Electronic Supplementary Material (ESI) for New Journal of Chemistry.

SUPPLEMENTARY INFORMATION

Aggregating distryrylpyridinium dye as a bimodal structural probe for G-quadruplex DNA

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Experimental part

General remarks: All commercially available chemicals, used for synthesis, were reagent grade and used without further purification. NMR spectra were measured with a Bruker Avance 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) spectrometer at 25 °C; chemical shifts are given in ppm (δ) values. Multiplicities of ^{13}C NMR signals were determined by means of DEPT-135 experiments. The melting points were determined in open-end capillaries with a digital melting point instrument (SMP30, Stuart). Elemental microanalysis of the new compound was performed by the *Service de Microanalyse*, CNRS–ICSN, Gif-sur-Yvette, France. Mass spectra (ESI in the positive-ion mode) were recorded with a Waters ZQ instrument (source voltage 50–75 kV). The purity of the dye was assessed by LC/MS analysis (Waters Alliance 2525 equipped with a Waters XTerra MS C18-5 µm column and Waters 2996 photodiode array detector; eluent A: water with 0.1% HCOOH, eluent B: MeCN, gradient elution with 10 to 100% of eluent B). Absorption spectra were recorded with an Agilent Cary 300 Bio double-beam spectrophotometer equipped with a temperature controller, in quartz cells (path length 1 cm), using slit widths of 2 nm and a scan rate of 150 nm min^{-1}. Fluorescence spectra were recorded with an Agilent Cary Eclipse Bio fluorimeter equipped with a temperature controller or a HORIBA Jobin Yvon FluoroMax-3 fluorimeter in quartz cells with a cross-section of 1 × 0.5 cm, using slit widths of 2 nm. Fluorescence quantum yield of **BCVP** was determined by comparative method using Rhodamine 6G as a reference (ϕ = 0.95 in EtOH).[1]

Synthesis of 2,4-bis((*E*)-2-(7-diethylaminocoumarin-3-yl)vinyl)-1-methylpyridinium iodide (BCVP): A solution of 1,2,4-trimethylpyridinium iodide (0.349 g, 1.40 mmol), 7-diethylamino-3-formylcoumarin[2] (1.03 g, 4.20 mmol) and piperidine (0.277 mL, 2.80 mmol) in abs. EtOH (14 mL) was heated under reflux for 3.5 h, and cooled to room temperature. The precipitate was collected, washed with EtOH and Et_2O, and recrystallized from MeCN–H_2O, to give **BCVP** (0.493 g, 0.701 mmol, 50%) as dark-red crystals with a bronze shine, m.p. > 260 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.14 (m, 12H), 3.47 (m, 8H), 4.16 (s, 3H), 6.56 (s, 1H), 6.60 (s, 1H), 6.71–6.86 (m, 2H), 7.47–7.58 (m, 2H), 7.60–7.86 (m, 4H), 7.89 (d, *J* = 6.6 Hz, 1H), 8.15 (s, 1H), 8.26 (s, 1H), 8.38 (s, 1H), 8.67 (d, *J* = 6.6 Hz, 1H) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 12.4 (CH_3), 44.4 (CH_2), 44.6 (CH_3), 96.1 (CH), 96.2 (CH), 108.3 (C_q), 108.4 (C_q), 109.9 (CH), 110.1 (CH), 113.4 (C_q), 113.9 (C_q), 116.4 (CH), 119.4 (CH), 120.3 (CH), 123.2 (CH), 130.6 (CH), 130.8 (CH), 135.9 (CH), 138.0 (CH), 145.0 (CH), 145.2 (CH), 145.8 (CH), 151.8 (C_q), 152.0 (C_q), 152.1 (C_q), 156.2 (C_q), 156.4 (C_q), 159.6 (C_q), 159.7 (C_q) ppm; MS (ESI$^+$): *m*/*z* (%) = 576.3 (100) [M$^+$]; UV/Vis (*c* = 10 µM, MeOH): λ_{max} = 527 nm (ε = 95300 cm^{-1} M^{-1}); anal. calcd. (%) for $C_{36}H_{38}IN_3O_4$ (703.6): C 61.45, H 5.44, N 5.97; found: C 61.16, H 5.47, N 5.98; purity (LC/MS): > 97%.

