Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Fluorescent Surrogate of Thymidine in Duplex DNA

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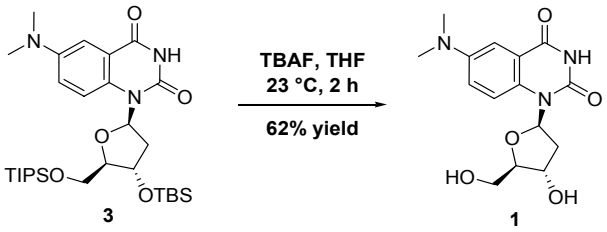
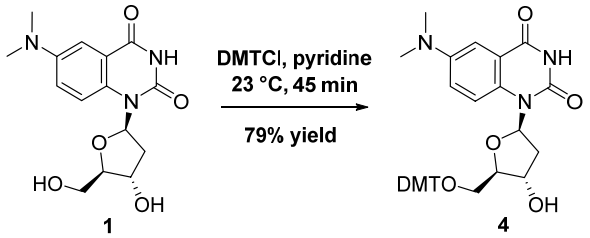
Synthetic Procedures and Characterizations

Starting materials were obtained in the highest commercial grades and used without further purification. Commercially-available 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribose was purified by flash column chromatography on silica gel (Hexane/EtOAc, 6:4) prior to use. All reactions sensitive to moisture and/or air were carried out under an atmosphere of argon and in dry solvents using oven-dried glassware. Commercially-available dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) were purified by a solvent purification system under an atmosphere of argon immediately prior to use. Commercially-available anhydrous dioxane was used directly without further drying. Analytical thin-layer chromatography was performed on pre-coated 250 µm layer thickness silica gel 60 F_{254} plates. Visualization was performed by ultraviolet light and staining with a 15% H_2SO_4 solution in EtOH/H_2O. Flash column chromatography was performed using 40-63 µm silica gel using compressed air. ^{1}H NMR spectra were recorded on 400 and 500 MHz spectrometers; residual solvent peaks were used as internal standards: DMSO (quint, δ^H = 2.50 ppm), $CHCl_3$ (s, δ^H = 7.26 ppm). ^{13}C-NMR spectra were recorded on 400 and 500 MHz spectrometers; δ relative to DMSO (δ 40.5 ppm) or $CHCl_3$ (δ 77.23 ppm). Coupling constants (*J*) are reported in hertz (Hz). The following abbreviations were used to describe the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, dd = doublet-doublet, ddd = doublet-doublet-doublet, dt = doublet-triplet, dq =doublet-quartet, br = broad. Mass spectra were obtained on a quadrupole ion trap instrument equipped with an atmospheric pressure ion (API) source. High-resolution electrospray mass spectra (HR-ESI MS) were recorded on a QTOF-MS instrument. Infrared spectra were recorded on a FT/IR-4100 spectrometer.



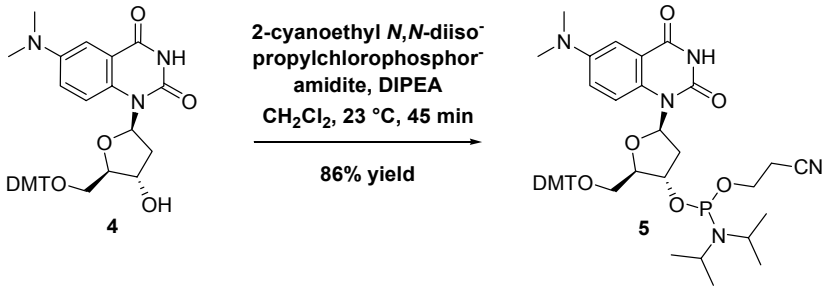
1'-(2'-deoxy-β-D-ribofuranoside)-6-(*N*,*N*-dimethylamino)-quinazoline-2,4-(3*H*)-dione (1). To a stirring solution of the nucleoside **3** (1.50 g, 2.53 mmol) in THF (36 mL), TBAF (2.65 g, 10.14 mmol) pre-dissolved in THF was added. The mixture was stirred at room temperature for 2h and evaporated *in vacuo*. The residue was washed with MeOH (6.0 mL), aqueous HOAc (6.0 mL), and H_2O (6.0 mL) and dried under high vacuum to obtain nucleoside **1** (0.500, 62%) as a yellow solid.

R_f (CH_2Cl_2/MeOH, 94:6): 0.23; **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 11.49 (s, br, 1H), 7.72 (d, J_1 = 9.3 Hz, 1H), 7.19 (d, J_1 = 3.2 Hz, 1H), 7.11 (dd, J_1 = 9.3 Hz, J_2 = 3.2 Hz, 1H), 6.64 (t, J_1 = 7.8 Hz, 1H, $H_{1'}$), 5.23 (d, J_1 = 5.0 Hz, 1H), 4.93 (t, J_1 = 5.0 Hz, 1H), 4.38-4.35 (m, 1H, $H_{3'}$), 3.71-3.61 (m, 3H, $H_{4'}$ and $2H_{5'}$), 2.92 (s, 6H), 2.63-2.56 (m, 1H, $H_{2'}$), 1.89 (ddd, J_1 = 13.3 Hz, J_2 = 7.3 Hz, J_3 = 3.5 Hz, 1H, $H_{2'}$); **^{13}C NMR** (100 MHz, DMSO-d_6) δ: 161.8, 149.7, 146.9, 129.8, 119.7, 118.2, 117.1, 108.1, 86.3, 83.2, 69.6, 60.9, 40.5, 36.1; **IR** (neat) ν: 3474, 3411, 3197, 3067, 2885, 2812, 1668, 1581, 1515, 1483, 1387, 1387, 1308, 1069, 1051, 1040, 1014, 1005, 810, 769, 626, 540, 522 cm^{-1}; **UV-Vis** (H_2O): λ_{max} = 357 nm and ε = 2900 M^{-1} cm^{-1}; **HR-ESI MS** (*m*/*z*): $[M + Na]^+$ calcd for $C_{15}H_{19}N_3O_5Na$ 344.12169, found 344.12193.



1'-[5'-*O*-(4,4-dimethoxytrityl)-2'-deoxy-β-D-ribofuranoside]-6-(*N*,*N*-dimethylamino)-quinazoline-2,4-(3*H*)-dione (3). Nucleoside **1** (0.150 g, 0.467 mmol) was co-evaporated with pyridine (2 x 1.5 mL) and suspended in pyridine (2.0 mL). To the stirring solution a solution of DMT-Cl (0.190 g, 0.560 mmol) pre-dissolved in pyridine was added. A clear red reaction mixture was observed, and it was stirred at room temperature for 45 min. The reaction was then quenched with a sat. solution of aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 and the combined organic layer was dried over $MgSO_4$, filtered, evaporated *in vacuo* and co-evaporated with toluene. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/Et_3N, 99:0.5:0.5 → 98.5:1:0.5) to obtain nucleoside **4** (0.229 g, 79%, containing 1% of Et_3N) as a green-yellow solid.

R_f (CH_2Cl_2/MeOH/Et_3N, 95:5:0.5): 0.39; **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 11.48 (s, br, 1H), 7.81 (d, J_1 = 9.3 Hz, 1H), 7.42 (d, J_1 = 7.2 Hz, 2H), 7.30-7.27 (m, 6H), 7.24-7.19 (m, 2H), 6.87-6.83 (m, 4H), 6.71 (t, J_1 = 7.6 Hz, 1H, $H_{1'}$), 6.48 (dd, J_1 = 9.4 Hz, J_2 = 3.2 Hz, 1H), 5.38 (s, br, 1H), 4.65-4.63 (m, 1H, $H_{3'}$), 3.88-3.86 (m, 1H, $H_{4'}$), 3.70 (s, 6H), 3.39-3.31 (m, 2H, $2H_{5'}$), 2.79 (s, 6H), 2.76-2.71 (m, 1H, $H_{2'}$), 1.98 (ddd, J_1 = 13.4 Hz, J_2 = 7.2 Hz, J_3 = 3.4 Hz, 1H, $H_{2'}$); **^{13}C NMR** (100 MHz, DMSO-d_6) δ: 161.9, 158.11, 158.09, 149.7, 146.2, 144.6, 135.5, 135.4, 129.85, 129.83, 129.79, 127.96, 127.8, 126.8, 119.5, 118.0, 117.2, 113.1, 108.2, 85.6, 84.6, 83.3, 69.6, 62.6, 55.0, 45.6, 40.0, 36.4, 10.7; **IR** (neat) v: 3412, 3183, 3055, 2940, 1682, 1508, 1481, 1316, 1249, 1176, 1076, 1032, 827, 731 cm^{-1}; **HR-ESI MS** (*m*/*z*): $[M + Na]^+$ calcd for $C_{36}H_{37}N_3O_7Na$ 646.25237, found 646.25220.



1'-[3'-*O*-[2-cyanoethoxy-(*N*,*N*-diisopropylamino)-phosphino]-(5'-*O*-(4,4-dimethoxytrityl)-2'-deoxy-β-D-ribofuranoside]-6-(*N*,*N*-dimethylamino)-quinazoline-2,4-(3*H*)-dione (4). To a stirring solution of nucleoside **4** (0.130 g, 0.208 mmol) in CH_2Cl_2 (1.2 mL) at 0 °C, freshly distilled diisopropylethylamine (DIPEA, 91 µL, 0.521 mmol) was added, and the reaction was stirred 5 min at 0 °C. To this solution 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (93 µL, 0.416 mmol) was added, and the reaction stirred at room temperature for 45 min. The reaction was quenched with a sat. sol. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (Hexane/EtOAc/Et_3N, 60:40:0.5 → 55:45:0.5) to obtain phosphoramidite **5** (0.147 g, 86%, diastereomeric mixture) as a yellow foam.

R_f (Hexane/EtOAc/Et_3N, 40:60:0.5): 0.32 and 0.42; **^{1}H NMR** (500 MHz, DMSO-d_6) δ: 11.48 (s, br, 2H), 7.81-7.78 (m, 2H), 7.42-7.38 (m, 4H), 7.29-7.20 (m, 16H), 6.85-6.79 (m, 8H), 6.71-6.67 (m, 2H, $H_{1'}$), 6.57-6.53 (m, 2H), 4.92-4.86 (m, 2H, $H_{3'}$), 4.05-4.00 (m, 2H, $H_{4'}$), 3.76-3.71 (m, 2H), 3.70 (s, 12H), 3.59-3.47 (m, 4H), 3.37-3.35 (m, 2H, $H_{5'}$), 2.89-2.84 (m, 2H, $H_{2'}$), 2.80 (s, 12H), 2.74 (t, J_1 = 5.9 Hz, 2H), 2.62 (t, J_1 = 5.9 Hz, 2H), 2.26-2.15 (m, 2H, $H_{2'}$), 1.34-1.25 (m, 4H), 1.12 (d, J_1 = 6.8 Hz, 6H), 1.10-1.08 (m, 12H), 0.95 (d, J_1 = 6.8 Hz, 6H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ: 170.2, 166.9, 161.85, 161.84, 158.14, 158.13, 158.11, 149.60, 149.57, 146.2, 144.45, 144.41, 135.4, 135.3, 135.2, 135.1, 131.7, 131.5, 129.96, 129.95, 129.81, 129.79, 128.6, 127.94, 127.91, 127.75, 127.74, 126.78, 126.77, 119.6, 119.5, 118.9, 118.7, 117.67, 117.65, 117.2, 117.1, 113.07, 113.06, 108.2, 85.72, 85.71, 83.69, 83.66, 83.50, 83.39, 72.4, 72.3, 71.6, 71.5, 67.3, 62.3, 62.1, 59.7, 58.45, 58.38, 58.31, 58.23, 54.95, 54.94, 54.93, 54.86, 42.59, 42.58, 42.49, 42.47, 40.0, 38.1, 35.8, 35.5, 29.8, 28.3, 24.3, 24.23, 24.21, 24.18, 24.16, 24.10, 23.2, 22.4, 20.7, 19.8, 19.78, 19.77, 19,71, 14.0, 13.8, 10.8; **^{31}P NMR** (202 MHz, DMSO-d_6) δ: 147.8, 147.3; **IR** (neat) ν: 3178, 3069, 2964, 2930, 2873, 1698, 1684, 1509, 1379, 1319, 1251, 1178, 1073, 1031, 977, 829 cm^{-1}; **HR-ESI MS** (*m*/*z*): $[M + Na]^+$ calcd for $C_{45}H_{54}N_5O_8PNa$ 846.36022, found 846.36021.

