

# Aggregation-Induced Emission Controlled by DNA Hybridization

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## 1. Synthetic and analytical procedures

Oligonucleotides were prepared via automated oligonucleotide synthesis by a standard synthetic procedure ('trityl-off' mode) on a 394-DNA/RNA synthesizer (Applied Biosystems). Coupling times for the DATPE phosphoramidites **6** and **7** were elongated to 120 seconds and 1,2-dichloroethane (0.1M building blocks) was used as the solvent instead of acetonitrile. Cleavage from the solid support and final deprotection was done by treatment with 30%  $\text{NH}_4\text{OH}$  solution at 55 °C overnight. All oligonucleotides were purified by reverse phase HPLC (Li Chrospher 100 RP-18, 5  $\mu\text{m}$ , Merck, Bio-Tek Instruments); eluent A =  $(\text{Et}_3\text{NH})\text{OAc}$  (0.1 M, pH 7.4); eluent B = MeCN; elution at 20°C; gradient 20-40% B over 30 min. Mass spectrometry of oligonucleotides was performed with a Sciex QSTAR pulsar (hybrid quadrupole time-of-flight mass spectrometer, Applied Biosystems). The method used: ESI-MS in negative mode,  $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{TEA}$ .

Extinction coefficient  $35000 \text{ M}^{-1}\text{cm}^{-1}$  (at 260 nm) for DATPE units was used for concentration determination.

Thermal denaturation experiments were carried out on Varian Cary-100 Bio-UV/VIS spectrophotometer equipped with a Varian Cary-block temperature controller and data were collected with Varian Win UV software at 260 nm and 335 nm (cooling-heating-cooling cycles in the temperature range of 20-70 °C, temperature gradient of 0.5 °C/min). Melting temperature ( $T_m$ ) values were determined as the maximum of the first derivative of the smoothed melting curve. Temperature dependent UV spectra were collected with an optic path of 1 cm over the range of 210-500 nm at 20-70 °C with a 10 °C interval on Varian Cary-100 Bio-UV/VIS spectrophotometer equipped with a Varian Cary-block temperature controller. The oligonucleotide concentration used for UV measurements was 1  $\mu\text{M}$  single strand in all cases.

CD spectra were recorded on a JASCO J-715 spectrophotometer using quartz cuvettes with an optical path of 1 cm.

Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller using 1 cm x 1 cm quartz cuvettes. The oligonucleotide concentration used for fluorescence measurements was 1  $\mu$ M single strand in all cases.

Quantum yields were calculated by using quinine sulphate in 0.05 M H<sub>2</sub>SO<sub>4</sub> at 20 °C as the standard.

The crystals were mounted in air and used for X-ray structure determination at ambient conditions. All measurements were made on a Oxford Diffraction SuperNova area-detector diffractometer using mirror optics monochromated Mo K $\alpha$  radiation ( $\lambda$  = 0.71073 Å) and Al filtered. Data reduction was performed using the *CrysAlisPro* program. The structure was solved by direct methods using *SHELXS-97*.

## 2. Synthesis and characterization of building blocks

### 1,2-bis(4-bromophenyl)-1,2-diphenylethene (1)

Under an N<sub>2</sub> atmosphere, a two-necked flask was charged with 4-bromobenzophenone (2.61 g, 10 mmol), zinc dust power (1.96 g, 30 mmol) and 60 mL anhydrous THF. The suspension was cooled down to 0 °C, and TiCl<sub>4</sub> (1.65 mL, 15 mmol) was slowly added by a syringe, then warmed to room temperature and refluxed overnight. Afterward, the reaction mixture was cooled to room temperature, and the reaction was treated with 50 mL 10% K<sub>2</sub>CO<sub>3</sub> aqueous solution, then filtrated and extracted by DCM three times. The organic layers were combined and dried over MgSO<sub>4</sub>. After filtration and solvent evaporation, the oil residue was poured in to 50 mL methanol, and the precipitation was collected and dried. A *E*-/*Z*- mixture of 1,2-bis(4-bromophenyl)-1,2-diphenylethene was obtained as a white solid in 76 % yield (1.85 g).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, ppm): δ7.11-7.18 (m, 4H), 7.00-7.06 (m, 6H), 6.88-6.94 (m, 4H), 6.77-6.82 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, ppm): δ143.0, 142.9, 142.4, 142.3, 140.3, 132.9, 131.3, 131.2, 131.0, 128.1, 127.9, 127.0, 126.9, 120.9, 120.7. ESI-MS: Calcd for C<sub>26</sub>H<sub>19</sub>Br<sub>2</sub> 490.9 [M+H]<sup>+</sup>, Found 490.9 [M+H]<sup>+</sup>.

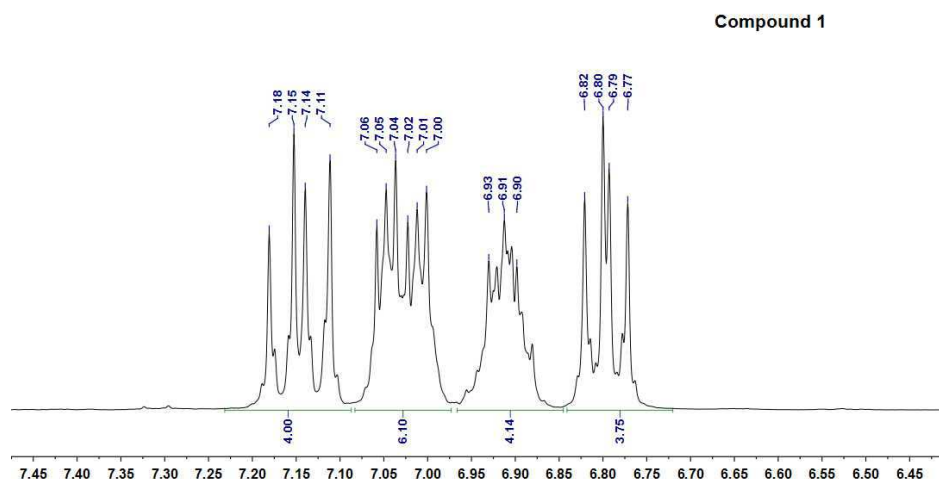


Fig.S1 <sup>1</sup>H-NMR of compound 1

**(E)-4,4'-((1,2-diphenylethene-1,2-diyl)bis(4,1-phenylene))bis(but-3-yn-1-ol)**  
**(2)**  
**(Z)-4,4'-((1,2-diphenylethene-1,2-diyl)bis(4,1-phenylene))bis(but-3-yn-1-ol)**  
**(3)**

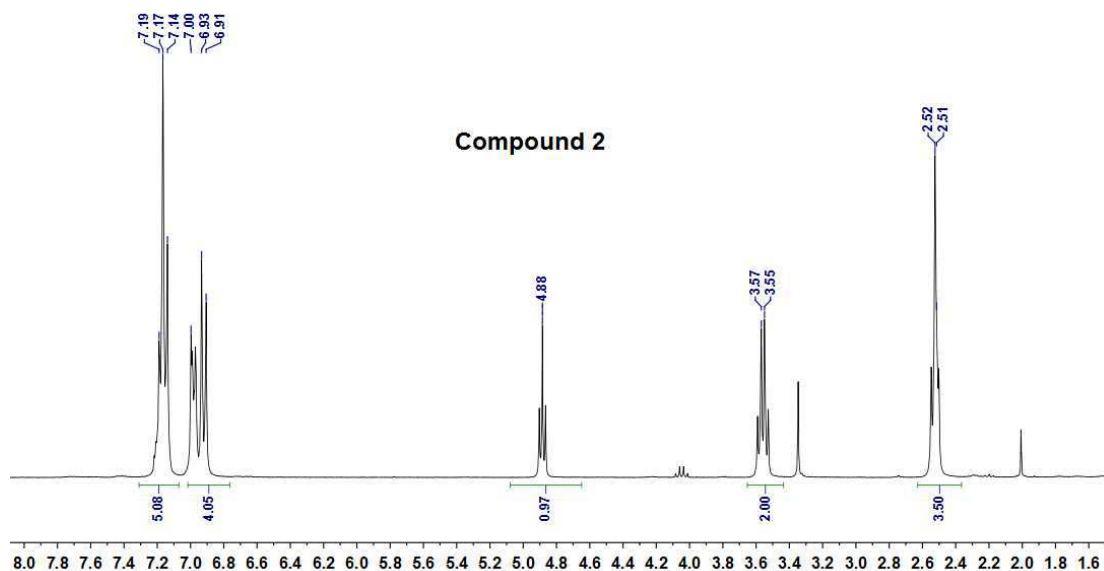
The mixture of 1,2-bis(4-bromophenyl)-1,2-diphenylethene (2.00 g, 4.08 mmol), bis(triphenylphosphine)palladium(II) chloride (69.0 mg, 0.096 mmol) and copper(I) iodide (9.2 mg, 0.048 mmol) were suspended in THF (50 mL) under argon and heated to 70 °C. After addition of freshly degassed triethylamine (30 mL), 3-butyne-1-ol (1.60 mL, 24.5 mmol) was added to the mixture which was stirred at this temperature overnight. After cooling to room temperature, the solvents were removed under reduced pressure. The residue was dissolved in THF (10 mL), filtered through celite, evaporated to dryness, re-dissolved in ethyl acetate (10 mL), washed with aqueous citric acid (10%) and saturated NaHCO<sub>3</sub> solution. After drying with MgSO<sub>4</sub> and filtration, the solution was evaporated to dryness. After adsorption to silica, the product was purified by column chromatography (SiO<sub>2</sub>; CH<sub>2</sub>Cl<sub>2</sub>/ Hexane 1:1 + 1% MeOH) furnished **2** (370 mg, R<sub>f</sub>=0.35 in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99:1 ) and **3** (360 mg, R<sub>f</sub>=0.30 in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99:1). Both isomers were obtained as light yellow solids.

The crystals of **2** or **3** were obtained by diffusion of hexane into their respective DCM solutions, and have been determined by X-ray single crystal diffraction analysis.

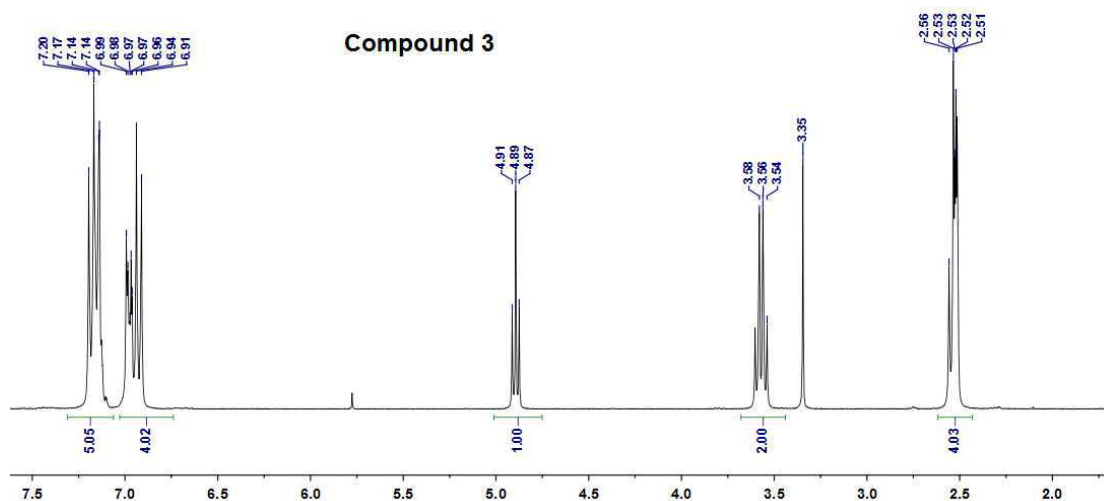
Analytical data for **2**: <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): δ 7.14-7.19 (m, 10H), 6.99 (d, *J*=2.4 Hz, 2H), 6.97 (d, *J*=1.5 Hz, 2H), 6.92 (d, *J*=8.4 Hz, 4H), 4.89 (t, *J*=5.4 Hz, 2H), 3.56 (m, 4H), 2.52 (m, 4H). <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>, ppm): δ 142.6, 140.4, 130.8, 130.7, 130.6, 128.0, 126.8, 121.4, 89.0, 80.9, 59.7, 23.3. ESI-MS: Calcd for C<sub>34</sub>H<sub>29</sub>O<sub>2</sub> 469.2 [M+H]<sup>+</sup>, Found 469.22 [M+H]<sup>+</sup>.

Analytical data for **3**: <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): δ 7.14-7.20 (m, 10H), 6.99 (d, *J*=2.4 Hz, 2H), 6.96 (d, *J*=1.8 Hz, 2H), 6.93 (d, *J*=8.4 Hz,

4H), 4.88 (t,  $J=5.7$  Hz, 2H), 3.57 (m, 4H), 2.52 (m, 4H).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ , ppm):  $\delta$  142.6, 140.4, 130.8, 130.6, 127.9, 126.8, 121.4, 89.0, 80.9, 59.7, 23.3. ESI-MS: Calcd for  $\text{C}_{34}\text{H}_{29}\text{O}_2$  469.2  $[\text{M}+\text{H}]^+$ , Found 469.22  $[\text{M}+\text{H}]^+$ .