Nucleic acid samples: Oligonucleotides (see sequences in Table S1), purified by RP-HPLC, were purchased from Eurogentec. Lyophilized oligonucleotides were dissolved in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer (pH 7.2) to a strand concentration of 100 or 500 µM, and the double-stranded structures (Table S1) were prepared by mixing equal volumes of solutions of the corresponding single strands (except for self-complementary duplexes). All samples were then heated to 95 °C for 5 min and left to cool to ambient temperature, to assure hybridization of double-stranded structures and folding of quadruplexes. Calf thymus DNA solution (10 mg mL^{-1}) was purchased from Invitrogen and diluted in the same buffer (see above) to a phosphate concentration of 2.2 mM (as checked by photometry, using the extinction coefficient of 6412 cm^{-1} M^{-1} at 260 nm). DNA samples were stored at 4 °C without freezing.

Spectrophotometric and spectrofluorimetric titrations: Stock solution of the dye was prepared in DMSO at a concentration of 4 mM. Spectrophotometric and spectrofluorimetric titrations were performed at a dye concentration of 5 µM (absorption) or 2.5 µM (fluorescence) in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer (pH 7.2) by adding aliquots of stock solutions of oligonucleotides (500 µM) or ct DNA.

Microplate-based absorption and fluorescence measurements: A 96-well transparent-bottom quartz microplate (Hellma) was filled with samples (200 µL per well) containing the dye (5 µM) in the absence or in the presence of DNA samples (oligonucleotides: final strand concentration of 25 µM, ct DNA: final phosphate concentration of 550 µM) in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer (pH 7.2). After mixing for 1 min, the microplate was incubated for 3 h at 25 °C and then read with a multi-mode reader (BMG FLUOstar Omega) in absorption and fluorescence modes. In the absorption mode, spectra were recorded from 300 to 800 nm using 50 flashes per well. In the fluorescence mode, emission intensity was measured using an excitation band-pass filter of 544 nm and an emission filter of 670 nm (bandwidth 10 nm) using 100 flashes per well. Fluorescence data were corrected to the signal of "blank" wells containing only buffer solution (Equation S1):

$$S = \frac{F(\text{DNA+dye}) - F_0}{F(\text{dye}) - F_0}, \qquad \text{(S1)}$$

where F(DNA+dye) is the emission intensity of the dye in the presence of a DNA sample, F(dye) is the average emission intensity of the wells containing the dye but no DNA, and F_0 is the average emission intensity of the wells containing only the buffer solution.

Thermal difference spectra and thermal denaturation of DNA samples: Thermal difference spectra (TDS) and thermal denaturation experiments were performed with samples containing 5 µM DNA (strand concentration) in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer (pH 7.2). For TDS, absorption spectra were recorded at 20 °C and then at 90 °C [ΔAbs = A(90 °C) – A(20 °C)]. In thermal denaturation experiments, samples were heated from 15 °C to 95 °C at a rate of 0.2 deg min^{-1}, while the absorption was continuously monitored at 260 nm at 295 nm.

Circular dichroism: Circular dichroism (CD) spectra of MTB2.1 and MTB2.2 were recorded with a JASCO J-710 spectropolarimeter equipped with a Peltier temperature controller, using quartz cells with a path length of 10 mm. The samples contained 5 µM DNA (strand concentration) in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer (pH 7.2). The scans were recorded at 20 °C from 210 to 330 nm using the following parameters: data pitch, 1 nm; bandwidth, 2 nm; response, 2 s; scan speed, 50 nm min^{-1}; the scans are the result of eight accumulations. The CD spectra were blank-subtracted and converted to molar dichroic absorption ($\Delta\varepsilon$, cm^{-1} M^{-1}) based on nucleoside concentration (Equation S2):

$$\Delta\varepsilon = \frac{\theta}{32980 \times c \times \ell}, \qquad \text{(S2)}$$

where θ is the ellipticity (millidegrees), c is the total nucleoside concentration in sample (M), and ℓ is the path length (cm).

Table S1. DNA sequences used in this work.