Photophysical Properties of ^{DMA}T

For all photophysical experiments, the fluorescent nucleoside ^{DMA}**T** (**1**) was prepared as 20 mM DMSO stock solution and stored at -20 °C. All measurements were collected using a Molecular Devices Spectra spectrophotometer in a quartz cuvette (1 cm path length) and were performed in duplicate or more at a final nucleoside concentration of 40 µM. Measurements were performed with spectrophotometric-grade dioxane and deionized H_2O. For all values reported, final DMSO concentrations after dilution were always less than 0.2%. E_T^{30} values for dioxane / water mixtures were determined by dissolving a small amount of Reichardt's dye in each solution and measuring the most red-shifted absorption maximum (Table S1).[1]

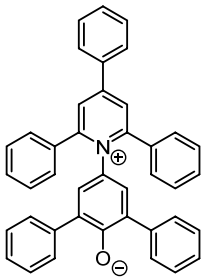
Reichardt's dye	Solvent	λ_{abs} [a]	λ_{abs} [b]	E_T^{30} [c]	E_T^{30} [d]	E_T^{30} [e]
	Dioxane	788	782	36.28	36.56	**36.42**
	0.90 / 0.10	615	642	46.49	44.53	**45.51**
	0.70 / 0.30	562	562	50.87	50.87	**50.87**
	0.30 / 0.70	500	500	57.18	57.18	**57.18**
	Water	453	453	63.11	63.11	**63.11**

Table S1. Absorbance maxima of Reichardt's dye in various mixtures of dioxane / water. The value in pure water was taken from a previous study.[1] λ_{abs} are reported at the most red-shifted absorbance wavelengths (in nm) from two trials [a] and [b]. E_T^{30} are reported in kcal mol^{-1} and calculated from [c] first trial and [d] the second trial. [e] The final E_T^{30} value is given as an average of the two independent measurements.

Molar extinction coefficient measurements were conducted in deionized H_2O using Beer's Law plots of concentration versus absorbance. Quantum yield measurements were performed in triplicate at the most red-shifted absorbance wavelength of each nucleoside using optical densities of 0.1 ± 0.05. Quinine hemisulfate (ϕ = 0.546 at λ_{ex} = 370 nm) in 0.5 M H_2SO_4 (n = 1.432) was used as the fluorescent standard for the relative quantum yields (ϕ) of compound **1**.[2] 2-Aminopyridine (ϕ = 0.60 at λ_{ex} = 285 nm) in 0.5 M H_2SO_4 (n = 1.432) was used as cross-reference standard with an apparent ϕ = 0.59, in good agreement with a previously-reported value.[3] Quantum yields were calculated according to the following equation:

$$\phi = \phi_R \frac{F}{F_R} \frac{A_R}{A} \frac{n^2}{n_R^2}$$

where ϕ_R is the quantum yield of the reference, F and F_R are the integrated emission intensities of the probe and the reference, respectively. A and A_R are the optical densities of the probe and the reference, respectively. n and n_R are the refractive indexes of the solvent in which the measurement was performed for the probe and the reference, respectively. ^{DMA}**T** (ϕ = 0.03 at pH = 7.35 with λ_{ex} = 370 nm) in H_2O was used as the fluorescent standard for the relative quantum yields (ϕ) of ^{DMA}**T** in DNA.

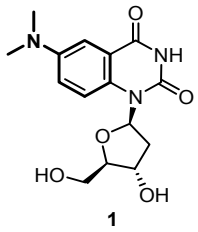
Structure	Solvent	λ_{abs}[a]	λ_{em}[b]	Stokes[c]	ε_{260}[d]	ε_{abs}[d]	ϕ[e]
 **1**	Dioxane	372	440	4.2	14.1	4.0	0.60
	0.90 / 0.10	373	465	5.3	13.5	3.7	0.47
	0.70 / 0.30	374	485	6.2	14.3	3.6	0.30
	0.30 / 0.70	368	510	7.4	16.3	3.1	0.09
	Water	357	522	8.9	15.0	2.9	0.03
	Acetonitrile	375	458	4.8	13.7	3.8	0.32

Table S2. Photophysical data of ^{DMA}**T** (**1**) in various mixtures of dioxane / water. [a] λ_{abs} are reported at the most red-shifted absorbance wavelength in nm. [b] λ_{em} in nm. [c] Stokes shifts are reported in 10^3 cm^{-1}. [d] Extinction coefficients (ε) are reported in 10^3 M^{-1} cm^{-1} and are given at λ_{abs} = 260 nm and at the most red-shifted absorbance. [e] Quinine hemisulfate (ϕ = 0.55 at λ_{ex} = 370 nm) in 0.5 M H_2SO_4 was used as the fluorescent standard for the relative quantum yields (ϕ)[2]. Errors associated are ±10% of the given values.

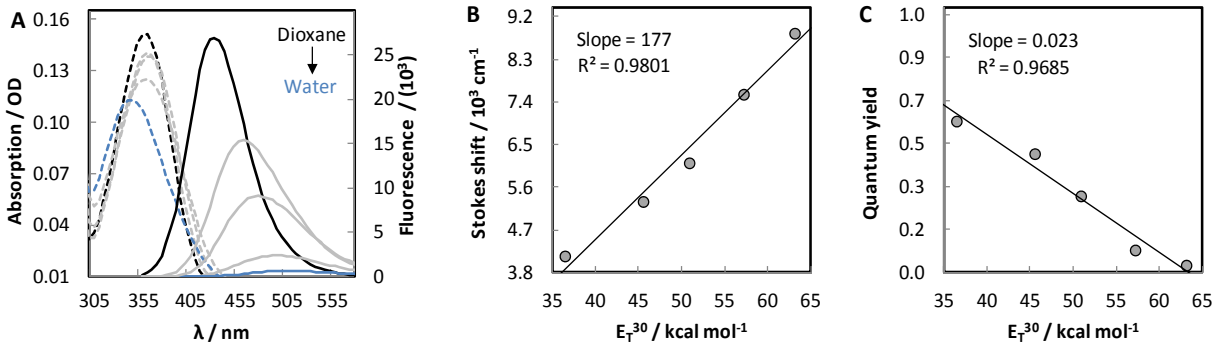


Figure S1. (A) Absorbance (---) and emission (—) spectra (λ_{ex} = 370 nm) of ^{DMA}**T** (**1**) at 40 µM in dioxane (black), water (blue), and various mixtures (grey). (B) Linear relationship between the Stokes shift and the E_T^{30}. (C) Linear relationship between the quantum yield and the E_T^{30}.

pK_a of ^{DMA}T

The absorbance and emission spectra of ^{DMA}**T** were recorded in a buffer containing 0.2 M Na_2HPO_4, 0.1 M citric acid and 0.1 M NaCl in different ratios to give different pH values (from pH = 7.55 to 11.50). The absorbance intensity (λ_{abs} = 345 nm) and fluorescence intensity (λ_{em} = 470 nm with λ_{ex} = 360 nm at the isobestic point) were plotted against the pH values.

Oligonucleotide Synthesis and Purification

The wild-type DNA sequences were obtained from Sigma-Aldrich as HPLC purified products (**Tables S3, S4 and S5**). ^{DMA}**T**-modified oligonucleotides were synthesized on a 1 µmol scale using a DNA synthesizer according to the standard Trityl-off procedure, except that 5% dichloroacetic acid in CH_2Cl_2 was used for Trityl deprotection. Two coupling reactions were performed for the site-specific introduction of the modified nucleoside into oligonucleotides. The phosphoramidite **5** (0.160 g, 0.185 mmol) was dissolved in dry acetonitrile (1.6 mL) and loaded onto the DNA synthesizer. The synthesis of the oligonucleotide was monitored using DMT deprotection. Upon completion of the sequences, the oligonucleotides were cleaved from the solid support and deprotected by treatment with 1.2 mL of 33% aqueous ammonium hydroxide at 55 °C for 12 hours in a 1.5 mL screw-top cap tube. The resulting products were lyophilized, and HPLC-purified using a semi-prep C-18 reverse-phase column (YMCbasic B-22-10P 150 x 10 mm) and a *Varian Pro Star* HPLC system. A gradient of 5% to 10% acetonitrile was applied over 50 minutes at 3 mL/min in 0.1 M triethylammonium acetate (TEAA, pH 7.0). Peaks were collected, lyophilized to dryness and HPLC-analyzed with an analytical column (XBridge Phenyl 3.5 µm 150 x 4.16 mm) using a gradient of 5% to 18% acetonitrile over 40 minutes at 0.5 mL/min in TEAA (**Figure S2**). The purified DNA samples were analyzed using HR-ESI mass spectroscopy (**Table S6**).

Oligonucleotide stock solutions were prepared at 100 µM in pure water and quantified using their calculated molar extinction coefficients (ε) at 260 nm. The extinction coefficients of the non-modified DNA were calculated using the nearest-neighbor model at 260 nm. The extinction coefficients of the ^{DMA}**T**-modified oligonucleotides were calculated using the base composition method which sums the coefficients of the isolated nucleosides which is then multiplied by a factor of 0.9 to account for the base stacking interactions in the oligonucleotide. The following value was used for all the ^{DMA}**T**-modified oligonucleotides: ε(**Telo**^{DMA}**T**) = 193 410 cm^{-1} M^{-1}.

Oligonucleotide Sequences and Masses

Name	Duplex Sequences
T2	5′-C**T**C TAA CCC TAA CCC TAA CCC-3′
T·A	3′-G**A**G ATT GGG ATT GGG ATT GGG-5′
T·T	3′-G**T**G ATT GGG ATT GGG ATT GGG-5′
C2	5′-C**C**C TAA CCC TAA CCC TAA CCC-3′
C·A	3′-G**A**G ATT GGG ATT GGG ATT GGG-5′
C·T	3′-G**T**G ATT GGG ATT GGG ATT GGG-5′
A2	5′-C**A**C TAA CCC TAA CCC TAA CCC-3′
A·A	3′-G**A**G ATT GGG ATT GGG ATT GGG-5′
X2	5′-C**X**C TAA CCC TAA CCC TAA CCC-3′
X·A	3′-G**A**G ATT GGG ATT GGG ATT GGG-5′
X·T	3′-G**T**G ATT GGG ATT GGG ATT GGG-5′

Name	Duplex Sequences
T14	5′-CCC TAA CCC TAA C**T**C TAA CCC-3′
T·A	3′-GGG ATT GGG ATT G**A**G ATT GGG-5′
T·T	3′-GGG ATT GGG ATT G**T**G ATT GGG-5′
C14	5′-CCC TAA CCC TAA C**C**C TAA CCC-3′
C·A	3′-GGG ATT GGG ATT G**A**G ATT GGG-5′
C·T	3′-GGG ATT GGG ATT G**T**G ATT GGG-5′
A14	5′-CCC TAA CCC TAA C**A**C TAA CCC-3′
A·A	3′-GGG ATT GGG ATT G**A**G ATT GGG-5′
X14	5′-CCC TAA CCC TAA C**X**C TAA CCC-3′
X·A	3′-GGG ATT GGG ATT G**A**G ATT GGG-5′
X·T	3′-GGG ATT GGG ATT G**T**G ATT GGG-5′

T13	5′-CCC TAA CCC TAA **T**CC TAA CCC-3′
T·A	3′-GGG ATT GGG ATT **A**GG ATT GGG-5′
T·T	3′-GGG ATT GGG ATT **T**GG ATT GGG-5′
C13	5′-CCC TAA CCC TAA CCC TAA CCC-3′
C·A	3′-GGG ATT GGG ATT **A**GG ATT GGG-5′
C·T	3′-GGG ATT GGG ATT **T**GG ATT GGG-5′
A13	5′-CCC TAA CCC TAA **A**CC TAA CCC-3′
A·A	3′-GGG ATT GGG ATT **A**GG ATT GGG-5′
X13	5′-CCC TAA CCC TAA **X**CC TAA CCC-3′
X·A	3′-GGG ATT GGG ATT **A**GG ATT GGG-5′
X·T	3′-GGG ATT GGG ATT **T**GG ATT GGG-5′
X·G	3′-GGG ATT GGG ATT **G**GG ATT GGG-5′
X·C	3′-GGG ATT GGG ATT **C**GG ATT GGG-5′

T15	5′-CCC TAA CCC TAA CC**T** TAA CCC-3′
T·A	3′-GGG ATT GGG ATT GG**A** ATT GGG-5′
T·T	3′-GGG ATT GGG ATT GG**T** ATT GGG-5′
C15	5′-CCC TAA CCC TAA CC**C** TAA CCC-3′
C·A	3′-GGG ATT GGG ATT GG**A** ATT GGG-5′
C·T	3′-GGG ATT GGG ATT GG**T** ATT GGG-5′
A15	5′-CCC TAA CCC TAA CC**A** TAA CCC-3′
A·A	3′-GGG ATT GGG ATT GG**A** ATT GGG-5′
X15	5′-CCC TAA CCC TAA CC**X** TAA CCC-3′
X·A	3′-GGG ATT GGG ATT GG**A** ATT GGG-5′
X·T	3′-GGG ATT GGG ATT GG**T** ATT GGG-5′
X·G	3′-GGG ATT GGG ATT GG**G** ATT GGG-5′
X·C	3′-GGG ATT GGG ATT GG**C** ATT GGG-5′

Table S3. Names and sequences of DNAs (X = ^{DMA}**T**).

