Supplementary Material (ESI) for Chemical Communications

Modulation of chiroptical properties by DNA-guided assembly of fluorenes.

Daniel Wenger, Vladimir L. Malinovskii and Robert Häner*

Electronic Supplementary Information

Content

1. Synthetic and analytical procedures

The required 2,7-dibromofluorene was purchased from TCI Europe. Nucleoside phosphoramidites from Transgenomic (Glasgow, UK) were used for oligonucleotide synthesis. Oligonucleotides **ON1-8** were prepared via automated oligonucleotide synthesis by a standard synthetic procedure ('trityl-off' mode) on a 394-DNA/RNA synthesizer (Applied Biosystems). Coupling time for the fluorene phosphoramidites 5 and 6 were elongated to 120 seconds and 1,2-dichloroethane (0.1M) was used as the solvent instead of acetonitrile. Cleavage from the solid support and final deprotection was done by treatment with 30% NH_4OH solution at 55°C overnight. All oligonucleotides were purified by reverse phase HPLC (LiChrospher 100 RP-18, 5μm, Merck), Bio-Tek Instruments); eluent A = $(Et_3NH)OAc$ (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, CH_3CN/H_2O/TEA.

All spectroscopic measurements were performed in potassium phosphate buffer (10 mM, 100 mM NaCl, pH 7.0). Extinction coefficients (at 260nm) used for concentration determination of modified oligonucleotides: 4500 $M^{-1}cm^{-1}$ was used for the fluorene unit as determined from the diol.

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 WinUV software at 260 nm and 330 nm (cooling-heating-cooling cycles in the temperature range of 10-90°C, temperature gradient of 0.5°C/min). Temperature melting (Tm) values were determined as the maximum of the first derivative of the smoothed melting curve. Temperature dependent UV-VIS spectra were collected with an optic path of 1 cm over the range of 210-500 nm at 10-90 ºC with a 10 ºC interval on Varian Cary-100 Bio-UV/VIS spectrophotometer equipped with a Varian Cary-block temperature controller. The oligonucleotide concentration used for UV-VIS measurements was 1μM single strand in all cases.

CD spectra were recorded on a *JASCO J-715* spectrophotometer using quartz cuvettes with an optic path of 1 cm. The oligonucleotide concentration used for the CD was 5μM single strand in all cases.
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 1μM single strand in all cases.

Quantum yields were calculated by using quinine sulphate in 0.1M H_2SO_4 at 20°C as the standard. Excitation wavelength used for **ON1,2,5,6**: 320nm, excitation wavelength used for **ON3,4,7,8**: 330nm. The absorption at the excitation wavelength was in the range of 0.05 and 0.1 units.

2. Synthesis and characterization of phosphoramidite building blocks

2,7-Bis(4-hydroxybut-1-yn-1-yl)fluorene **(1)**
1.00 g (3.085mmol) 2,7-dibromofluorene was dissolved in 30ml of THF. To this solution 7 mg (0.0375mmol) of copper(I) iodide and 53mg (0.075mmol) of $(PPh_3)_2Cl_2Pd$(II) was added. The solution was heated under reflux, once reaching 70°C, 20ml of freshly degassed triethylamine was added. Finally 1.19ml (18.3mmol) of 3-butyn-1-ol was added and the solution was stirred under reflux over night. After cooling to room temperature, THF and triethylamine was evaporated and the remaining solid was dissolved in 50ml of THF. This suspension was filtered over Celite® and THF was evaporated again. The solid was taken up in EtOAc after which the precipitate was filtered of and the solution was washed with citric acid (10%) and with $NaHCO_3$ (sat) and dried over $MgSO_4$. The volume of the organic phase was reduced until precipitation. The solution was then put in a cool ultrasonic bath and the resulting precipitate was filtered off and dried under reduced pressure resulting in 638mg (68%) of a light brownish solid.

^{1}H-NMR (300MHz, D_6-DMSO): 2.58 (4H, t, J= 7.0), 3.6 (4H, q, J= 6.6), 3.90 (2H, s), 4.91 (2H, t, J= 5.5), 7.41 (2H, d, J= 8.1), 7.60 (2H, s), 7.86 (2H, d, J= 7.9). ^{13}C-NMR (75MHz, D_6-DMSO): 23.4, 36.1, 59.8, 81.6, 88.7, 120.4, 121.7, 128.0, 130.2, 140.1, 143.5.

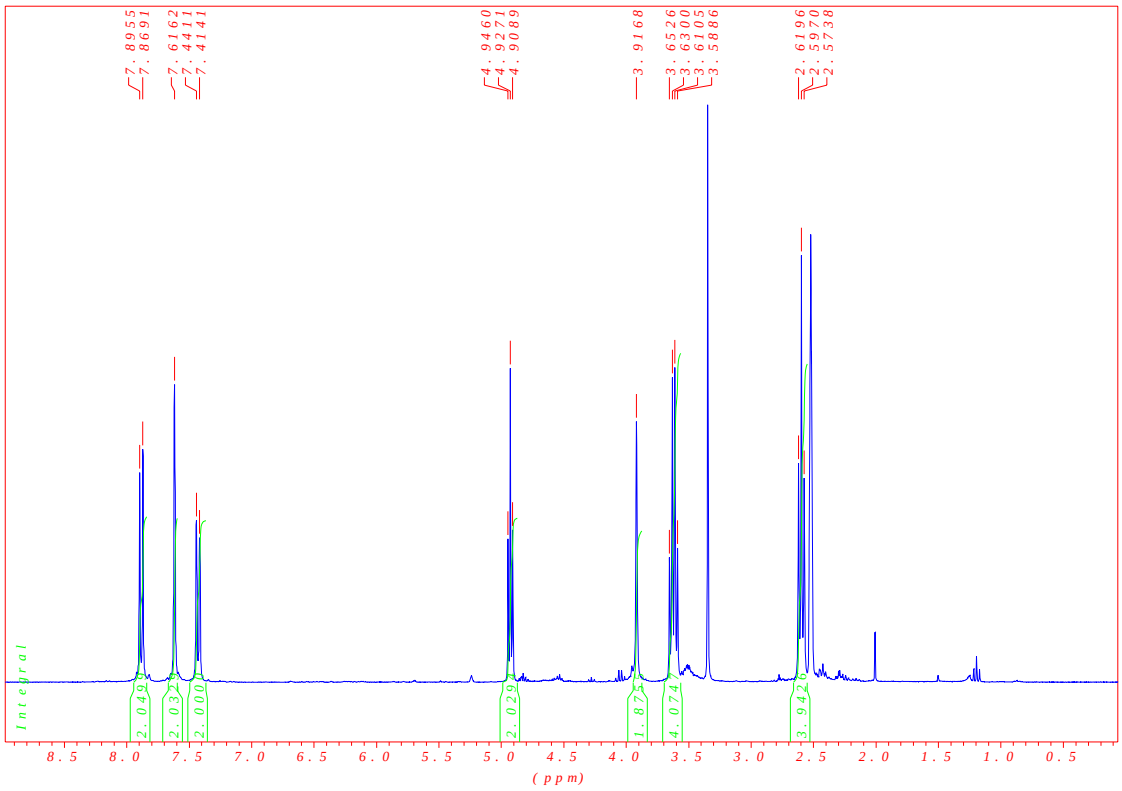

Figure 1. ^{1}H-NMR of compound **1**

2,7-Bis(6-hydroxyhex-1-yn-1-yl)fluorene (**2**)
1.00 g (3.085mmol) 2,7-dibromofluorene was dissolved in 30ml of THF. To this solution 30ml triethylamine filtered over aluminumoxide was added. The solution was degassed for 30 minutes. Afterwards 110mg (0.156mmol) of $(PPh_3)_2Cl_2Pd(II)$ and 29mg (0.156mmol) copper (I) iodide was added. Finally 0.77ml (6.9mmol) of 5-hexyn-1-ol was added the reaction mixture was heated under reflux overnight. After cooling to room temperature the mixture was filtered over celite, THF and triethylamine was evaporated and solid was dissolved in EtOAc. The residue was purified by column chromatography over silica gel with pure EtOAC. The product fractions were combined, EtOAc evaporated and the resulting solid was dried under high vacuum yielding in 250mg (28%) of a white yellow solid.

^{1}H-NMR (300MHz, D_6-DMSO): 1.5-1.7 (8H, m), 2.4-2.5 (4H, m), 3.4-3.5 (4H, m), 3.90 (2H, s), 4.43 (2H, t, J= 5.1), 7.40 (2H, d, J= 7.9), 7.58 (2H, s), 7.86 (2H, d, J= 7.9). ^{13}C-NMR (75MHz, D_6-DMSO): 18.6, 24.9, 31.7, 36.0, 60.2, 81.2, 90.9, 120.3, 121.8, 127.9, 130.1, 140.1, 143.5.

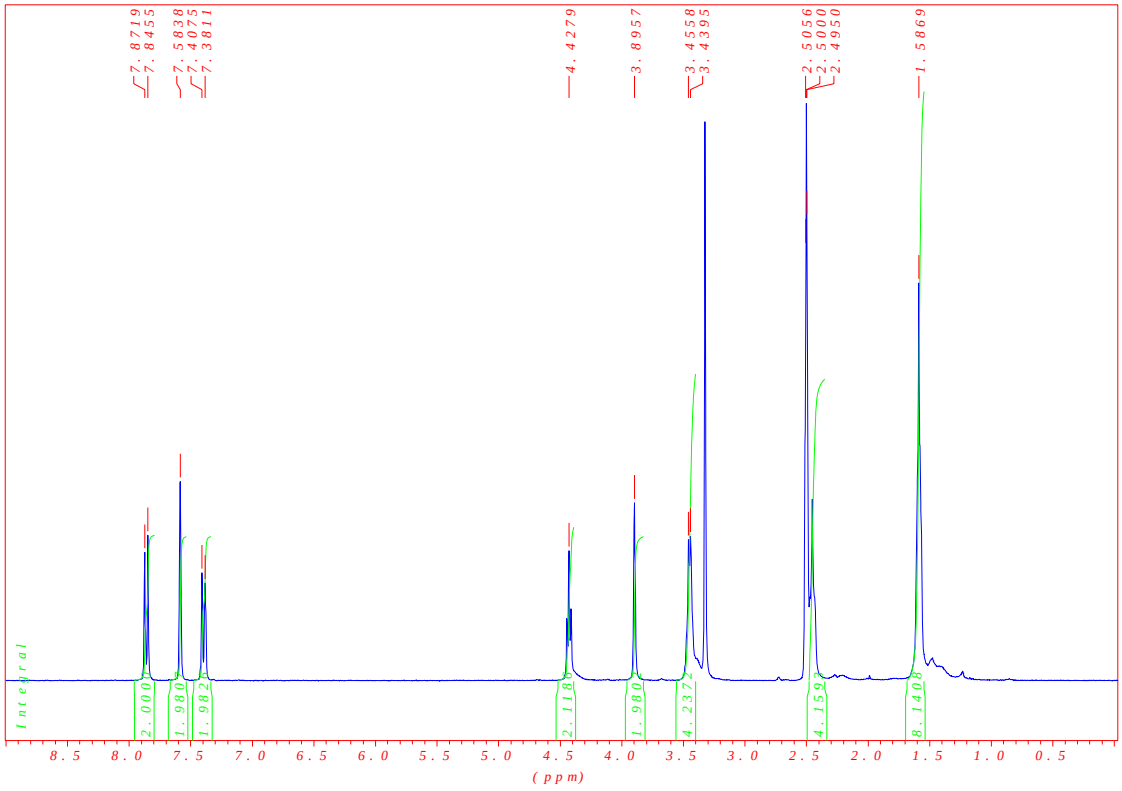

Figure 2. ^{1}H-NMR of compound **2**

2-[4-(4,4`-Dimethoxytriphenylmethyloxy)but-1-yn-1-yl]-7-(4-hydroxybut-1-yn-1-yl) fluorene (**3**)

To a solution of 490mg (1.62mmol) of **1** in 13ml THF and 7ml pyridine, a solution of 553mg (1.62mmol) 4,4`-dimethoxytrityl chloride in 3.5ml THF was added dropwise under argon over 2 hours at room temperature. THF and pyridine were then evaporated. The solid was then taken up in 150ml EtOAc, filtrated and washed with citric acid (10%) and $NaHCO_3$ (sat) and dried over $MgSO_4$. EtOAc was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (EtOAc/Hexane 1:1 + 2% triethylamine to EtOAc/Hexane 1:1 + 2% triethylamine + 4% methanol). The product fractions were combined, evaporated and dried under high vacuum to furnish 254mg (26%) of a yellow white foam.