Acronym	Sequence (5′ → 3′)	Bases	Structure	PDB	Ref.
22CTA	AGGGCTAGGGCTAGGGCTAGGG	22	G4 anti-parallel	2KM3	3
Bcl2Mid	GGGCGCGGGAGGAATTGGGCGGG	23	G4 hybrid	2F8U	4
93del	GGGGTGGGAGGAGGGT	16	G4 parallel, dimer	1Y8D	5
26CEB	AAGGGTGGGTGTAAGTGTGGGTGGGT	26	G4 parallel	2LPW	6
c-kit2-T12T21	CGGGCGGGCGCTAGGGAGGGT	21	G4 parallel	2KYP	7
TBA	GGTTGGTGTGGTTGG	15	G4 anti-parallel		8
c-kit87up	AGGGAGGGCGCTGGGAGGAGGG	22	G4 parallel	2O3M	9
c-myc	TGAGGGTGGGTAGGGTGGGTAA	22	G4 parallel	1XAV	10
c-kit2-T21	CGGGCGGGCGCGAGGGAGGGT	21	G4 parallel, dimer	2KYO	7
22AG	AGGGTTAGGGTTAGGGTTAGGG	22	G4 hybrid (mixture)		11
CEB1	AGGGGGGAGGGAGGGTGG	18	G4 parallel, dimer	2MB4	12
Bm-U16	TAGGTTAGGTTAGGTUAGG	19	G4 anti-parallel		13
TET4	TTGGGGTTGGGGTTGGGGTTGGGG	24	G4 hybrid	186D	14
HIV-PRO-1	TGGCCTGGGCGGGACTGGG	19	G4 anti-parallel		15
hras-1	TCGGGTTGCGGGCGCAGGGCACGGGCG	27	G4 anti-parallel		16
N-myc	TAGGGCGGGAGGGAGGGAA	19	G4 parallel	2LEE 2LED (dimer)	17
Pu24T	TGAGGGTGGTGAGGGTGGGGAAGG	24	G4 parallel	2MGN	18
MTB2.1	GGGGCTGGTGGGACGGGTGGGGC	23	putative G4		this work
MTB2.2	TGGGACGGGTGGGGCCGGCGGGC	23	putative G4		this work
ss 1	CACTAAACCTAACACTAACCAT	22	single-strand		
ss 2	ATGCCCTACGCGTCTTCTACTT	22	single-strand		
ss 3	GTCGCCGGGCCAGTCGTCCATAC	23	single-strand		
ss 4	GTATGGACGACTGGCCCGGCGAC	23	single-strand		
ss 5	CCCACACACACACTCTCTCTCC	22	single-strand		
ss 6	GACGTGTCGAAAGAGCTCCGATTA	24	single-strand		
ss 7	TAATCGGAGCTCTTTCGACACGTC	24	single-strand		
ss 8	GGAGAGAGAGTGTGTGTGTGGG	22	single-strand		
dT26	TTTTTTTTTTTTTTTTTTTTTTTTTT	26	single-strand		
ct DNA	calf thymus DNA	n/a	duplex		
duplex 1	1) ATGGTTAGTGTTAGGTTTAGTG 2) CACTAAACCTAACACTAACCAT	2×22	duplex		
duplex 2	1) GTCGCCGGGCCAGTCGTCCATAC 2) GTATGGACGACTGGCCCGGCGAC	2×23	duplex		
ds-lac	GAATTGTGAGCGCTCACAATTC	22	self-complementary duplex		
duplex 3	1) GACGTGTCGAAAGAGCTCCGATTA 2) TAATCGGAGCTCTTTCGACACGTC	2×24	duplex		
ds26	CAATCGGATCGAATTCGATCCGATTG	26	self-complementary duplex		