**Fig.S2**  $^1\text{H}$ -NMR of compound 2

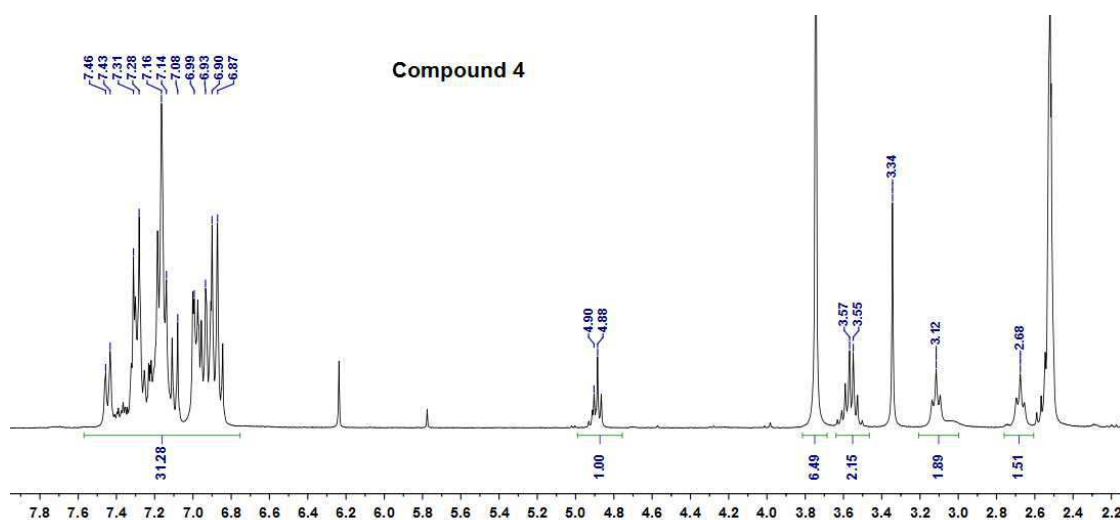


**Fig.S3**  $^1\text{H}$ -NMR of compound 3

**(*E*)-4-(4-(2-(4-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)but-1-yn-1-yl)phenyl)-1,2-diphenylvinyl)phenyl)but-3-yn-1-ol (4)**

Compound **2** (400 mg, 0.86 mmol) was co-evaporated with anhydrous pyridine (20 mL). The residue obtained was dissolved in pyridine (10 mL) under argon atmosphere. 4-dimethylaminopyridine (122 mg, 1.0 mmol), 4,4'-dimethoxytrityl chloride (305 mg, 0.9 mmol) in 5 mL THF was added dropwise and the reaction mixture was stirred at room temperature for 2 hours. Then pyridine and THF was removed under vacuum and the residue was then taken up in 40 mL EtOAc, filtrated and washed with citric acid (10%) and saturated NaHCO<sub>3</sub> and dried over MgSO<sub>4</sub>. EtOAc was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel and eluted with the mixture of CH<sub>2</sub>Cl<sub>2</sub> and hexane (1:1) plus with 1% MeOH and 2% triethylamine, then evaporated and dried under high vacuum to furnish 190 mg (29%) of **4** in light yellow foam.

<sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): 7.48-7.05 (m, 19H), 7.00-6.84(m, 12H), 4.88 (t, J=5.7 Hz, 2H), 3.74 (m, 6H), 3.57 (m, 2H), 3.12 (m, 2H), 2.68 (m, 2H), 2.52 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>, ppm): 158.1, 157.8, 144.9, 142.8, 142.6, 140.5, 140.4, 140.2, 135.7, 130.8, 130.7, 128.9, 127.6, 113.2, 112.8, 59.7, 55.0, 26.3, 23.2. ESI-MS: Calcd for C<sub>55</sub>H<sub>47</sub>O<sub>4</sub>771.34 [M+H]<sup>+</sup>, Found 771.32 [M+H]<sup>+</sup>.

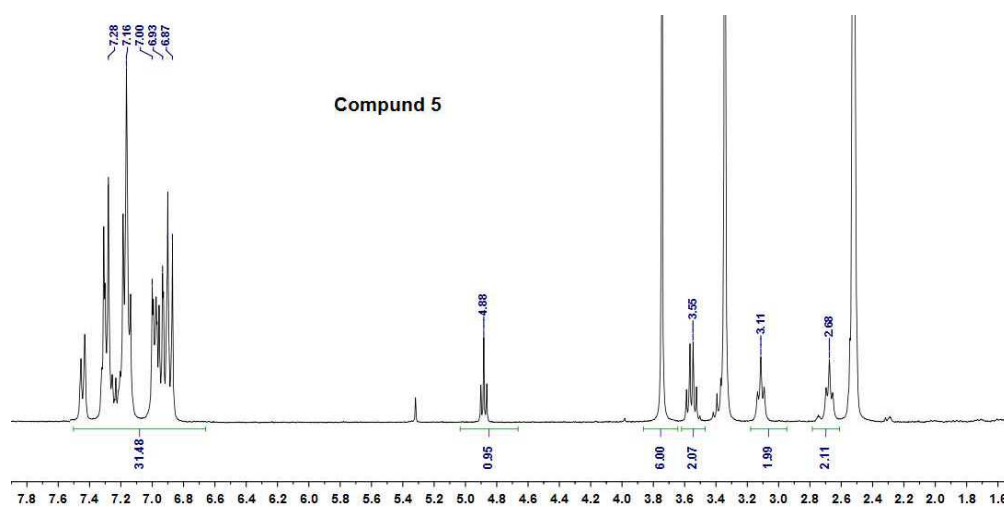


**Fig.S4** <sup>1</sup>H-NMR of compound **4**

**(Z)-4-(4-(2-(4-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)but-1-yn-1-yl)phenyl)-1,2-diphenylvinyl)phenyl)but-3-yn-1-ol (5)**

Compound **3** (300 mg, 0.64 mmol) was co-evaporated with anhydrous pyridine (15 mL). The residue obtained was dissolved in pyridine (7 mL) under argon atmosphere. 4-dimethylaminopyridine (85 mg, 0.7 mmol), 4,4'-dimethoxytrityl chloride (237 mg, 0.7 mmol) in 4 mL THF was added dropwise and the reaction mixture was stirred at room temperature for 2 hour. Then pyridine and THF was removed under vacuum and the residue was then taken up in 40 mL EtOAc, filtrated and washed with citric acid (10%) and saturated NaHCO<sub>3</sub> and dried over MgSO<sub>4</sub>. EtOAc was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel and eluted with the mixture of CH<sub>2</sub>Cl<sub>2</sub> and hexane (1:1) plus with 1% MeOH and 2% triethylamine. The product fractions were combined, evaporated and dried under high vacuum to furnish 113 mg (23%) of **5** as yellow white foam.

<sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): 7.48-7.08 (m, 19H), 7.02-6.84(m, 12H), 4.88 (t, J=5.7 Hz, 2H), 3.74 (m, 6H), 3.57 (m, 2H), 3.12 (m, 2H), 2.68 (m, 2H), 2.52 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>, ppm): 158.1, 157.8, 144.9, 142.8, 142.6, 140.5, 140.4, 140.2, 135.7, 130.8, 130.7, 128.9, 127.6, 113.2, 112.8, 59.7, 55.0, 26.3, 23.2. ESI-MS: Calcd for C<sub>55</sub>H<sub>47</sub>O<sub>4</sub> 771.34 [M+H]<sup>+</sup>, Found 771.32 [M+H]<sup>+</sup>.



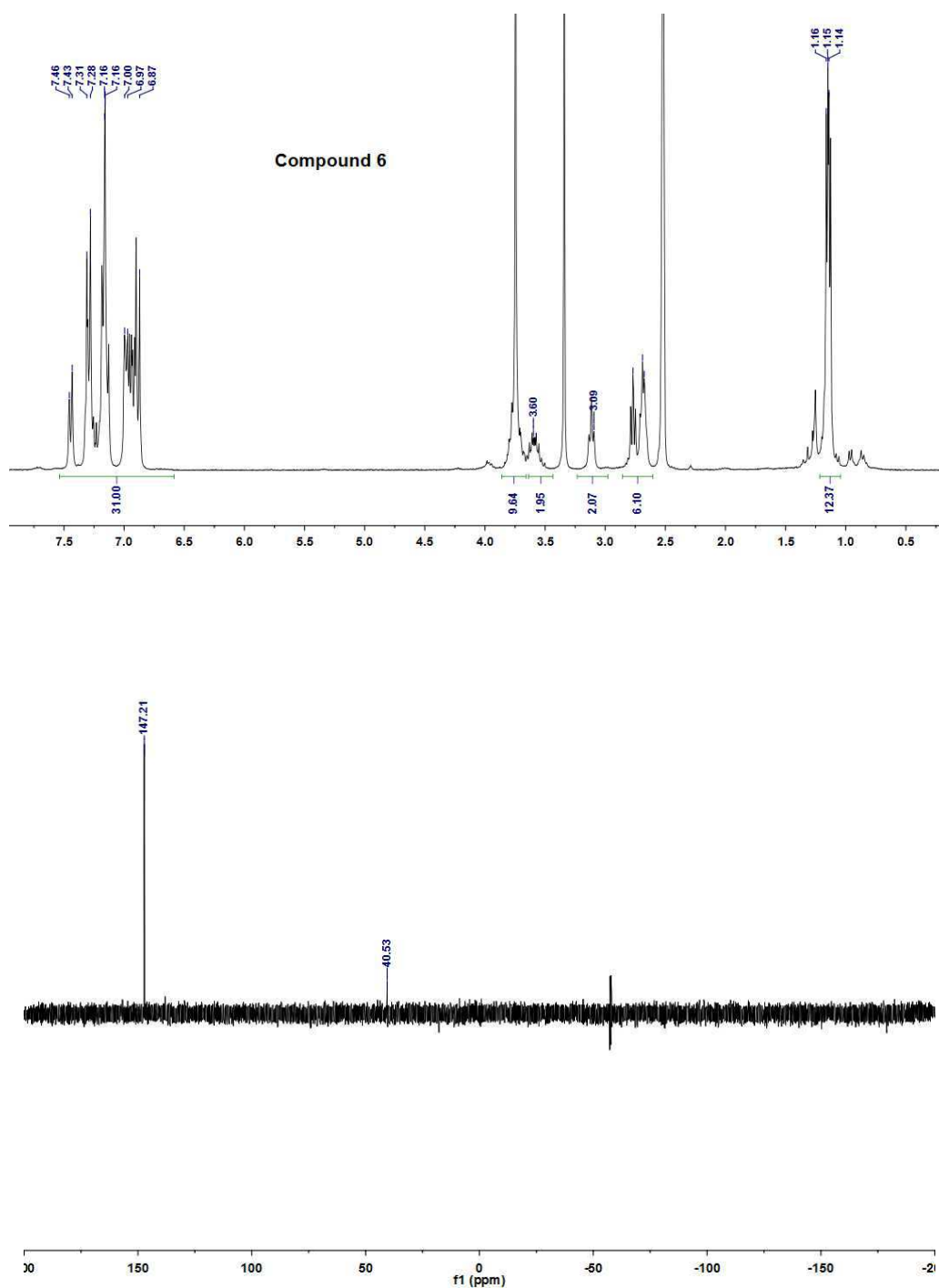
**Fig.S5** <sup>1</sup>H-NMR of compound **5**



**(*E*)-4-(4-(2-(4-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)but-1-yn-1-yl)phenyl)-1,2-diphenylvinyl)phenyl)but-3-yn-1-yl (2-cyanoethyl) diisopropylphosphoramidite (**6**)**

400 mg (0.52 mmol) of **4** was dissolved in 10 mL CH<sub>2</sub>Cl<sub>2</sub> and 268 µL (1.56 mmol) diisopropylethylamine. 130 mg (0.55 mmol) of 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite was then added dropwise at room temperature under argon. The reaction mixture was stirred for 1 hour. The volume of CH<sub>2</sub>Cl<sub>2</sub> was then reduced and a column chromatography over silica gel was directly performed with pure CH<sub>2</sub>Cl<sub>2</sub> with 2% triethylamine. After evaporated and dried, 309 mg (61%) of **6** yields as yellow white foam.

<sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): δ7.48-7.13 (m, 19H), 7.00-6.87 (m, 12H), 3.74 (m, 8H), 3.61 (m, 2H), 3.11 (m, 2H), 2.79-2.68 (m, 6H), 1.15 (m, 12H). <sup>31</sup>P-NMR (121.5MHz, DMSO-d<sub>6</sub>): δ147.21. ESI-MS: Calcd for C<sub>64</sub>H<sub>63</sub>N<sub>2</sub>O<sub>5</sub>PK 1010.41 [M+K]<sup>+</sup>, Found 1009.3 [M+K]<sup>+</sup>.