A	Name	Sequence (5'-3')	Molecular Formula	Calculated 10x charged *m/z*	Measured 10x charged *m/z*
	X2	CXC TAA CCC TAA CCC TAA CCC	$C_{204}H_{252}N_{72}O_{122}P_{20}$	628.10535	628.10560
	X13	CCC TAA CCC TAA XCC TAA CCC	$C_{204}H_{252}N_{72}O_{122}P_{20}$	628.10535	628.10894
	X14	CCC TAA CCC TAA CXC TAA CCC	$C_{204}H_{252}N_{72}O_{122}P_{20}$	628.10535	628.10680
	X15	CCC TAA CCC TAA CCX TAA CCC	$C_{204}H_{252}N_{72}O_{122}P_{20}$	628.10535	628.10842

Table S4. Calculated and measured high resolution HR-ESI MS for the ^{DMA}**T**-containing DNA (**X** = ^{DMA}**T**). Monoisotopic masses obtained for the 10 times negatively charged DNAs. MS values are reported in *m*/*z*.

HPLC Analyses of Purified Oligonucleotides

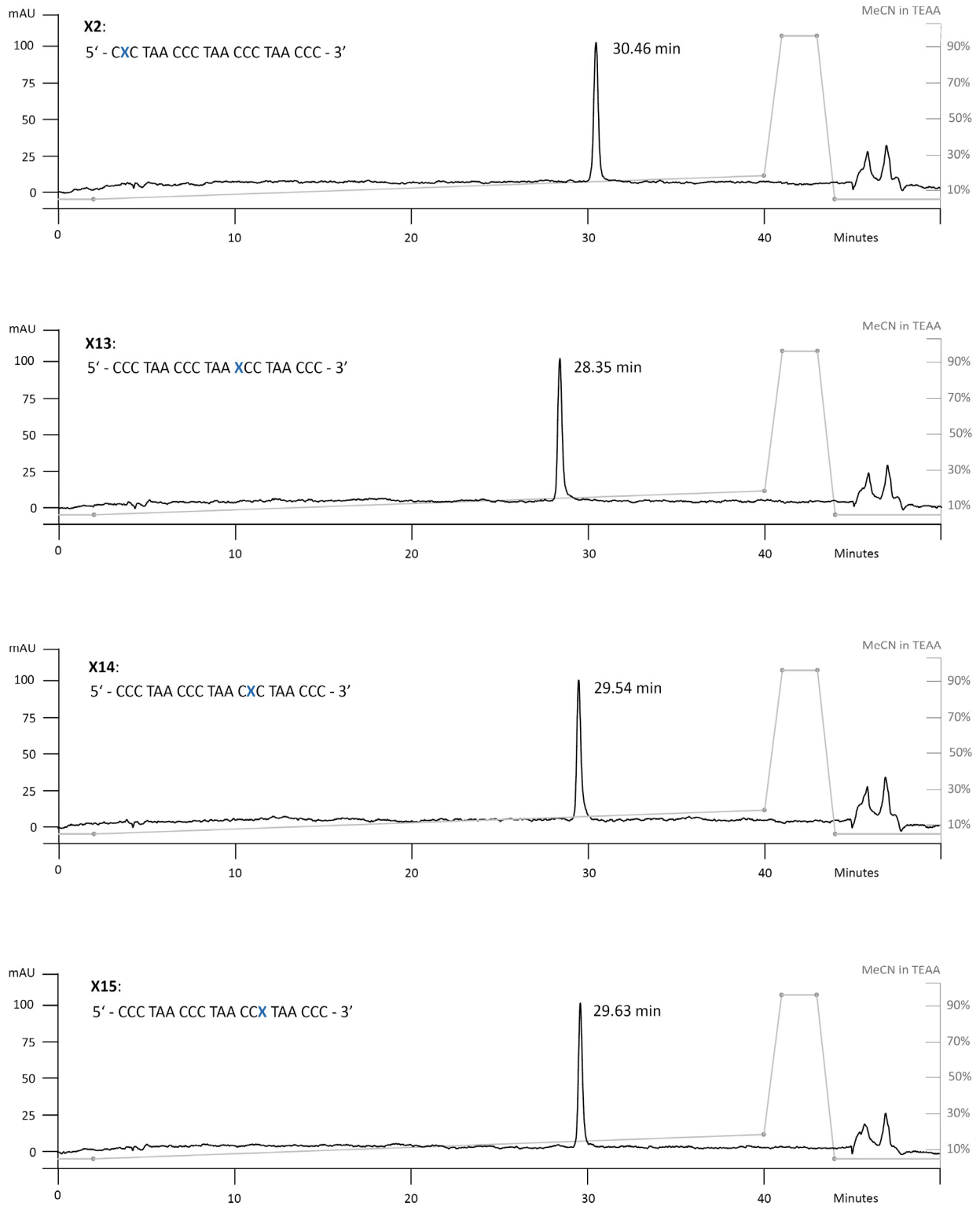


Figure S2. HPLC chromatograms of purified oligonucleotides **X2**, **X13**, **X14** and **X15** analyzed with a gradient of 5% to 18% acetonitrile over 40 minutes at 0.5 mL/min in TEAA buffer (**X** = ^{DMA}**T**).

DNA Folding and Buffer Conditions

Oligonucleotide stock solutions were prepared in water at a concentration of 100 µM and then diluted into the desired buffer at the indicated final concentration. DNA studies were performed in a buffer containing 0.2 M Na_2HPO_4, 0.1 M Citric acid and 0.1 M NaCl or 0.1M $NaNO_3$. Duplex DNA studies were performed at pH = 7.35 in the presence of 1.1 equivalents of the complementary strand. Before measurements, oligonucleotides were heated at 90 °C for 5 minutes, and slowly cooled to room temperature overnight.

Circular Dichroism (CD) Studies

CD melting experiments were carried out using a 0.1 cm path length thermo-controlled CD quartz cell. Spectra were collected at 25 °C between 220 and 340 nm with 0.1 nm steps, 2 nm band width, and a scanning rate of 50 nm/min. Three scans were measured and averaged for each reported spectrum. A concentration of 5 µM of pre-folded DNA was used for all CD experiments.

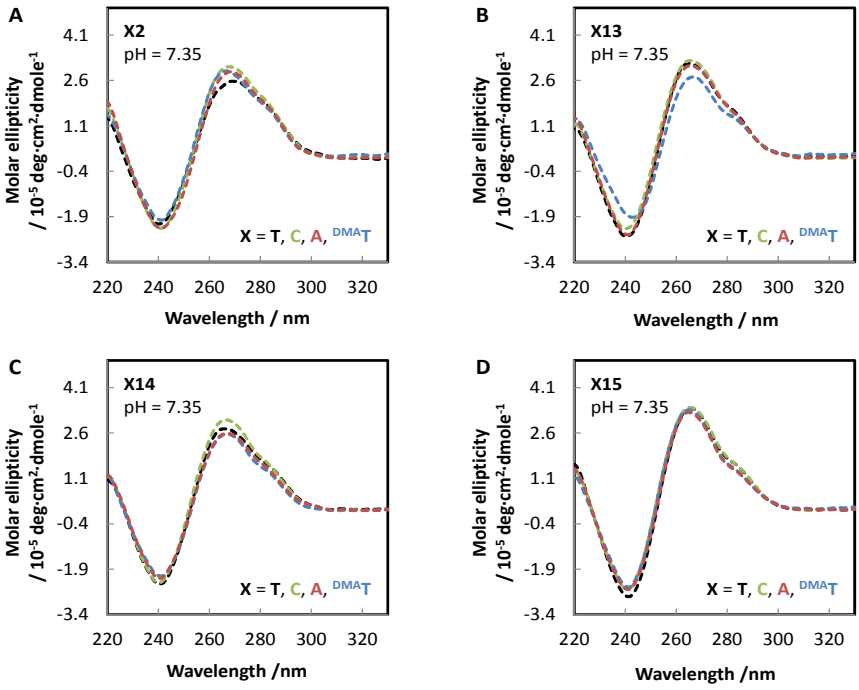


Figure S3. CD spectra of duplexes at 25 °C for: (A) **X2**, (B) **X13**, (C) **X14** and (D) **X15** with **X** = **T**, **C**, **A** and ^{DMA}**T** at pH = 7. All samples contained 5 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM NaCl).

Thermal Denaturation Studies

CD melting experiments were carried out using a 0.1 cm path length thermo-controlled CD quartz cell. A 5 µM solution of pre-folded DNA was equilibrated for 15 minutes at 5 °C and slowly ramped to 95 °C at a rate of 10 °C/h. The molar ellipticity of each sample was monitored at λ = 262 nm. The melting temperatures were determined by plotting the molar ellipticity of the sample versus the temperature using a dose-response non-linear regression. T_m values were calculated from the averaged values of heating and cooling curves for duplex DNA, and the heating curve for *i*-motif DNA. The reproducibility of the measurements is within 0.3 °C.

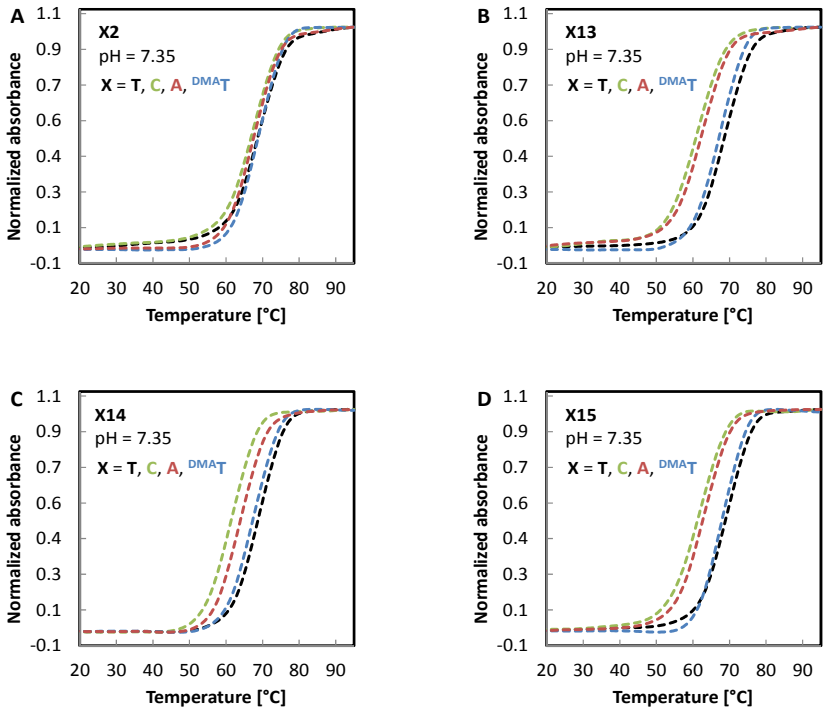


Figure S4. Thermal denaturation spectra of duplexes: (A) **X2**, (B) **X13**, (C) **X14** and (D) **X15** with **X** = **T**, **C**, **A** and ^{DMA}**T** at pH = 7.35. All samples contained 5 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM NaCl).

