

Novel interstrand communication systems within DNA duplexes based on 1-, 2- and 4-(phenylethynyl)pyrenes attached to 2'-amino-LNA: high-affinity hybridization and fluorescence sensing

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SUPPORTING INFORMATION

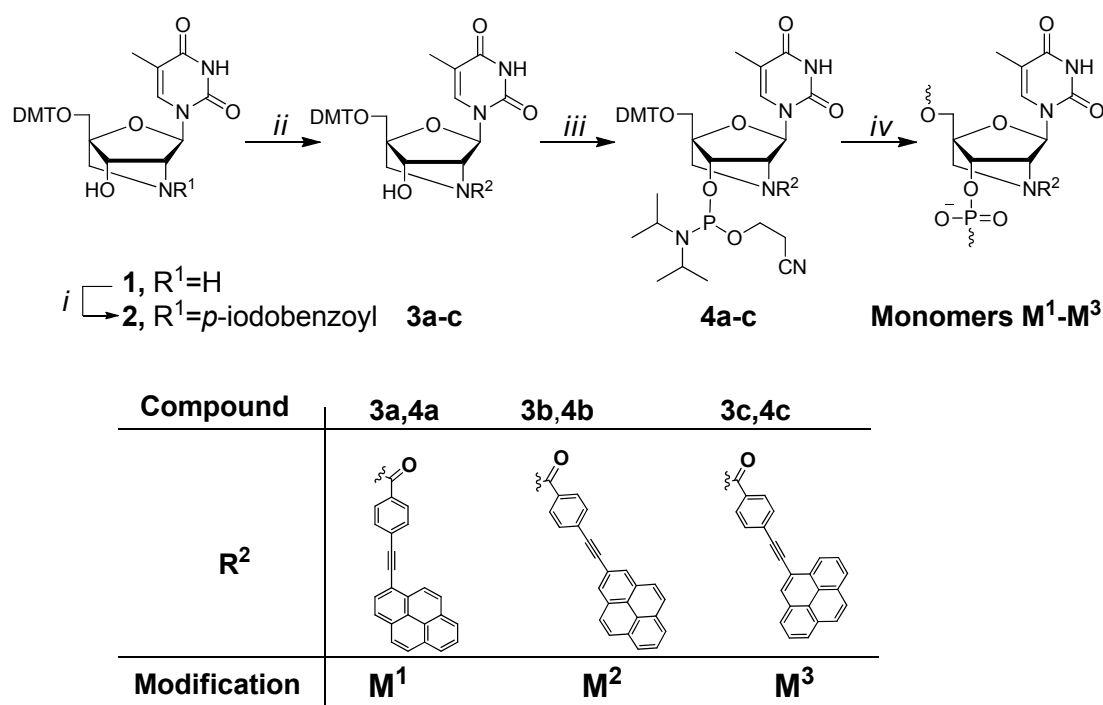
- S2. Synthesis of monomers; HMQC- and HMBC-based interpretation of ^1H and ^{13}C NMR spectra (Figs.S1–S4).
- S11. Synthesis and purification of oligonucleotides; MALDI-MS of synthesized oligonucleotides (Table S1).
- S12. UV-visible absorption and thermal denaturation studies; selected absorption spectra and thermal denaturation curves of modified conjugates (Figs. S5–S6).
- S14. Fluorescence steady-state emission studies and quantum yield determinations.
- S15. Representative fluorescence steady-state emission spectra of modified ONs and their duplexes (Figs. S7–S9).
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Synthesis of monomers

General. Reagents obtained from commercial suppliers were used as received. 2- And 4-ethynylpyrenes,¹ 5-(pyren-1-ylethynyl)-2'-deoxyuridine² and diisopropylammonium tetrazolide³ were synthesized as described. 5-(Pyren-1-ylethynyl)-2'-deoxyuridine and 9,10-diphenylanthracene were used as standards for emission quantum yield measurements after recrystallization. HPLC grade toluene and acetone were distilled and stored over activated 4Å molecular sieves. DCM was always used freshly distilled over CaH₂. Other solvents were used as received. Photochemical studies were performed using spectroquality cyclohexane and abs. ethanol. NMR spectra were recorded at 303 K on a Bruker DRX 500 MHz instrument. Chemical shifts are reported in ppm, relative to solvents peaks (CDCl₃: 7.26 ppm for ¹H and 77.0 ppm for ¹³C; DMSO-*d*₆: 2.50 ppm for ¹H and 39.5 ppm for ¹³C; 85% aq. H₃PO₄: 0.00 ppm for ³¹P). ¹H NMR coupling constants are reported in Hz and refer to apparent multiplicities. ESI high resolution mass spectra were recorded in positive ion mode using a PE SCIEX QSTAR pulsar mass spectrometer. Analytical thin-layer chromatography was performed on Kieselgel 60 F₂₅₄ precoated aluminium plates (Merck). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm.

Synthesis of modified LNA phosphoramidites. The attachment of various (pyrenylethynylphenyl)methyl moieties to 2'-amino-LNA was carried out as shown in Scheme S1. Nucleoside **1**⁴ was acylated with 4-iodobenzoic acid in the presence of HATU (*N,N,N',N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate)⁵ as coupling reagent to give the 4-iodobenzoyl derivative **2** in quantitative yield. This was coupled with 1-, 2- or 4-ethynylpyrenes¹ in condition of Pd/Cu-catalyzed Sonogashira reaction⁶ to afford compounds **3a–c**. These nucleoside derivatives were then phosphitylated with bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine in DCM in the presence of diisopropylammonium tetrazolide to give phosphoramidites **4a–c**.

Structures of nucleoside derivatives **2–4** were confirmed by NMR and ESI-HRMS, compounds **2**, **3a–c** were also confirmed by HMBC- and HMQC-NMR spectra.