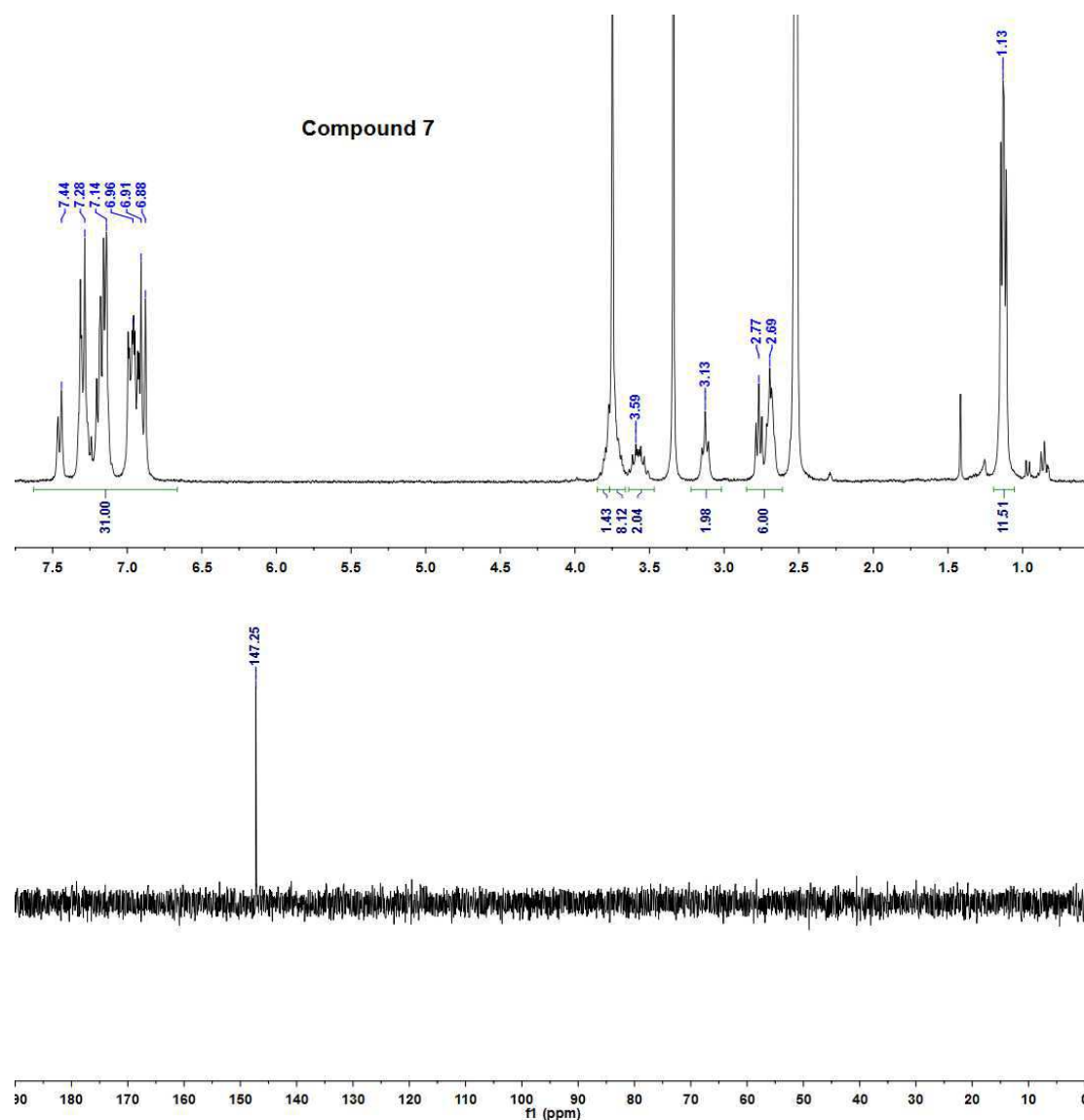
**Fig.S6.**  $^1\text{H}$ -NMR and  $^{31}\text{P}$ -NMR of compound **6**

**(Z)-4-(4-(2-(4-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)but-1-yn-1-yl)phenyl)-1,2-diphenylvinyl)phenyl)but-3-yn-1-yl (2-cyanoethyl) diisopropylphosphoramidite (7)**

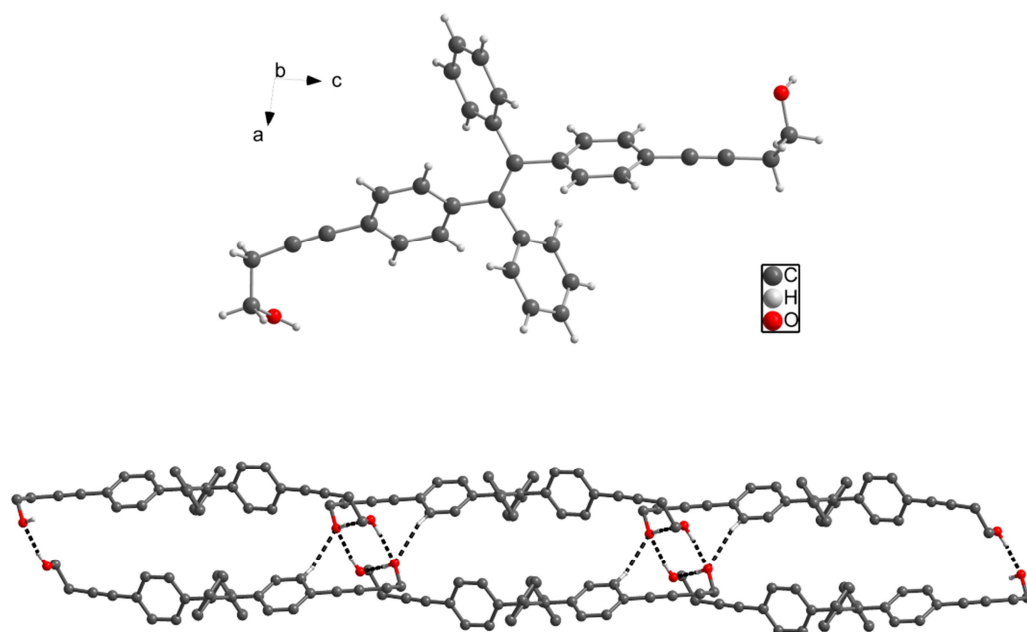
The synthesis and separations are keeping consistent with compound **6**. 260 mg of **5** was used and resulted 232 mg of **7** (71%) as yellow foam.

<sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>, ppm): 7.46-7.14 (m, 19H), 7.00-6.87 (m, 12H), 3.74 (m, 8H), 3.61 (m, 2H), 3.11 (m, 2H), 2.79-2.68 (m, 6H), 1.15 (m, 12H).

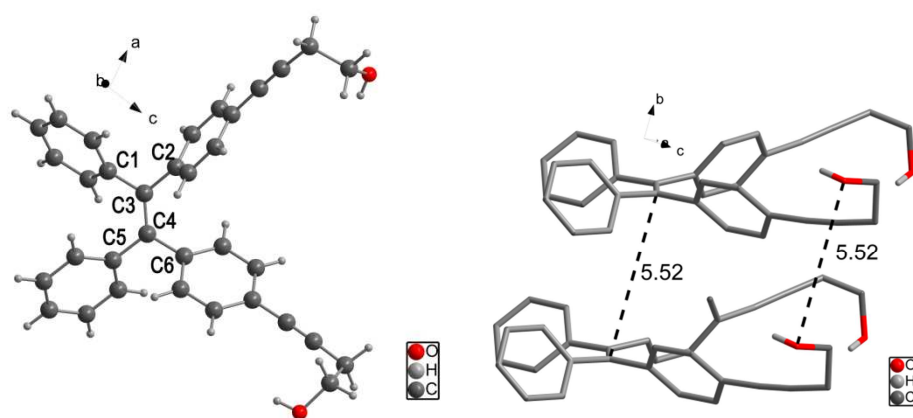
<sup>31</sup>P-NMR (121.5 MHz, DMSO-d<sub>6</sub>): 147.25. ESI-MS: Calcd for C<sub>64</sub>H<sub>63</sub>N<sub>2</sub>O<sub>5</sub>PK 1010.41 [M+K]<sup>+</sup>, Found 1009.3 [M+K]<sup>+</sup>.



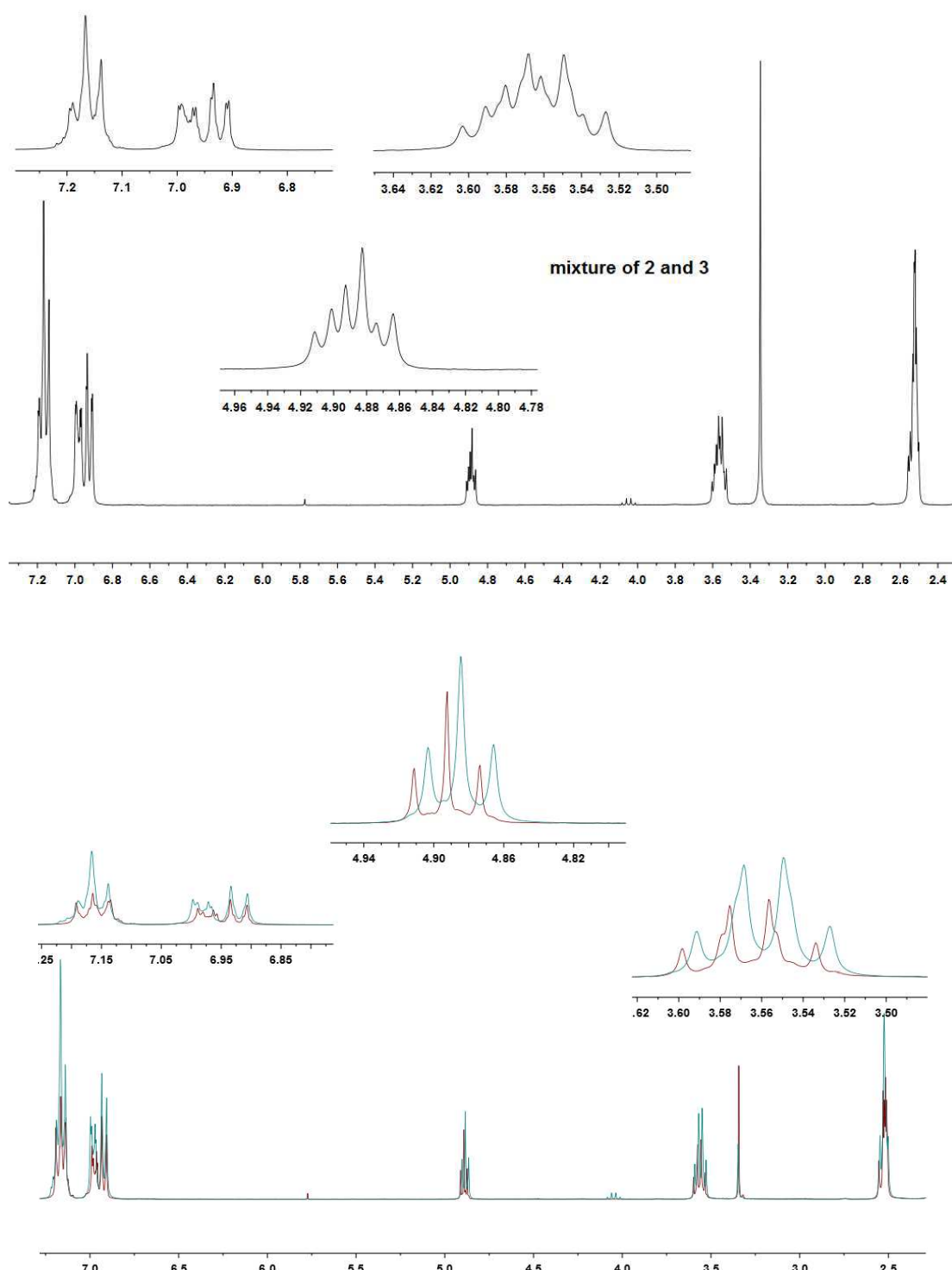
**Fig.S7** <sup>1</sup>H-NMR and <sup>31</sup>P-NMR of compound **7**