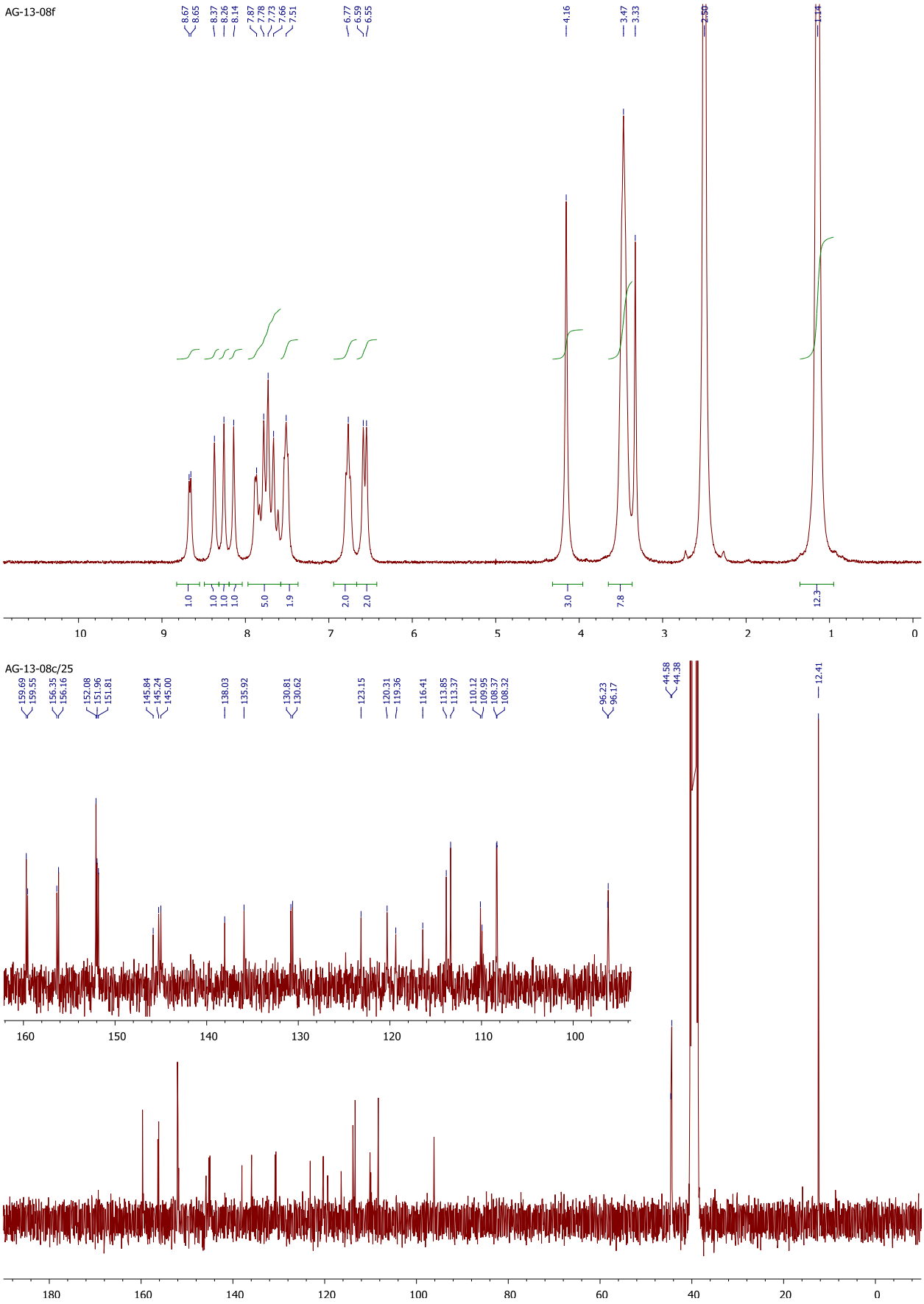


Fig. S1 ^{1}H (300 MHz, top) and ^{13}C (75 MHz, bottom) NMR spectra of **BCVP** in [D_6]DMSO.

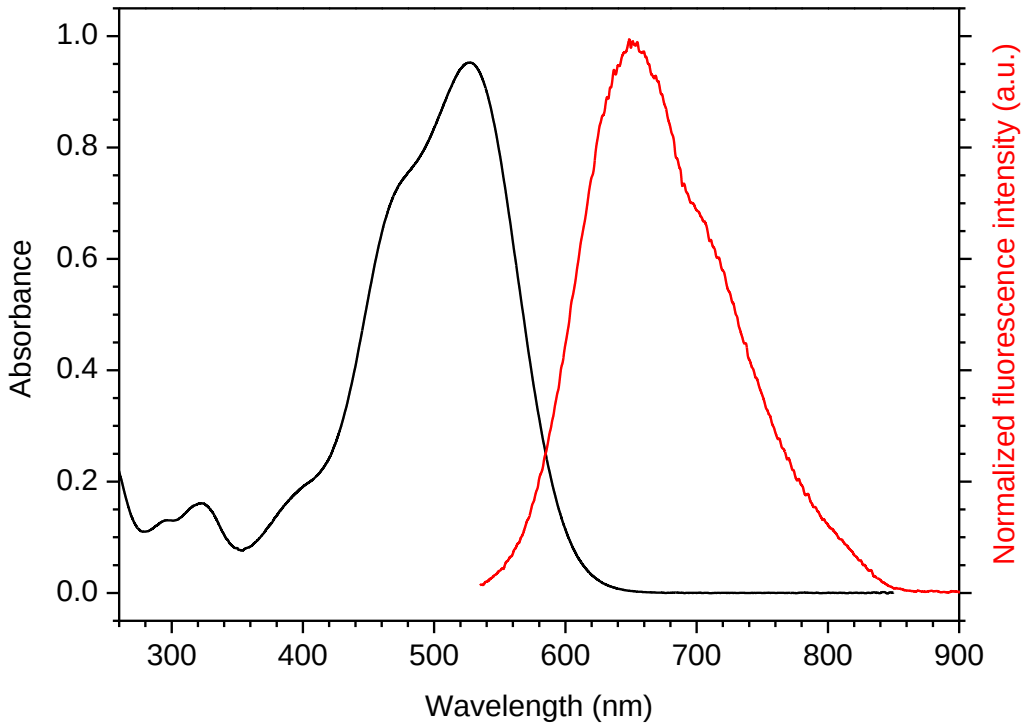


Fig. S2 Absorption (black, c = 10 µM) and emission (red, c = 2 µM, λ_{ex} = 527 nm) spectra of **BCVP** in MeOH.