Scheme S1. Reaction conditions and yields: (i) 4-iodobenzoic acid, HATU, DIPEA, DMF, rt (100%); (ii) 1-ethynylpyrene, Pd(PPh₃)₄, CuI, Et₃N, DMF, rt (**3a**: 77%), 2-ethynylpyrene, Pd(PPh₃)₄, CuI, Et₃N, DMF, rt (**3b**: 89%), 4-ethynylpyrene, Pd(PPh₃)₄, CuI, Et₃N, DMF, rt (**3c**: 71%); (iii) (*i*-Pr₂N)₂PO(CH₂)₂CN, diisopropylammonium tetrazolide, DCM, rt (**4a**: 90%; **4b**: 69%, **4c**: 73%); (iv) DNA synthesizer.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-5-(4-iodobenzoyl)-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (2). To a stirred solution of 4-iodobenzoic acid (96 mg, 0.39 mmol) and HATU (136 mg, 0.36 mmol) in DMF (3 mL) was added diisopropylethylamine (132 μ L, 0.76 mmol) in one portion. The mixture was stirred for 10 min at room temperature and then added dropwise to a stirred solution of **1** (200 mg, 0.35 mmol) in DMF (3 mL). After stirring for 30 min TLC showed completion of the reaction. The reaction mixture was diluted with EtOAc (100 mL) and successively washed with water (2 $\times$ 150 mL), 5% NaHCO₃ (2 $\times$ 150 mL), and water (3 $\times$ 150 mL). The organic layer was dried over Na₂SO₄ and evaporated. The residue was chromatographed on silica gel using gradient elution with 0 to 10% MeOH in CHCl₃, containing 1% Et₃N (v/v/v). Yield 280 mg (100%), white foam. *R_f* 0.48 (MeOH–toluene–Et₃N, 15:84:1 (v/v/v)). ¹H NMR (500 MHz, DMSO-*d*₆; the signals are given for the major rotamer) δ 11.29 (br. s, 1H), 7.79 (d, 2H, *J* = 8.3 Hz), 7.53 (br. s, 1H), 7.48 (d, 2H, *J* = 8.3 Hz), 7.44 (m, 2H), 7.37–7.23 (m, 7H), 6.92 (m, 4H), 5.86 (d, 1H, *J* = 5.1 Hz), 5.75 (s, 1H), 4.26 (s, 1H), 4.17 (m, 1H), 3.76 (s, 6H), 3.63 (d, 1H, ²*J* = 11.5 Hz), 3.50 (d, 1H, ²*J* = 11.0 Hz), 3.43 (d, 1H, ²*J* = 11.0 Hz), 3.33 (d, 1H, ²*J* = 11.5 Hz), 1.50 (s, 3H). ¹³C NMR (125.7 MHz, DMSO-*d*₆) δ 168.58, 163.79, 158.30 (2C), 150.20, 144.67, 137.04 (2C), 135.38, 135.08, 134.43, 134.22, 130.15 (2C), 129.88 (2C), 129.79 (2C), 128.04 (2C), 127.74 (2C), 126.94, 113.40 (4C), 108.66, 97.65, 87.29, 86.62, 85.88, 69.12,

65.83, 59.26, 55.14 (2C), 51.68, 12.37. ESI-HRMS: m/z 824.1430 ($[M+Na]^+$, $C_{39}H_{36}IN_3O_8Na^+$ calcd. 824.1439).

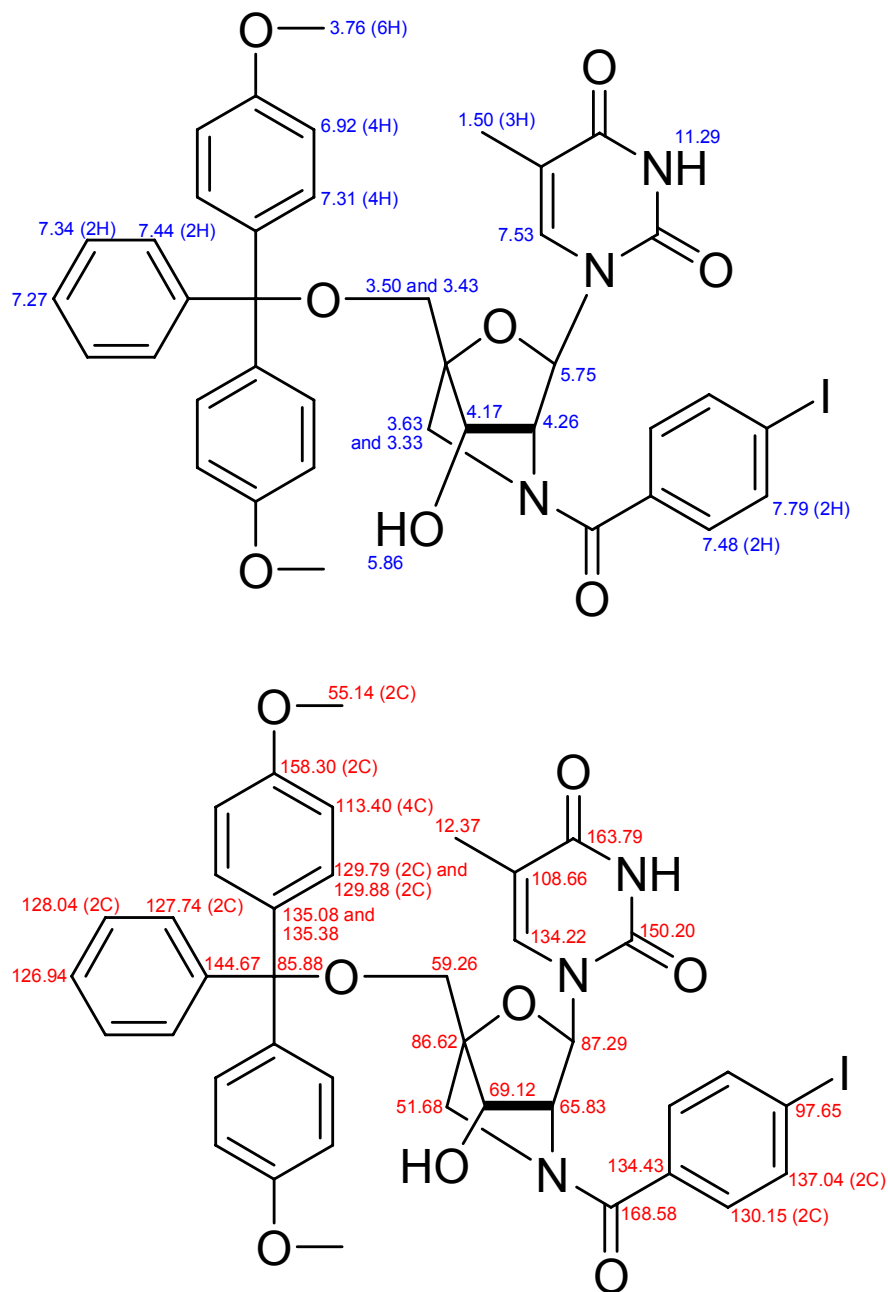


Figure S1. HMBC- and HMQC-based characterization of 1H and ^{13}C NMR spectra, compound 2.

General procedure for the preparation of the compounds 3a–c. To a solution of **2** (280 mg, 0.35 mmol) and the corresponding ethynylpyrene (97 mg, 0.43 mmol) in DMF (20 mL) under argon were successively added Pd(PPh₃)₄ (20 mg, 0.017 mmol), CuI (3.2 mg, 0.017 mmol) and triethylamine (125 μ L, 0.9 mmol), and the reaction mixture was stirred for 16 h at room temperature. The disappearance of the starting iodide was checked by TLC (MeOH–CHCl₃–Et₃N, 5:94:1, (v/v/v)). The mixture was then diluted with CHCl₃ (200 mL), washed with 0.1 M EDTA-(NH₄)₂ (2 $\times$ 200 mL) and water (5 $\times$ 200 mL), dried over Na₂SO₄, and evaporated to dryness. The residue was chromatographed on a silica gel column in an appropriate solvent system. The compounds described below were obtained.