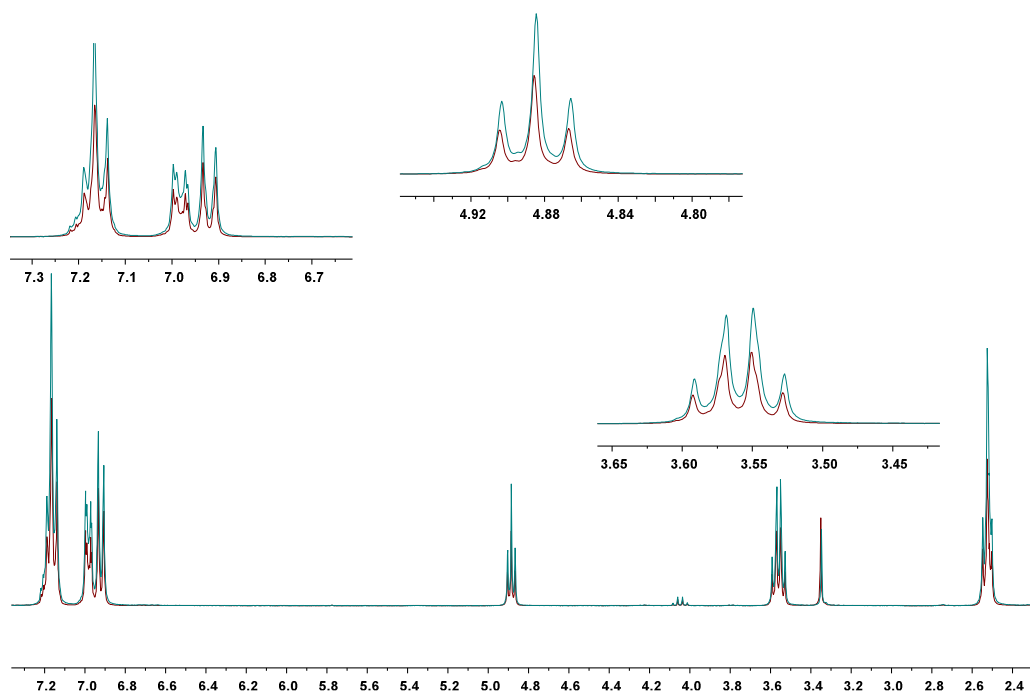
**Fig.S8** The crystal structure of compound **2** (CCDC 932688) and its packing mode



**Fig. S9** The crystal structure of compound **3** (CCDC 932687) and its packing mode

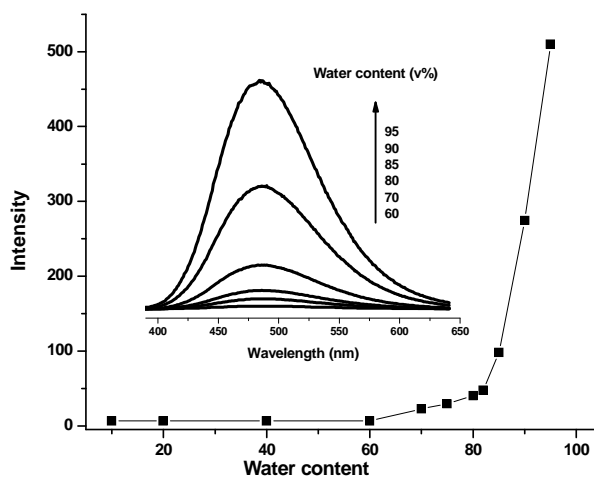


**Fig. S10** <sup>1</sup>H-NMR spectra of the mixture of *E/Z*-isomers (upper); superposition of the <sup>1</sup>H-NMR spectra for the pure compounds **2** and **3** (**2** in green and **3** in red).



**Fig. S11** Superposition of the  $^1\text{H}$  NMR spectra of compound **2** before (green) and after irradiation (365nm, 8W, 30 min) followed by thermal treatment (90 °C, 120 min) in  $\text{DMSO}-d_6$ . The experiment shows that irradiation or thermal treatment does not result in *E/Z*-isomerization.

### 3. AIE properties of the building block *E*-DATPE



**Fig.S12** Changes in the fluorescence intensity of *E*-DATPE with variation of the water/THF ratio; (insert) fluorescence spectra of *E*-DATPE in THF/water mixtures with different contents of water;  $\lambda_{\text{ex}} = 335 \text{ nm}$ ,  $[\text{DATPE}] = 10 \mu\text{M}$ .

## 4. MS data of oligonucleotides

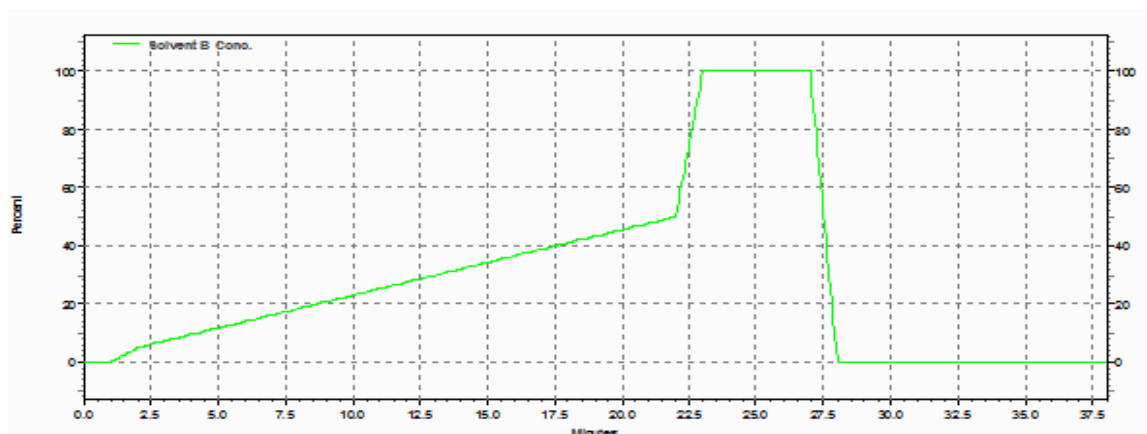
Table S1. DNA sequences used in this work

		Sequence
<b>D<sub>R</sub></b>	<b>R1</b>	5' -AGC TCG GTC ATC GAG AGT GCA
	<b>R2</b>	3' -TCG AGC CAG TAG CTC TCA CGT
<b>D1</b>	<b>ON1</b>	5' -AGC TCG GTC <b>AM<sub>E</sub>C</b> GAG AGT GCA
	<b>ON2</b>	3' -TCG AGC CAG <b>TM<sub>E</sub>G</b> CTC TCA CGT
<b>D2</b>	<b>ON3</b>	5' -AGC TCG GTC <b>AM<sub>Z</sub>C</b> GAG AGT GCA
	<b>ON4</b>	3' -TCG AGC CAG <b>TM<sub>Z</sub>G</b> CTC TCA CGT
<b>D3</b>	<b>ON5</b>	5' -AGC TCG GTC <b>M<sub>E</sub>M<sub>E</sub>C</b> GAG AGT GCA
	<b>ON6</b>	3' -TCG AGC CAG <b>M<sub>E</sub>M<sub>E</sub>G</b> CTC TCA CGT
<b>D4</b>	<b>ON7</b>	5' -AGC TCG GTC <b>M<sub>Z</sub>M<sub>Z</sub>C</b> GAG AGT GCA
	<b>ON8</b>	3' -TCG AGC CAG <b>M<sub>Z</sub>M<sub>Z</sub>G</b> CTC TCA CGT

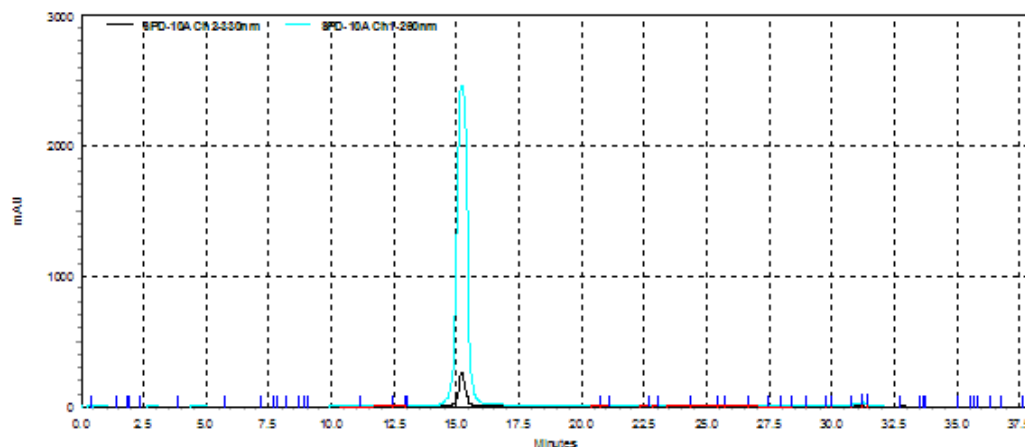
Number	Molecular formula	Calc. mass	Exp. mass
<b>ON1</b>	C <sub>229</sub> H <sub>271</sub> N <sub>81</sub> O <sub>120</sub> P <sub>20</sub>	6697.22	6697.20
<b>ON2</b>	C <sub>227</sub> H <sub>275</sub> N <sub>71</sub> O <sub>124</sub> P <sub>20</sub>	6599.15	6599.16
<b>ON3</b>	C <sub>229</sub> H <sub>271</sub> N <sub>81</sub> O <sub>120</sub> P <sub>20</sub>	6697.22	6697.20
<b>ON4</b>	C <sub>227</sub> H <sub>275</sub> N <sub>71</sub> O <sub>124</sub> P <sub>20</sub>	6599.15	6599.15
<b>ON5</b>	C <sub>253</sub> H <sub>286</sub> N <sub>76</sub> O <sub>119</sub> P <sub>20</sub>	6914.19	6914.28
<b>ON6</b>	C <sub>251</sub> H <sub>291</sub> N <sub>69</sub> O <sub>121</sub> P <sub>20</sub>	6825.12	6825.30
<b>ON7</b>	C <sub>253</sub> H <sub>286</sub> N <sub>76</sub> O <sub>119</sub> P <sub>20</sub>	6914.19	6914.32
<b>ON8</b>	C <sub>251</sub> H <sub>291</sub> N <sub>69</sub> O <sub>121</sub> P <sub>20</sub>	6825.12	6825.30
<b>R1</b>	C <sub>205</sub> H <sub>257</sub> N <sub>83</sub> O <sub>123</sub> P <sub>20</sub>	6471.24	6471.28
<b>R2</b>	C <sub>203</sub> H <sub>258</sub> N <sub>76</sub> O <sub>125</sub> P <sub>20</sub>	6382.18	6382.22

## 5. Analysis of oligonucleotides by HPLC

Column: Merck LiChroCART 250-4 (Li Chrospher 100, RP-18, 5  $\mu$ m); 0% (1 min), 0-4% (1 min) and 4-50% (20 min) MeCN in 0.1 M  $\text{NEt}_3$ \*HOAc @ 1 mL/min, 20  $^\circ\text{C}$ ; detection at 260 and 330 nm.