^{1}H-NMR (300MHz, D_6-DMSO): 2.58 (2H, t, J=7.0), 2.73 (2H, t, J=6.2), 3.16 (2H, t, J=6.2), 3.60 (2H, q, J_1=5.8, J_2=6.6), 3.91 (2H, s), 3.73 (6H, s), 4.91 (1H, t, J= 5.7), 6.88 (4H, d, J=8.9), 7.18-7.27 (1H, m), 7.27-7.36 (6H, m), 7.4-7.5 (4H, m), 7.60 (2H, d, J=5.1), 7.88 (2H, m).

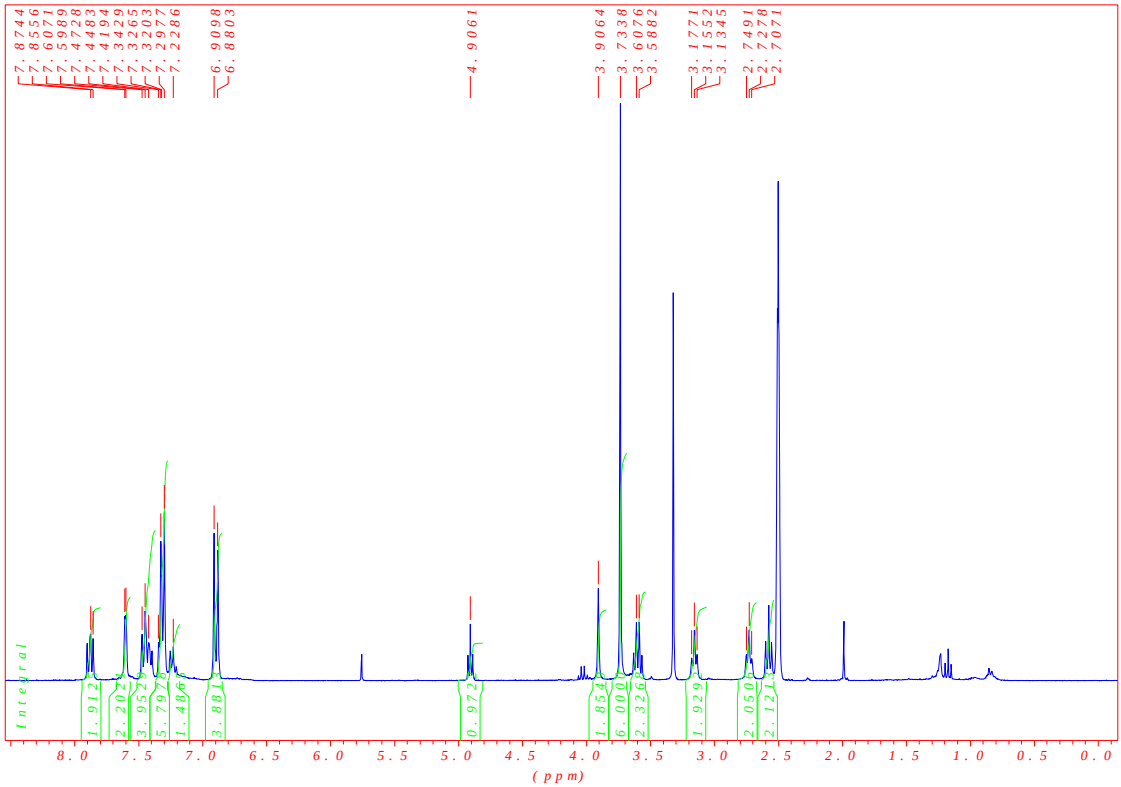

Figure 3. ^{1}H-NMR of compound **3**

2-[6-(4,4`-Dimethoxytriphenylmethyloxy)hex-1-yn-1-yl]-7-(6-hydroxyhex-1-yn-1-yl) fluorene (**4**)

To a solution of 250mg (0.697mmol) of **2** in 15ml THF and 6ml pyridine, a solution of 238mg (0.7mmol) 4,4`-dimethoxytrityl chloride in 5ml THF was added dropwise under argon over 2 hours at room temperature. THF and pyridine were then evaporated. The solid was then taken up in 80ml EtOAc, filtrated and washed with citric acid (10%) and $NaHCO_3$ (sat) and dried over $MgSO_4$. EtOAc was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (EtOAc/Hexane 1:1 + 2% triethylamine). The product fractions were combined, evaporated and dried under high vacuum to furnish 95mg (22%) of a yellow white foam.

^{1}H-NMR (300MHz, D_6-DMSO): 1.5-1.8 (8H, m), 2.35-2.47 (4H, m), 3.03 (2H, t, J=6.0), 3.4-3.5 (2H, m), 3.72 (6H, s), 3.89 (2H, s), 4.43 (1H, t, J= 5.3), 6.88 (4H, d, J=8.9), 7.2-7.35 (7H, m), 7.35-7.45 (4H, m), 7.60 (2H, d, J=5.1), 7.88 (2H, d, J=7.9).

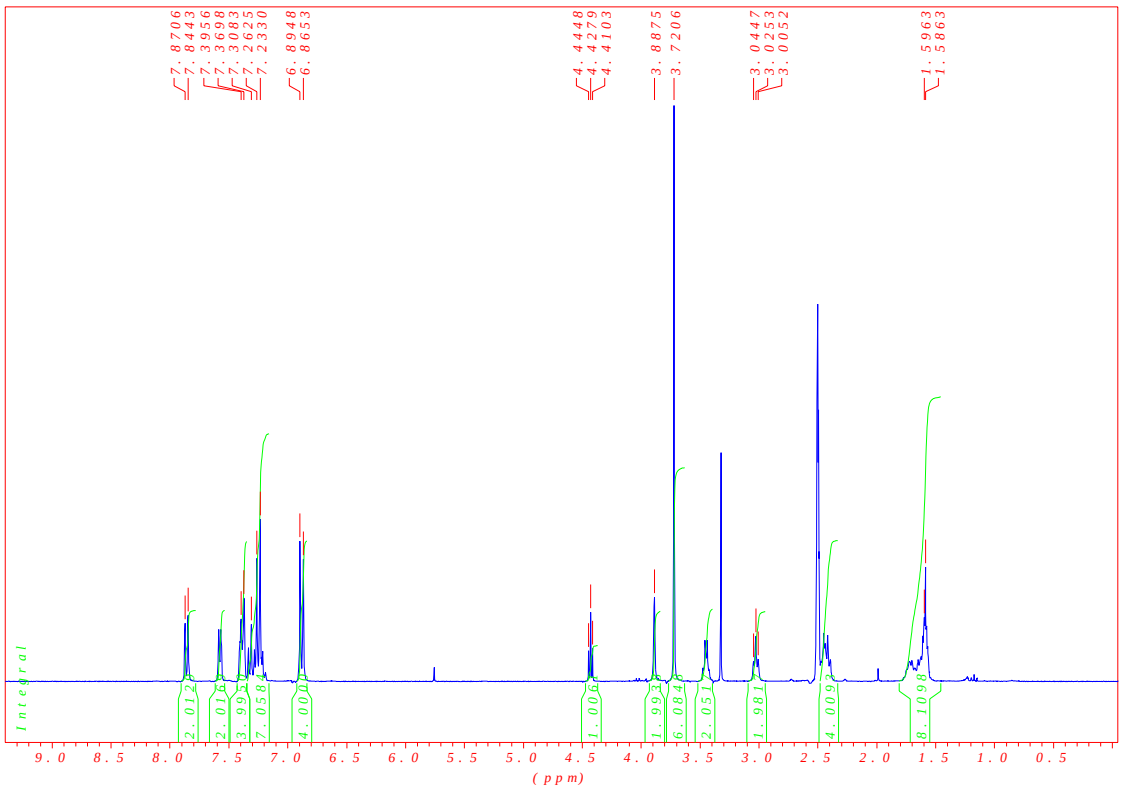

Figure 4. ^{1}H-NMR of compound **4**

O-2-Cyanoethyl-O`-{[4-(4,4`-Dimethoxytriphenylmethyloxy)but-1-yn-1-yl)fluorene-1-yl] - but-3-yn-1-yl) fluorene-N,N-diisopropyl phosphoramidite (**5**)

450mg (0.74mmol) of **3** was dissolved in 15ml CH_2Cl_2 and 383µL (2.23mmol) diiosopropylethylamine. 194mg (0.81mmol) of 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite was then added dropwise at roomtemperature under argon. The reaction mixture was stirred for 1 hour. The volume of CH_2Cl2 was then reduced and a column chromatography over silica gel was directly performed with pure CH_2Cl_2 with 2% triethylamine. After evaporation and drying under high vacuum, 401mg (67%) of a yellow white foam resulted.