(1R,3R,4R,7S)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-5-[4-(pyren-1-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (3a) was chromatographed on silica gel gradient elution with 0 to 5% MeOH in CHCl₃, containing 1% Et₃N (v/v/v). Yield 249 mg (77%), yellow foam. *R_f* 0.46 (MeOH–CHCl₃–Et₃N, 3:96:1 (v/v/v)). ¹H NMR (500 MHz, DMSO-*d*₆; the signals are given for the major rotamer) δ 11.42 (br. s, 1H), 8.64 (d, 1H, *J* = 9.1 Hz), 8.41 (m, 3H), 8.35 (m, 1H, *J* = 8.0 Hz), 8.32 (m, 1H, *J* = 8.0 Hz), 8.29 (m, 1H, *J* = 9.1 Hz), 8.24 (m, 1H, *J* = 9.1 Hz), 8.16 (t, 1H, *J* = 7.6 Hz), 7.84 (s, 4H), 7.58 (br. s, 1H), 7.46 (m, 2H), 7.39–7.23 (m, 7H), 6.94 (m, 4H), 6.00 (d, 1H, *J* = 3.8 Hz), 5.83 (s, 1H), 4.35 (s, 1H), 4.22 (m, 1H), 3.76 (s, 6H), 3.68 (d, 1H, ²*J* = 11.2 Hz), 3.52 (d, 1H, ²*J* = 11.0 Hz), 3.44 (d, 1H, ²*J* = 11.0 Hz), 3.37 (d, 1H, ²*J* = 11.2 Hz), 1.48 (s, 3H). ¹³C NMR (125.7 MHz, DMSO-*d*₆) δ 168.65, 163.84, 158.31 (2C), 150.21, 144.69, 135.40, 135.10, 135.03, 134.27, 131.34 (2C), 131.29 (2C), 130.83, 130.55, 129.89 (3C), 129.81 (2C), 129.10, 128.68 (2C), 128.65, 128.05 (2C), 127.75 (2C), 127.30, 126.94, 126.89, 126.21, 126.16, 125.04, 124.83, 124.50, 123.70, 123.44, 116.36, 113.40 (4C), 108.67, 94.59, 89.96, 87.31, 86.68, 85.89, 69.18, 65.87, 59.31, 55.14 (2C), 51.74, 12.37. ESI-HRMS: *m/z* 922.3092 ([M+Na⁺], C₅₇H₄₅N₃O₈Na⁺ calcd. 922.3099).

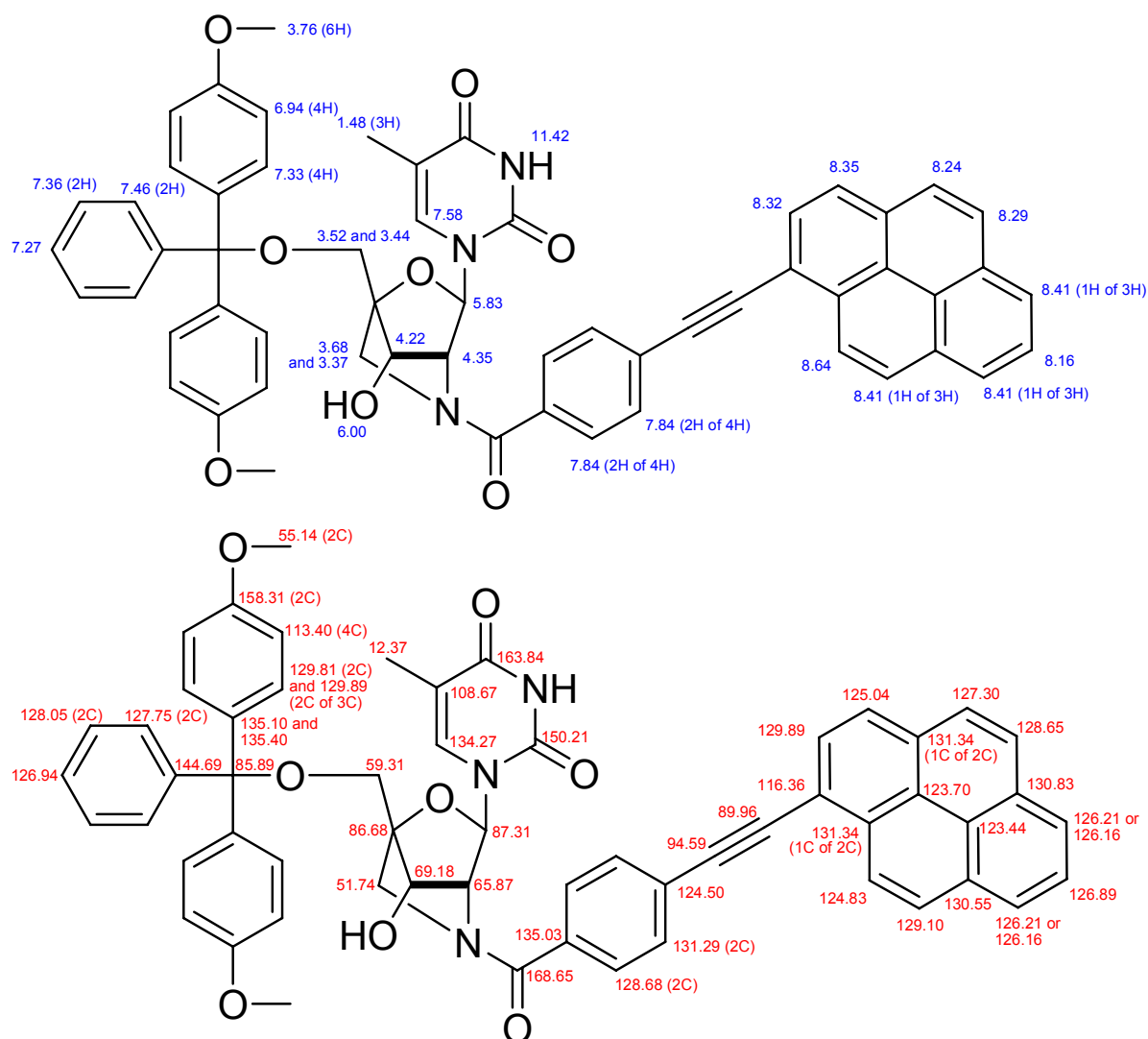


Figure S2. HMBC- and HMQC-based characterization of ^1H and ^{13}C NMR spectra, compound **3a**.

