitro-1*H-*imidazol-1-yl)uracil* (**3a** [10]; 0.45 g, 2.00 mmol), $(NH_4)_2SO_4$ (20 mg, 0.15 mmol), and hexamethyldisilazane (HMDS; 10.0 ml, 47.0 mmol) was heated under reflux with stirring for 14 h. The excess of HMDS was evaporated under reduced pressure, and the resulting oily residue was dissolved in anh. MeCN (25 ml) and cooled to −15°. *1,3-Di*-O-*acetyl-2-deoxy-5*-O-*propanoyl*-D-*ribofuranose* (**4** [13]; 0.70 g, 2.50 mmol) was added, followed by dropwise addition of trimethylsilyl triflate (TMSOTf; 0.17 ml, 1.0 mmol). The mixture was stirred at −15° for 1 h and warmed to 0° during 1.5 h. Then, 5% aq. $NaHCO_3$ (25 ml) was added, and the resulting mixture was extracted with CH_2Cl_2 (3 × 25 ml). The combined org. phase was dried (Na_2SO_4), evaporated to dryness, and the resulting brownish solid was purified by CC (0–5% MeOH in $CHCl_3$ (*v*/*v*)) to furnish an anomeric mixture of nucleosides **5a** (α/β 1:1 based on ^{1}H-NMR signals). White solid (0.82 g, 93%). R_f (10% MeOH in CH_2Cl_2 (*v*/*v*)) 0.44. ^{1}H-NMR ($CDCl_3$, Me_4Si): 1.08 (*t*, *J* = 7.5, Me); 1.16 (*t*, *J* = 7.5, Me); 2.00 (*s*, Me); 2.12 (*s*, Me); 2.25–2.43 (*m*, CH_2, H_a–C(2′)); 2.56–2.64 (*m*, 1 H_b–C(2′)); 2.80–2.91 (*m*, 1 H_b–C(2′)); 4.21 (*d*, *J* = 4.2, CH_2(5′)); 4.26–4.31 (*m*, CH_2(5′)); 4.48–4.54 (*m*, 1 H–C(4′)); 4.74–4.78 (*m*, 1 H–C(4′)); 5.17–5.25 (*m*, 1 H–C(3′)); 5.25–5.29 (*m*, 1 H–C(3′)); 6.21–6.30 (*m*, 2 H–C(1′)); 7.72 (*d*, *J* = 1.5, 1 H–C(2″)); 7.84 (*d*, *J* = 1.5, 1 H–C(2″)); 8.03 (*s*, 1 H–C(6)); 8.08 (*d*, *J* = 1.5, 1 H–C(5″)); 8.12 (*s*, 1 H–C(6)); 8.21 (*d*, *J* = 1.5, 1 H–C(5″)); 11.57 (br. *s*, 2 NH). ^{13}C-NMR ($CDCl_3$, Me_4Si): 8.9; 8.9; 20.8; 21.0; 27.3 (2 C); 37.8; 38.5; 63.3; 63.4; 73.7; 74.1; 83.1; 84.9; 86.0; 87.9; 111.9; 112.7; 120.2; 120.3; 134.9; 135.4; 136.4 (2C); 147.3 148.9; 149.0; 158.6; 158.9; 169.7; 170.3; 173.7; 174.1. HR-ESI-MS: 460.1080 ($[M+Na]^+$, $C_{17}H_{19}N_5NaO_9^+$; calc. 460.1075).