^{1}H-NMR (300MHz, D_6-DMSO): 1.16 (12H, dd, J=4.5), 3.15 (2H, t, J=6.6), 3.73 (6H, s), 3.91 (2H, s), 6.90(4H, d, J=8.85), 7.2-7.5 (11H, m), 7.60 (2H, d, J=6.39), 7.9 (2H, dd, J=3.75).
^{31}P-NMR (121.5MHz, $CDCl_3$): 147.25

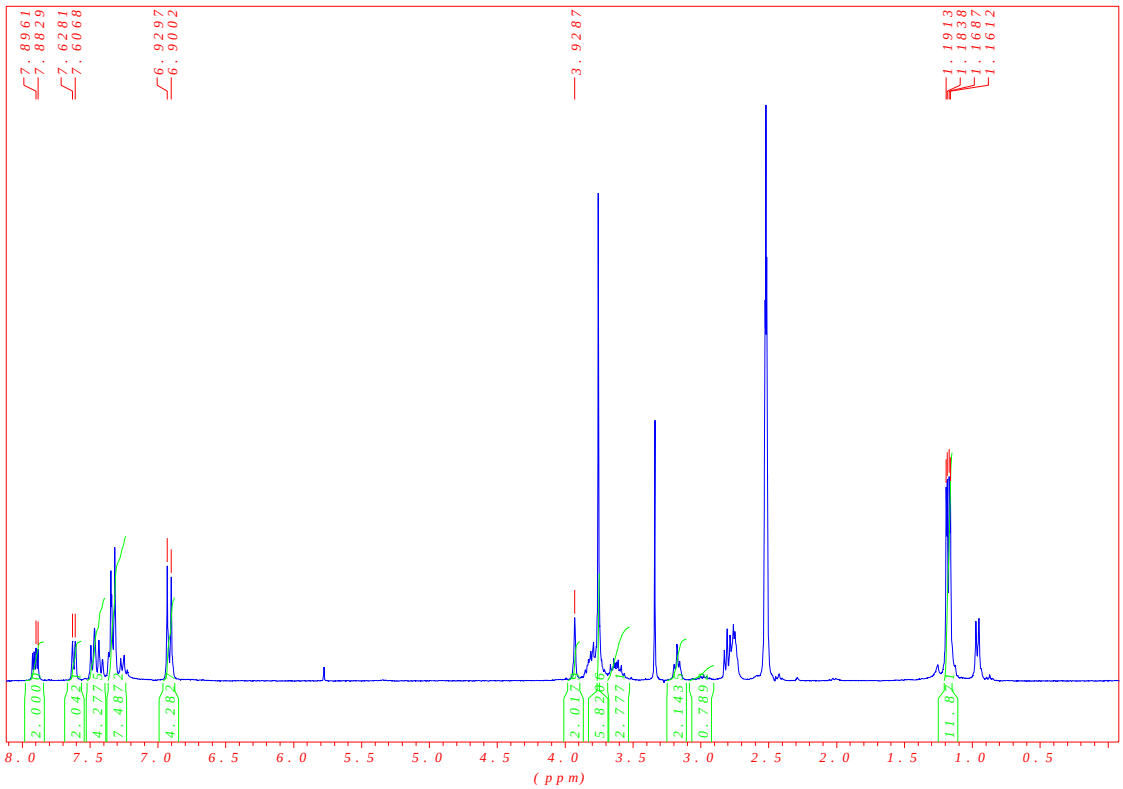

Figure 5. ^{1}H-NMR of compound **5**

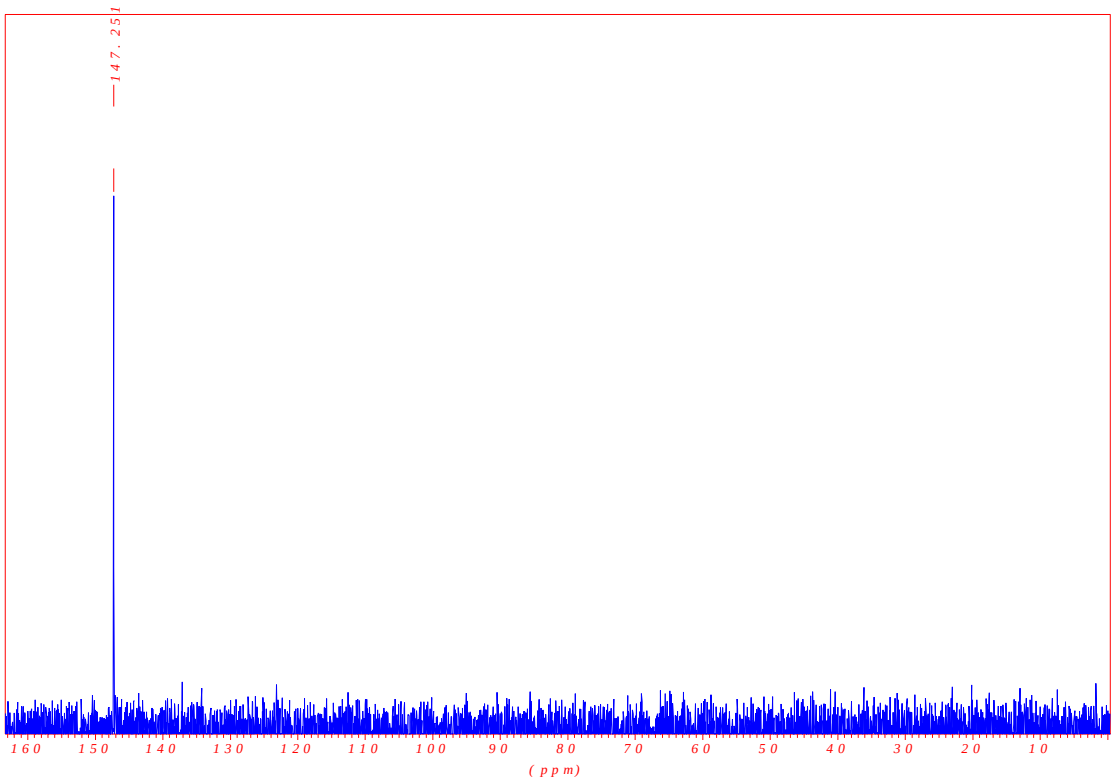

Figure 6. ^{31}P-NMR of compound **5**

O-2-Cyanoethyl-O`-{[4-(4,4`-Dimethoxytriphenylmethyloxy)hex-1-yn-1-yl)fluorene-1-yl]- hex-5-yn-1-yl) fluorene-N,N-diisopropyl phosphoramidite (**6**)

272mg (0.41mmol) of **5** was dissolved in 15ml CH_2Cl_2 and 211µL (1.23mmol) diisopropylethylamine. 108mg (0.45mmol) of 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite was then added dropwise at roomtemperature under argon. The reaction mixture was stirred for 1 hour. The volume of CH_2Cl_2 was then reduced and a column chromatography over silica gel was directly performed with pure CH_2Cl_2 with 2% triethylamine. After evaporation and drying under high vacuum, 280mg (79%) of a yellow white foam resulted.

^{1}H-NMR (300MHz, D_6-DMSO): 1.1 (12H, d, J=6.8), 1.55-1.8 (8H, m), 2.45-2.5 (2H, m), 2.76 (2H, t, J=5.82), 3.03 (2H, t, J=6.03), 3.5-3.8 (12H, m), 3.89 (2H, s), 6.88 (4H, d, J=9.03), 7.15-7.45 (11H, m), 7.57 (2H, d, J=3.39), 7.86 (2H, d, J=7.92).
^{31}P-NMR (121.5MHz, $CDCl_3$): 146.59