(1R,3R,4R,7S)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-5-[4-(pyren-2-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (3b) was purified in the same solvent system as described for **3a**. Yield 281 mg (89%), yellow foam. R_f 0.42 (MeOH– CHCl_3 – Et_3N , 3:96:1 (v/v/v)). ^1H NMR (500 MHz, $\text{DMSO}-d_6$; the signals are given for the major rotamer) δ 11.42 (br. s, 1H), 8.54 (s, 2H), 8.35 (d, 2H, $J = 7.6$ Hz), 8.26 (m, 2H, $J = 8.9$ Hz), 8.22 (m, 2H, $J = 8.9$ Hz), 8.13 (t, 1H, $J = 7.6$ Hz), 7.82 (d, 2H, $J = 7.9$ Hz), 7.72 (d, 2H, $J = 7.9$ Hz), 7.58 (br. s, 1H), 7.46 (d, 2H, $J = 7.7$ Hz), 7.38–7.25 (m, 7H), 6.94 (m, 4H), 5.99 (d, 1H, $J = 3.7$ Hz), 5.82 (s, 1H), 4.32 (s, 1H), 4.21 (m, 1H), 3.76 (s, 6H), 3.67 (d, 1H, $^2J = 11.1$ Hz), 3.51 (d, 1H, $^2J = 11.0$ Hz), 3.44 (d, 1H, $^2J = 11.0$ Hz), 3.37 (d, 1H, $^2J = 11.1$ Hz), 1.48 (s, 3H). ^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$) δ 168.63, 163.85, 158.29 (2C), 150.17, 144.68, 135.38, 135.08, 135.04, 134.26, 131.29 (2C), 130.85 (4C), 129.88 (2C), 129.79 (2C), 128.65

(2C), 128.35 (2C), 128.04 (2C), 127.74 (2C), 127.58 (2C), 126.92 (4C), 125.74 (2C), 124.32, 123.60, 123.44, 119.55, 113.40 (4C), 108.65, 91.55, 89.20, 87.31, 86.67, 85.88, 69.16, 65.84, 59.30, 55.13 (2C), 51.71, 12.36. ESI-HRMS: m/z 922.3098 ($[M+Na]^+$, $C_{57}H_{45}N_3O_8Na^+$ calcd. 922.3099).