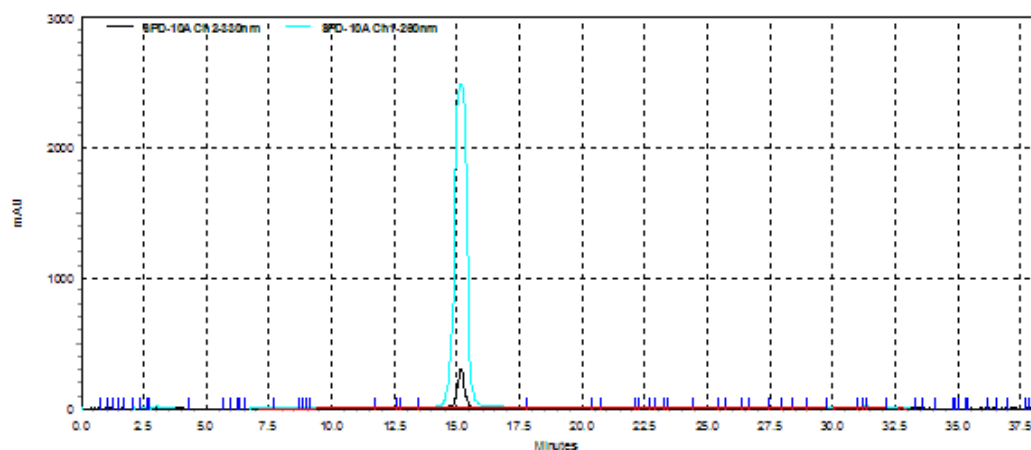


**Fig. S13** The method used in the HPLC.

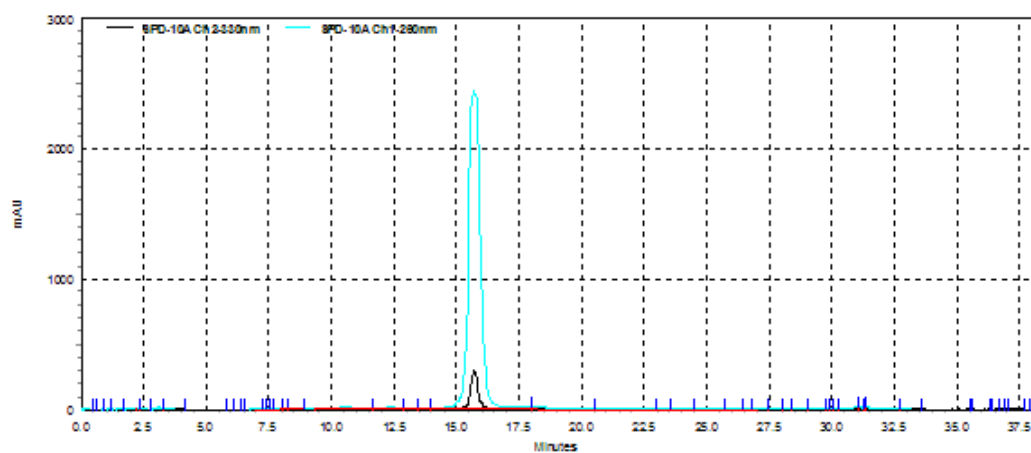


**Fig. S14** ON1 analyzed by HPLC (cyan: 260 nm; black: 330 nm)

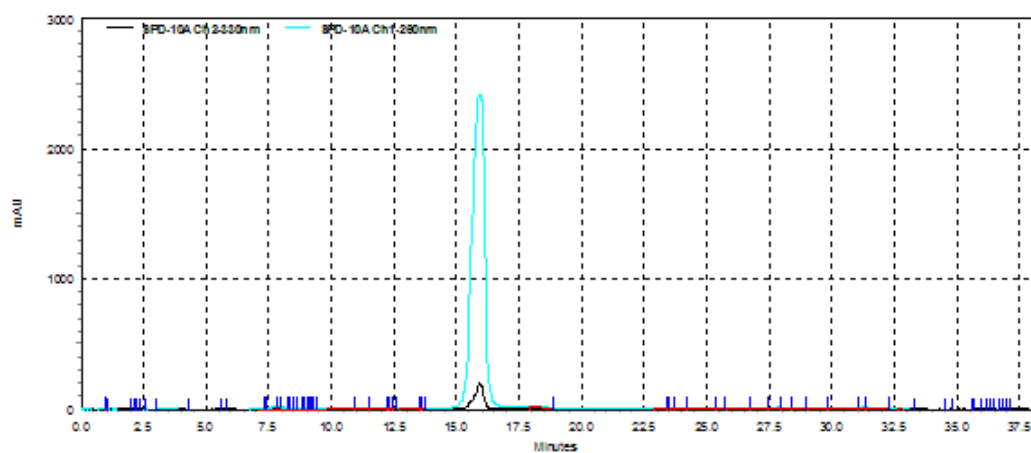




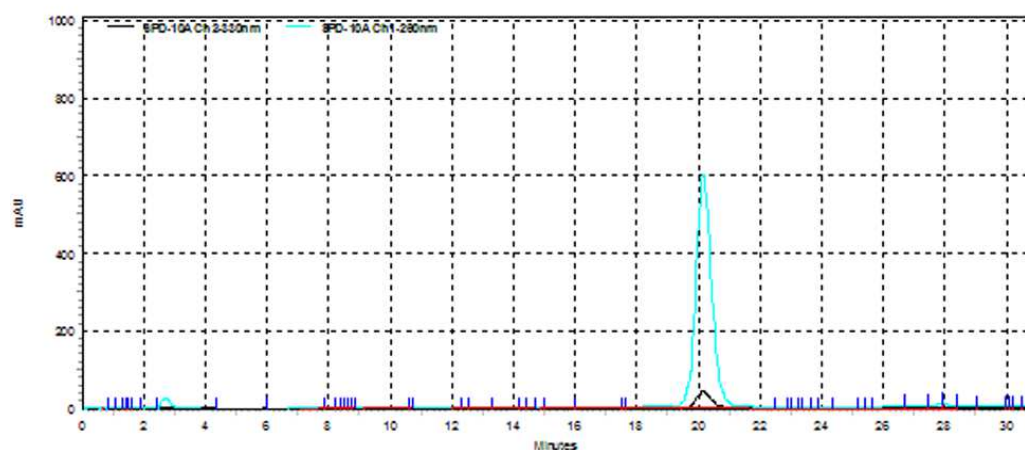
**Fig. S15** ON2 analyzed by HPLC (cyan: 260 nm; black: 330 nm)



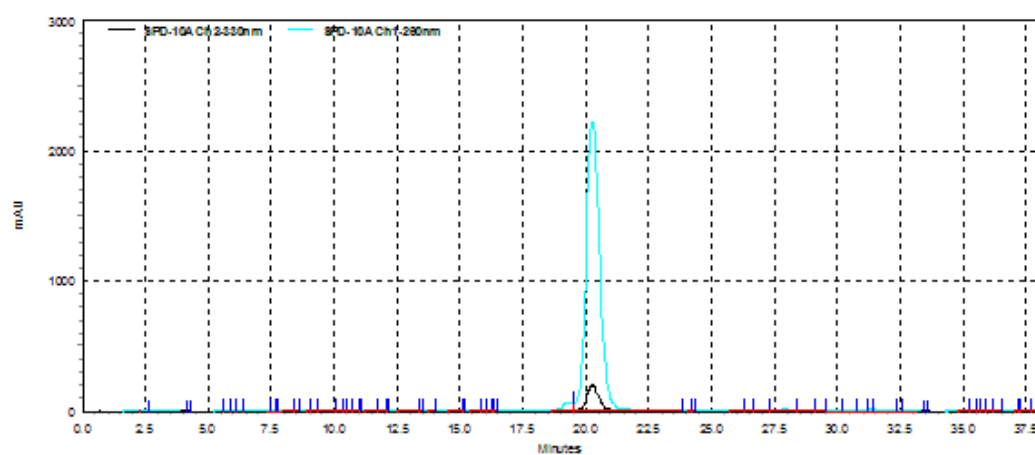
**Fig. S16** ON3 analyzed by HPLC (cyan: 260 nm; black: 330 nm)



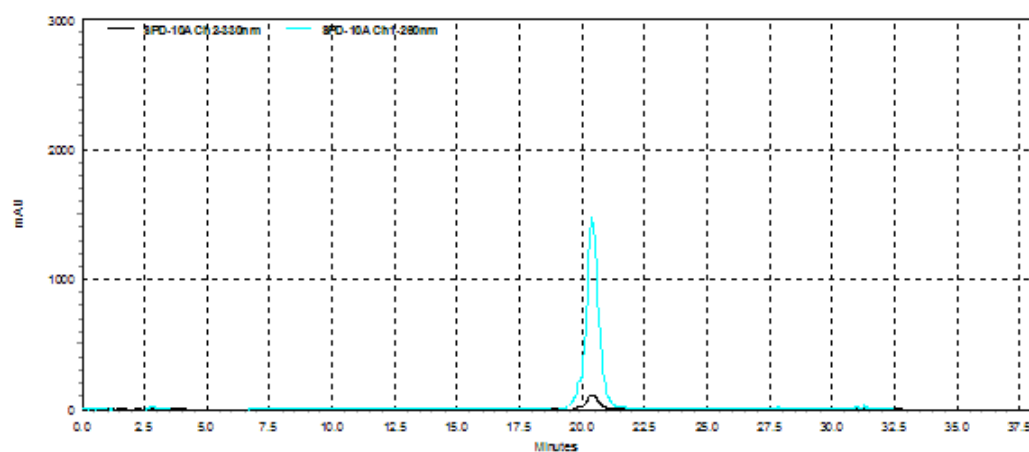
**Fig. S17** ON4 analyzed by HPLC (cyan: 260 nm; black: 330 nm)



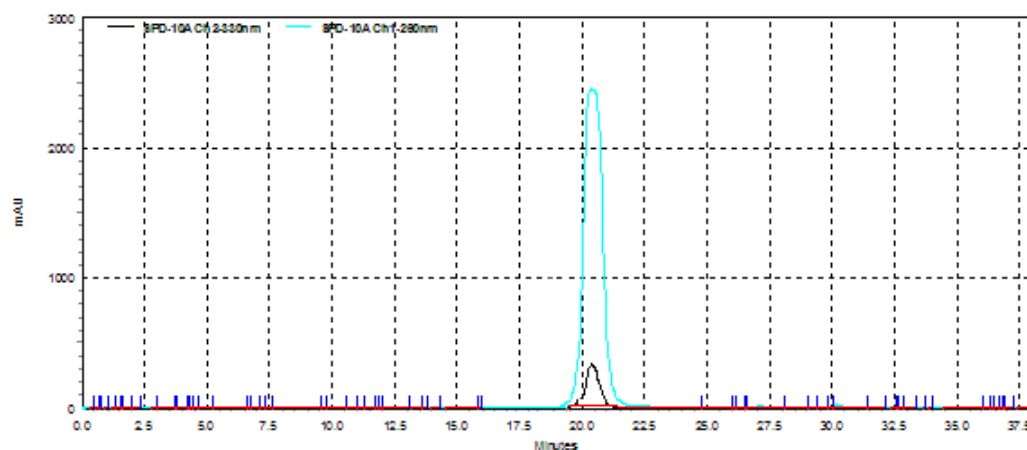
**Fig. S18 ON5** analyzed by HPLC (cyan: 260 nm; black: 330 nm)



**Fig. S19 ON6** analyzed by HPLC (cyan: 260 nm; black: 330 nm)

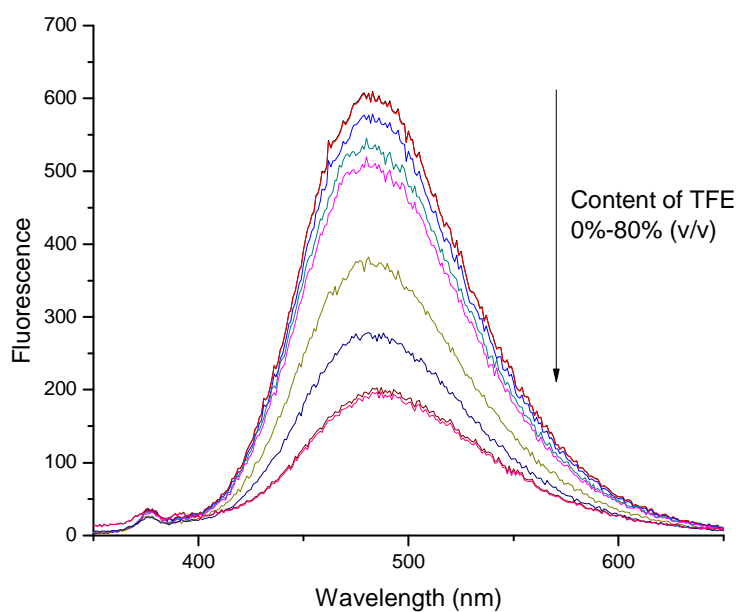


**Fig. S20 ON7** analyzed by HPLC (cyan: 260 nm; black: 330 nm)

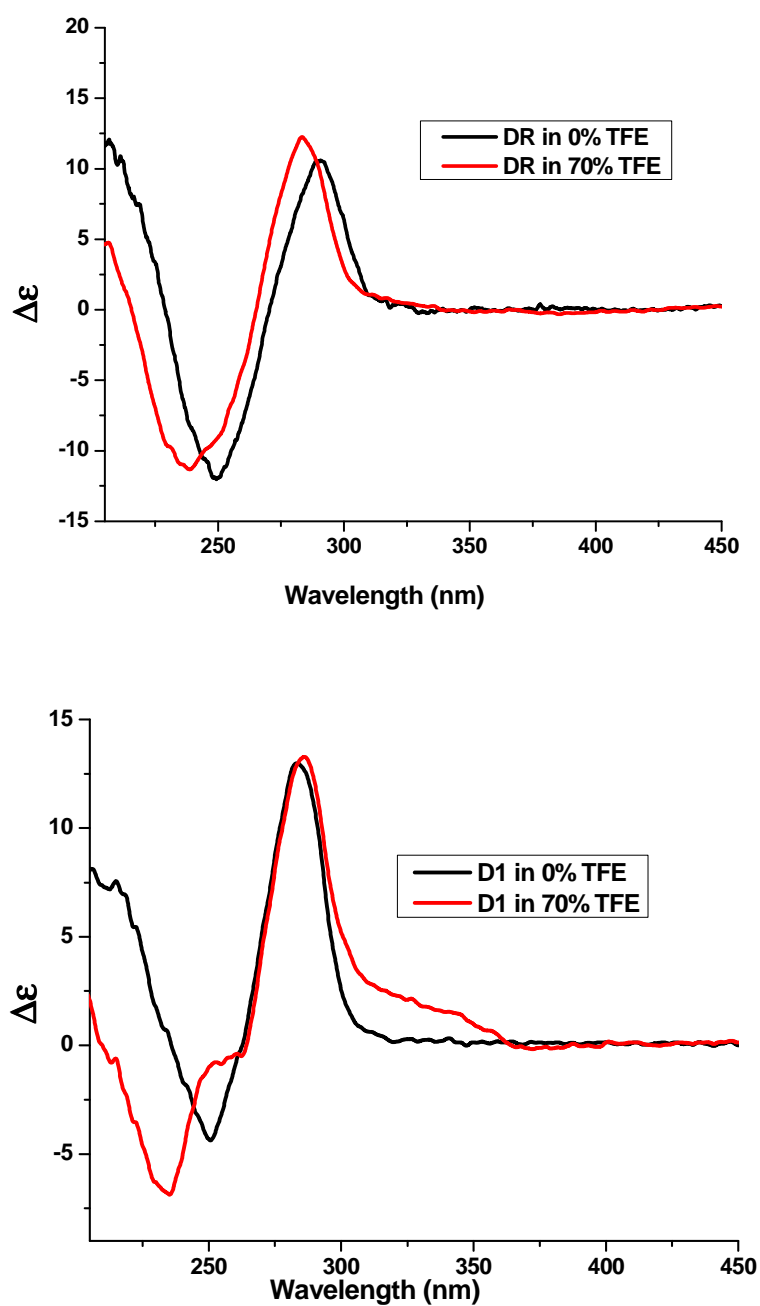


**Fig. S21** ON8 analyzed by HPLC (cyan: 260 nm; black: 330 nm)

## 6. The fluorescence and CD measurements in TFE-water



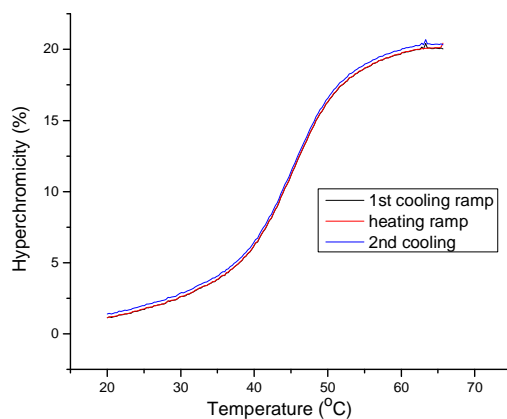
**Fig. S22.** The fluorescence emission of single strand **ON1** decreases with TFE content (1  $\mu$ M each strand, 100mM NaCl, 10mM sodium phosphate buffer, pH=7.4,  $\lambda_{\text{ex}}$ =335 nm; PMT=700V).