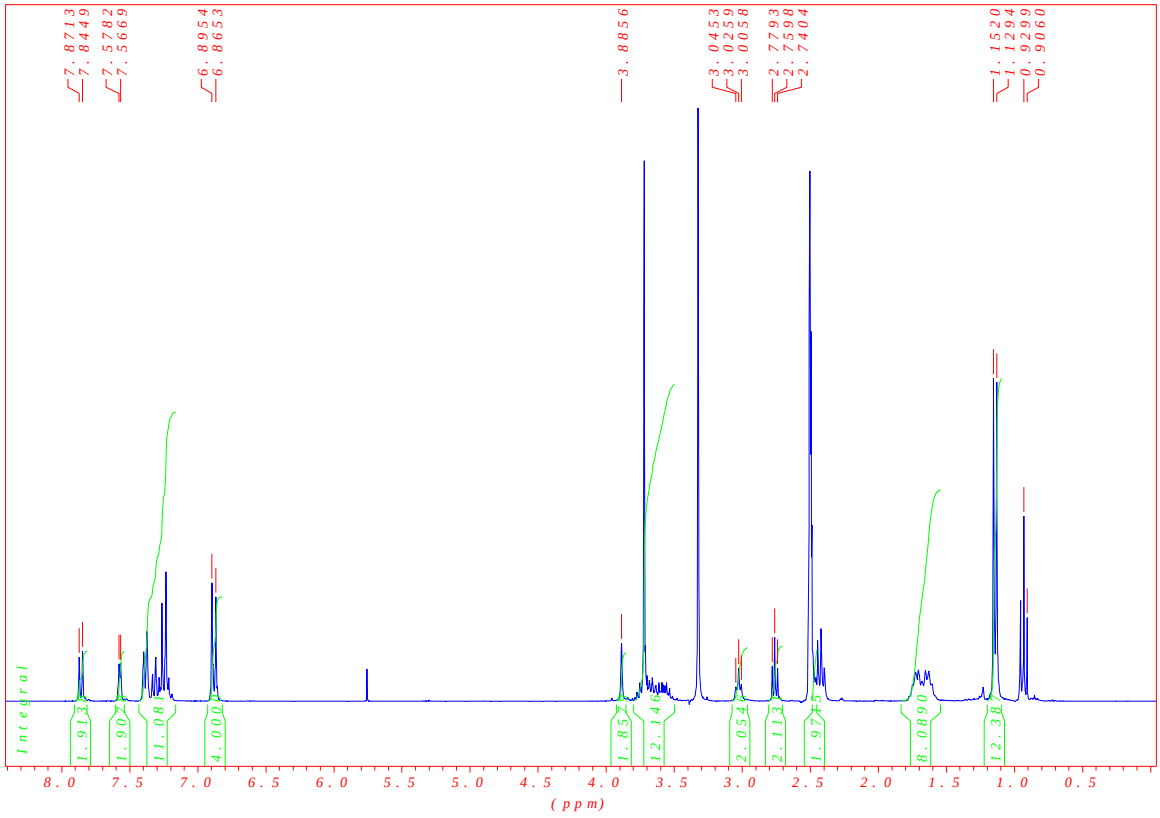

Figure 7. ^{1}H-NMR of compound **6**

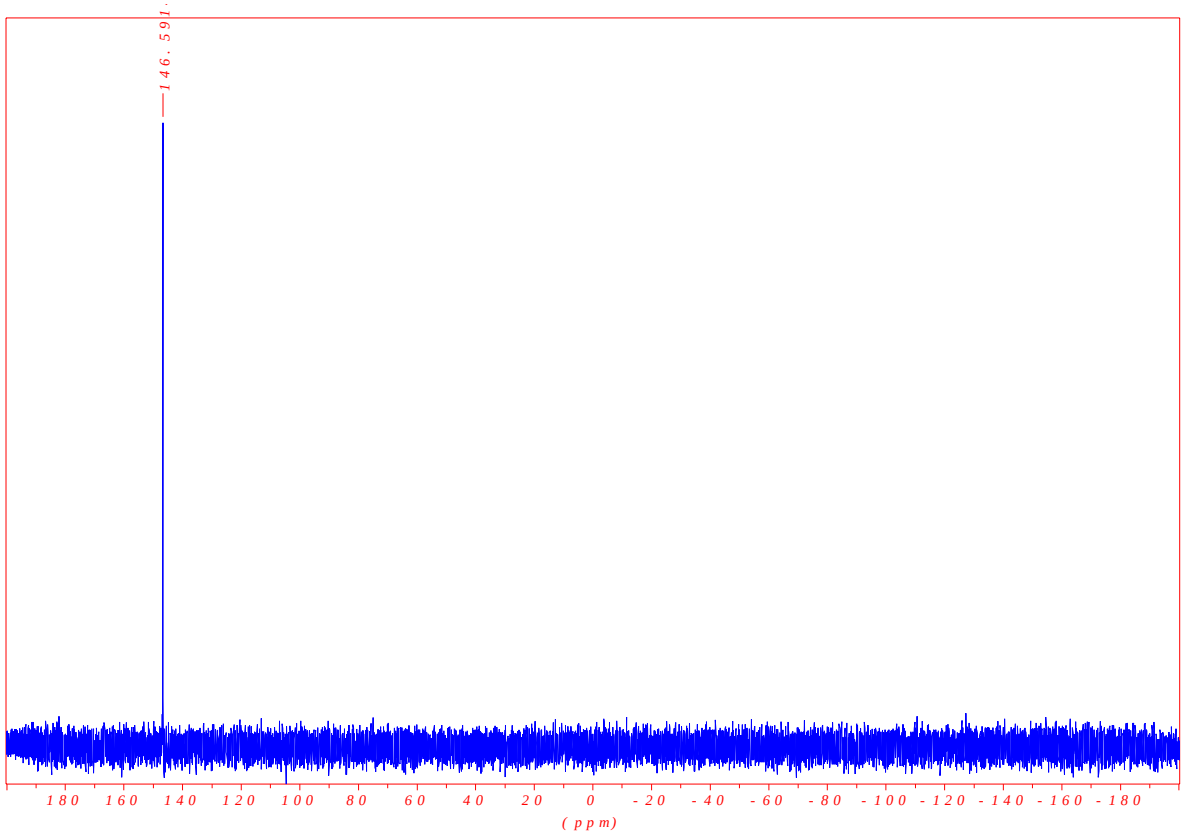

Figure 8. ^{31}P-NMR of compound **6**

3. MS Data of oligonucleotides **ON1-ON8**

Number	Brutto formula	Calc. mass	Exp. mass
ON1	$C_{216}H_{261}N_{81}O_{120}P_{20}$	6531.1	6532.4
ON2	$C_{214}H_{263}N_{71}O_{124}P_{20}$	6433.1	6433.7
ON3	$C_{227}H_{266}N_{76}O_{119}P_{20}$	6582.0	6582.5
ON4	$C_{225}H_{267}N_{69}O_{121}P_{20}$	6493.0	6492.7
ON5	$C_{220}H_{269}N_{81}O_{120}P_{20}$	6587.2	6587.9
ON6	$C_{218}H_{271}N_{71}O_{124}P_{20}$	6489.1	6490.0
ON7	$C_{235}H_{282}N_{76}O_{119}P_{20}$	6694.2	6695.3
ON8	$C_{225}H_{267}N_{69}O_{121}P_{20}$	6605.1	6606.1

4. Melting profiles of hybrids

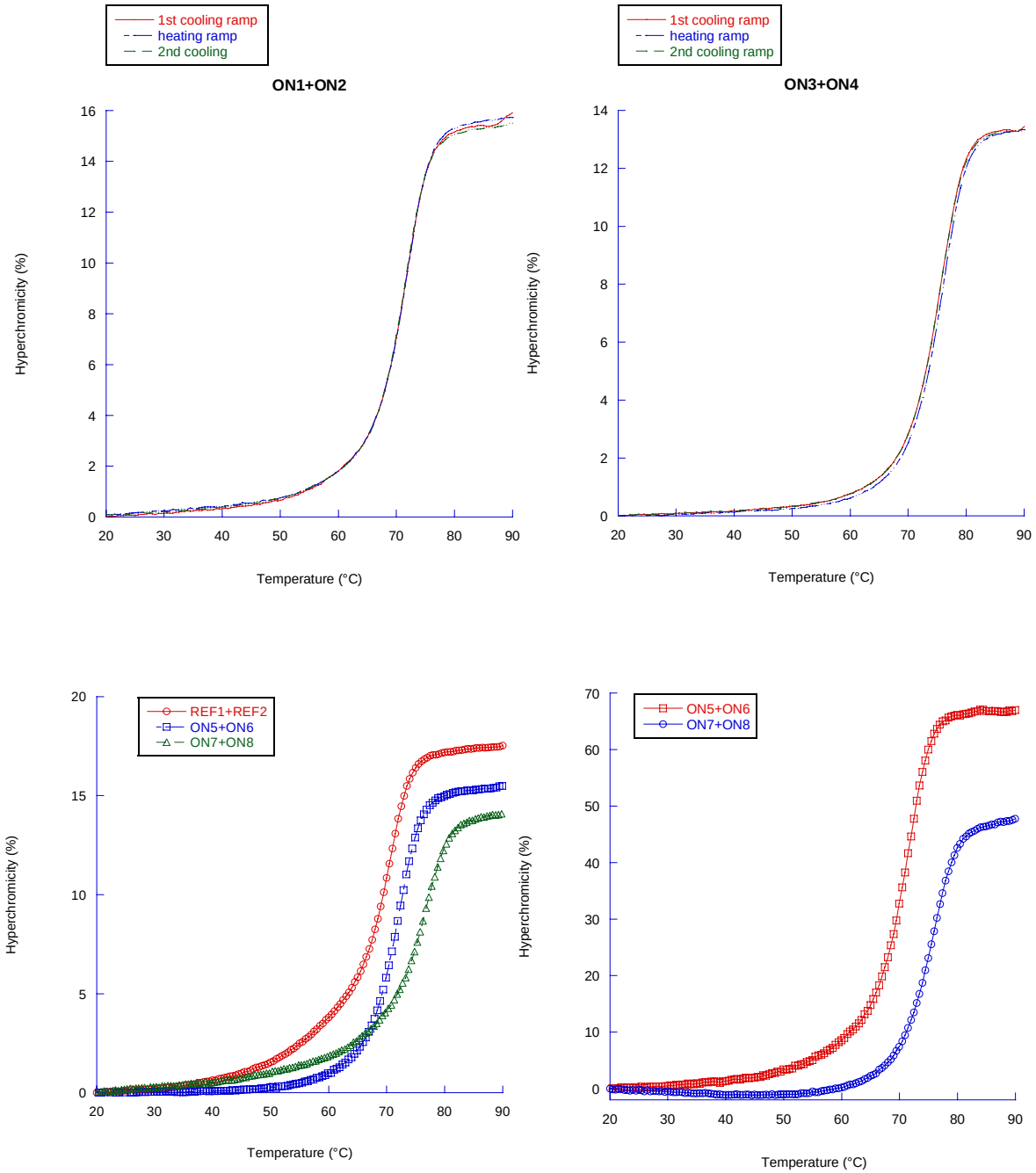

Figure 9. Melting profiles of the modified hybrids with 3 ramps monitored at 260 nm (top), in comparison with the fully natural duplex (bottom left) and a melting profile monitored at 330 nm. Conditions: 1µM single strand concentration, 10mM phophatebuffer, 100mM NaCl, heating/ cooling rate 0.5°C/min.

5. Temperature dependent UV-VIS of hybrids

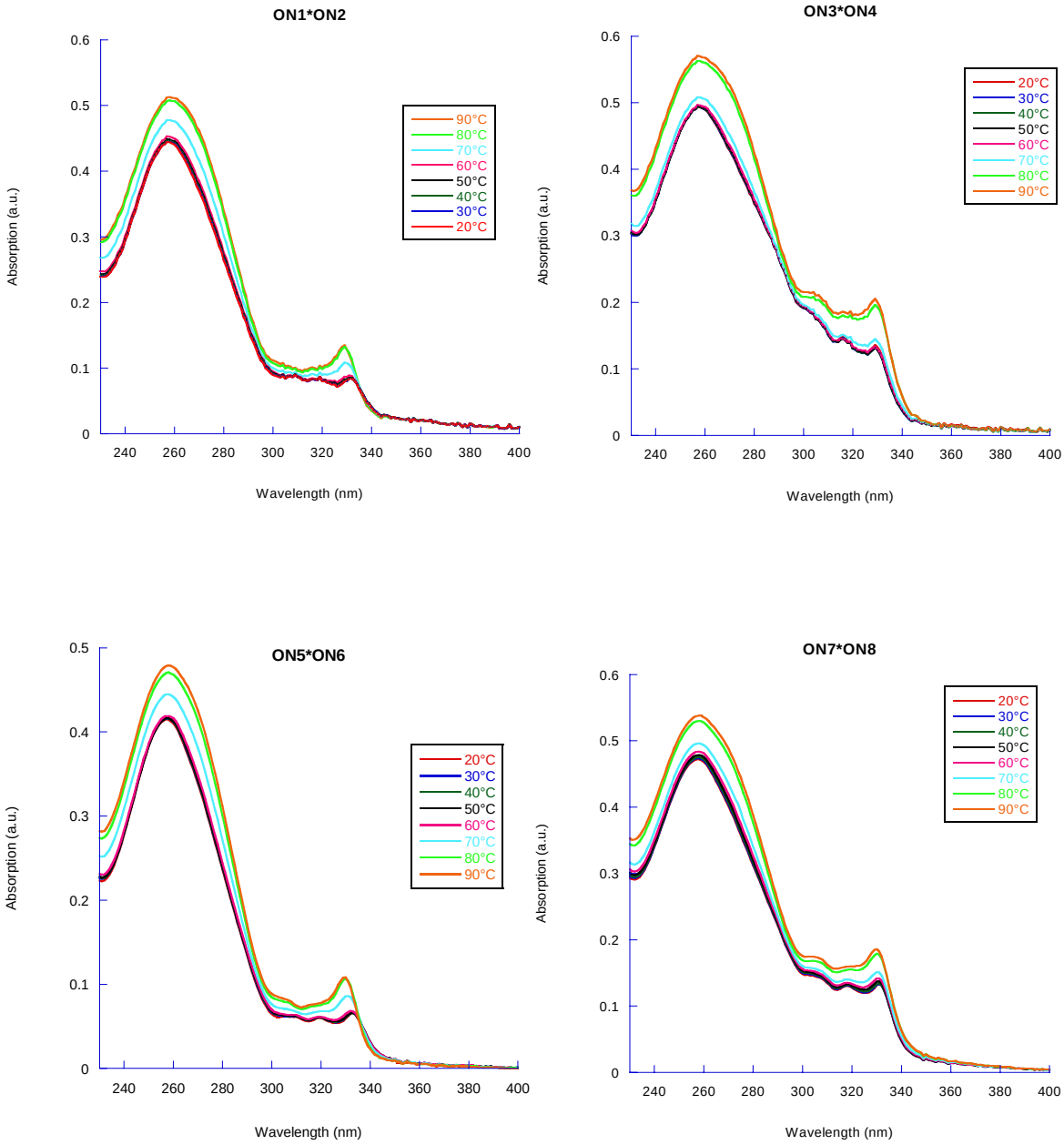

Figure 10. Temperature dependent UV-vis spectra of the different hybrids. Conditions: 1µM single strand concentration, 10mM phosphatebuffer, 100mM NaCl.