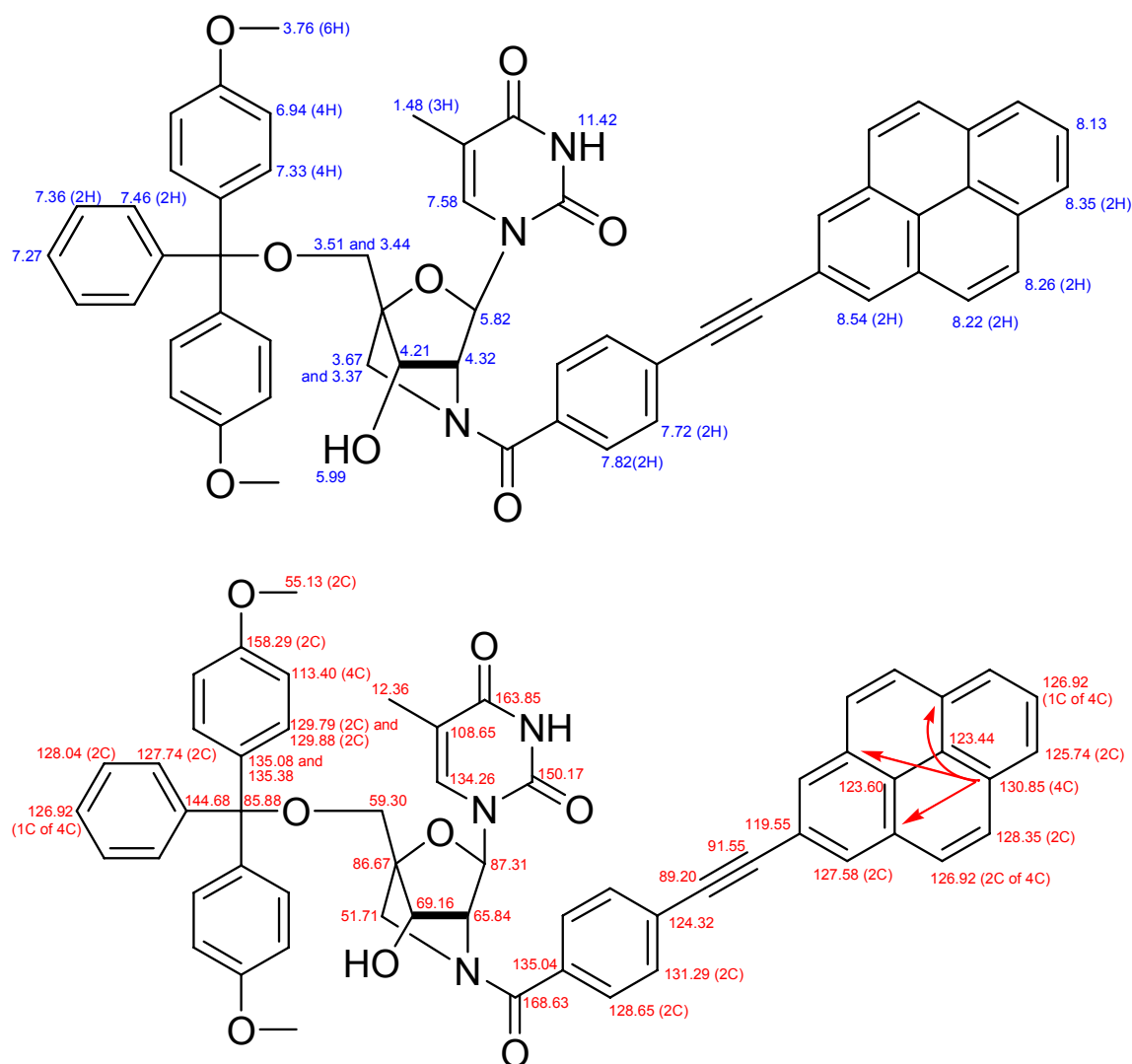


Figure S3. HMBC- and HMQC-based characterization of ^1H and ^{13}C NMR spectra, compound **3b**.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-5-[4-(pyren-4-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (3c) was chromatographed on silica gel using gradient elution with 10 to 25% EtOAc in toluene, containing 1% Et₃N (v/v/v). Yield 224 mg (71%), yellow foam. *R_f* 0.25 (acetone–toluene–Et₃N, 30:59:1 (v/v/v)). ¹H NMR (500 MHz, DMSO-*d*₆; the signals are given for the major rotamer) δ 11.42 (br. s, 1H), 8.75 (d, 1H, *J* = 7.8 Hz), 8.66 (s, 1H), 8.43 (d, 1H, *J* = 7.3 Hz), 8.39 (m, 2H), 8.29–8.22 (m, 3H, *J* = 9.1 Hz), 8.14 (t, 1H, *J* = 7.7 Hz), 7.85 (s, 4H), 7.58 (br. s, 1H), 7.46 (m, 2H), 7.39–7.23 (m, 7H), 6.94 (m, 4H), 6.01 (d, 1H, *J* = 3.8 Hz), 5.83 (s, 1H), 4.35 (s, 1H), 4.22 (m, 1H), 3.76 (s, 6H), 3.68 (d, 1H, ²*J* = 11.3 Hz), 3.52 (d, 1H, ²*J* = 11.0 Hz), 3.44 (d, 1H, ²*J* = 11.0 Hz), 3.38 (d, 1H, ²*J* = 11.3 Hz), 1.48 (s, 3H). ¹³C NMR (125.7 MHz, DMSO-*d*₆) δ 168.63, 163.84, 158.30 (2C), 150.20, 144.68, 135.39, 135.18, 135.09, 134.26, 132.70, 131.44 (2C), 130.99, 130.57, 129.88 (2C), 129.80 (2C), 129.73, 129.37, 128.69 (2C), 128.65, 128.04 (2C), 127.74 (2C), 127.62, 127.45, 126.95, 126.87, 126.81, 126.60, 126.19, 125.79, 124.30, 123.67 (2C), 123.47, 118.97, 113.40 (4C), 108.67, 93.55, 89.07, 87.31, 86.68, 85.88, 69.18, 65.86, 59.30, 55.13 (2C), 51.74, 12.37. ESI-HRMS: *m/z* 922.3089 ([M+Na⁺], C₅₇H₄₅N₃O₈Na⁺ calcd. 922.3099).

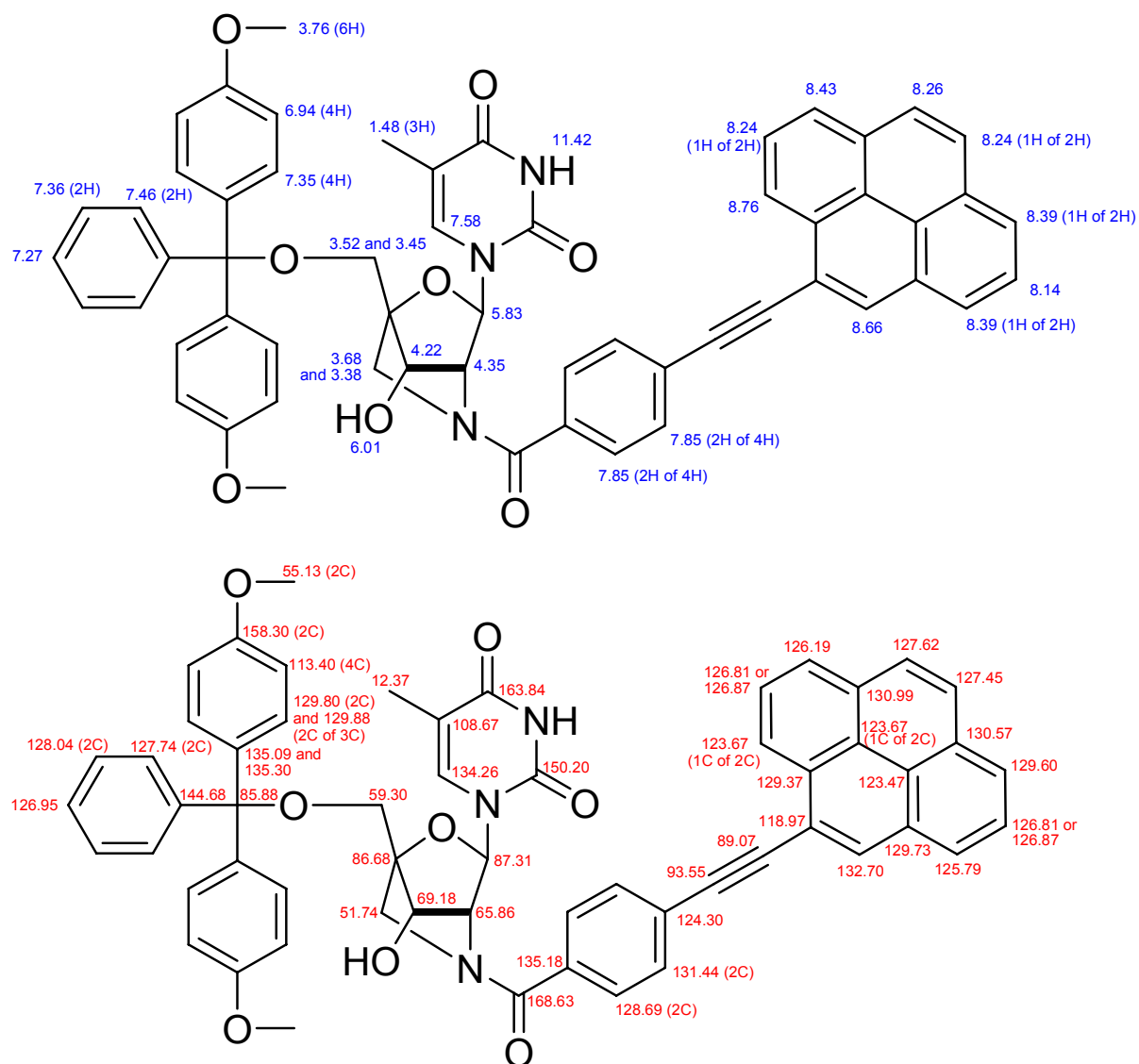


Figure S4. HMBC- and HMQC-based characterization of ¹H and ¹³C NMR spectra, compound **3c**.

General procedure for the preparation of the compounds 4a–c. The LNA nucleoside derivative **16a–c** (216 mg, 0.24 mmol) was evaporated to dryness after addition of anhydrous DCM (2×35 mL), dissolved in anhydrous DCM (20 mL), and diisopropylammonium tetrazolide (92 mg, 54 mmol) and bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine (219 μ L, 0.69 mmol) were added under argon. The reaction mixture was stirred at room temperature for 12 h. After complete conversion of the starting compound **16** (monitoring by TLC, acetone–toluene–Et₃N, 30:59:1 (v/v/v)), the mixture was diluted with EtOAc (200 mL) and washed with saturated solutions of NaHCO₃ (2×200 mL) and brine (150 mL). The organic layer was dried over Na₂SO₄, evaporated, and the residue was purified by chromatography on silica gel using gradient elution with 20% to 30% acetone in toluene, containing 1% Et₃N (v/v/v).