Fluorescence properties in DNA

All fluorescence spectra were measured using pre-folded DNA in 96-well plates. Quantum yields of ^{DMA}**T** (**1**) in the context of oligonucleotides were determined as described above. The emission spectra of the probe in the context of the DNA were collected using the following wavelengths of excitation: λ_{ex} = 370 nm in single- and double-stranded DNA. The excitation spectra of the probe in the context of the DNA were collected using the following wavelengths of emission: λ_{em} = 515 nm in single- and double-stranded DNA. ^{DMA}**T** (ϕ = 0.03 at pH = 7.35 with λ_{ex} = 370 nm) in H_2O was used as the fluorescent standard for the relative quantum yields (ϕ) of ^{DMA}**T** in DNA. Fluorescence spectra were measured using 4 µM of DNA in a buffer mixture containing 20 mM of Na_2HPO_4 and 10 mM of citric acid supplemented with 10 mM NaCl at pH = 7.35. The relatively high absorbance of DNA samples at 260 nm (< 0.4 AU) caused a significant attenuation of the excitation beam that reduced the fluorescence intensities of the probe. This was evidenced by non-linear relationships between raw fluorescence intensities $F_{raw}(\lambda_{ex})$ and DNA concentrations above 50 nM when samples were photoexcited at 260 nm. $F_{raw}(\lambda_{ex})$ values at each excitation wavelength λ_{ex} were therefore corrected by multiplication with the following correction factor (CF) to obtain corrected $F(\lambda_{ex})$ values,[4] where $A(\lambda_{ex})$ is the raw absorbance of the sample at each wavelength of excitation:

$$\mathrm{CF} = 2.303 * \frac{A_{(\lambda ex)}}{1 - 10^{-A(\lambda ex)}}$$

Anisotropy measurements were performed with pre-folded DNA in 96-well plate and corrected for background and buffer contributions. Fluorescence anisotropy was calculated according to the following equation:

$$r = \frac{\mathrm{I}_{\parallel} - \mathrm{I}_{\perp}}{\mathrm{I}_{\parallel} + 2\mathrm{I}_{\perp}}$$

where $I_{\parallel}$ and $I_{\perp}$ refers to the vertically and horizontally polarized light, respectively.

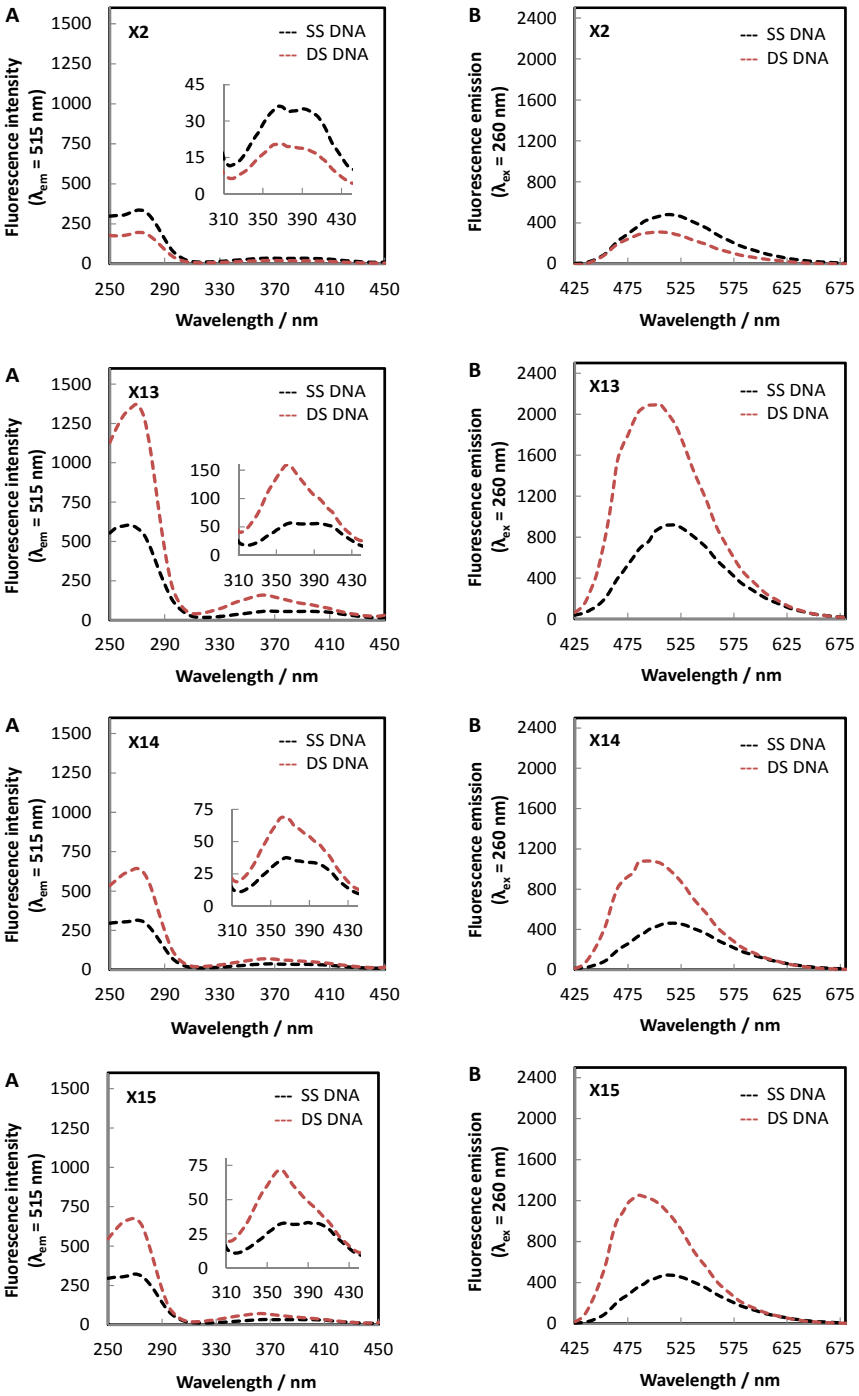


Figure S5. (A) Excitation (λ_{em} = 515 nm) and (B) emission spectra (λ_{ex} = 260 nm) of **X2**, **X13**, **X14** and **X15** prepared as unstructured, "SS" DNA (black) and double-stranded, "DS" DNA (red) with (**X** = ^{DMA}**T**). All samples contained 4 µM of DNA in phosphate citric acid buffer (20 mM of Na_2HPO_4, 10 mM of citric acid and 10 mM NaCl at pH = 7.35).

Metal Binding Studies

Oligonucleotide stock solutions were prepared in water at a concentration of 100 µM and then diluted into a buffer containing 0.2 M Na_2HPO_4, 0.1 M Citric acid and 0.1M $NaNO_3$ (pH = 7.35). One equiv. of ^{DMA}**T** DNA was mixed with 1.1 equiv. of the complementary strand and 0 – 3.0 equiv. of $Hg(ClO_4)_2$, $ZnCl_2$, $CuCl_2$, $MgCl_2$, $FeCl_2$, $CaCl_2$, $CdCl_2$, $PdCl_2$, $NiCl_2$, or $AgNO_3$, heated at 90 °C for 5 minutes and slowly cooled to room temperature overnight.

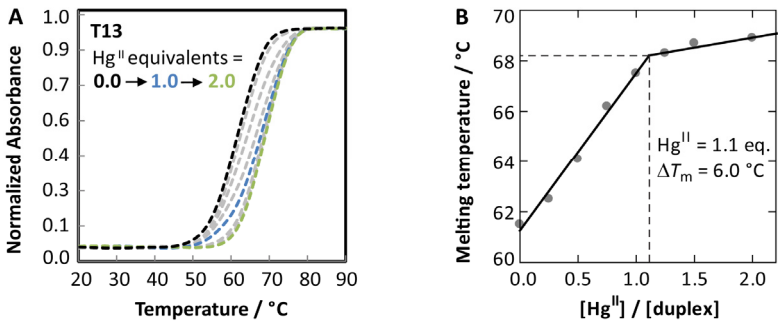


Figure S6. (A) Thermal denaturation curves of duplex **T13** in the absence (black) and in the presence of $Hg(ClO_4)_2$ (1.0 equiv. (blue), 2.0 equiv. (green) and intermediate (grey)). (B) Plot of the melting temperature versus equivalents of Hg^{II} ions. The complementary strand contained a "**T**" mismatched opposite to the "**T13**" base. All samples contained 5 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM $NaNO_3$) at pH = 7.35.

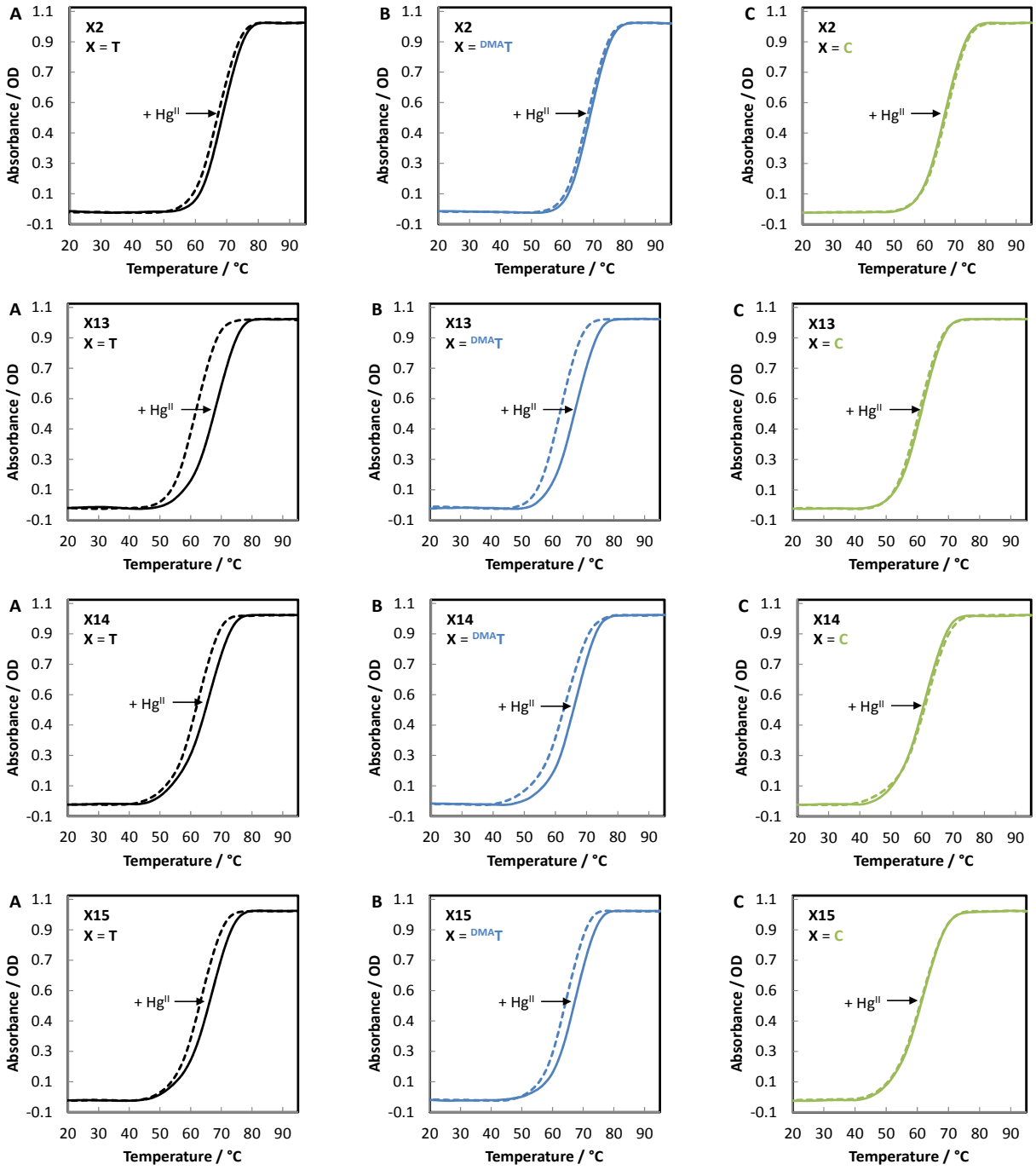


Figure S7. Thermal denaturation curves of duplex DNA containing **X2**, **X13**, **X14** or **X15** with **X** = (A) **T**, (B) ^{DMA}**T** and (C) **C** in the absence (---) and in the presence (—) of $Hg(ClO_4)_2$ (1.0 equiv.). The complementary strand a "**T**" mismatched opposite to the "**X**" base. All samples contained 5 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM $NaNO_3$) at pH = 7.35.