6. UV-VIS of single strands, 20°C vs 90°C.

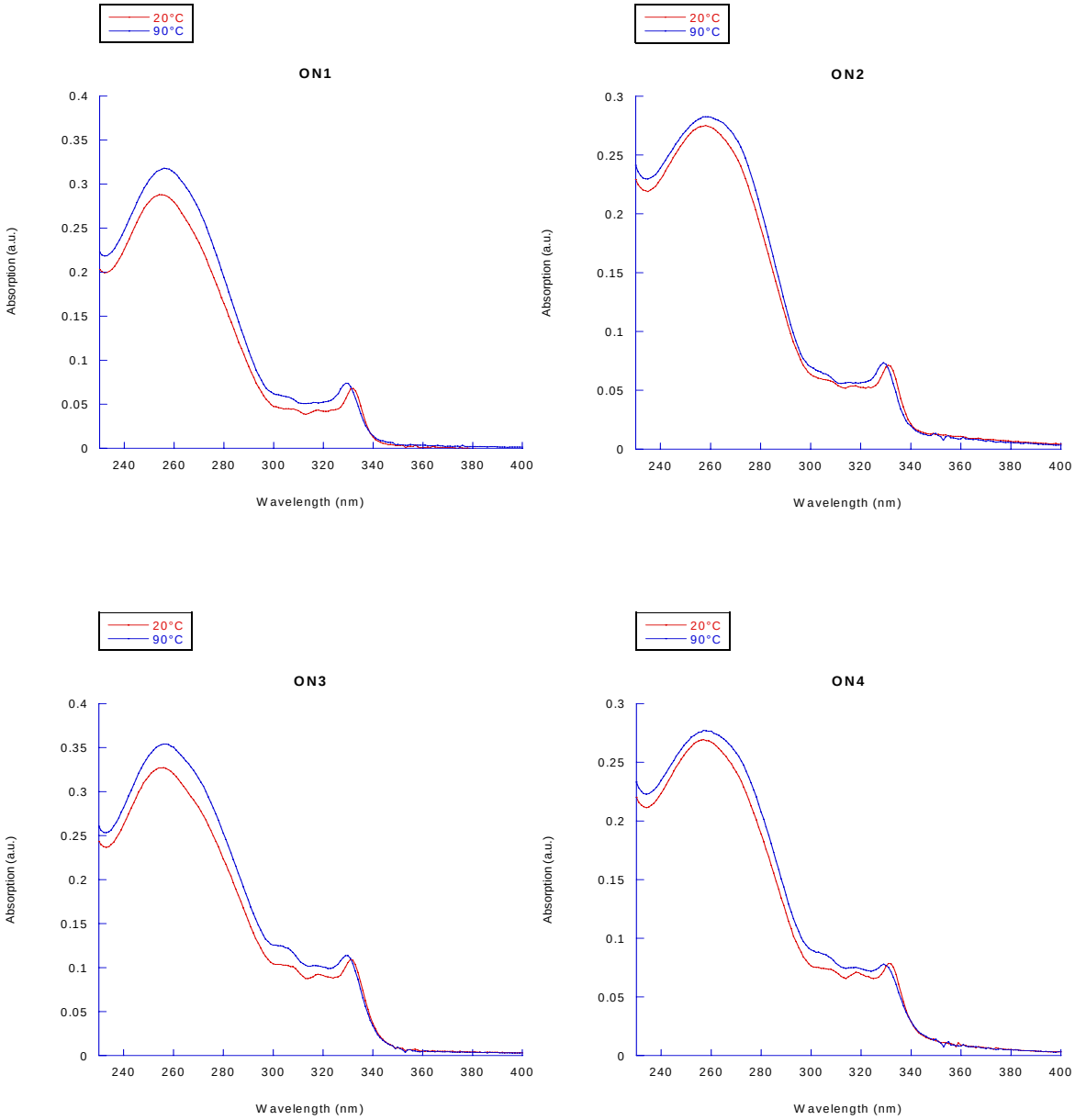

Figure 11. UV-vis spectra of the single strands recorded at 20°C and 90°C. Conditions: 1µM single strand concentration, 10mM phosphatebuffer, 100mM NaCl.

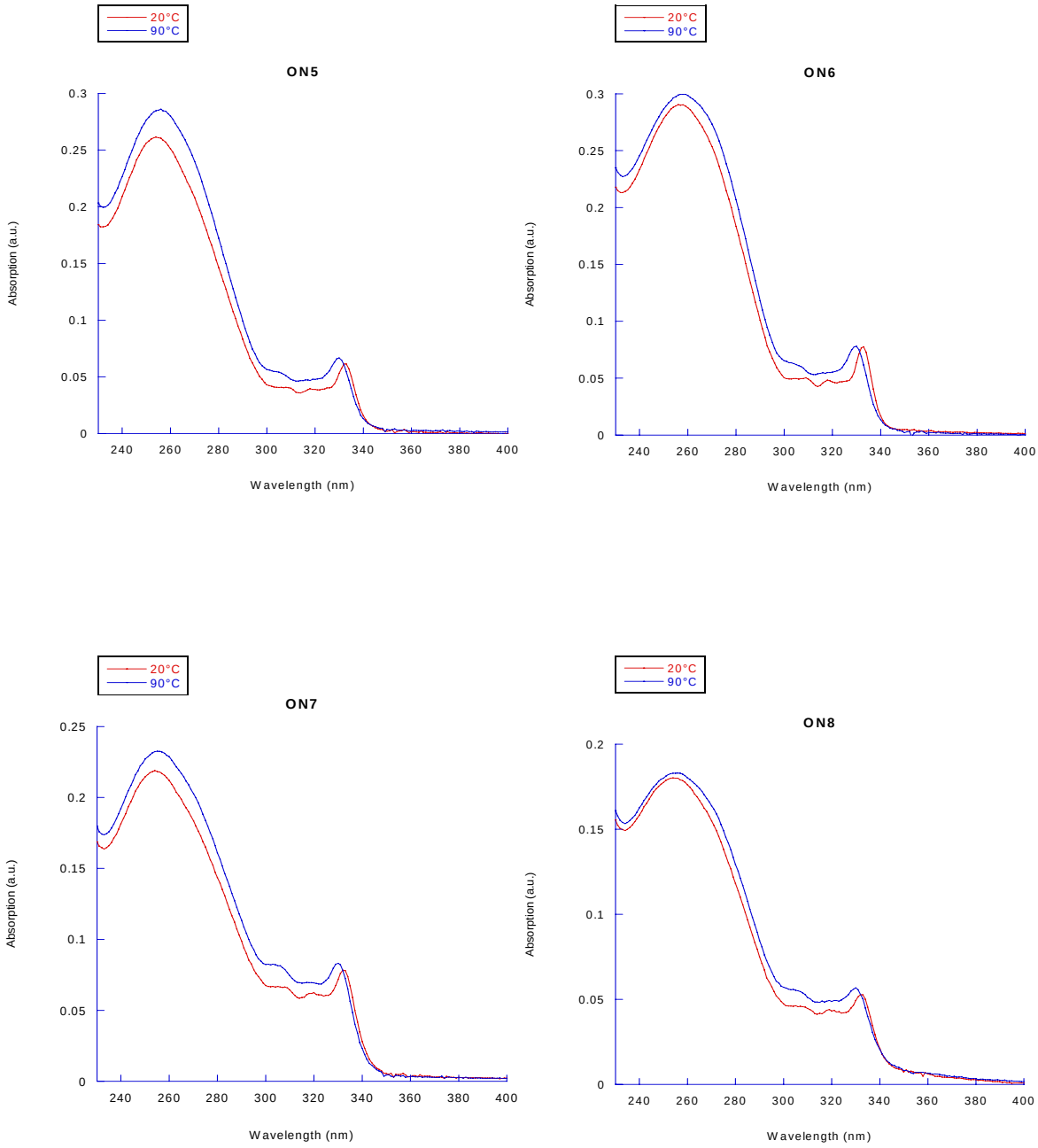

Figure 11 (continued)

7. Temperature dependent fluorescence of hybrids

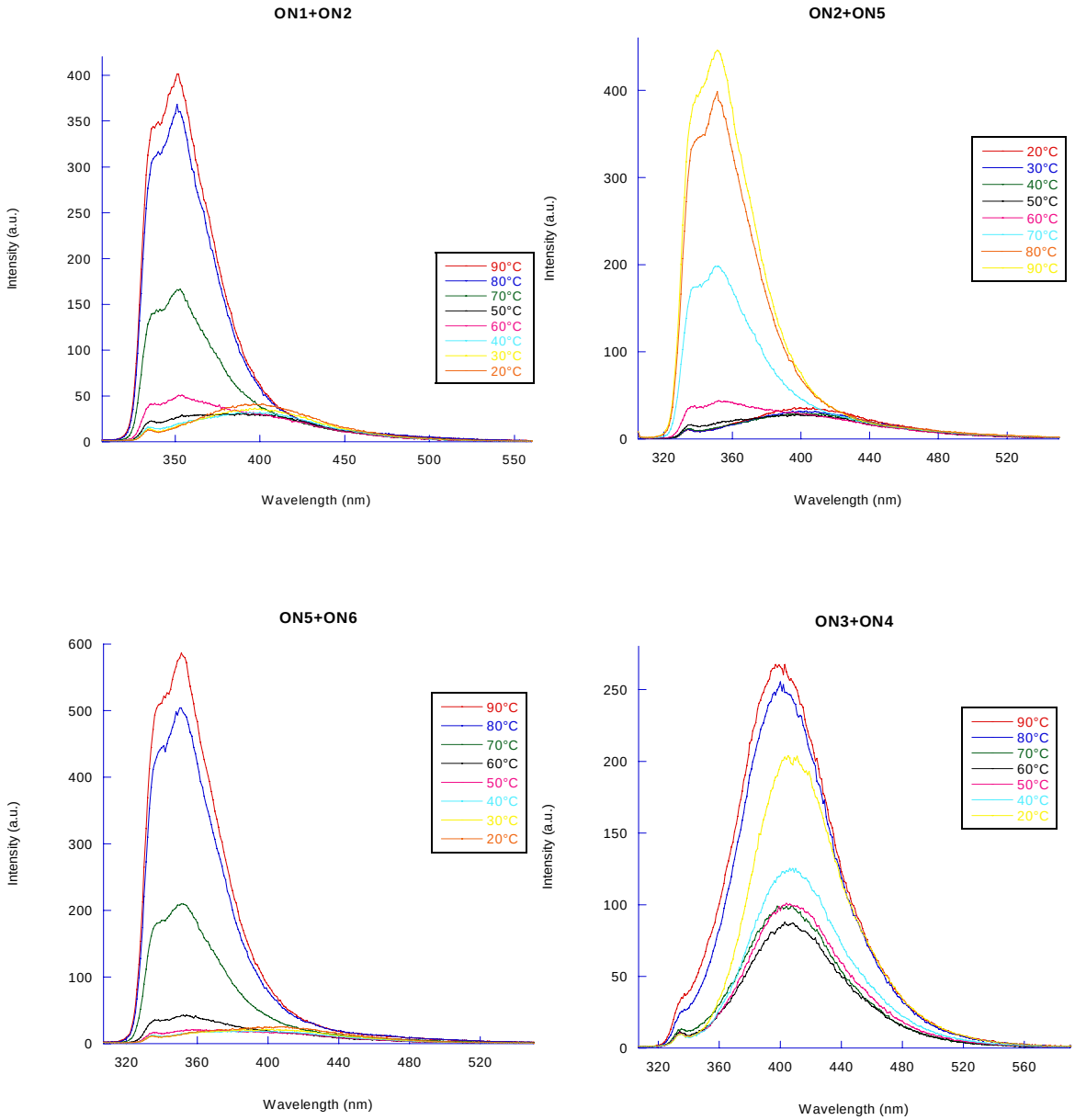

Figure 12. Temperature dependent fluorescence spectra of the hybrids. Condidtions: 1µM single strand concentration, 10mM phosphate buffer, 100mM NaCl. Instrumental setup: Excitation wavelength (300 nm), excitation slitwidth: 2.5nm, emission slitwidth: 2.5nm, detector voltage: 800V.