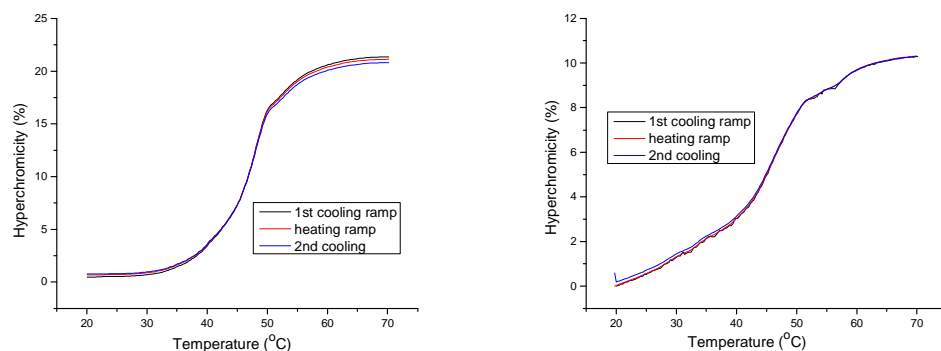
**Fig. S23.** Influence of TFE (70%, v/v) on the CD spectra of hybrids (top: **DR**; bottom: **D1**; 3  $\mu$ M each strand, 100 mM NaCl, 10 mM sodium phosphate buffer, pH=7.4).

## 7. Melting profiles of hybrids

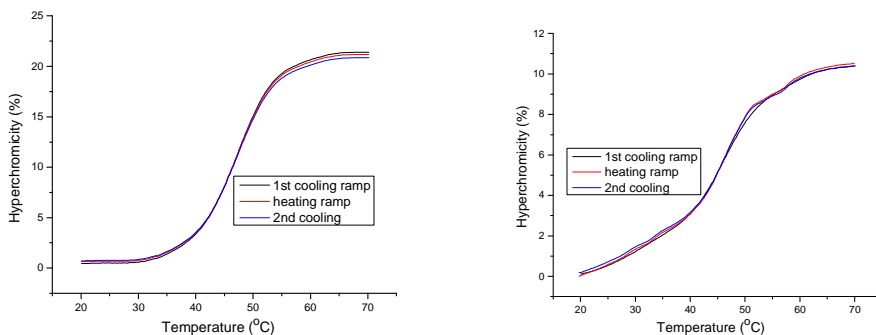
Conditions: TFE/water (70/30 v/v), 100mM NaCl, 10mM sodium phosphate buffer, pH=7.4; 0.5 °C/min.



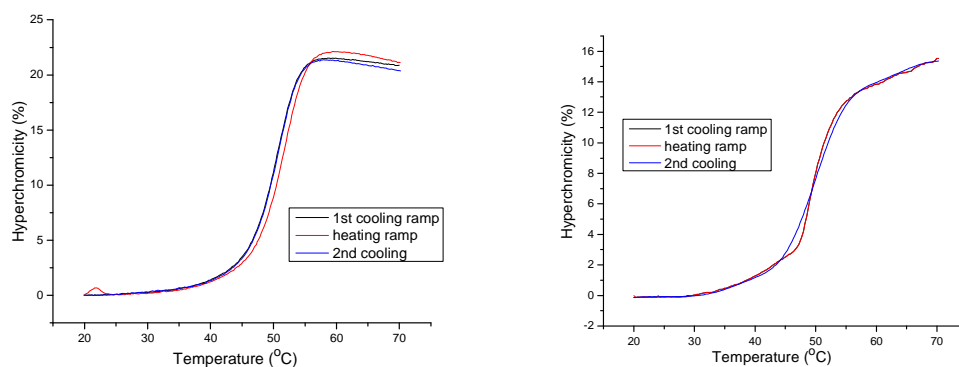
**Fig. S24** Cooling-heating-cooling cycle of hybrid **D<sub>R</sub>** monitored at 260 nm.



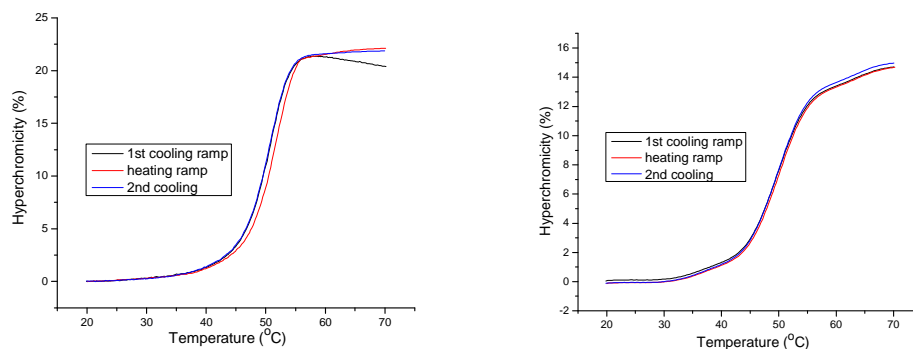
**Fig. S25** Cooling-heating-cooling cycle of hybrid **D1** monitored at 260 nm (left) and 335 nm (right).



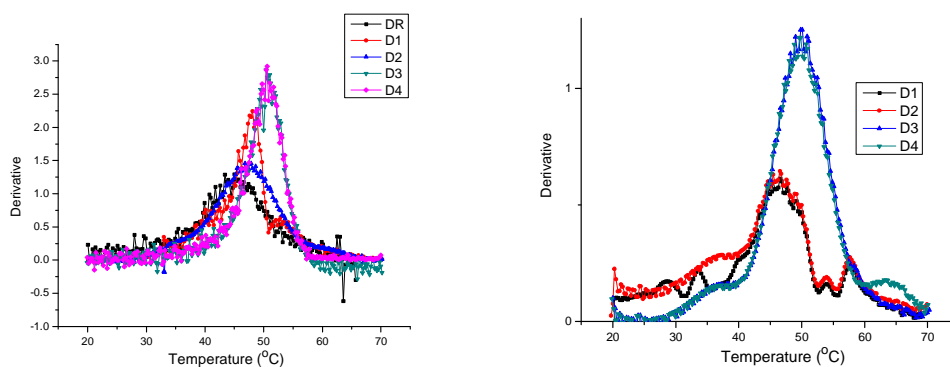
**Fig. S26** Cooling-heating-cooling cycle of hybrid **D2** monitored at 260 nm (left) and 335 nm (right).



**Fig. S27** Cooling-heating-cooling cycle of hybrid **D3** monitored at 260 nm (left) and 335 nm (right).



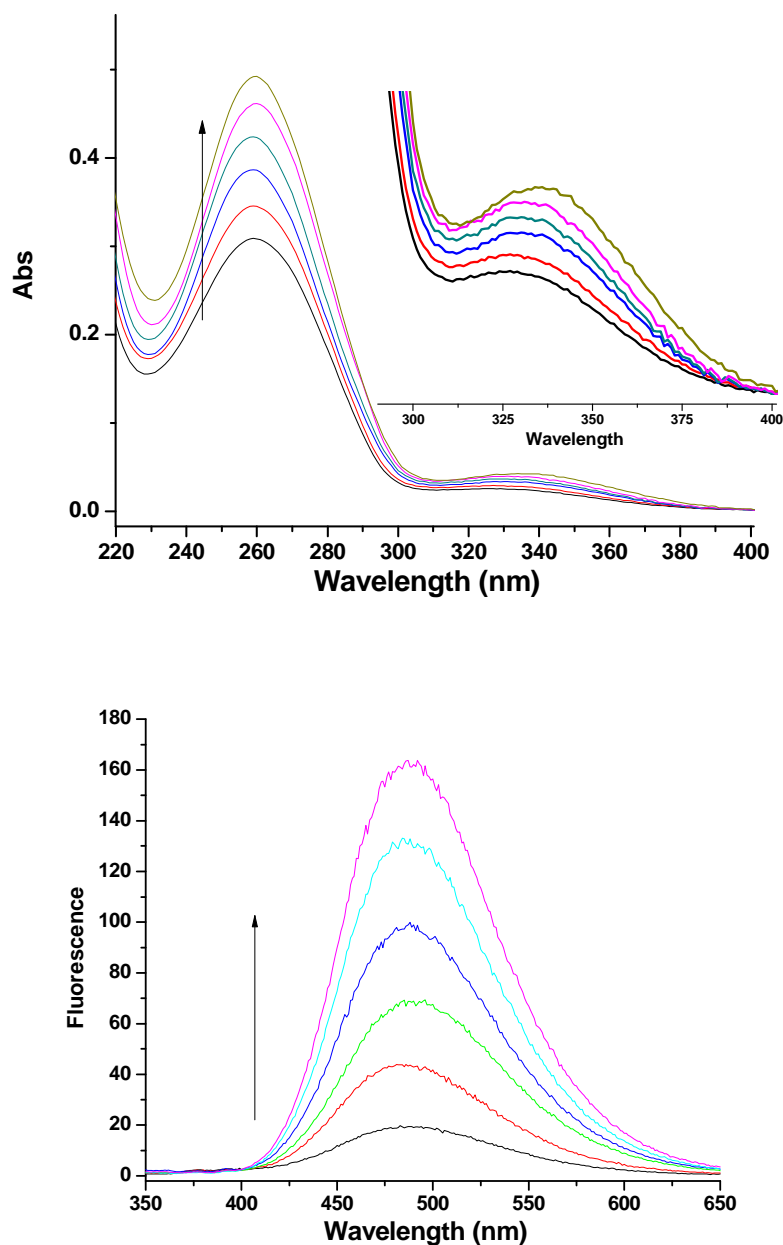
**Fig. S28** Cooling-heating-cooling cycle of hybrid **D4** monitored at 260 nm (left) and 335 nm (right).