*1-(2′-Deoxy-β-*D-*ribofuranosyl-1′-yl)-5-(4″-nitro-1*H-*imidazol-1″-yl)-pyrimidine-2,4-(1*H,*3*H*)-dione* (**6a**). Nucleoside **5a** (0.73 g, 1.73 mmol) was suspended in anh. MeOH (25 ml), followed by addition of MeONa (0.40 g, 7.40 mmol). The mixture was stirred for 1.5 h, whereupon H_2O (5 ml) and *Amberlist IRC* 120 (*ca*. 1 g) were added to obtain neutral pH. The resulting suspension was decanted, and the resin was washed with MeOH (10 ml). The combined MeOH phase was evaporated to dryness. Fractional crystallization of the residue from MeOH gave pure β-anomer **6a** (168 mg). An additional portion of **6a** (40 mg) was isolated from the evaporated filtrate by CC (10% MeOH/CH_2Cl_2). Compound **6a** was obtained as a white solid material in a combined yield of 208 mg (36%). R_f (5% MeOH in CH_2Cl_2 (*v*/*v*)) 0.11. ^{1}H- and ^{13}C-NMR spectra were identical to published data [10]. HR-ESI-MS: 362.0713 ($[M+Na]^+$, $C_{12}H_{13}N_5NaO_7^+$; calc. 362.0707).

1-{5′-O-*[Bis(4-methoxyphenyl)(phenyl)methyl]-2′-deoxy-β-*D-*ribofuranosyl-1′-yl}-5-(4″-nitro-1*H-*imidazol-1″-yl)-pyrimidine-2,4-(1*H,*3*H*)-dione* (**7a**). Compound **6a** (0.08 g, 0.23 mmol) was co-evaporated with anh. pyridine (2 × 5 ml) and dissolved in anh. pyridine (1 ml). 4,4′-Dimethoxytrityl chloride (DMTrCl; 100 mg, 0.3 mmol) was added, and the mixture was stirred at r.t. for 3 h, whereupon MeOH (0.2 ml) was added, and stirring was continued for 20 min. The mixture was diluted with CH_2Cl_2 (10 ml) and washed with 5% aq. $NaHCO_3$ (1 × 5 ml). The aq. phase was extracted with CH_2Cl_2 (2 × 10 ml), and the combined org. phase was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by silica-gel CC (0–5% MeOH in CH_2Cl_2, containing 0.5% Et_3N (*v*/*v*/*v*)) to obtain **7a** (111 mg, 80%). White solid. R_f (5% MeOH in CH_2Cl_2 (*v*/*v*)) 0.21. ^{1}H-NMR ($CDCl_3$, Me_4Si): 2.35–2.47 (*m*, H_a–C(2′)); 2.55–

2.67 (*m*, H_b–C(2′)); 3.30–3.40 (*m*, 2 CH_2(5′)); 3.719 (*s*, 2 MeO); 3.12–3.17 (*m*, H–C(4′)); 4.66–4.72 (*m*, H–C(3′)); 6.29 (*t*, *J* = 6.3, H–C(1′)); 6.70–6.74 (*m*, 4 arom. H); 7.00–7.23 (*m*, 11 arom. H, H–C(2″)); 7.48 (*s*, H–C(5″)); 8.21 (*s*, H–C(6)). ^{13}C-NMR ($CDCl_3$, Me_4Si): 44.9; 55.3; 55.4; 63.4; 72.2; 86.7; 87.7 (2 C); 112.4; 113.3 (2 C); 113.4 (2 C); 120.7; 127.4; 127.9 (2 C); 128.0 (2 C); 129.9 (4 C); 134.9; 135.1; 136.7; 143.9; 147.6; 149.7; 158.7 (2 C); 159.9. HR-ESI-MS: 664.2019 ($[M+Na]^+$, $C_{33}H_{31}N_5NaO_9^+$; calc. 664.2014).