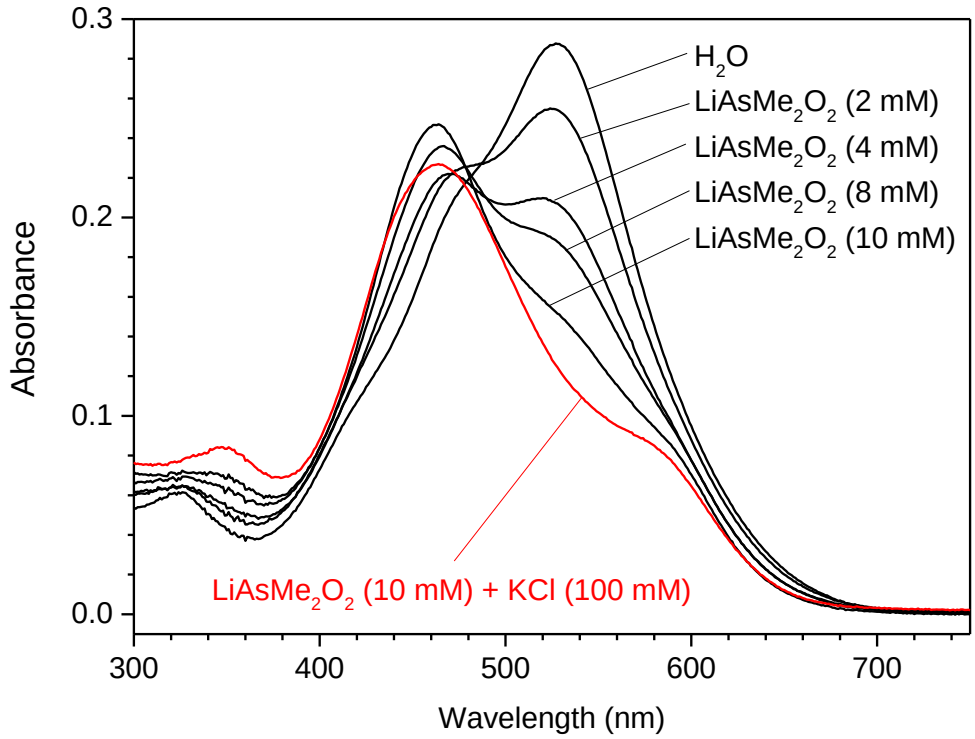


Fig. S3 Absorption spectra of **BCVP** (c = 5 µM) in ultrapure water, $LiAsMe_2O_2$ buffer (2, 4, 8 and 10 mM), as well as in $LiAsMe_2O_2$ (10 mM) + KCl (100 mM) buffer.