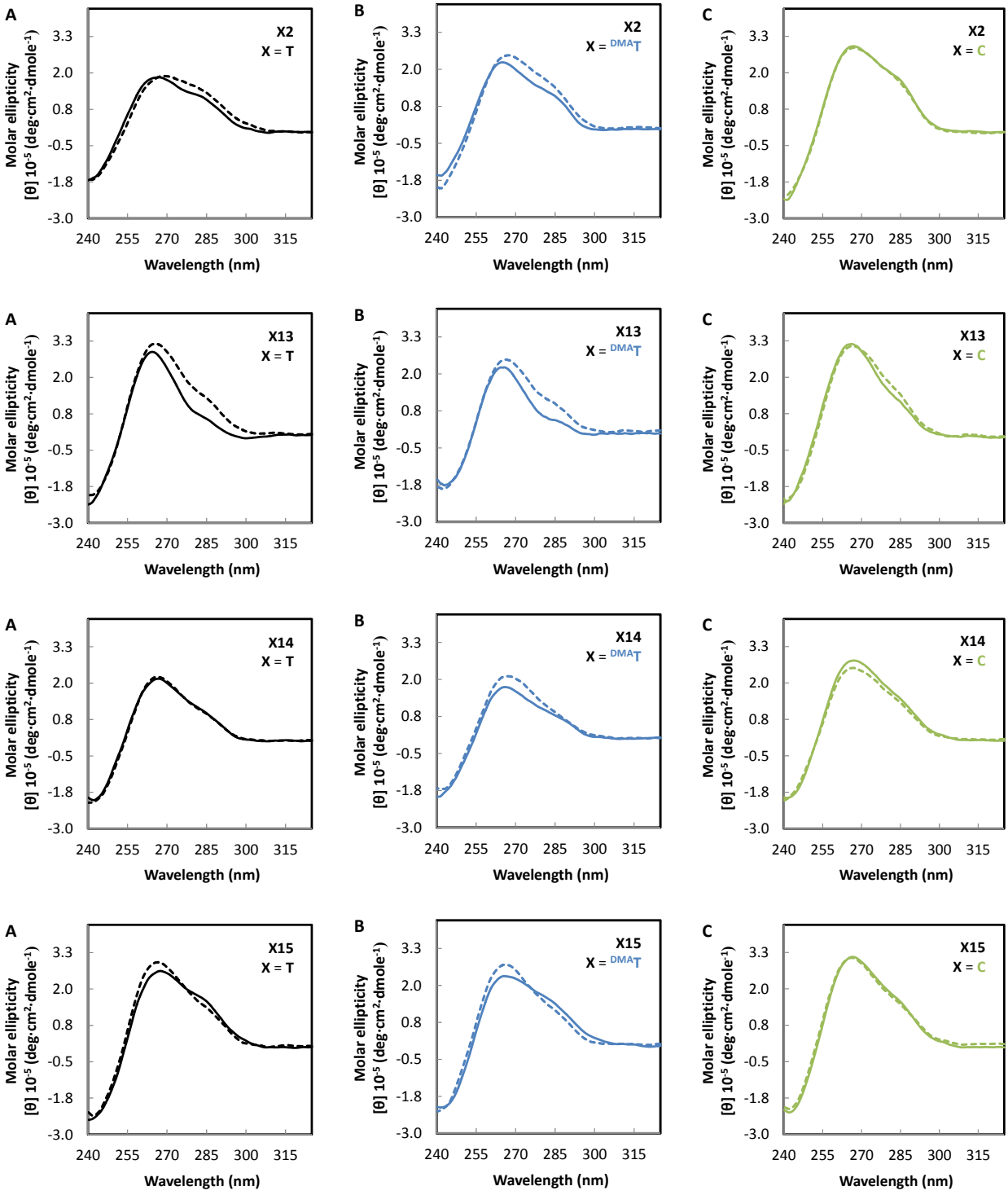


Figure S8. CD spectra of duplex DNA containing **X2**, **X13**, **X14** or **X15** at 5 °C with **X** = (A) **T**, (B) ^{DMA}**T** and (C) **C** in the absence (---) and in the presence (—) of $Hg(ClO_4)_2$ (1.0 equiv.). The complementary strand contained a "**T**" mismatched opposite to the "**X**" base. All samples contained 5 μM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM $NaNO_3$) at pH = 7.35.

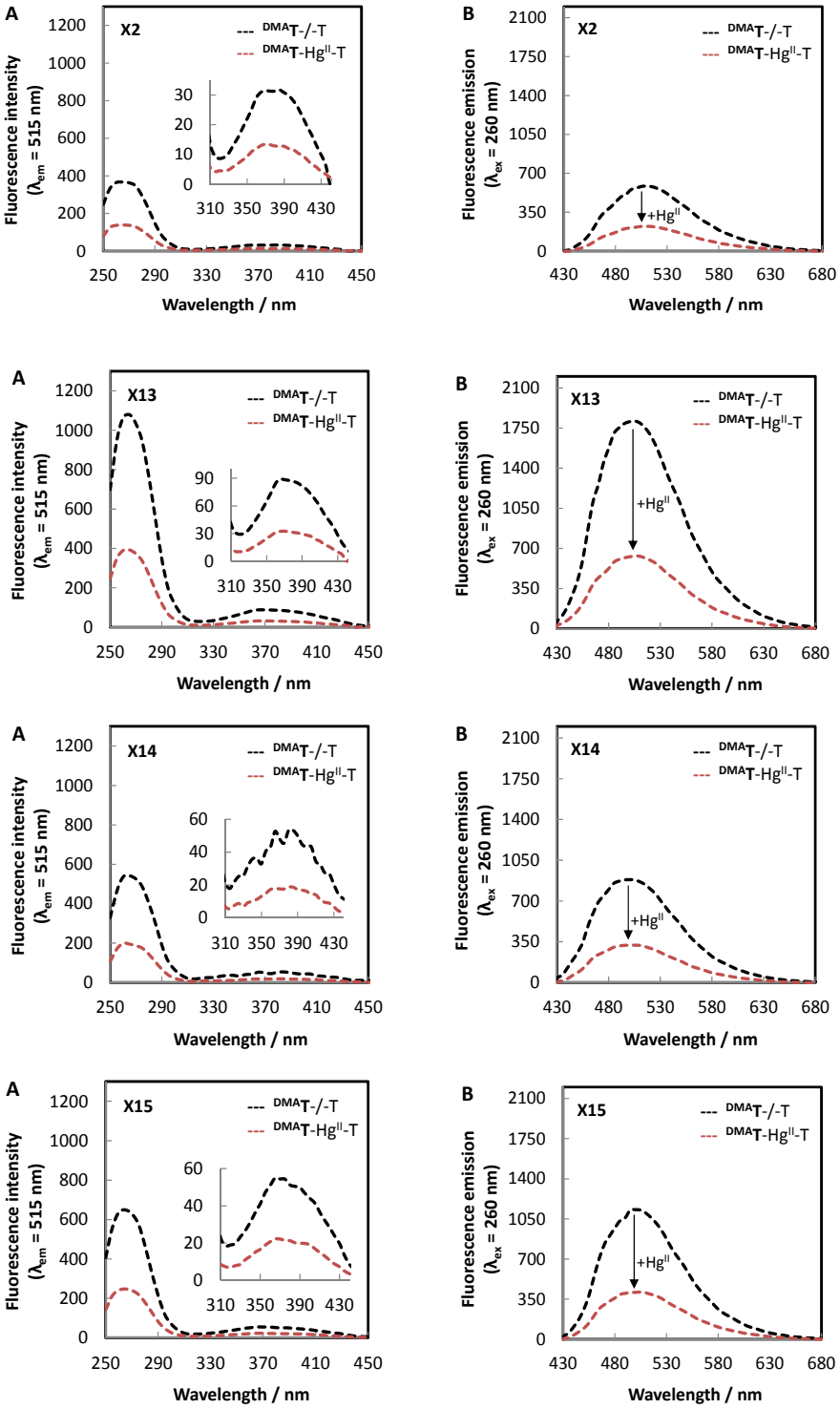


Figure S9. (A) Excitation (λ_{em} = 515 nm) and (B) fluorescence spectra (λ_{ex} = 260 nm) of **X2**, **X13**, **X14** and **X15** as double-stranded DNA with a T-/-T mismatch (black) and a T-Hg^{II}-T match (red) with (**X** = ^{DMA}**T**). All samples contained 4 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM $NaNO_3$) at pH = 7.35 with or without 1.0 eq. of Hg^{II}. The complementary strand contained a T mismatched opposite to the ^{DMA}**T** base.

Base Pairing	Sequence	λ_{abs} [a]	λ_{em} [b]	r [c]	ϕ [d]	$\Delta\phi$ [e]
^{DMA}**T**-T	**X2**	375	510	0.10	0.04	-
	X13	365	505	0.09	0.13	-
	X14	385	501	0.13	0.07	-
	X15	365	500	0.12	0.08	-
^{DMA}**T**-Hg^{II}-T	**X2**	360	512	0.10	0.01	-69%
	X13	360	507	0.09	0.05	-63%
	X14	360	504	0.15	0.03	-61%
	X15	360	505	0.11	0.03	-66%

Table S5. Photophysical data of ^{DMA}**T** in duplex DNA. [a] λ_{abs} are reported at the most red-shifted absorbance maxima in nm, [b] λ_{em} in nm, [c] Fluorescence anisotropy (r) were calculated at pH = 7.35 (λ_{ex} = 375 nm. λ_{em} = 500 nm). [d] ^{DMA}**T** (ϕ = 0.03 at pH = 7.35 with λ_{ex} = 370 nm) was used as the fluorescent standard for calculating the relative quantum yields (ϕ) of ^{DMA}**T** in DNA. [e] Relative deviation of fluorescence quantum yield of ^{DMA}**T**–T mismatched duplex upon addition of Hg^{II} ions. ^{DMA}**T**–Hg^{II}–T base pairs were generated by the addition of 1.0 equiv. of $Hg(ClO_4)_2$ followed by heating to 90 °C for 5 min and cooled to room temperature overnight before reading. Reproducibility is within ± 30% of each reported ϕ value.

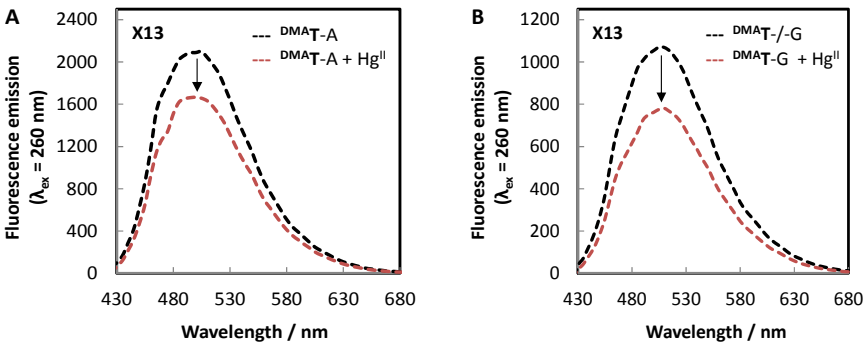


Figure S10. Fluorescence spectra (λ_{ex} = 260 nm) of **X13** as double-stranded DNA containing (A) a ^{DMA}**T**-A base pair (black) and (B) a ^{DMA}**T**-G mismatched pair (black) in the presence of Hg^{II} ions (red) with (**X** = ^{DMA}**T**). All samples contain 4 µM of DNA in phosphate citric acid buffer (200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM $NaNO_3$) at pH = 7.35 with or without 1.0 eq. of Hg^{II}.

Base Pairing	Sequence	λ_{abs} [a]	λ_{em} [b]	r [c]	ϕ [d]	$\Delta\phi$ [e]
^{DMA}**T**-A	**X13**	355	504	0.09	0.20	-
	X15	355	486	0.15	0.11	-
^{DMA}**T**-G	**X13**	365	508	0.10	0.09	-
	X15	370	505	0.12	0.06	-
^{DMA}**T**-A + Hg^{II}	**X13**	365	505	0.10	0.17	ca. -13%
	X15	370	490	0.14	0.09	ca. -18%
^{DMA}**T**-G + Hg^{II}	**X13**	365	510	0.11	0.07	ca. -23%
	X15	370	505	0.13	0.06	ca. -12%

Table S6. Photophysical data of ^{DMA}**T** in duplex DNA. [a] λ_{abs} are reported at the most red-shifted absorbance maxima in nm, [b] λ_{em} in nm, [c] Fluorescence anisotropy (r) were calculated at pH = 7.35 (λ_{ex} = 375 nm. λ_{em} = 500 nm). [d] ^{DMA}**T** (ϕ = 0.03 at pH = 7.35 with λ_{ex} = 370 nm) was used as the fluorescent standard for calculating the relative quantum yields (ϕ) of ^{DMA}**T** in DNA. [e] Relative deviation of fluorescence quantum yield of ^{DMA}**T**–T mismatched duplex upon addition of 1.0 eq. of Hg^{II} ions. ^{DMA}**T**–Hg^{II}–T base pairs were generated by the addition of 1.0 equiv. of $Hg(ClO_4)_2$ followed by heating to 90 °C for 5 min and cooled to room temperature overnight before reading. Reproducibility is within ± 30% of each reported ϕ value.