*1-[5-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2′-deoxy-β-*D*-ribofuranosyl-1′-yl]-5-(4″-nitro-1*H*-imidazol-1″-yl)-pyrimidine-2,4-(1*H*,3*H*)-dione* (**8a**). Nucleoside **7b** (110 mg, 0.165 mmol) was co-evaporated with anh. MeCN (2 × 5 ml) and dissolved in anh. MeCN (2 ml). To the obtained stirred soln. at r.t. was added $EtN(i\text{-}Pr)_2$ (170 µl, 9.9 mmol), followed by 2-cyanoethyl *N,N*′-(diisopropyl)phosphoramidochloridite (50 µl, 0.22 mmol). After 60 min, the mixture was diluted with CH_2Cl_2 (20 ml) and washed with 5% aq. $NaHCO_3$ (1 × 10 ml), and the aq. phase was extracted with CH_2Cl_2 (2 × 10 ml). The combined org. phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica-gel CC (0–2.5% MeOH in CH_2Cl_2 (*v*/*v*)) to afford **8a** (116 mg, 83%). White solid. R_f (50% AcOEt in petroleum ether (*v*/*v*)) 0.33. ^{31}P-NMR ($CDCl_3$, H_3PO_4): 149.52; 149.60. HR-ESI-MS: 864.3098 ($[M+Na]^+$, $C_{42}H_{48}N_7NaO_{10}P^+$; calc. 864.3092).

*2-(2′,4′-Dioxo-1*H*,3*H*-pyrimidin-5′-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**3b**). A suspension of **1** (0.127 g, 1.00 mmol), *naphthalene-1,8-dicarboxylic anhydride* (**2b**; 0.200 g, 0.10 mmol), Et_3N (1.00 ml, 7.20 mmol), and DMAP (50.0 mg, 0.41 mmol) was heated under reflux in a mixture of DMSO (5.0 ml) and EtOH (5.0 ml) for 96 h. The mixture was then poured onto ice (25 g), and the precipitate formed was filtered off, washed successively with H_2O and MeOH, and dried in the air to afford **3b** (0.218 g, 71%). R_f (15% MeOH in CH_2Cl_2 (*v*/*v*)) 0.47. ^{1}H-NMR ((D_6)DMSO, Me_4Si): 7.82 (*s*, H–C(6′)); 7.91 (*t*, *J* = 7.7, H–C(6), H–C(7)); 8.53 (*d*, *J* = 7.7, H–C(4), H–C(5), H–C(8), H–C(9)); 11.30 (*s*, NH); 11.59 (*s*, NH). ^{13}C-NMR ((D_6)DMSO, Me_4Si): 109.5; 121.8; 127.4; 127.7; 131.2; 131.4; 134.9; 142.3; 151.0; 160.8; 163.2. EI-MS: 307 (M^+, $C_{16}H_9N_3O_4^+$).

*2-[1′-(3″-*O*-Acetyl-2″-deoxy-5″-*O*-propanoyl-*D*-ribofuranosyl-1″-yl)-2′,4′-dioxo-1*H*,3*H*-pyrimidin-5′-yl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**5b**). A mixture of **3b** (0.614 g, 2.00 mmol), $(NH_4)_2SO_4$ (20 mg, 0.15 mmol), and HMDS (10.0 ml, 47.0 mmol) was heated under reflux with stirring for 14 h. The excess of HMDS was evaporated and the resulting oily residue was dissolved in anh. MeCN (25 ml). Compound **4** [13]; 0.70 g, 2.50 mmol) was added, and the mixture was cooled to −15° whereupon TMSOTf (0.17 ml, 1.00 mmol) was added dropwise. The mixture was stirred at −15° for 1 h and then stirred for 1.5 h, while the temp. was allowed to reach 0°. Then, 5% aq. $NaHCO_3$ (25 ml) was added, and the resulting mixture was extracted with CH_2Cl_2 (3 × 25 ml). The combined org. phase was dried (Na_2SO_4) and evaporated to dryness. The resulting residue was purified by CC (0–5% MeOH in $CHCl_3$ (*v*/*v*)) to yield an anomeric mixture of nucleosides **5b** (α/β 1:1 based on ^{1}H-NMR signals) as a white solid material (0.89 g, 86%). R_f (5% MeOH in $CHCl_3$ (*v*/*v*)) 0.23. ^{1}H-NMR ((D_6)DMSO, Me_4Si): 0.84 (*t*, *J* = 7.5, Me); 1.02 (*t*, *J* = 7.5, Me); 1.97 (*s*, Me); 2.07 (*s*, Me); 2.20–2.42 (*m*, 7 H, CH_2, H_a–C(2″), H_b–C(2″)); 2.73–2.83 (*m*, 1 H_b–C(2″)); 4.10–4.24 (*m*, 5 H, CH_2(5″), H–C(4″)); 4.72 (*t*, *J* = 4.8, 1 H–C(4″)); 5.08 (*d*, *J* = 5.7, 1 H–C(3″)); 5.17–5.22 (*m*, 1 H–C(3″)); 6.16 (*d*, *J* = 6.6, 1 H–C(1″)); 6.24 (*t*, *J* = 7.1, 1 H–C(1″)); 7.90–7.95 (*m*, 4 arom. H); 8.09 (*s*, 1 H–C(6′)); 8.10 (*s*, 1 H–C(6′)); 8.48–8.56 (*m*, 8 arom. H); 11.86 (br. *s*, NH); 11.99 (br. *s*, NH). ^{13}C-NMR ((D_6)DMSO, Me_4Si): 8.6; 8.8; 20.6; 20.7; 26.5; 26.6; 36.5; 37.6; 63.3; 63.5; 73.8; 79.2; 81.5; 84.5; 85.1; 87.8; 109.6; 110.9; 121.7 (2 C); 122.0; 122.1; 127.4 (2 C); 127.4 (2 C); 127.7; 127.7; 130.9; 131.1; 131.2; 131.3; 131.5; 131.5; 134.8; 134.8; 135.1 (2 C); 140.5; 140.8; 149.7; 149.8; 159.7; 160.2; 163.1; 163.1; 163.2; 163.3; 170.0; 170.5; 173.4 (2C). HR-ESI-MS: 544.1332 ($[M+Na]^+$, $C_{26}H_{23}N_3NaO_9^+$; calc. 544.1326).