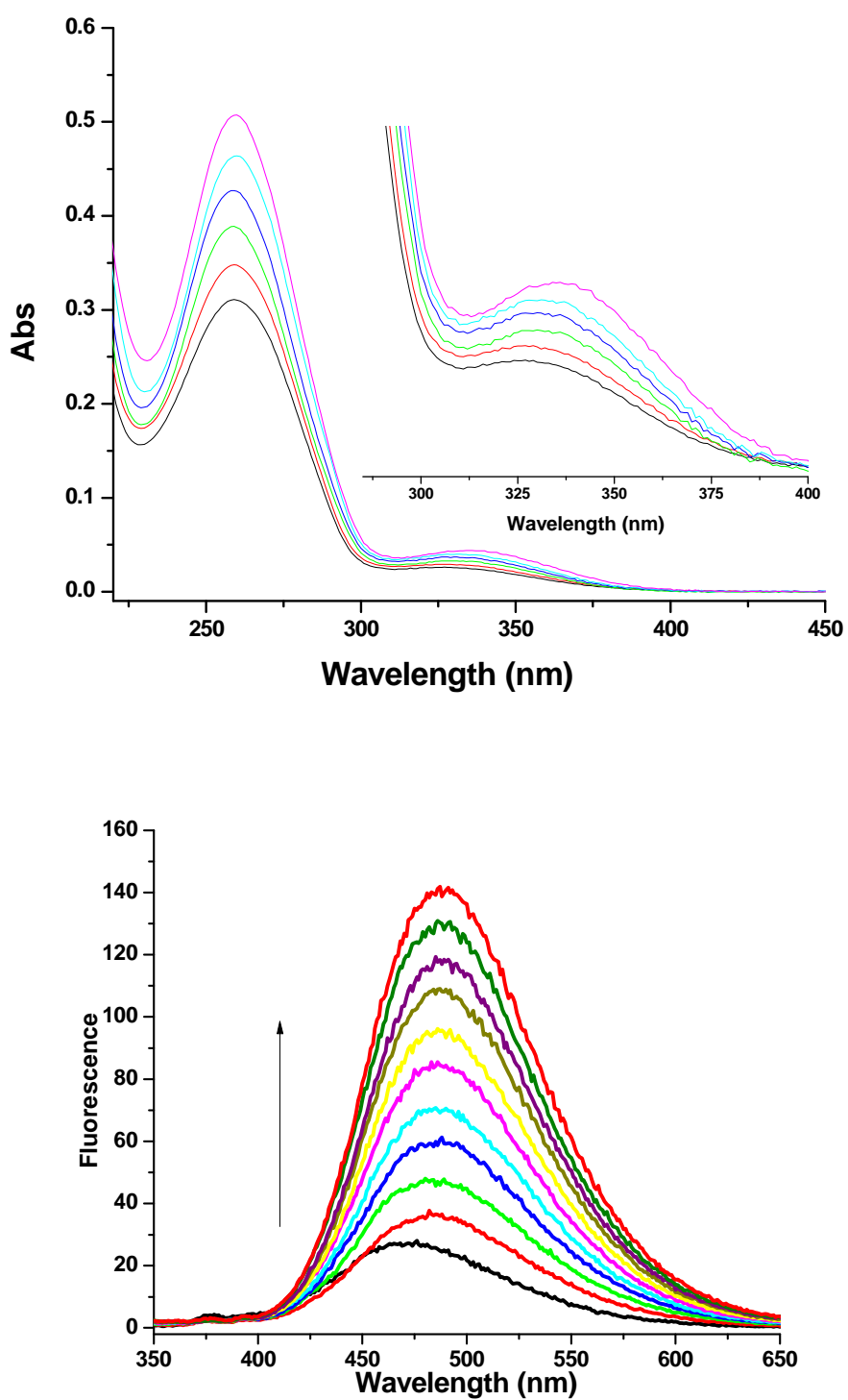
**Fig. S29** The 1<sup>st</sup> derivative of the second cooling ramp (cooling-heating-cooling cycle) monitored at 260 nm (left) and 335 nm (right) for all hybrids. The T<sub>m</sub> values are: **DR** = 45.0 °C, **D1** = 47.0 °C, **D2** = 47.0 °C, **D3** = 49.5 and **D4** = 50.0 °C.

## 8. UV/fluorescence titrations of hybrids D1-D4

Conditions: 1.0  $\mu\text{M}$  oligonucleotide (each strand), 10 mM sodium phosphate buffer (pH 7.4), 100 mM NaCl, TFE/water (70 /30 v/v).

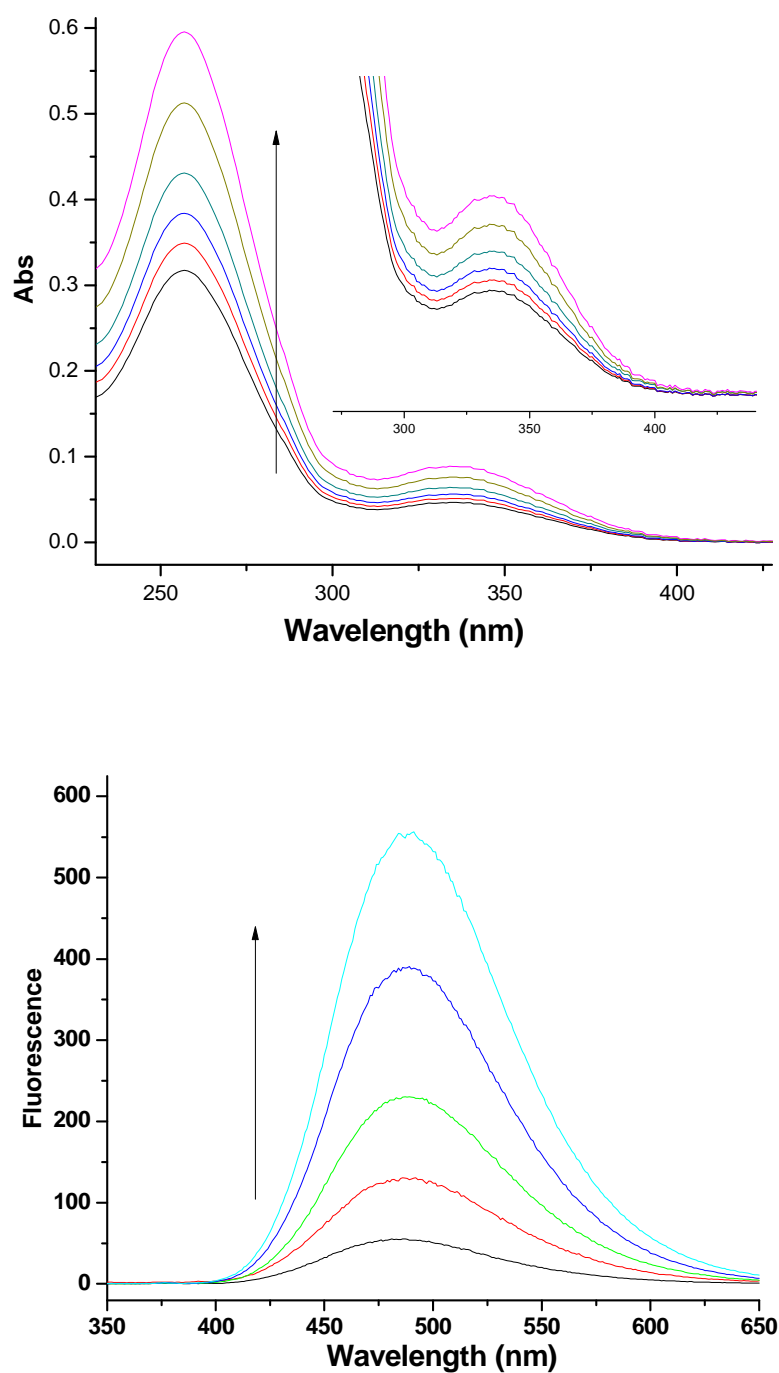


**Fig. S30** UV and fluorescence titration of **ON1** by addition of **ON2** (individual steps = 0.2  $\mu\text{M}$ ); arrows indicate increasing **ON2** concentrations.

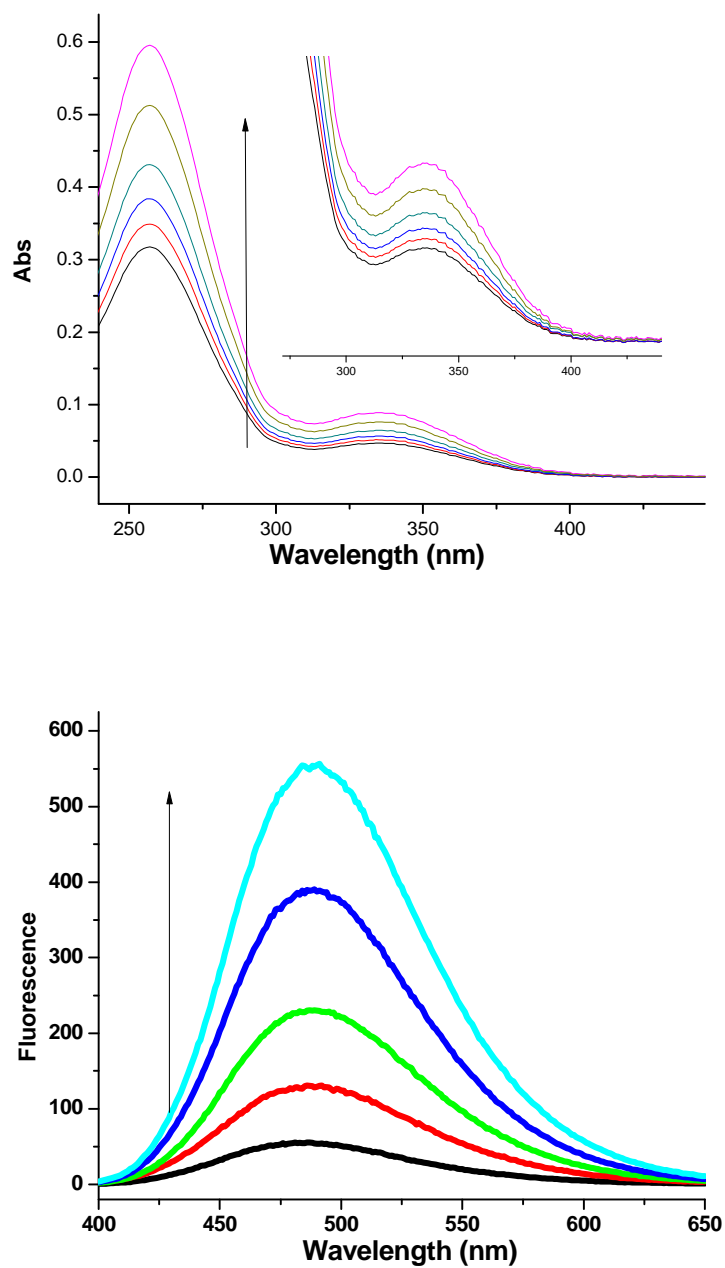


**Fig. S31** UV and fluorescence titration of **ON3** by addition of **ON4** (individual steps = 0.2  $\mu\text{M}$ , top or 0.1  $\mu\text{M}$ , bottom); arrow indicates increasing **ON4** concentrations.

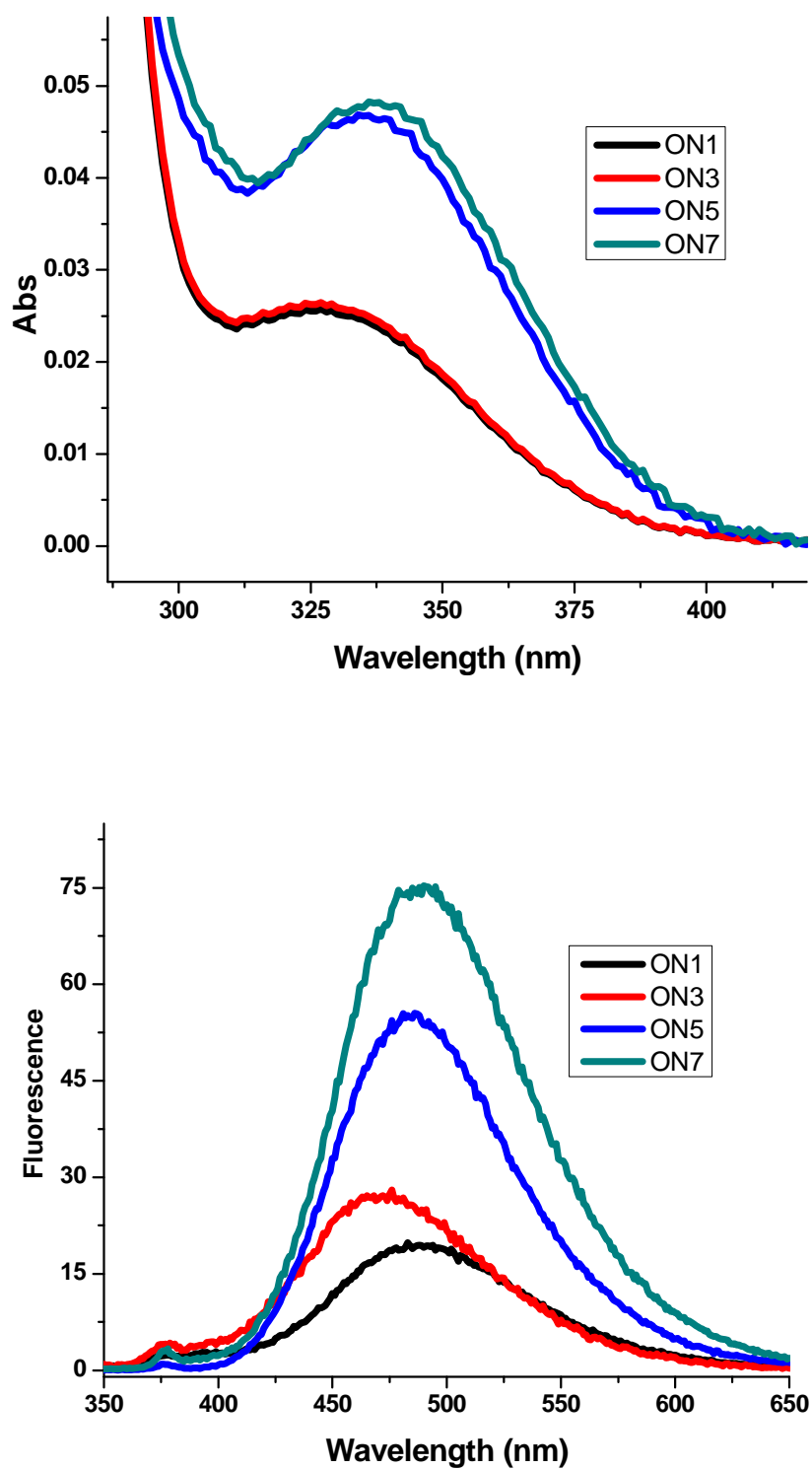




**Fig. S32** UV and fluorescence titration of **ON5** by addition of **ON6** (individual steps = 0.2  $\mu\text{M}$ ); arrows indicate increasing **ON6** concentrations.