(1*R*,3*R*,4*R*,7*S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityl-oxymethyl)-5-[4-(pyren-1-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (4a), yield 238 mg (90%), yellow foam. *R*_f 0.49, 0.54 (acetone–toluene–Et₃N, 30:59:1 (v/v/v)). ³¹P NMR (202.4 MHz, CDCl₃): δ 151.01 (0.43P), 149.71 (0.57P), diastereomers. ESI-HRMS: *m/z* 1122.4171 ([M+Na⁺], C₆₆H₆₂N₅O₉PNa⁺ calcd. 1122.4177).

(1*R*,3*R*,4*R*,7*S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityl-oxymethyl)-5-[4-(pyren-2-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (4b), yield 181 mg (69%), yellow foam. *R*_f 0.37, 0.43 (acetone–toluene–Et₃N, 30:59:1 (v/v/v)). ³¹P NMR (202.4 MHz, CDCl₃): δ 151.01 (0.45P), 149.71 (0.55P), diastereomers. ESI-HRMS: *m/z* 1122.4166 ([M+Na⁺], C₆₆H₆₂N₅O₉PNa⁺ calcd. 1122.4177).

(1*R*,3*R*,4*R*,7*S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityl-oxymethyl)-5-[4-(pyren-4-ylethynyl)benzoyl]-3-(thymine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (4c), yield 193 mg (73%), yellow foam. *R*_f 0.52, 0.60 (acetone–toluene–Et₃N, 30:59:1 (v/v/v)). ³¹P NMR (202.4 MHz, DMSO-*d*₆): δ 149.56 (0.47P), 148.30 (0.53P), diastereomers. ESI-HRMS: *m/z* 1122.4175 ([M+Na⁺], C₆₆H₆₂N₅O₉PNa⁺ calcd. 1122.4177).

Synthesis and purification of oligonucleotides

Oligonucleotide synthesis was carried out on a PerSpective Biosystems Expedite 8909 instrument in 200 nmol scale using manufacturer's standard protocols. In case of LNA phosphoramidites (monomers $^{Me}C^L$, T^L , G^L) the coupling step time was extended to 15 min. To accomplish incorporation of monomers M^1-M^3 extended coupling time (35 min) was applied using 1*H*-tetrazole solution in CH_3CN as activator. Step-wise coupling yields of 85-95% for monomers M^1-M^3 were obtained based on the absorbance of the dimethoxytrytyl cation released after each coupling. The coupling efficiencies of standard DNA amidites varied between 98% and 100%. Cleavage from solid support and removal of nucleobase protecting groups was performed using standard conditions (32% aqueous ammonia for 12 h at 55 °C). Unmodified DNA/RNA strands (**ON1–ON4**) were obtained from commercial suppliers and used without further purification, while all the modified oligonucleotides were purified by DMT-ON RP-HPLC using the Waters Prep LC 4000 equipped with Xterra MS C18-column (10 μm , 300 mm $\times$ 7.8 mm). Elution was performed starting with an isocratic hold of A-buffer for 5 min followed by a linear gradient to 55% B-buffer over 75 min at a flow rate of 1.0 mL/min (A-buffer: 95% 0.1 M NH_4HCO_3 , 5% CH_3CN ; B-buffer: 25% 0.1 M NH_4HCO_3 , 75% CH_3CN). RP-purification was followed by detritylation (80% aq. AcOH, 20 min), precipitation (abs. EtOH, -18 °C, 12 h) and washing with abs. EtOH three times. The identity and purity of ONs were verified by MALDI-TOF mass spectrometry (Table S1) and IC HPLC.

Table S1. MALDI-MS of synthesized ONs.^a

#	Sequence, 5'→3'	Found m/z [M-H] ⁻	Calc. m/z [M-H] ⁻
ON5	GTG A <u>M</u> ¹ A TGC	3108	3109
ON6	GCA <u>M</u> ¹ AT CAC	3035	3038
ON7	GCA TA <u>M</u> ¹ CAC	3038	3038
ON8	GTG A <u>M</u> ² A TGC	3109	3109
ON9	GCA <u>M</u> ² AT CAC	3035	3038
ON10	GCA TA <u>M</u> ² CAC	3038	3038
ON11	GTG A <u>M</u> ³ A TGC	3109	3109
ON12	GCA <u>M</u> ³ AT CAC	3037	3038
ON13	GCA TA <u>M</u> ³ CAC	3036	3038

^asee Scheme S1 for the structures of monomers M^1-M^3 .

UV-visible absorption and thermal denaturation studies

UV-visible absorption spectra and thermal denaturation experiments were performed on a Perkin Elmer Lambda 35 UV/VIS spectrometer equipped with a PTP 6 (peltier temperature programmer) in medium salt buffer (100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0). Concentrations of ONs were calculated using the following extinction coefficients ($OD_{260}/\mu\text{mol}$): G, 10.5; A, 13.9; T/U, 7.9; C, 6.6; $\mathbf{M}^1$, 14.8; $\mathbf{M}^2$, 42.2; $\mathbf{M}^3$, 11.4. ONs (0.5 μM each strand) were thoroughly mixed, denaturated by heating and subsequently cooled to the starting temperature of experiment. Thermal denaturation temperatures (T_m values, $^{\circ}\text{C}$) were determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} vs. temperature). Reported T_m values are an average of two measurements within ± 1.0 $^{\circ}\text{C}$.