*2-[1′-(2″-Deoxy-β-*D*-ribofuranosyl-1″-yl)-2′,4′-dioxo-1*H*,3*H*-pyrimidin-5′-yl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**6b**). Nucleoside **5b** (0.80 g, 1.53 mmol) was suspended in anh. MeOH (25 ml), and MeONa (0.5 g, 9.25 mmol) was added under stirring. After stirring for 100 min, H_2O (5 ml) and *Amberlist IRC* 120 (*ca.* 1 g) were added to reach neutral pH. The resulting suspension was decanted, and the resin was washed with MeOH (3 × 10 ml). The combined MeOH phase was evaporated to dryness. Fractional crystallization from MeOH gave pure β-anomer **6b** (0.218 g, 33%). White crystalline material. R_f (10% MeOH in CH_2Cl_2 (*v*/*v*)) 0.20. ^{1}H-NMR ((D_6)DMSO, Me_4Si): 2.03–2.12 (*m*, H_a–C(2″)); 2.21–

2.28 (*m*, H_b–C(2″)); 3.44–3.57 (*m*, CH_2(5″)); 3.80 (*q*, *J* = 3.8, H–C(4″)); 4.19–4.24 (*m*, H–C(3″)); 4.39 (*t*, *J* = 5.3, OH–C(5″)); 5.31 (*d*, *J* = 4.3, OH–C(3″)); 6.23 (*t*, *J* = 6.6, H–C(1″)); 7.92, (*t*, *J* = 7.8, 2 arom. H); 8.26, (*s*, H–C(6′)); 8.51–8.55 (*m*, 4 arom. H); 11.90 (br. *s*, NH). ^{13}C-NMR ((D$_6$)DMSO, Me_4Si): 40.1; 61.2; 70.1; 84.6; 87.6; 110.5; 121.7; 127.4 (2 C); 127.7; 131.3 (2 C); 131.6; 135.0 (2 C); 140.6; 149.9; 159.8; 163.0; 163.1. HR-ESI-MS: 446.0964 ([*M* + Na]$^+$, $C_{21}H_{17}N_3NaO_7^+$; calc. 446.0959).