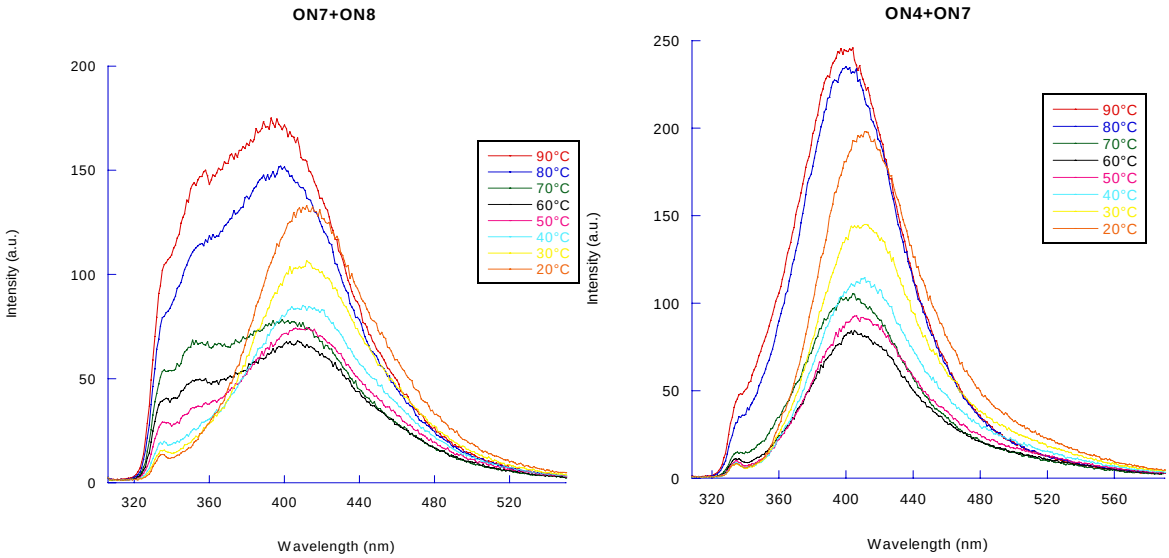

Figure 12 (continued)

8. Fluorescence of single strands **ON1-ON8**

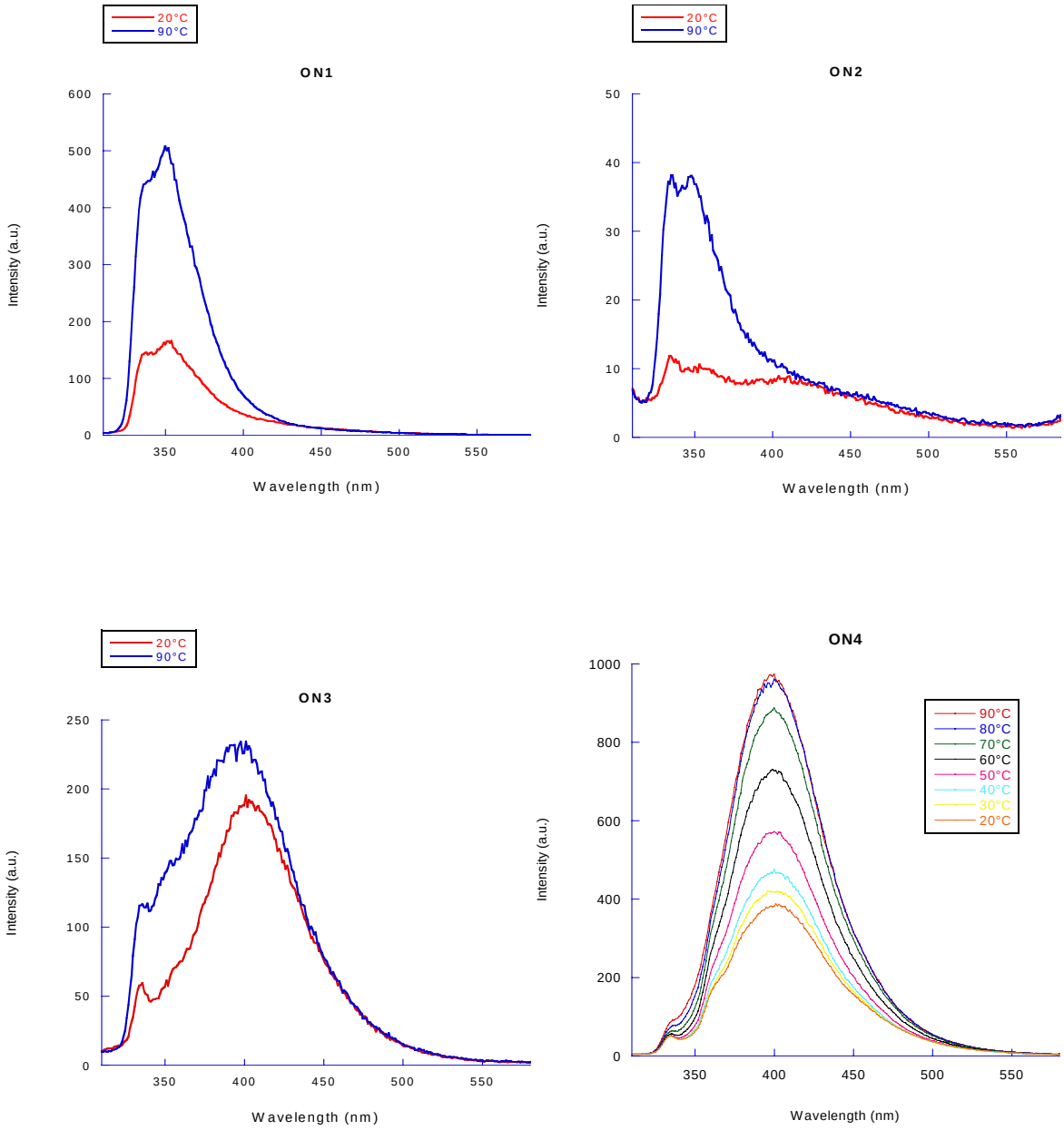

Figure 13. Temperature dependent fluorescence spectra of the hybrids. Condidtions: 1µM single strand concentration, 10mM phosphate buffer, 100mM NaCl. Instrumental setup for ON1,2,3,5,6,7,8: Excitation wavelength (300 nm), excitation slitwidth: 5nm, emission slitwidth: 5nm, detector voltage: 700V, Instrumental setup for ON4: Excitation wavelength (300 nm), excitation slitwidth: 2.5nm, emission slitwidth: 2.5nm, detector voltage: 800V.

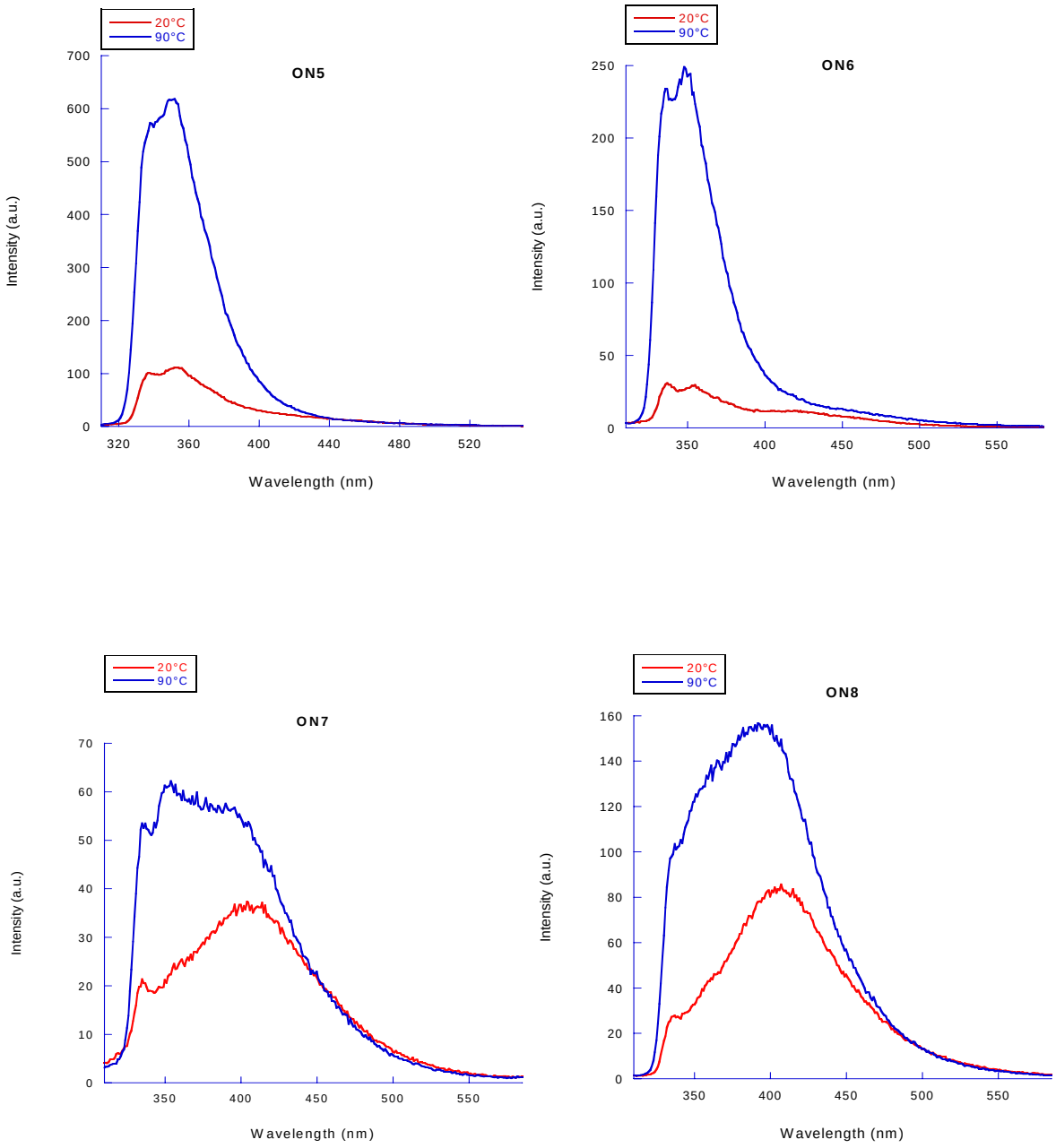

Figure 13 (continued)