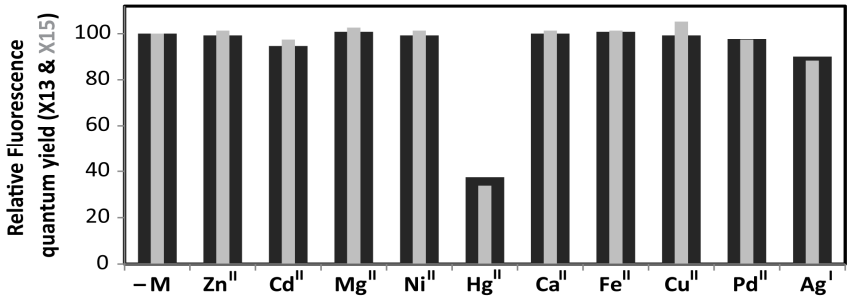
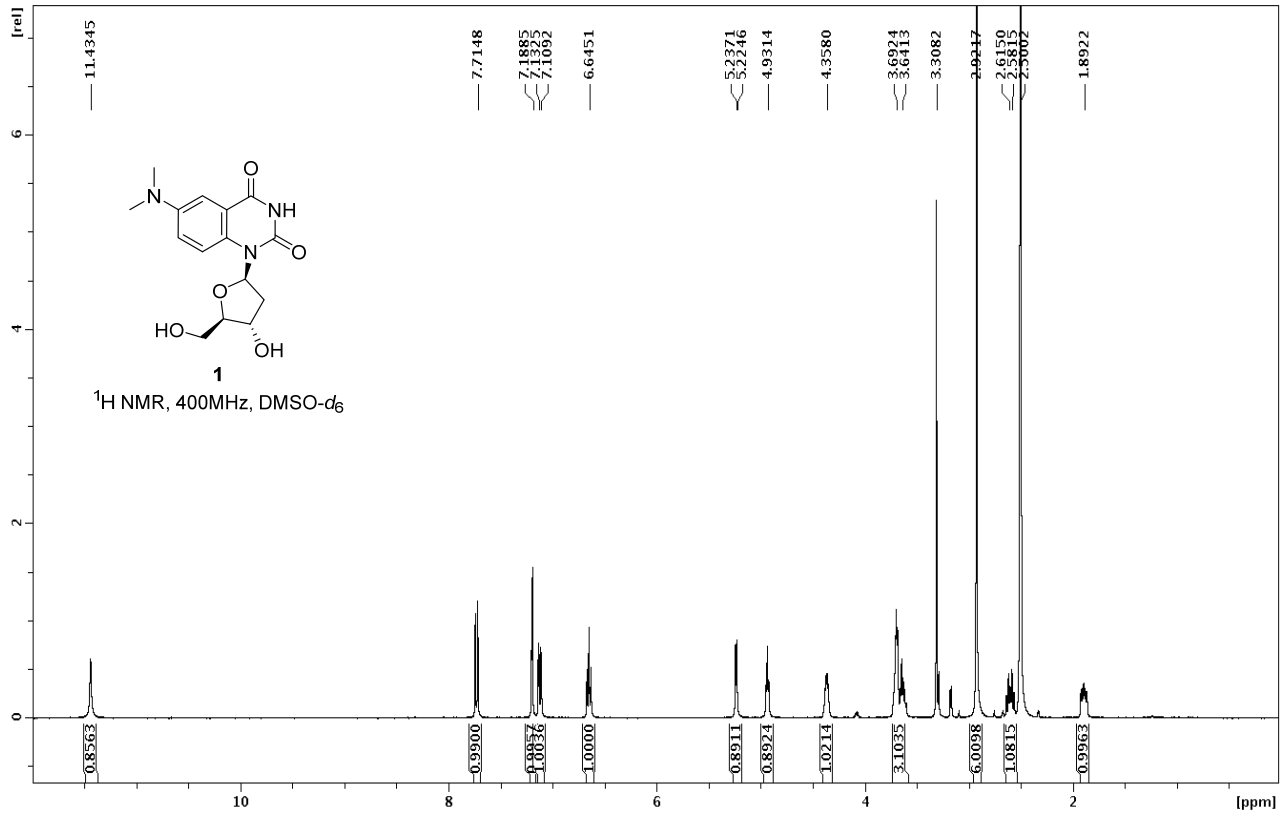


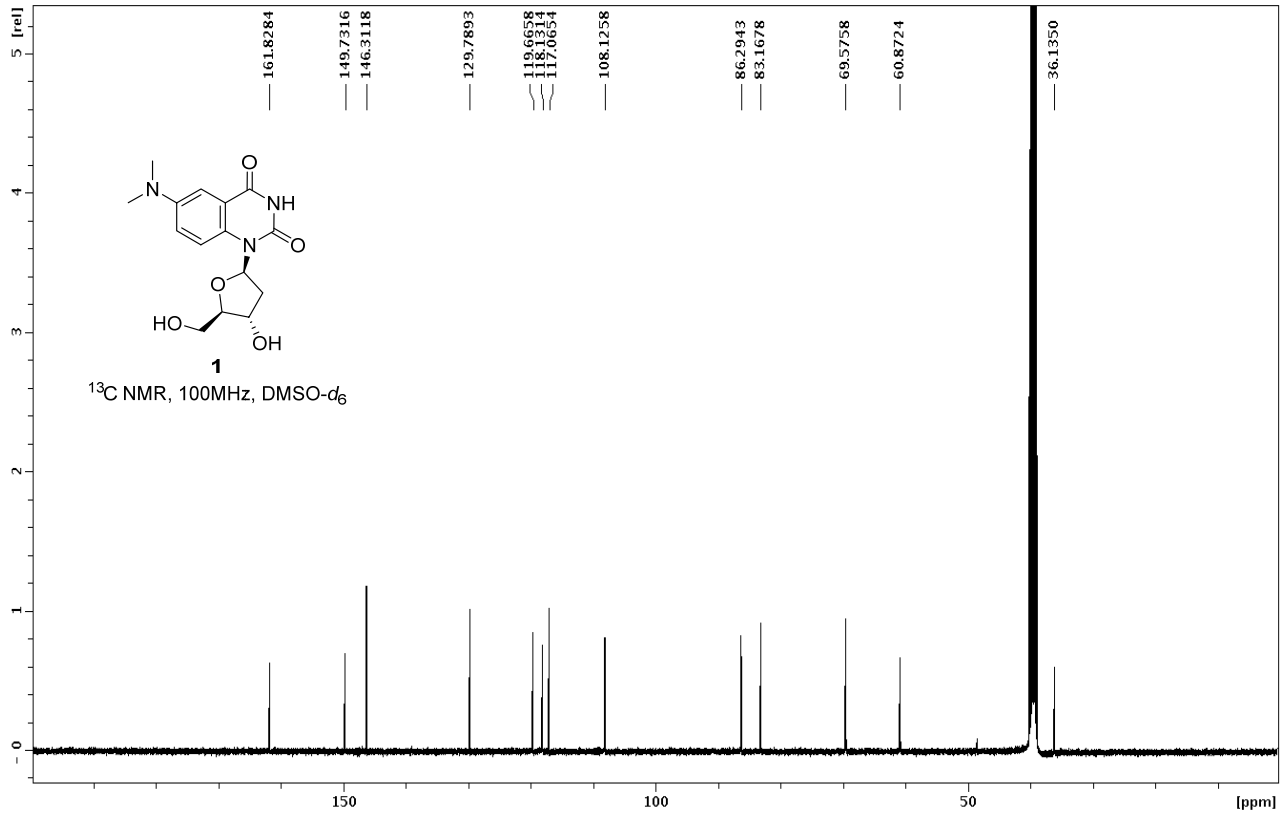
Figure S11. Relative quantum yield of ^{DMA}**T**-**T** mismatched duplex DNA **X13** (black) or **X15** (grey) upon addition of metal ions (1.0 equiv.): $Hg(ClO_4)_2$, $ZnCl_2$, $CuCl_2$, $MgCl_2$, $FeCl_2$, $CaCl_2$, $CdCl_2$, $PdCl_2$, $NiCl_2$, or $AgNO_3$. All samples contained 4 µM of DNA in a buffer containing 200 mM of Na_2HPO_4, 100 mM of citric acid and 100 mM NaCl or $NaNO_3$ (pH = 7.35).

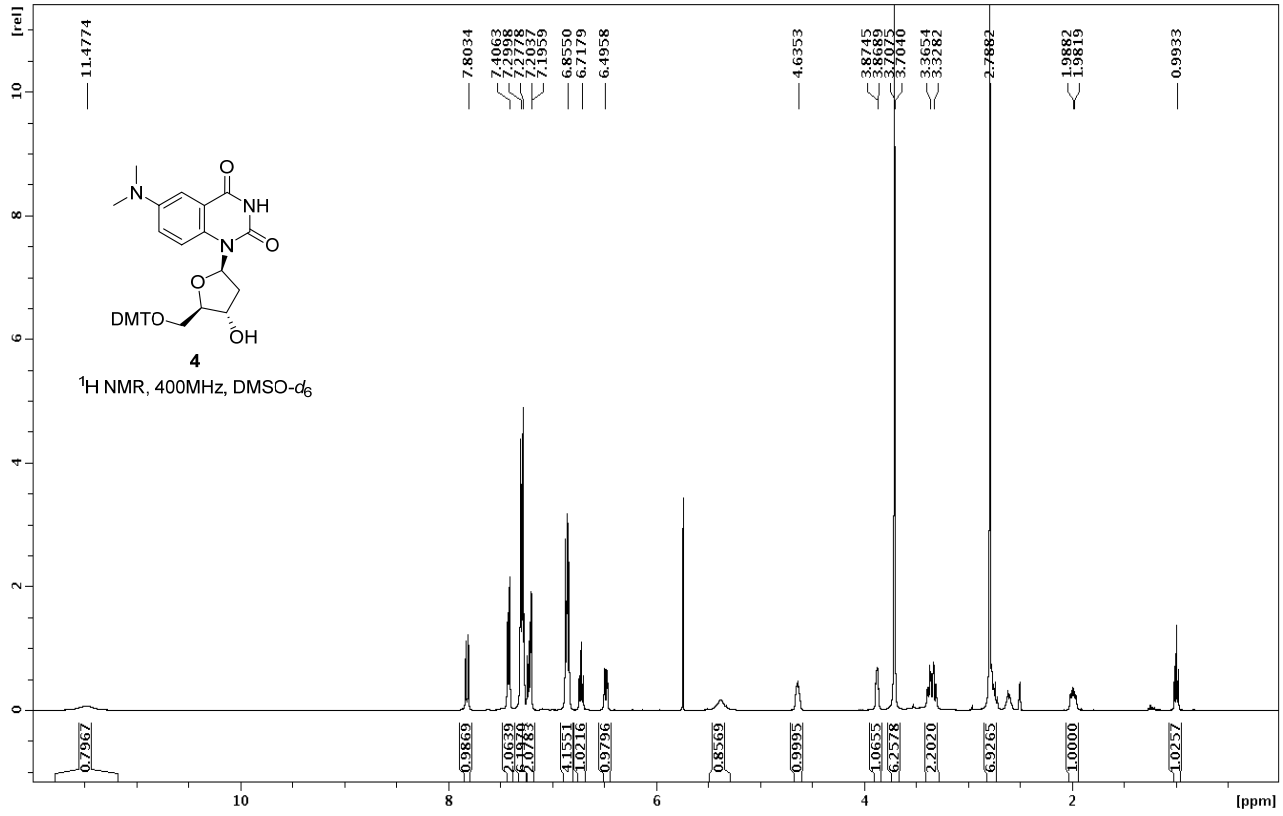
References

(1) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.
(2) Melhuish, W. H. *J. Phys. Chem.* **1961**, *65*, 229.
(3) Rusakowi.R; Testa, A. C. *J. Phys. Chem.* **1968**, *72*, 2680.
(4) Xu, D. G.; Nordlund, T. M. *Biophys. J.* **2000**, *78*, 1042.