A

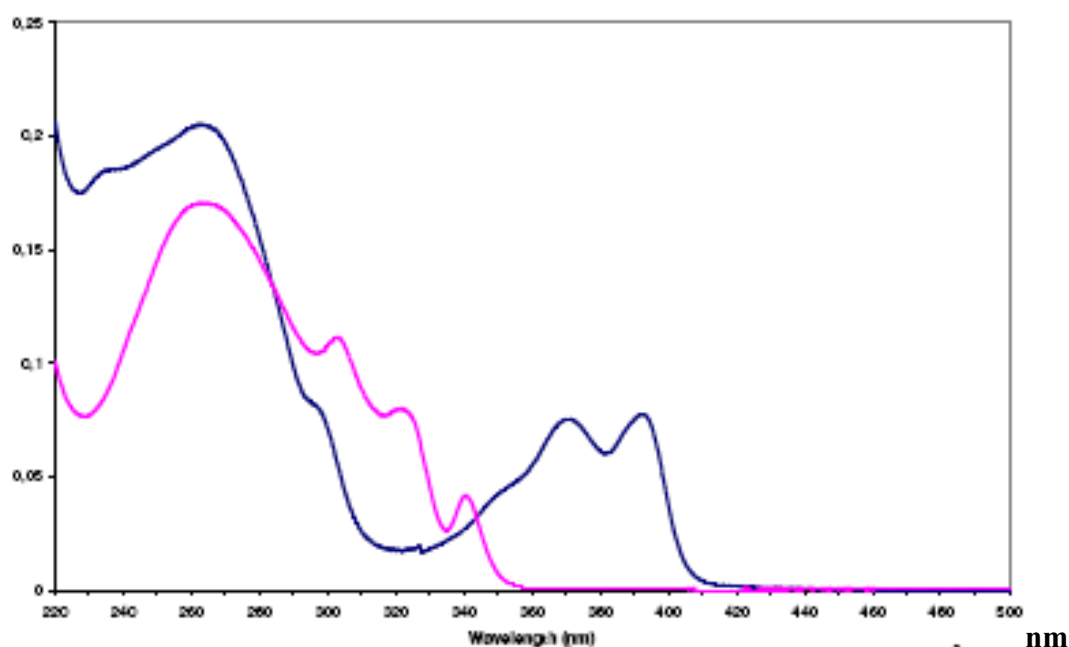


Figure S5. Absorption spectra (not normalized) of **ON5** and **ON8** containing monomers $\mathbf{M}^1$ and $\mathbf{M}^2$ (shown in *blue* and *pink*, respectively) recorded in medium salt buffer at 19 $^{\circ}\text{C}$ using 0.5 μM concentration of ONs.

Absorbance at 260 nm

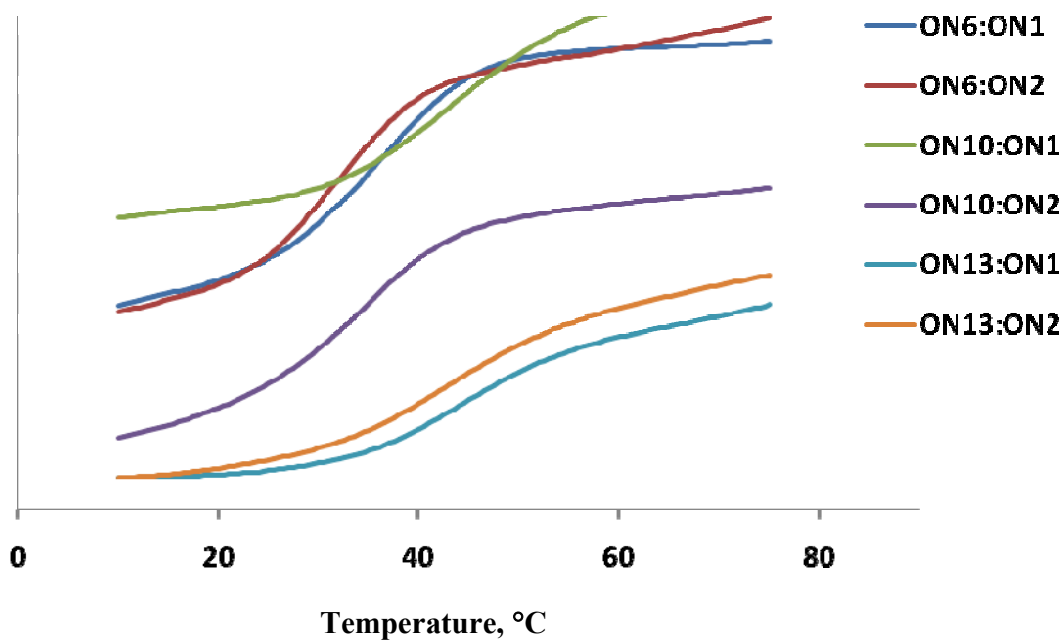


Figure S6. Thermal denaturation curves of duplexes containing monomers M^1 – M^3 recorded in medium salt buffer using 0.5 μ M concentration of ONs.

Fluorescence steady-state emission studies and quantum yield determinations

Fluorescence spectra were obtained in a medium salt buffer using a PerkinElmer luminiscence spectrometer LS 55 equipped with a Peltier temperature controller. For recording of fluorescence spectra 0.1 μM concentrations of the ss probe or corresponding duplex were used. The fluorescence quantum yields (Φ_F) were measured by the relative method using standards of highly diluted solution of 9,10-diphenylanthracene (Φ_F 0.95)⁷ in cyclohexane as a first standard and 5-(pyren-1-ylethynyl)-2'-deoxyuridine² in abs. EtOH as a second standard. For fluorescence quantum yield determinations 0.5 μM solutions were used. In all cases, absorption in the range 310-520 nm did not exceed 0.1, and was not less than 0.01. Control experiments with an elimination of dissolved oxygen in the buffer solution did not show significant change of fluorescence intensity compared to non-degassed solutions. Thus, the samples used in quantum yield measurements were not degassed. Steady-state fluorescence emission spectra were obtained as an average of 5 scans using an excitation wavelength of 360 nm (**M**¹), 315 nm (**M**²) and 345 nm (**M**³), excitation slit of 4.0 nm, emission slit of 2.5 nm and scan speed of 120 nm/min. The fluorescence quantum yield of 5-(pyren-1-ylethynyl)-2'-deoxyuridine² in abs. EtOH relative to 9,10-diphenylanthracene in cyclohexane (lit. (7) Φ_F 0.95) in these experimental settings were measured to be 0.43 and 0.91, respectively.

Emission quantum yields of modified ONs Φ_F (ON) were determined according to:⁸

$$\Phi_F(\text{ON}) = \Phi_F(\text{ref}) \times \frac{I(\text{ON})}{I(\text{ref})} \times \frac{OD_{\lambda_{\text{ex}}}(\text{ref})}{OD_{\lambda_{\text{ex}}}(\text{ON})} \times \frac{n_{\text{buffer}}^2}{n_{\text{ref}}^2}$$

where Φ_F (ref) is the cross-calibrated (by using of 9,10-diphenylanthracene in cyclohexane as a first standard) value of the fluorescence quantum yield of 5-(pyren-1-ylethynyl)-2'-deoxyuridine in abs. EtOH; I (ON) is the area of the fluorescence emission spectrum of the sample, I (ref) is the area of the second standard's emission spectrum at the same region, n_{buffer} and n_{ref} are the refractive indexes of the buffer (1.334) and ethanol (1.361), respectively. High quantum yields ($\Phi_F > 0.2$) were determined as the average of two measurements within $\pm 10\%$. Determinations of low quantum yields ($\Phi_F < 0.1$) may be associated with considerably larger error than determination of higher quantum yields.