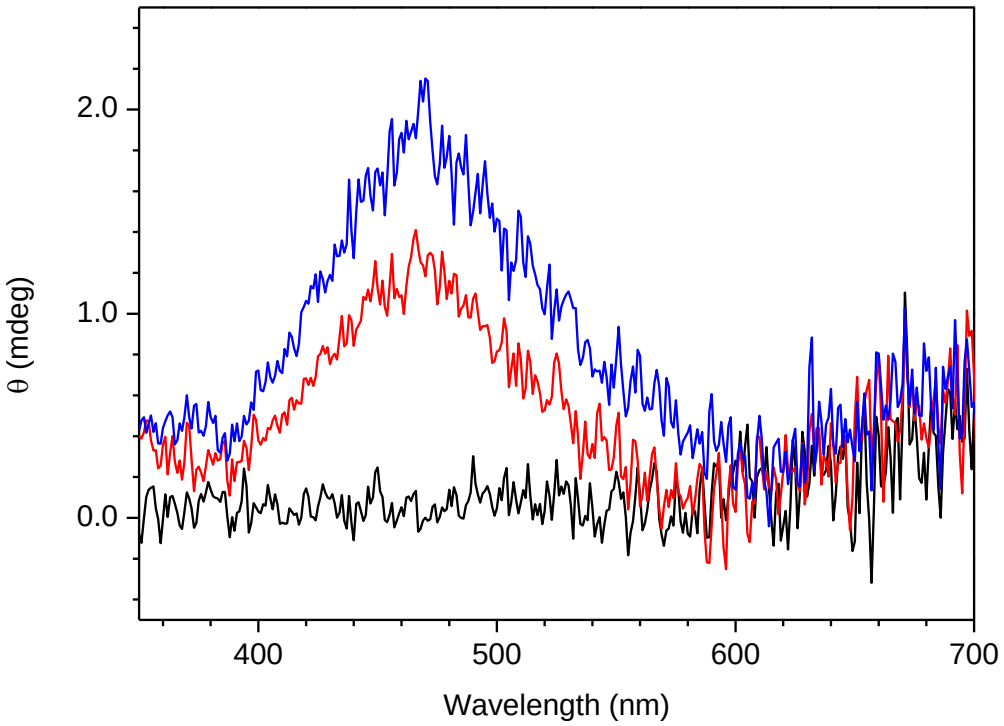


Fig. S4 Circular dichroism spectra of ct DNA (c = 20 µM bp in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2), in the absence (black) and in the presence of **BCVP** at concentrations of 5 µM (red) and 20 µM (blue curve), demonstrating the appearance of induced CD bands of the dye.