*2-[1′-{5-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-2″-deoxy-β-*D*-ribofuranosyl-1″-yl}-2′,4′-dioxo-1*H,*3*H*-pyrimidine-5′-yl)-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione* (**7b**). Compound **6b** (60 mg, 0.188 mmol) was co-evaporated with anh. pyridine (2 × 5 ml) and dissolved in anh. pyridine (2 ml). DMTrCl (72.0 mg, 0.283 mmol) was added, and the resulting mixture was stirred at r.t. for 20 h and then evaporated to dryness. The residue was dissolved in AcOEt (20 ml), and the resulting org. phase was successively washed with sat. aq. $NaHCO_3$ (10 ml) and brine (2 × 10 ml). The org. phase was evaporated to dryness, and the residue was purified by CC (0–5% MeOH in CH_2Cl_2 containing 0.5% Et_3N (*v*/*v*/*v*)) to furnish **7b** (87 mg, 86%). White solid. R_f (10% MeOH in CH_2Cl_2 (*v*/*v*)) 0.38. ^{1}H-NMR ($CDCl_3$, Me_4Si): 2.32–2.55 (*m*, CH_2(2″)); 3.26–3.38 (*m*, CH_2(5″)); 3.65 (*s*, MeO); 3.65 (*s*, MeO); 3.99–4.04 (*m*, H–C(4″)); 4.53–4.59 (*m*, H–C(3″)); 5.5 (br. *s*, OH, NH); 6.43 (*t*, *J* = 6.5, H–C(1″)); 6.58–6.69 (*m*, 5 arom. H); 6.92 (*t*, *J* = 7.8, 2 arom. H); 7.07 (*dd*, *J* = 1.1, 8.9, 4 arom. H); 7.14 (*dd*, *J* = 1.1, 8.3, 2 arom. H); 7.61–7.73 (*m*, 2 arom. H); 8.01 (*s*, H–C(6′)); 8.14–8.20 (*m*, 2 arom. H); 8.39 (*dd*, *J* = 1.1, 7.3, 1 arom. H); 8.46 (*dd*, *J* = 1.1, 7.3, 1 arom. H). ^{13}C-NMR ($CDCl_3$; Me_4Si): 41.6; 55.2; 55.2; 63.4; 72.0; 85.4; 86.3; 86.8; 113.2 (4 C); 122.4; 122.4; 126.6; 126.8 (2 C); 127.7 (4 C); 128.7; 129.9 (2 C); 130.0 (2 C); 131.6; 131.7; 131.9; 134.3; 134.4; 135.1; 135.3; 140.4; 144.5; 150.0; 158.4; 158.5; 159.5; 163.3; 163.6. HR-ESI-MS: 748.2271 ([*M* + Na]$^+$, $C_{42}H_{35}N_3NaO_9^+$; calc. 748.2265).

*2-(1′-{5″-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-3″-*O*-[(2-cyanoethoxy)(diisopropylamino)-phosphino]-2″-deoxy-β-*D*-ribofuranosyl-1″-yl}-2′,4′-dioxo-1*H,*3*H*-pyrimidine-5′-yl)-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione* (**8b**). Nucleoside **7b** (110 g, 0.151 mmol) was co-evaporated with anh. MeCN (2 × 5 ml) and dissolved in anh. MeCN (2 ml) containing 20% of EtN^iPr_2 (*v*/*v*). To this soln. was added 2-cyanoethyl *N*,*N*′-(diisopropyl)phosphoramidochloridite (67 µl, 0.303 mmol), and the resulting mixture was stirred at r.t. for 4 h, whereupon abs. EtOH (1 ml) was added. The resulting mixture was diluted with CH_2Cl_2 (25 ml) and then successively washed with sat. aq. $NaHCO_3$ (10 ml) and brine (10 ml). The combined aq. phase was extracted with CH_2Cl_2 (20 ml) and evaporated to dryness. The residue was purified by silica-gel CC (0–50% acetone in petroleum ether (*v*/*v*)) to afford **8b** (103 mg, 73%). White solid material. R_f (50% acetone in petroleum ether (*v*/*v*)) 0.4. ^{31}P-NMR ($CDCl_3$; H_3PO_4): 149.71; 149.16. HR-ESI-MS: 948.3344 ([*M* + Na]$^+$, $C_{51}H_{52}N_5NaO_{10}P^+$; calc. 948.3344).