^{1}H, ^{13}C and ^{31}P NMR Spectra



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0.9933
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0.9869
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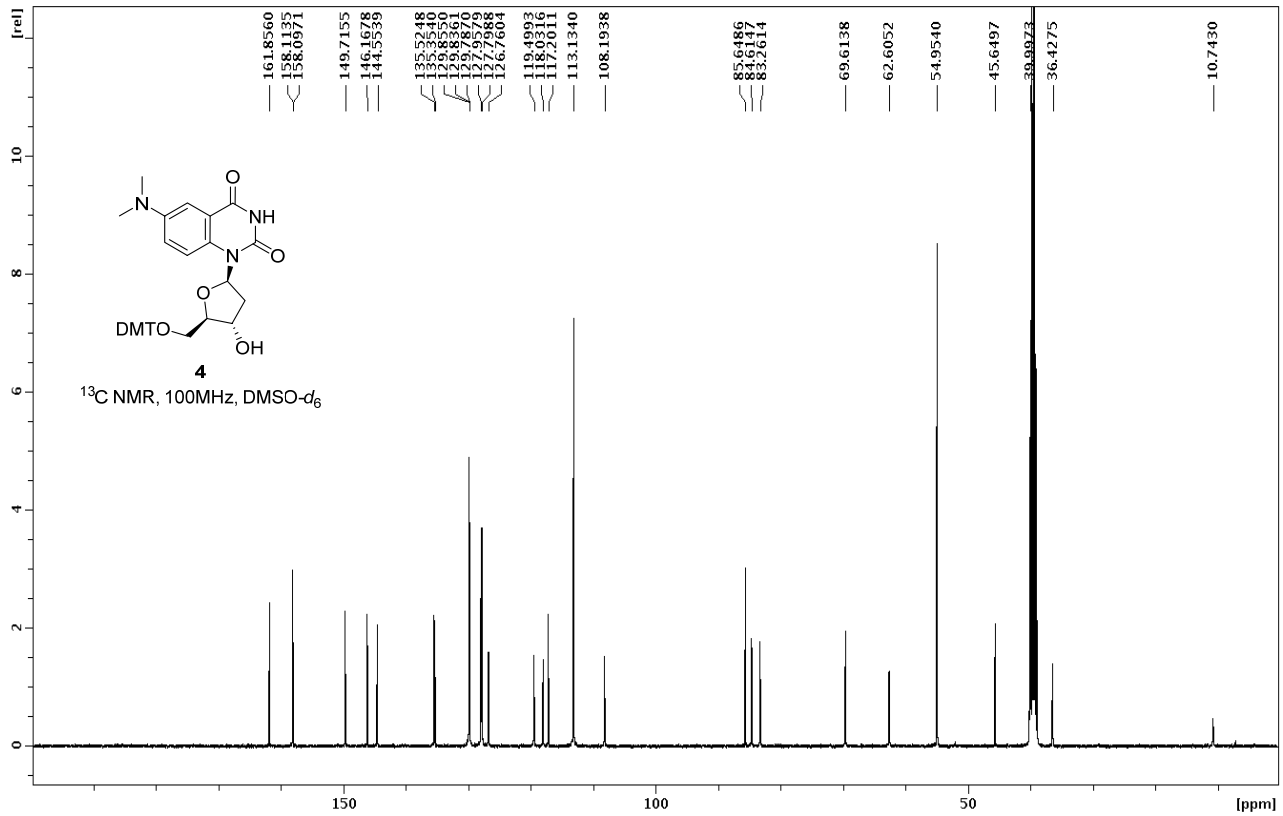
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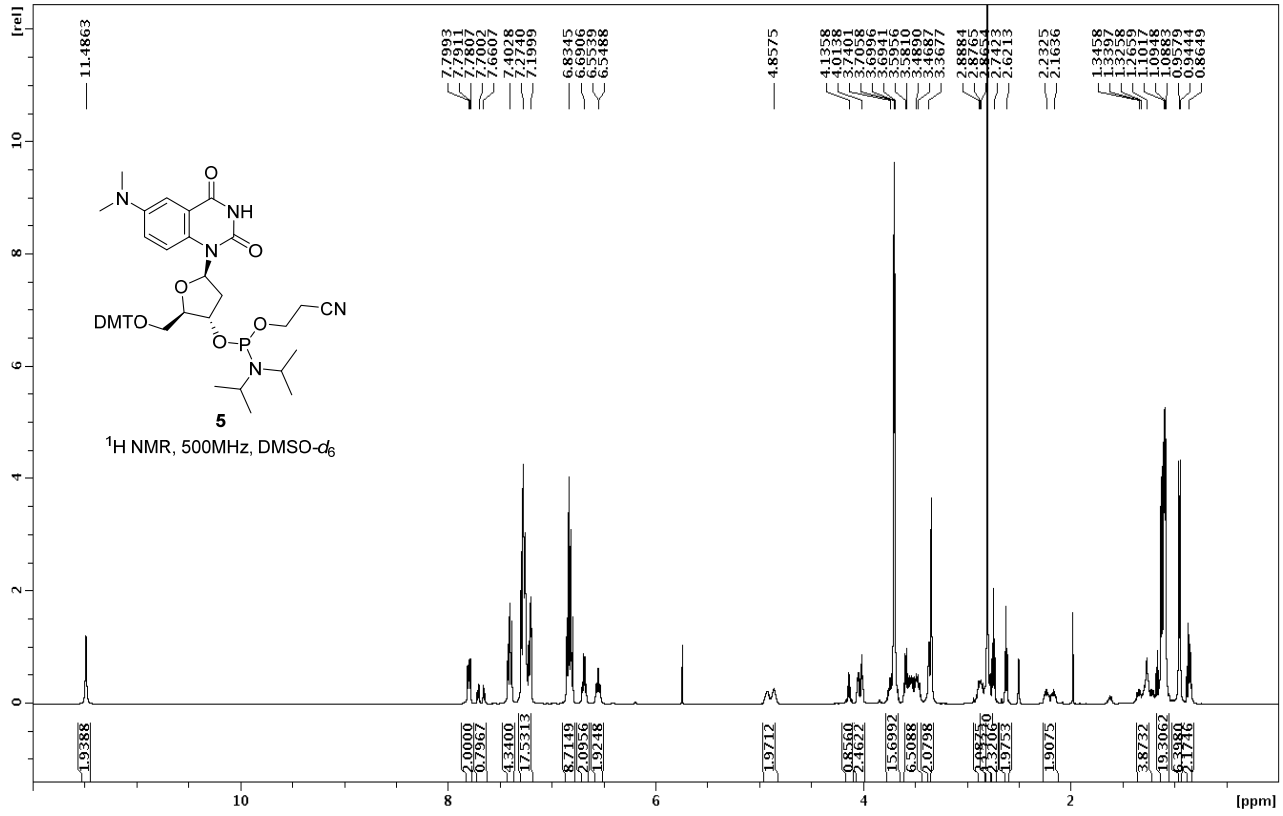
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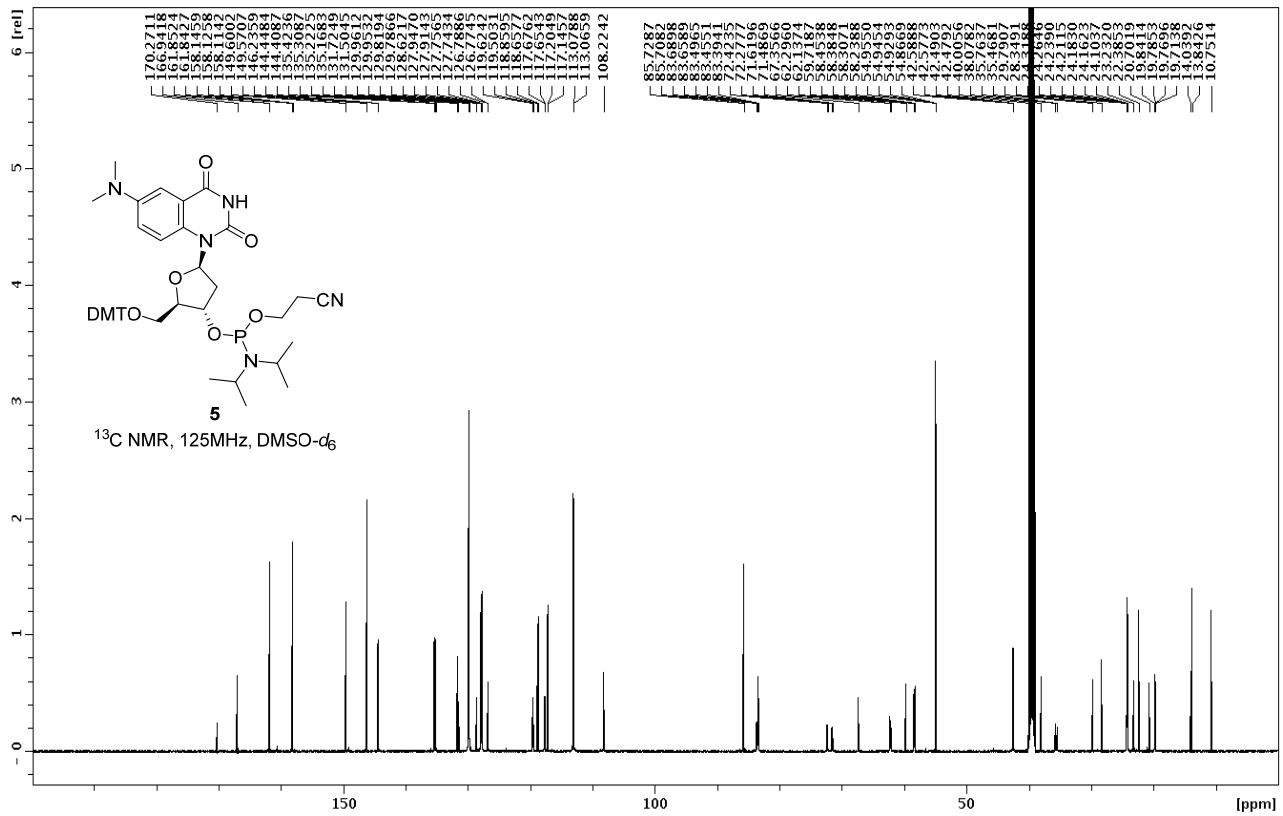
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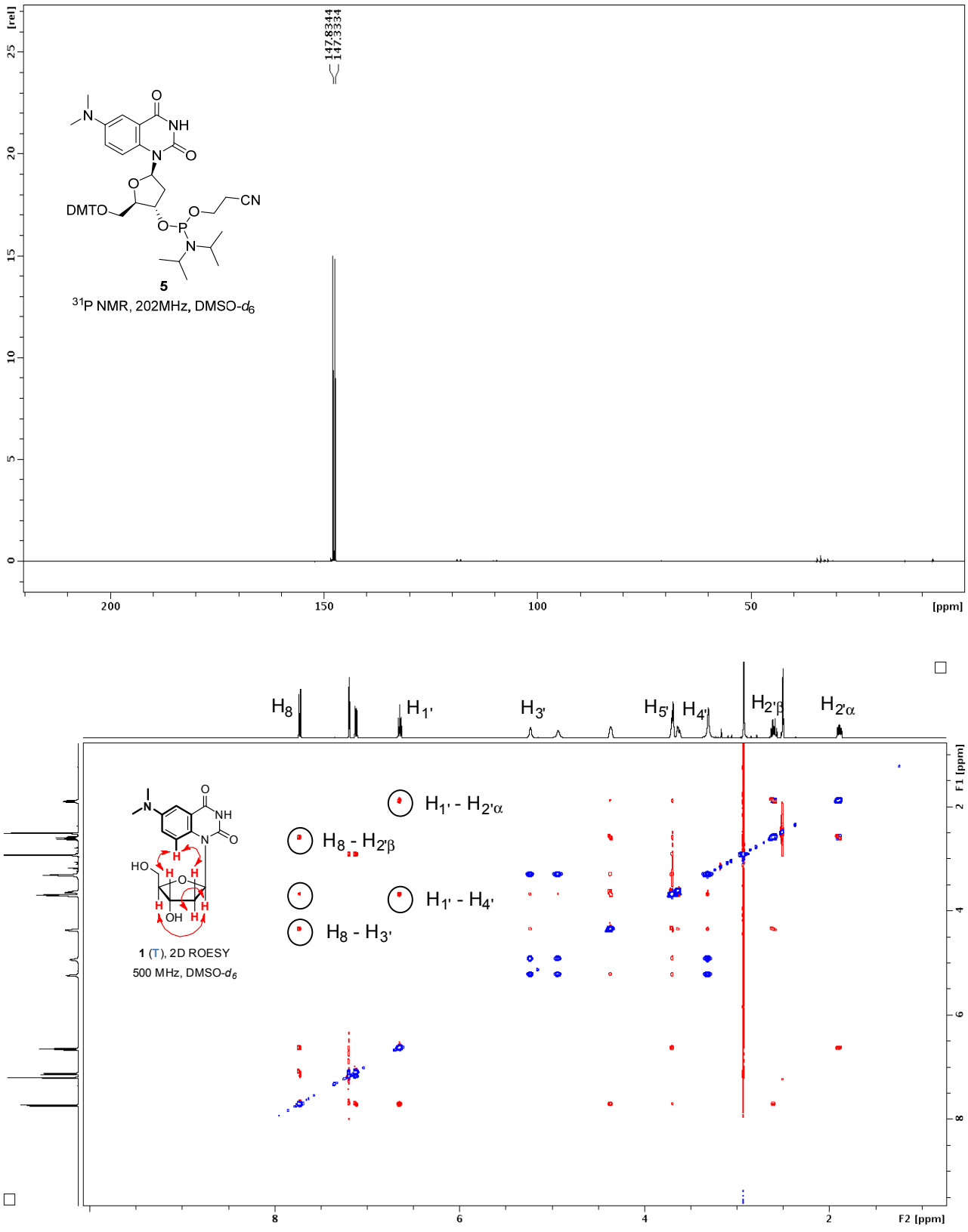