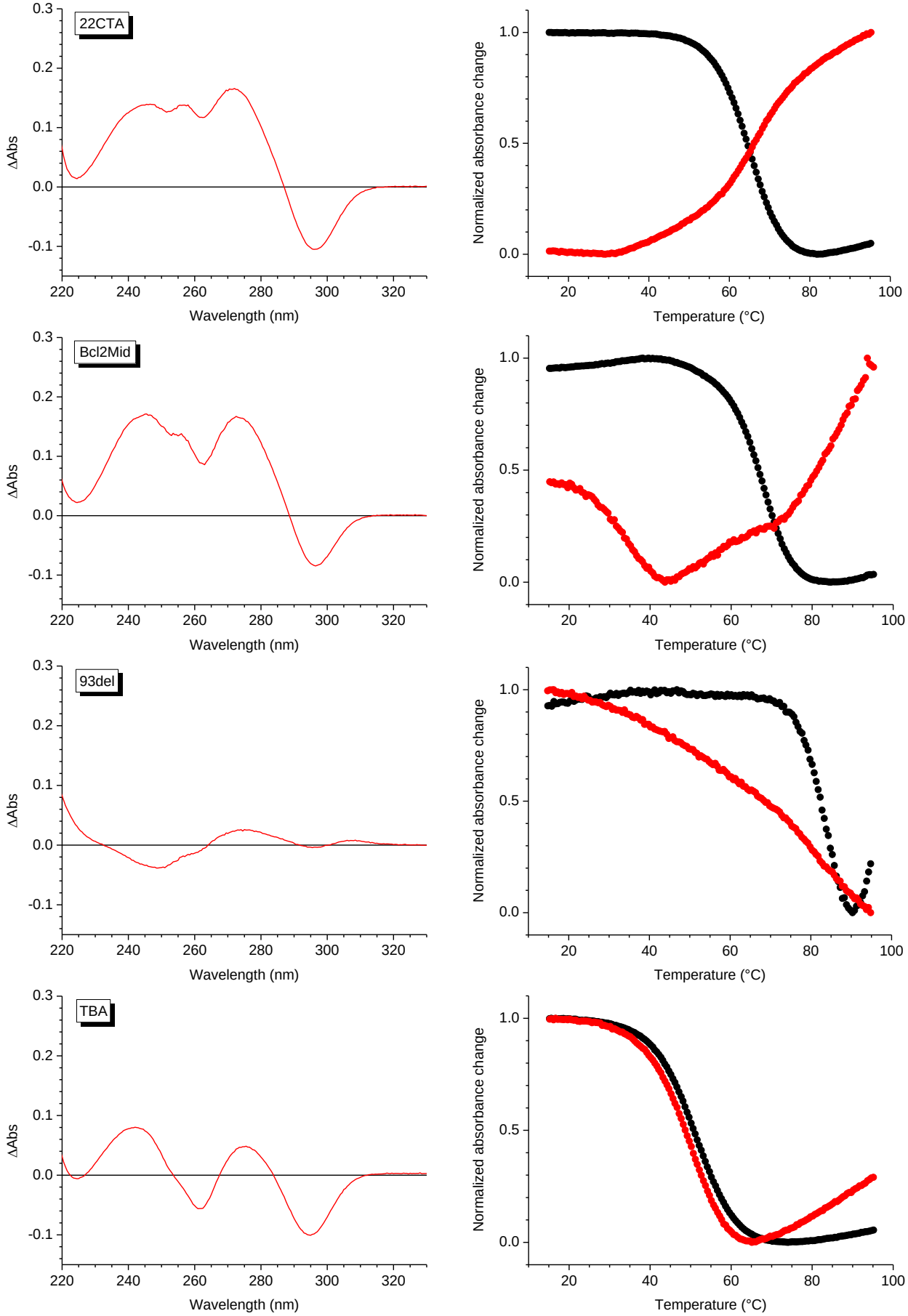


Fig. S5 TDS (left panels; ΔAbs = $A_{90\,°C} - A_{20\,°C}$) and UV melting profiles (right panels), monitored at 260 (red points) and 295 nm (black points), of selected nucleic acid samples (see legends). Experimental conditions: *c*(DNA) = 5 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2.

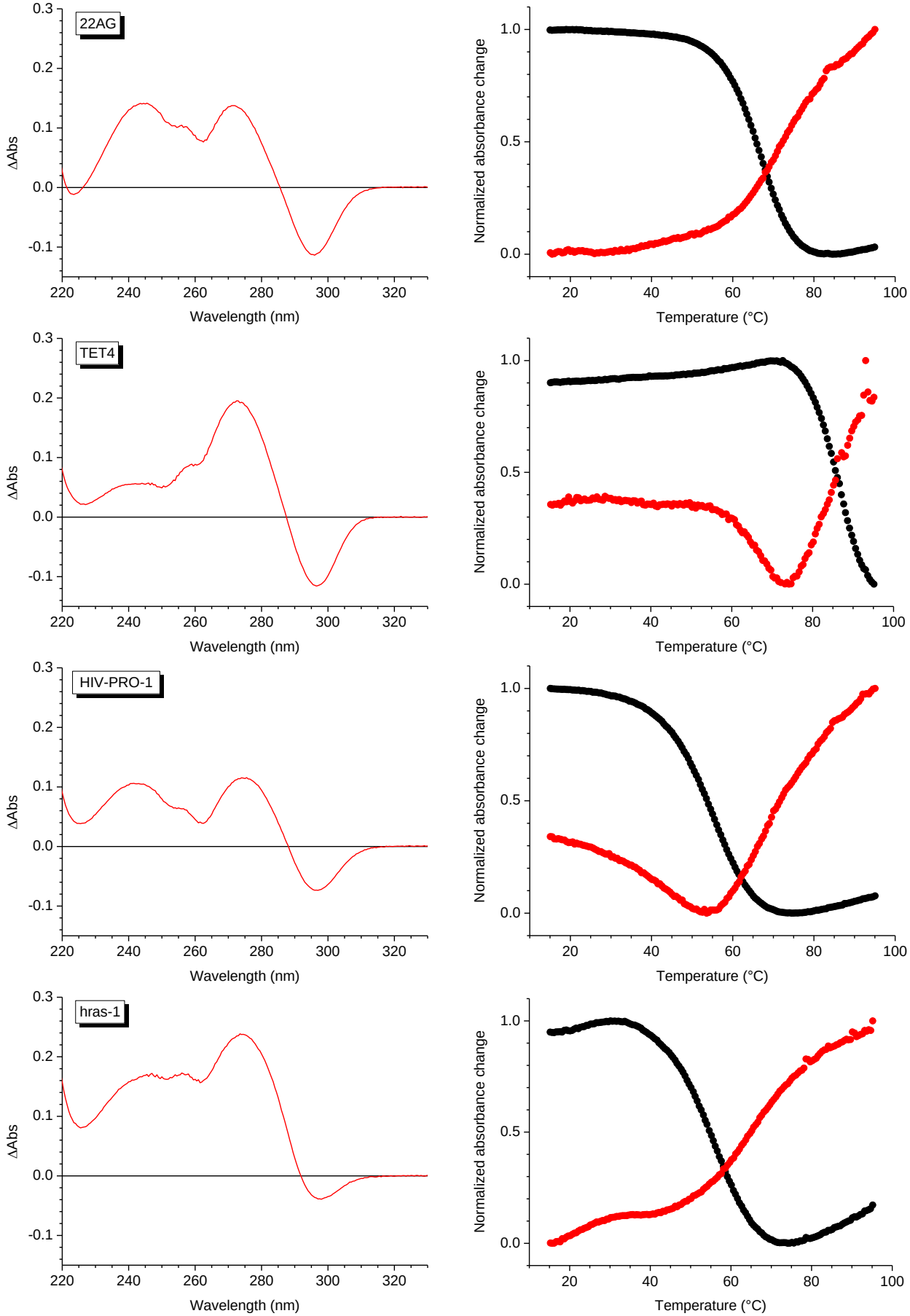


Fig. S6 TDS (left panels; ΔAbs = $A_{90\,°C} - A_{20\,°C}$) and UV melting profiles (right panels), monitored at 260 (red points) and 295 nm (black points), of selected nucleic acid samples (see legends). Experimental conditions: *c*(DNA) = 5 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2.