9. CD of fluorene-modified hybrids

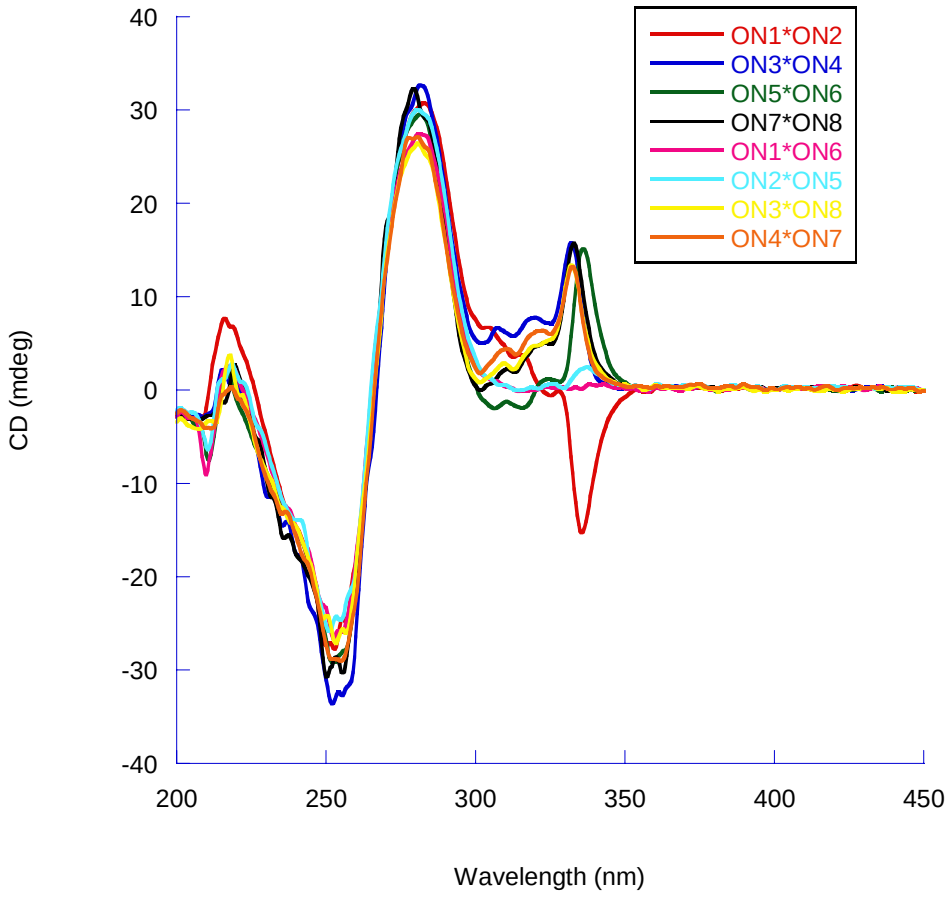

Figure 14. Overview on the circular dichroism spectra of the hybrids at 20°C. Conditions: 5µM single strandconcetration, 10mM phosphatebuffer, 100mM NaCl.

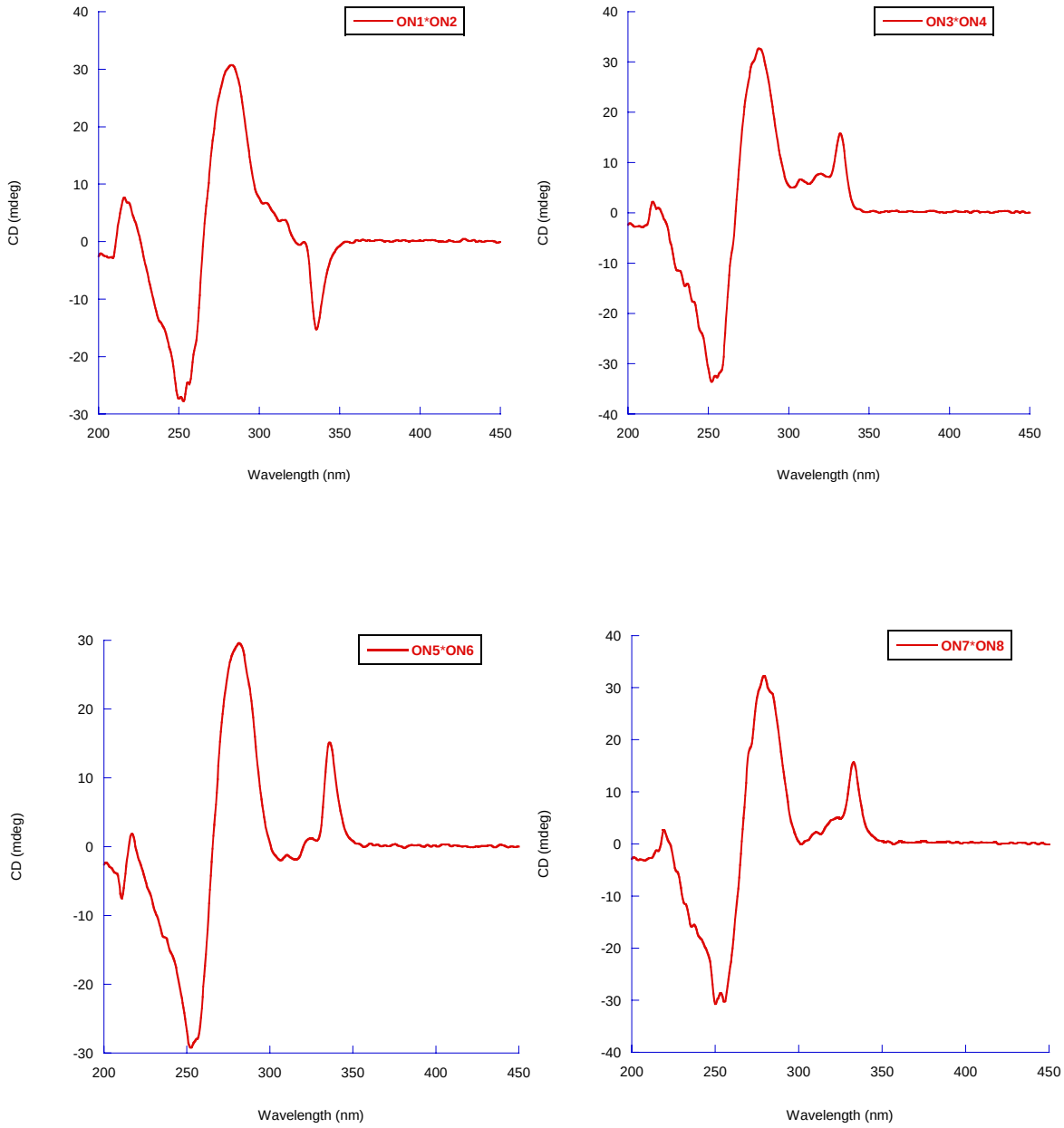

Figure 15. Circular dichroism spectra of the hybrids at 20°C. Conditions: 5µM single strandconcetration, 10mM phosphatebuffer, 100mM NaCl.

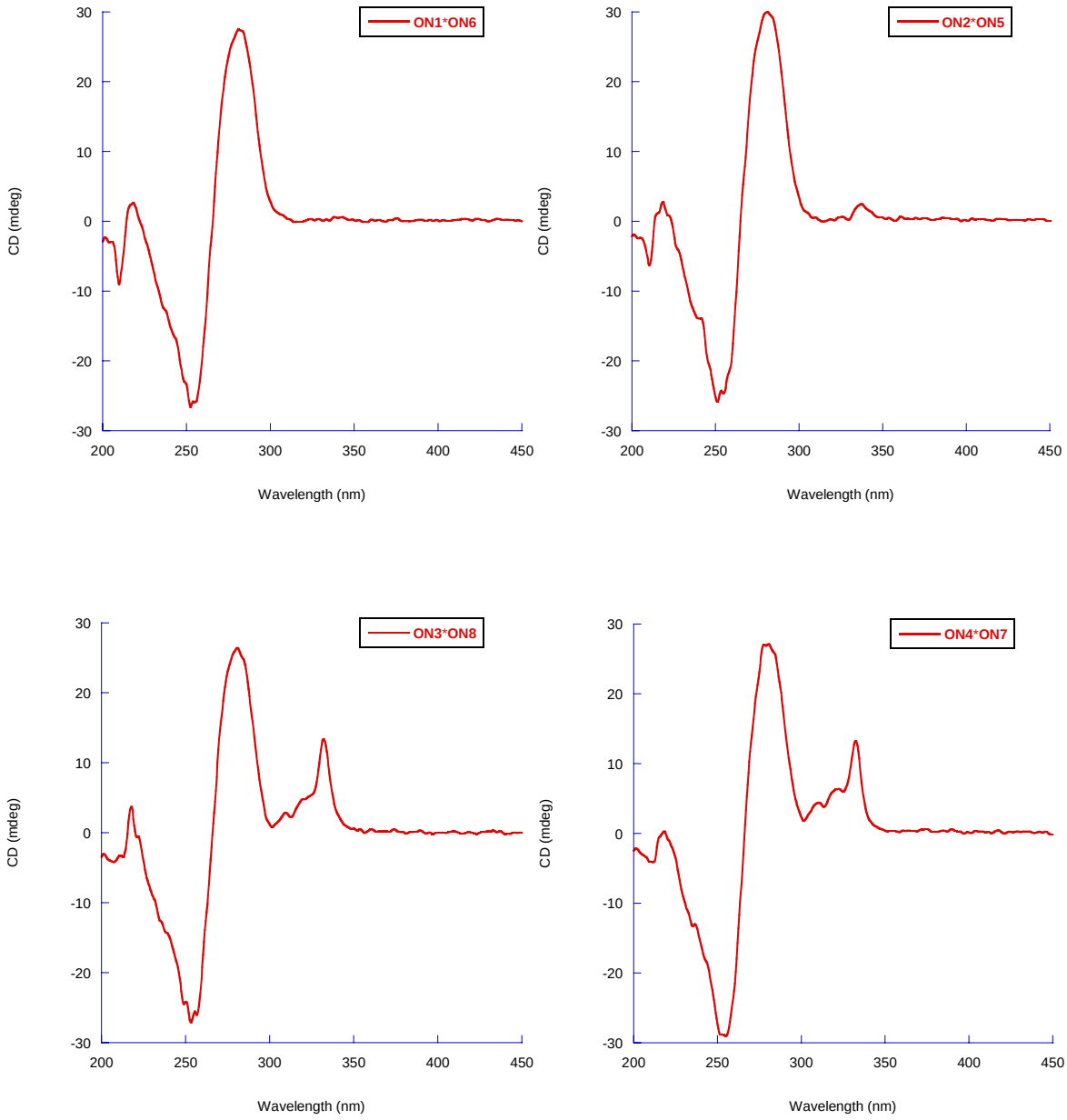

Figure 15 (continued)