Synthesis of Oligodeoxynucleotides. Oligodeoxynucleotides (ODNs) containing monomers **X** and **Y** (see *Scheme 1* for structures) were synthesized in 0.2-µmol scale on an automated DNA synthesizer using succinoyl linked LCAA-CPG (long-chain alkylamine-controlled pore glass) columns with a pore size of 500 Å. Standard procedures were used for DNA amidites, *i.e.*, Cl_3CCOOH in CH_2Cl_2 as detritylation reagent, 0.25M 4,5-dicyano-1*H*-imidazole (DCI) in MeCN as activator, Ac_2O in THF as cap *A* soln., 1-methyl-1*H*-imidazole in THF as cap *B* soln., and 0.02M I_2 in H_2O/pyridine/THF as oxidizing soln. For the amidites **8a** and **8b**, extended coupling times (0.05M phosphoramidites in MeCN, 15 min) and 1*H*-tetrazole as catalyst were used, resulting in stepwise coupling yields of *ca.* 99%. Removal of nucleobase-protecting groups and cleavage from the solid support for ODNs containing monomer **X** (*i.e.*, **ON2**, **ON3**, and **ON11**) was achieved using standard conditions (32% aq. NH_3 for 12 h at 55°), whereas ONs containing monomer **Y** (*i.e.*, **ON4** and **ON12**) were deprotected using 32% aq. NH_3 at r.t. for 24 h. Unmodified DNA and RNA strands were obtained from commercial suppliers. Purification to at least 80% purity of all modified ODNs was performed by RP-HPLC (using the system described below) on *O(5′)*-DMTr-containing ODNs, followed by detritylation (80% aq. AcOH, 20 min, r.t.) and precipitation (abs. EtOH), followed by washing with abs. EtOH (2 × 300 µl). The system used for purification was a *Waters 600* system equipped with an *Xterra MS C18* (10 µm, 7.8 × 10 mm) pre-column and an *Xterra MS C18* (10 µm, 7.8 × 150 mm) column using the representative gradient protocol depicted in *Table 4*. The composition of all synthesized ODNs was verified by MALDI-MS analysis (*cf. Table 6*) recorded in negative-ion mode on a *PerSeptive Voyager STR* using 3-hydroxypicolinic acid as a matrix, whereas the purity (> 80%) was verified by ion-exchange HPLC using an a *LaChrom L-7000* system equipped with a *Dionex PA100* column (4 × 250 mm) at pH 8 using the protocol shown in *Table 5*.

Table 4. *RP-HPLC Gradient Protocol*[a])

Time [min]	Buffer *A* [vol.-%]	Buffer *B* [vol.-%]
0	100	0
2	100	0
40	30	70
47	0	100
50	0	100
51	100	0
60	100	0

[a]) Buffer *A:* 0.05M TEAA (triethylammonium acetate), pH 7.4; Buffer *B:* 75% MeCN in H_2O (*v*/*v*); flow rate 2.5 ml/min.

Table 5. *Ion-Exchange HPLC Gradient Protocol*[a])

Time [min]	H_2O [vol.-%]	2M NaCl [vol.-%]	0.25M *Tris*·Cl [vol.-%]
0	88	2	10
2	88	2	10
20	55	35	10
25	10	80	10
30	10	80	10
35	88	2	10
45	88	2	10

[a]) Flow rate 1 ml/min.

Table 6. *MALDI-MS Data of Synthesized ODNs*[a])

ON	Sequence	*m*/*z*	
		Found	Calc.
ON2	5′-GTG A**X**A TGC	2752	2850
ON3	5′-G**X**G A**X**A **X**GC	3050	3044
ON4	5′-GTG A**Y**A TGC	2934	2935
ON11	5′-TGC ACT GTA **X**GT CTG TAC CAT	6482	6484
ON12	5′-TGC ACT GTA **Y**GT CTG TAC CAT	6568	6567

[a]) For structures of monomer **X** and **Y**, see *Scheme 1*.