Figure S12. ^{1}H-^{1}H 2D ROESY NMR spectra of nucleoside **1** (^{DMA}**T**) in DMSO-d_6 (mixing time = 250 ms).

HR-MS Spectra

X2: 5’-C**X**C TAA CCC TAA CCC TAA CCC-3’ (**X** = ^{DMA}**T**)

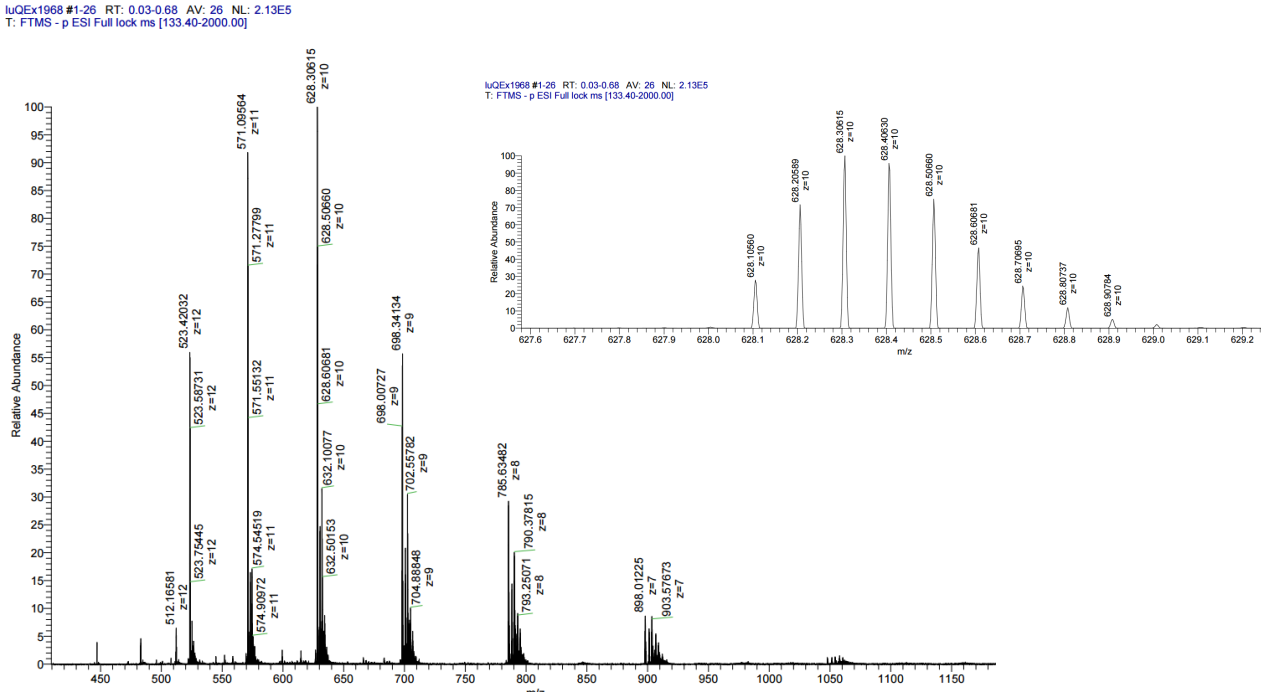

Figure S13. High resolution ESI mass spectra of the synthesized oligonucleotide **X2**.

X13: 5’-CCC TAA CCC TAA **X**CC TAA CCC-3’ (**X** = ^{DMA}**T**)

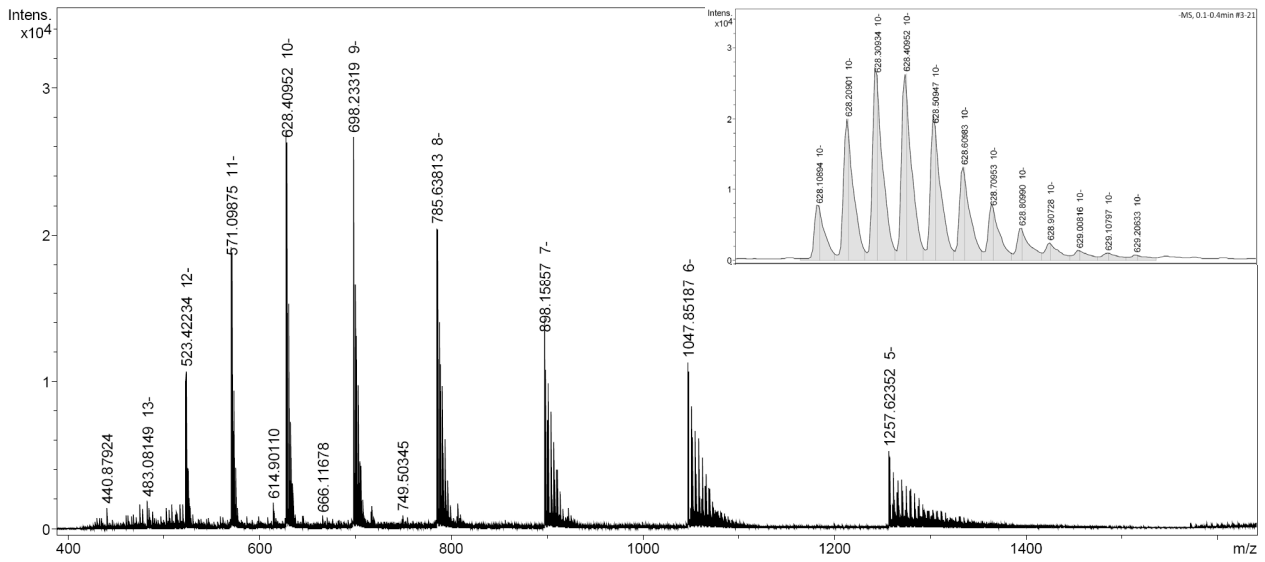

Figure S14. High resolution ESI mass spectra of the synthesized oligonucleotide **X13**.

X14: 5’-CCC TAA CCC TAA C**X**C TAA CCC-3’ (**X** = ^{DMA}**T**)

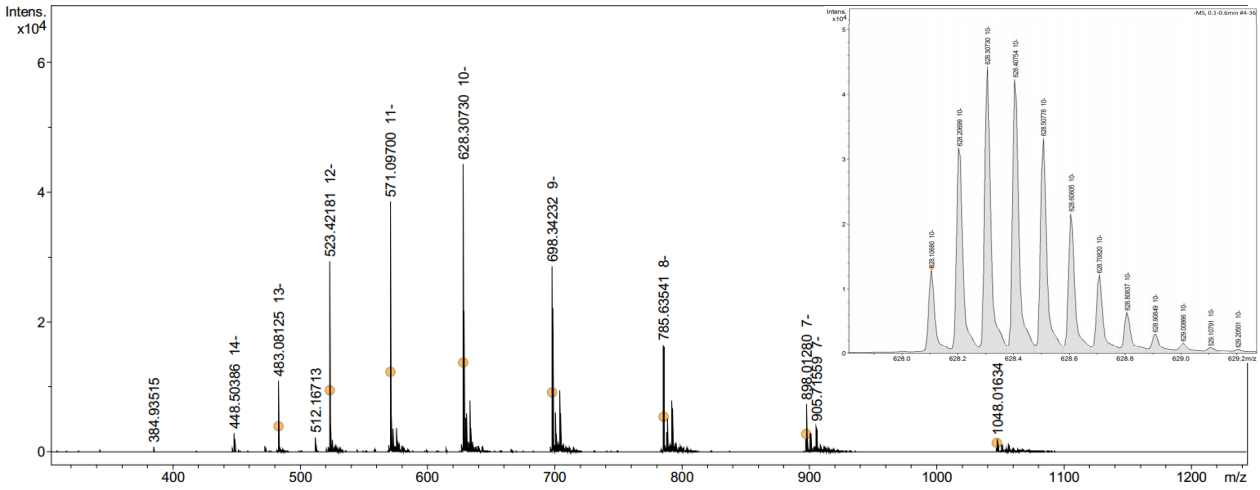

Figure S15. High resolution ESI mass spectra of the synthesized oligonucleotide **X14**.

X15: 5’-CCC TAA CCC TAA CC**X** TAA CCC-3’ (**X** = ^{DMA}**T**)

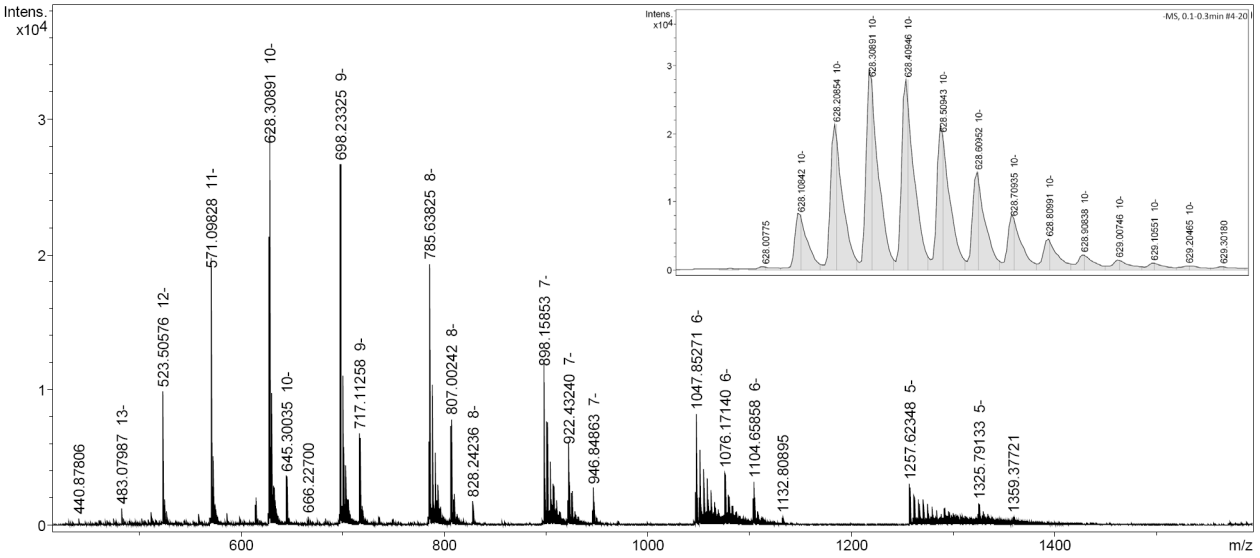

Figure S16. High resolution ESI mass spectra of the synthesized oligonucleotide **X15**.