I

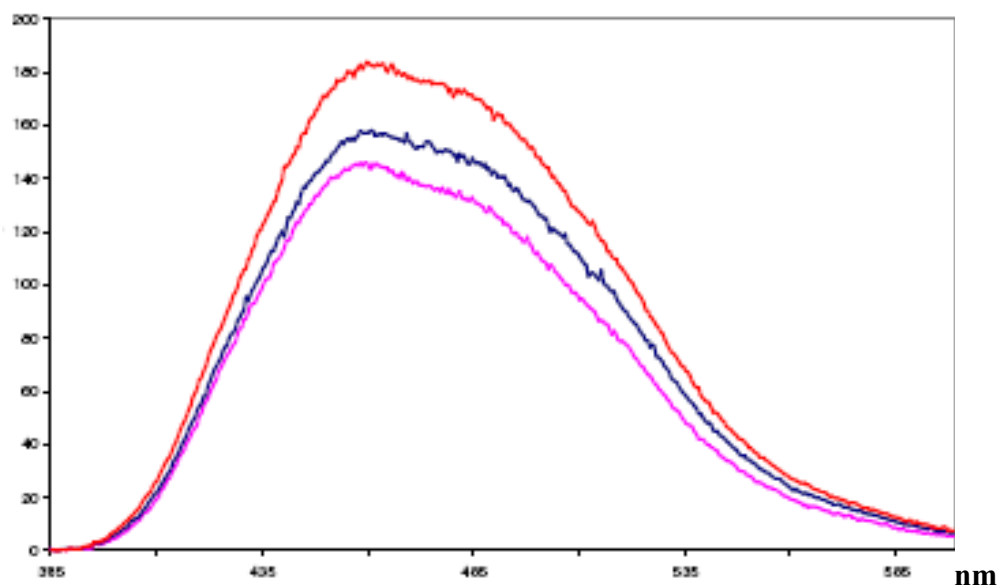


Figure S7. Steady-state fluorescence emission spectra of M^1 -containing conjugates: **ON5**, **ON5:ON1** and **ON5:ON2** (shown in *pink*, *red* and *blue*, respectively). Spectra were obtained in medium salt buffer at 19 °C using an excitation wavelength of 360 nm and 0.1 μ M concentrations of ONs and complementary strands.

I

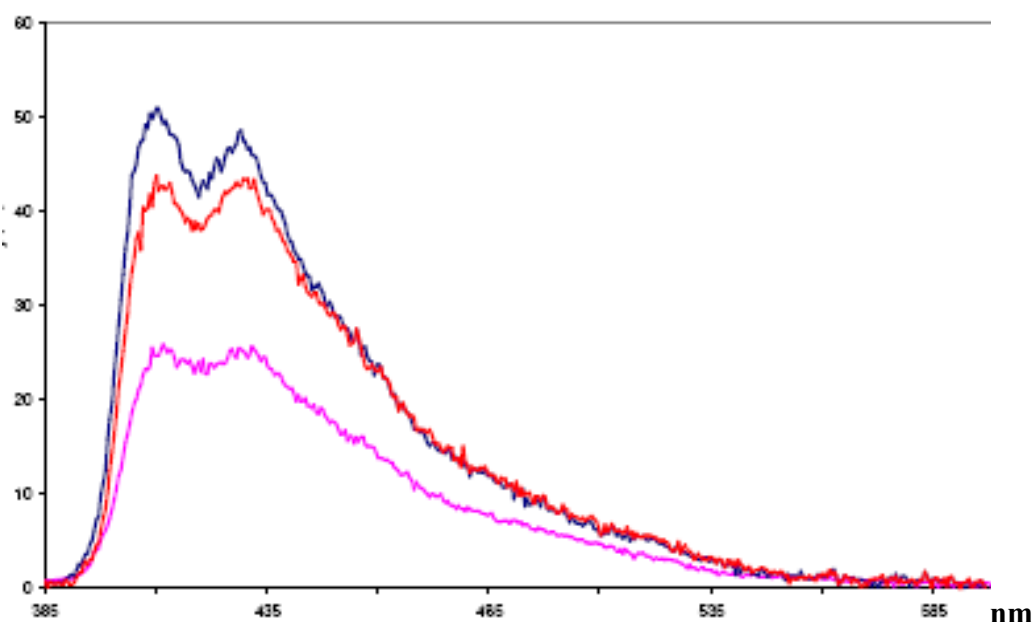


Figure S8. Steady-state fluorescence emission spectra of M^2 containing conjugates: **ON9**, **ON9:ON1** and **ON9:ON2** (shown in *pink*, *red* and *blue*, respectively). Spectra were obtained in medium salt buffer at 19 °C using an excitation wavelength of 315 nm and 0.1 μ M concentrations of ONs and

complementary strands.

I

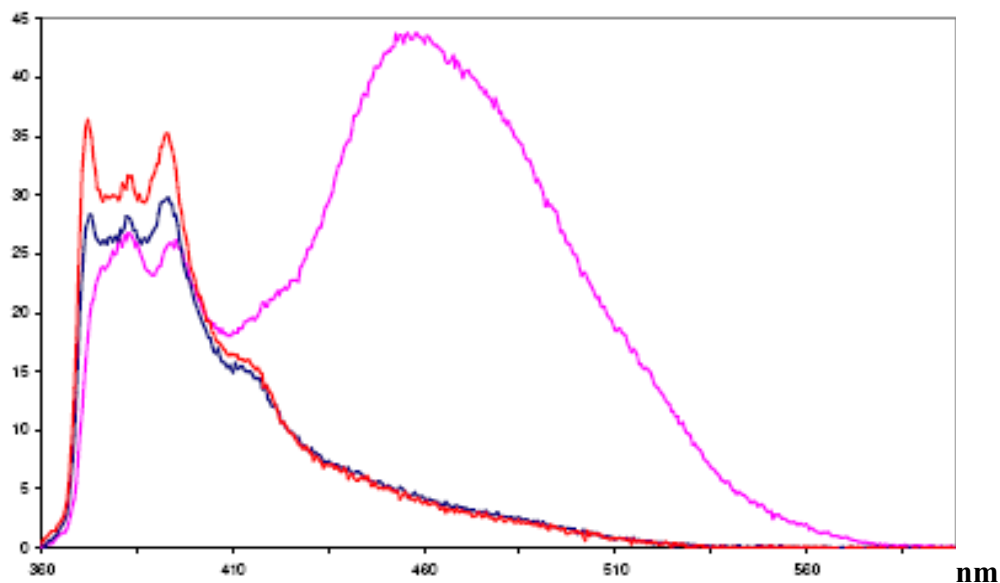


Figure S9. Steady-state fluorescence emission spectra of M^3 containing conjugates: **ON11**, **ON13** and **ON11:ON13** (shown in *red*, *blue* and *pink*, respectively). Spectra were obtained in medium salt buffer at 19 °C using an excitation wavelength of 345 nm and 0.1 μ M concentrations of ONs and complementary strands.

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