Thermal Denaturation Studies. Concentrations of ODNs were estimated using the following extinction coefficients (OD/μmol): for DNA: G (12.01), A (15.20), T (8.40), and C (7.05); for RNA: G (13.70), A (15.40), U (10.00), and C (9.00); for modified monomers: **X** (8.44) and **Y** (16.80). The ODNs (1.0 μM each strand) were thoroughly mixed, denatured by heating, and subsequently cooled to the starting temp. of the experiment. Quartz optical cells with a path length of 1.0 cm were used. Thermal denaturation temps. (T_m values/°C) were measured on a *Perkin-Elmer Lambda 35* UV/VIS spectrometer equipped with a *PTP-6 Peltier* temp. programmer and determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} *vs.* temp.) recorded in medium salt buffer (T_m buffer; 100 mM NaCl, 0.1 mM EDTA, and pH 7.0 adjusted with 10 mM NaH_2PO_4/5 mM Na_2HPO_4). A temp. ramp of 1.0°/min was used in all experiments. Reported thermal denaturation temps. are an average of two measurements within ±1.0°.

Protocol for Steady-State Fluorescence Studies. Fluorescence measurements were performed on a *Perkin-Elmer LS 55* luminescence spectrometer equipped with a temp. controller. Quartz optical cells with a path length of 1.0 cm were used. Measurements were conducted using 1.0 μM of strands in T_m buffer. Corrections were made for solvent background, but no attempts were made to eliminate dissolved oxygen in the buffer soln. Solns. were heated to 70–80° prior to measurements, followed by cooling to the temp. of the experiment. Steady-state fluorescence emission spectra (360–600 nm) were obtained at 10° (±0.1°) as an average of five scans at an excitation wavelength of 343 nm using an excitation slit of 10 nm, emission slit of 10 nm, and scan speed of 120 nm/min.

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REFERENCES

[1] D. K. Toubanaki, T. K. Christopoulos, P. C. Ioannou, C. S. Flordellis, *Anal. Chem.* **2009**, *81*, 218; Y. Wang. B. Liu, *Anal. Chem.* **2007**, *79*, 7214; A. Okamoto, K. Tainaka, Y. Ochi, K. Kanatani, I. Saito, *Mol. BioSyst.* **2006**, *2*, 122; D. Gerion, F. Chen, B. Kannan, A. Fu, W. J. Parak, D. J. Chen, A. Majumdar, A. P. Alivisatos, Anal. Chem. 2003, 75, 4766; H. Haga, Y. Yamada, Y. Ohnishi, Y. Nakamura, T. Tanaka, *J. Hum. Genet.* **2002**, *47*, 605; A. Chakravarti, *Nat. Genet. Suppl.* **1999**, *21*, 56; D. G. Wang, J.-B. Fan, C.-J. Siao, A. Berno, P. Young, R. Sapolsky, G. Ghandour, N. Perkins, E. Winchester, J. Spencer, L. Kruglyak, L. Stein, L. Hsie, T. Topaloglou, E. Hubbell, E. Robinson, M. Mittmann, M. S. Morris, N. Shen, D. Kilburn, J. Rioux, C. Nusbaum, S. Rozen, T. J. Hudson, R. Lipshutz, M. Chee, E. S. Lander, *Science* **1998**, *280*, 1077; D. N. Cooper, M. Krawczak, S. E. Antonarakis, in 'The Metabolic and Molecular Bases of Inherited Disease, Vol. 1', Eds. C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, McGraw-Hill, New York, 1995, p. 259.

[2] Y. Suh, J. Vijg, *Mutat. Res. Fundam. Mol. Mech. Mutagen*. **2005**, *573*, 41.