10. Circular dichroism of single strands **ON1-ON8**

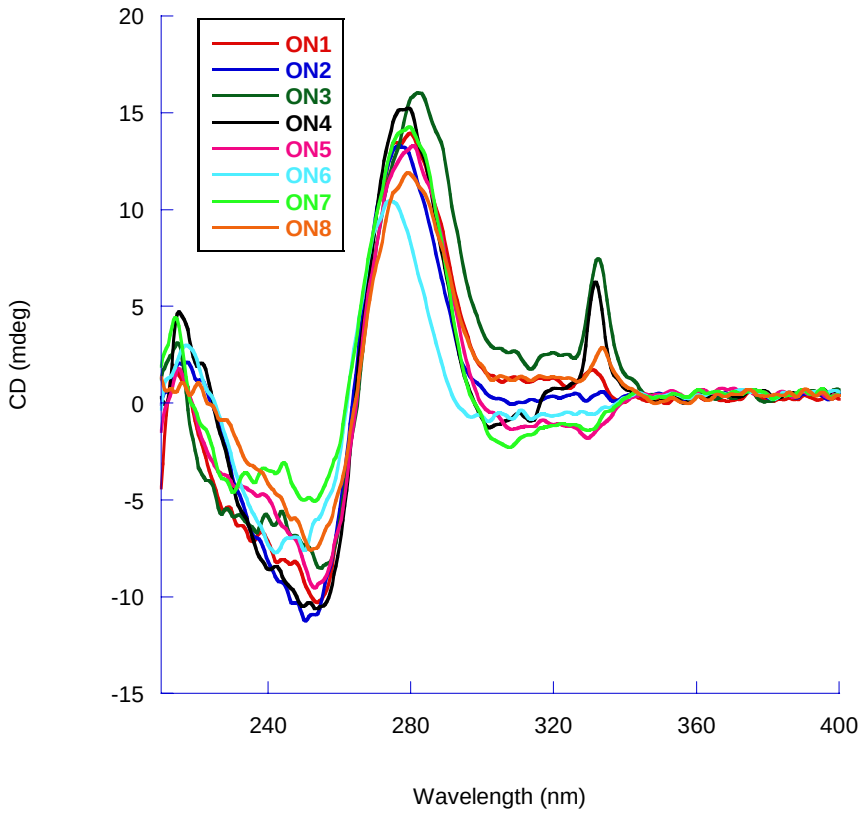

Figure 16. Overview on the circular dichroism spectra of the single strands at 20°C. Conditions: 5µM single strandconcetration, 10mM phosphatebuffer, 100mM NaCl.

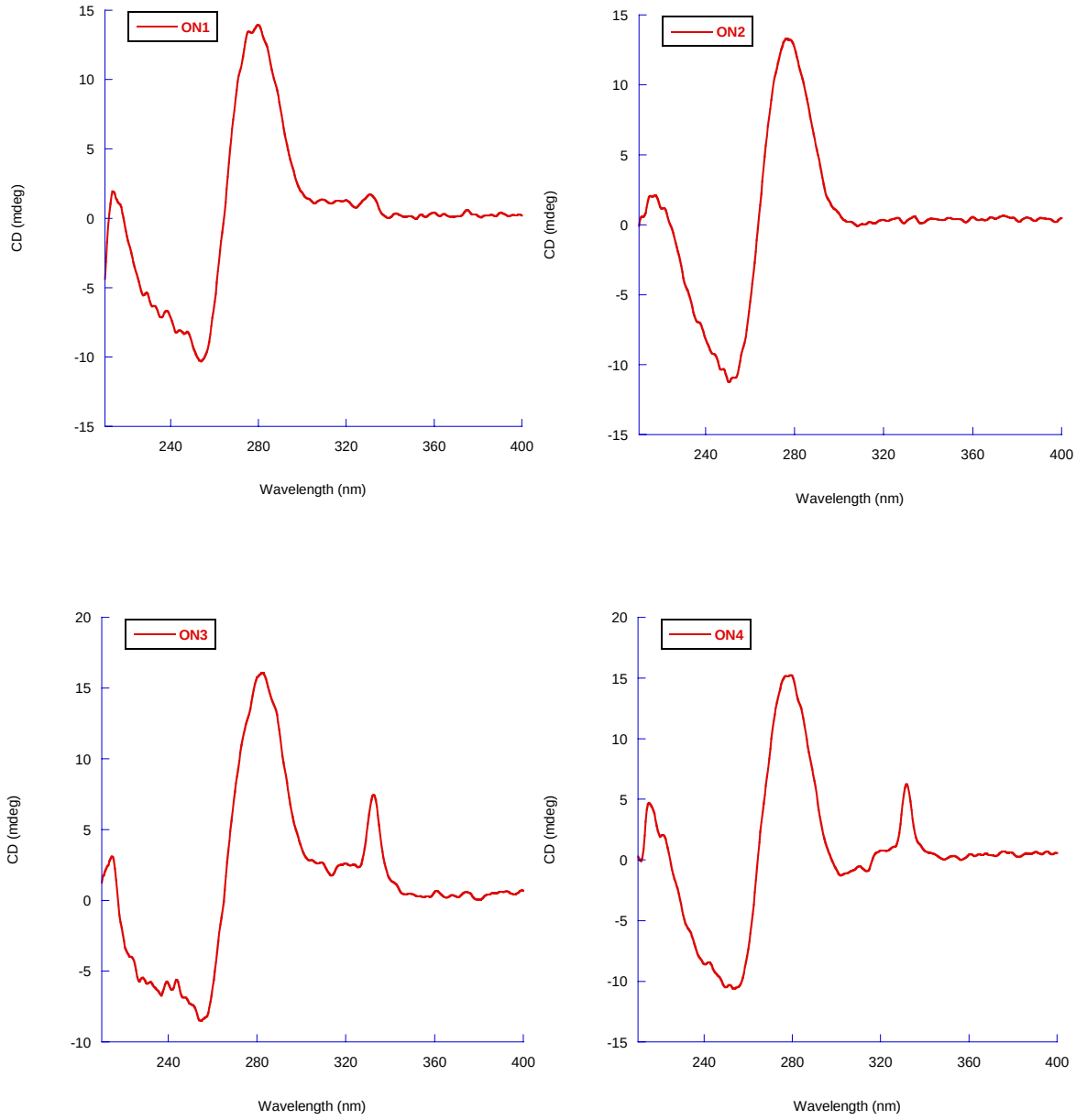

Figure 17. Circular dichroism spectra of the single strands at 20°C. Conditions: 5µM single strandconcetration, 10mM phosphatebuffer, 100mM NaCl.

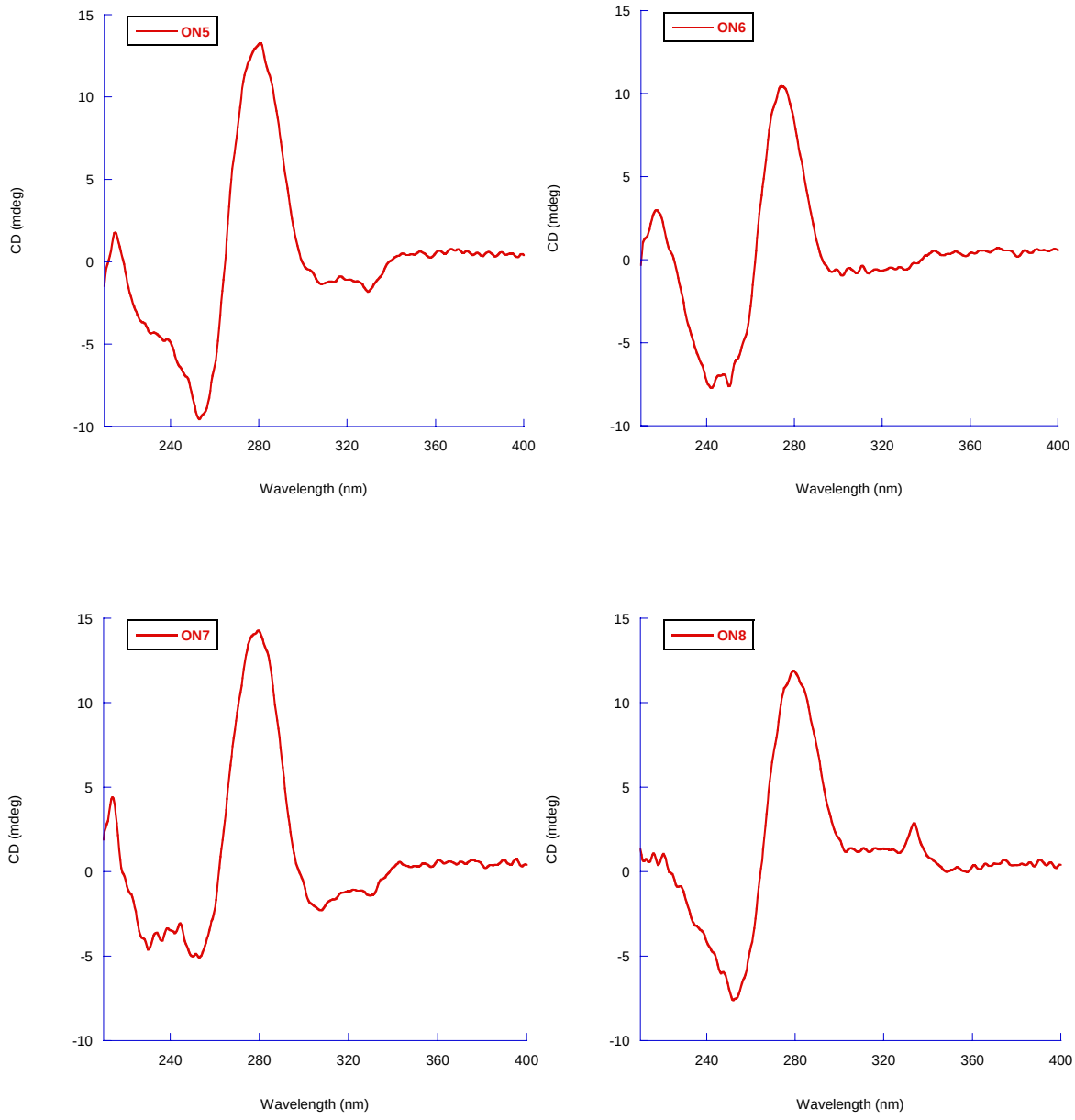

Figure 17 (continued)

11. Quantum Yields

Oligonucleotide	Quantum Yield 20°C	Quantum Yield 85°C
ON1	0.034	0.080
ON2	0.008	0.014
ON3	0.009	0.010
ON4	0.017	0.042
ON5	0.031	0.101
ON6	0.009	0.036
ON7	0.009	0.015
ON8	0.010	0.040

Hybrid	Quantum Yield 20°C
ON1*ON2	0.012
ON3*ON4	0.016
ON5*ON6	0.010
ON7*ON8	0.014
ON1*ON6	0.011
ON2*ON5	0.011
ON3*ON8	0.013
ON4*ON7	0.020