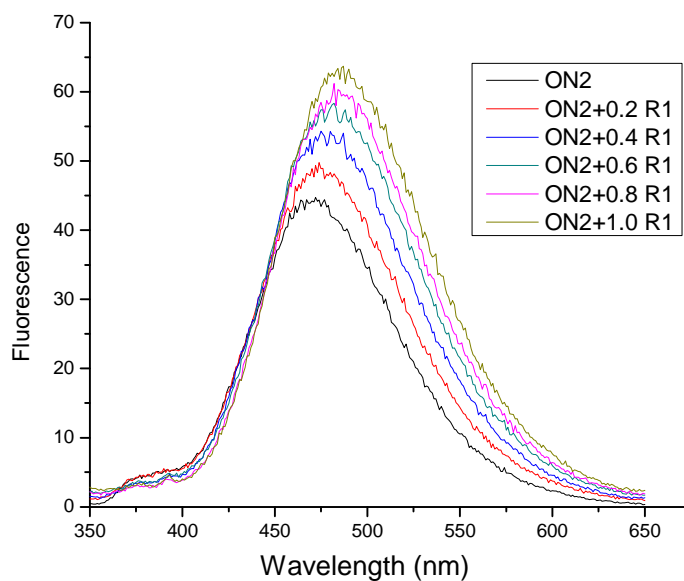


**Fig. S33** UV and fluorescence titration of **ON7** by addition of **ON8** (individual steps = 0.2  $\mu\text{M}$ ); arrows indicate increasing **ON8** concentrations.

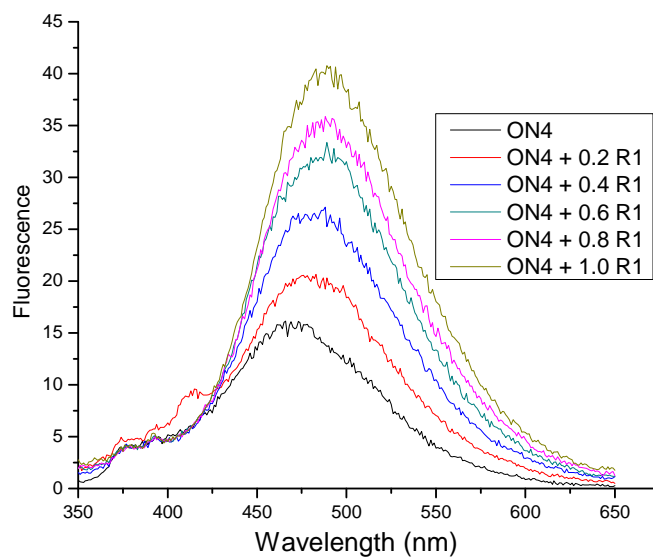


**Fig. S34** UV (top) and fluorescence (bottom,  $\lambda_{\text{ex}} = 335$  nm, slits: 5 nm) spectra of selected single strands.

## 9. Fluorescence spectra of hybrids R1\*ON2 and R1\*ON4

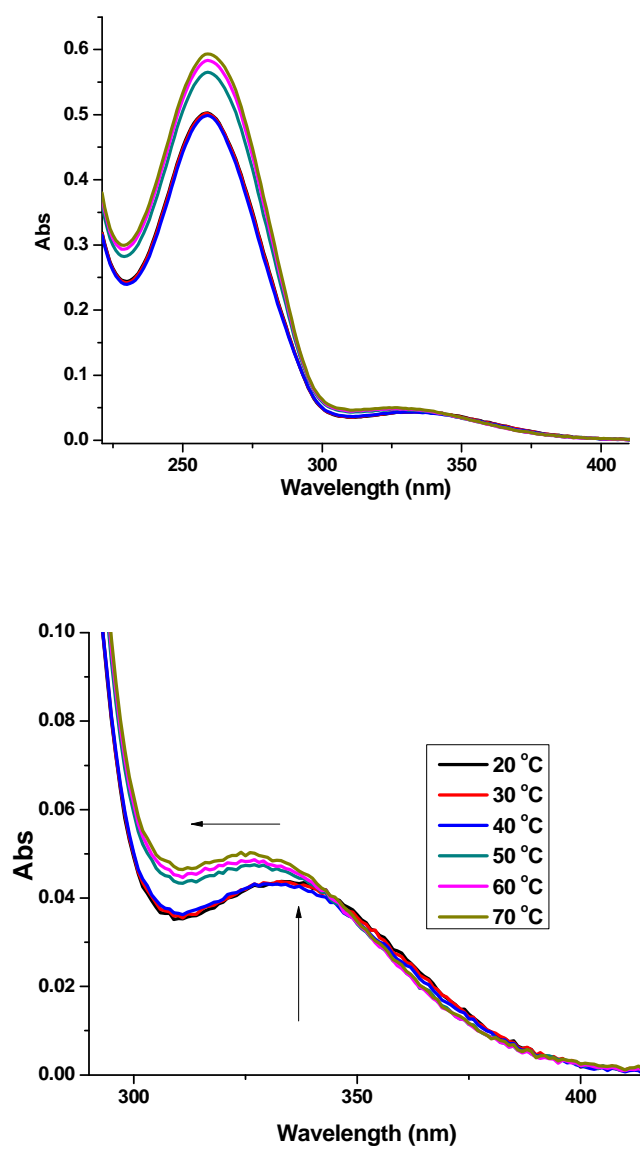


**Fig. S35** Fluorescence titration of **ON2** by addition of **R1** (individual steps = 0.2  $\mu\text{M}$ ;  $\lambda_{\text{ex}}$  = 335 nm, slits: 5 nm).



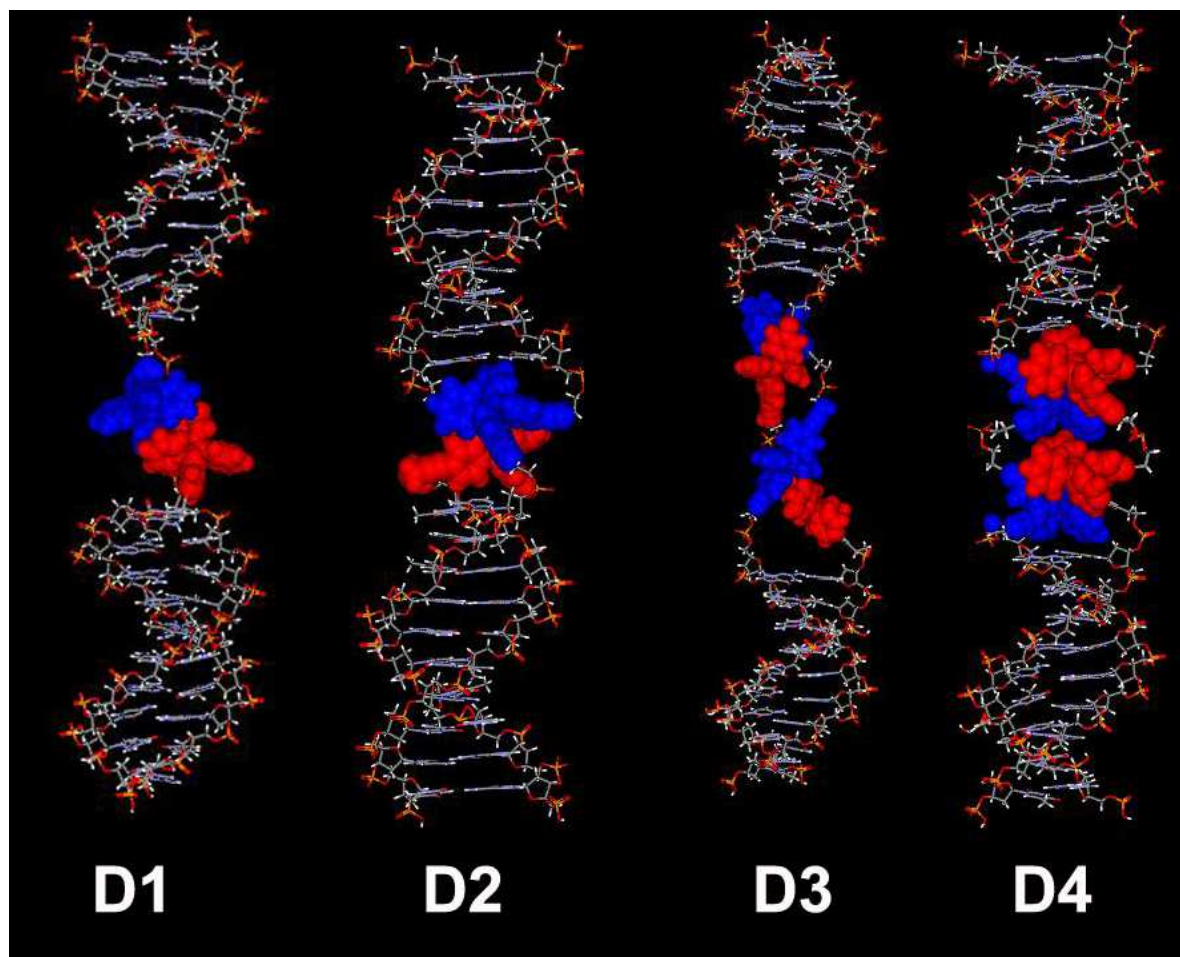
**Fig. S36** Fluorescence titration of **ON4** by addition of **R1** (individual steps = 0.2  $\mu\text{M}$ ;  $\lambda_{\text{ex}}$  = 335 nm, slits: 5 nm).

## 10. Temperature dependent UV-VIS of hybrid D1



**Fig. S37** Temperature-dependent UV-spectra of hybrid **D1**

## 11. Molecular models of D1-D4



**Fig. S38** AMBER-optimized hybrid models (*HyperChem*, release 7.5)

## 12. Quantum yield determination

Table S2. Quantum yields of *E/Z*-isomers, the single strands and duplexes were determined using quinine sulfate as standard.<sup>1</sup>

		Value	Quantum yield $\Phi(\%)$ <sup>[c]</sup>
<b>Quinine Sulfate</b>	Area <sup>[a]</sup>	45683.28	
	Abs. <sup>[b]</sup>	0.04761	54.6
<b><i>E</i>-DATPE<sup>d</sup></b>	Area <sup>[a]</sup>	80467.7	
	Abs. <sup>[b]</sup>	0.1146	39.9
<b><i>Z</i>-DATPE<sup>d</sup></b>	Area <sup>[a]</sup>	73917.1	
	Abs. <sup>[b]</sup>	0.1072	39.2
<b>ON1</b>	Area <sup>[a]</sup>	2213.93	
	Abs. <sup>[b]</sup>	0.02459	5.1
<b>ON2</b>	Area <sup>[a]</sup>	4773.54	
	Abs. <sup>[b]</sup>	0.02578	10.5
<b>D1</b>	Area <sup>[a]</sup>	16757.94	
	Abs. <sup>[b]</sup>	0.04248	22.4
<b>ON3</b>	Area <sup>[a]</sup>	2821.77	
	Abs. <sup>[b]</sup>	0.02599	6.8
<b>ON4</b>	Area <sup>[a]</sup>	1589.41	
	Abs. <sup>[b]</sup>	0.02437	3.7
<b>D2</b>	Area <sup>[a]</sup>	14857.14	
	Abs. <sup>[b]</sup>	0.04376	19.3
<b>ON5</b>	Area <sup>[a]</sup>	7981.96	
	Abs. <sup>[b]</sup>	0.04809	9.4
<b>ON6</b>	Area <sup>[a]</sup>	4853.72	
	Abs. <sup>[b]</sup>	0.04560	6.1
<b>D3</b>	Area <sup>[a]</sup>	41030.21	
	Abs. <sup>[b]</sup>	0.07625	30.6
<b>ON7</b>	Area <sup>[a]</sup>	5870.90	
	Abs. <sup>[b]</sup>	0.04672	7.1
<b>ON8</b>	Area <sup>[a]</sup>	8139.44	
	Abs. <sup>[b]</sup>	0.04693	9.9
<b>D4</b>	Area <sup>[a]</sup>	48937.65	
	Abs. <sup>[b]</sup>	0.07798	32.1

[a] Area under curve (excitation at 335 nm); [b] absorption maximum at 335 nm.

$$^{[c]} \Phi = \Phi_{ref} \times \frac{Area_{comp} \times Abs_{ref}}{Abs_{comp} \times Area_{ref}}$$

[d] Determined in water/THF (95/5, v/v)

1. Y. Makino, S. Uchiyama, K.-i. Ohno, H. Arakawa, *Anal. Chem.* **2010**, 82, 1213-1220.