[3] A. Russom, S. Haasl, A. J. Brookes, H. Andersson, G. Stemme, *Anal. Chem.* **2006**, *78*, 2220; A. Okamoto, K. Tanaka, T. Fukuta, I. Saito, *J. Am. Chem. Soc.* **2003**, *125*, 9296; W. M. Howell, M. Jobs, U. Gyllensten, A. J. Brookes, *Nat. Biotechnol*. **1999**, *17*, 87; J. G. Hacia, *Nat. Genet.* **1999**, *21*, 42; D. Whitcombe, J. Theaker, S. P. Guy, T. Brow, S. Little, *Nat. Biotechnol*. **1999**, *17*, 804; S. Tyagi, D. P. Bratu, F. R. Kramer, *Nat. Biotechnol*. **1998**, *16*, 49; D. Whitcombe, J. Brownie, H. L. Gillard, D. McKechnie, J. Theaker, C. R. Newton, S. Little, *Clin. Chem*. **1998**, *44*, 918.

[4] R. T. Ranasinghe, T. Brown, *Chem. Commun.* **2005**, 5487; A. Okamoto, Y. Saito, I. Saito, *Photochem. Photobiol. C: Photochem. Rev.* **2005**, *6*, 108.

[5] D. Honcharenko, C. Zhou, J. Chattopadhyaya, *J. Org. Chem.* **2008**, *73*, 2829; T. S. Kumar, A. S. Madsen, M. E. Østergaard, J. Wengel, P. J. Hrdlicka, *J. Org. Chem.* **2008**, *73*, 7060; K. Kawai, K. Kawabata, S. Tojo, T. Majami, *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2363.

[6] A. Okamoto, K. Tainaka, I. Saito, *J. Am. Chem. Soc*. **2003**, *125*, 4972; E. Mayer, L. Valis, R. Huber, N. Amann, H.-A. Wagenknecht, *Synthesis* **2003**, 2335; M. Manoharan, K. L. Tivel, M. Zhao, K. Nafisi, T. L. Netzel, *J. Phys. Chem*. **1995**, *99*, 17461.

[7] P. J. Hrdlicka, B. R. Babu, M. D. Sørensen, J. Wengel, *J. Am. Chem. Soc*. **2005**, *127*, 13293; T. Bryld, T. Højland, J. Wengel, *Chem. Commun*. **2004**, 1064; G. T. Hwang, Y. J. Seo, S. J. Kim, B. H. Kim, *Tetrahedron Lett.* **2004**, *45*, 3543.

[8] S. G. Srivatsan, H. Weizman, Y. Tor, *Org. Biomol. Chem.* **2008**, *6*, 133; P. Cekan, S. T. Sigurdsson, *Chem. Commun*. **2008**, 3393; K. Seio, M. Mizuta, K. Tasaki, K. Tamaki, A. Ohkubo, M. Sekine, *Bioorg. Med. Chem*. **2008**, *16*, 8287.

[9] D. Jacquemin, E. A. Perpète, G. Scalmani, M. J. Frisch, I. Ciofini, C. Adamo, *Chem. Phys. Lett.* **2007**, *448*, 3.

[10] K. Walczak, E. B. Pedersen, C. Nielsen, *Acta Chem. Scand.* **1998**, *52*, 513.

[11] M. Gütschow, T. Hecker, A. Thiele, S. Hauschildt, K. Eger, *Bioorg. Med. Chem*. **2001**, *9*, 1059.

[12] A. Dikshit, M. Chaddha, R. K. Singh, K. Misra, *Can. J. Chem*. **1988**, *66*, 2989.

[13] A. K. Prasad, M. D. Sørensen, V. S. Parmar, J. Wengel, *Tetrahedron Lett.* **1995**, *36*, 6163.
[14] U. Niedballa, H. Vorbrüggen, *J. Org. Chem.* **1974**, *39*, 3654.
[15] V. V. Demidov, M. D. Frank-Kamenetskii, *Trends Biochem. Sci.* **2004**, *29*, 62.
[16] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512.

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