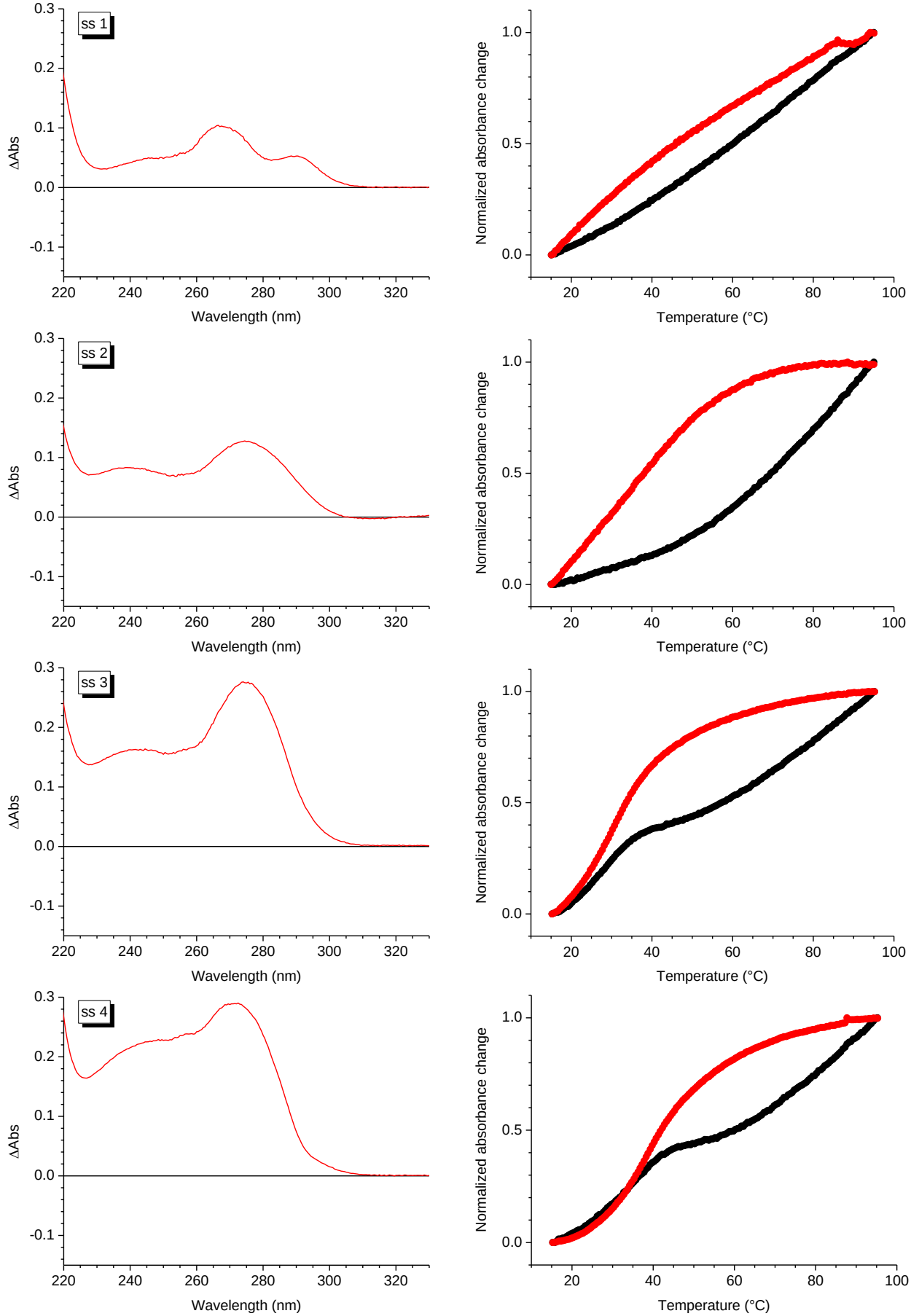


Fig. S7 TDS (left panels; ΔAbs = $A_{90\,°C} - A_{20\,°C}$) and UV melting profiles (right panels), monitored at 260 (red points) and 295 nm (black points), of selected nucleic acid samples (see legends). Experimental conditions: *c*(DNA) = 5 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2.

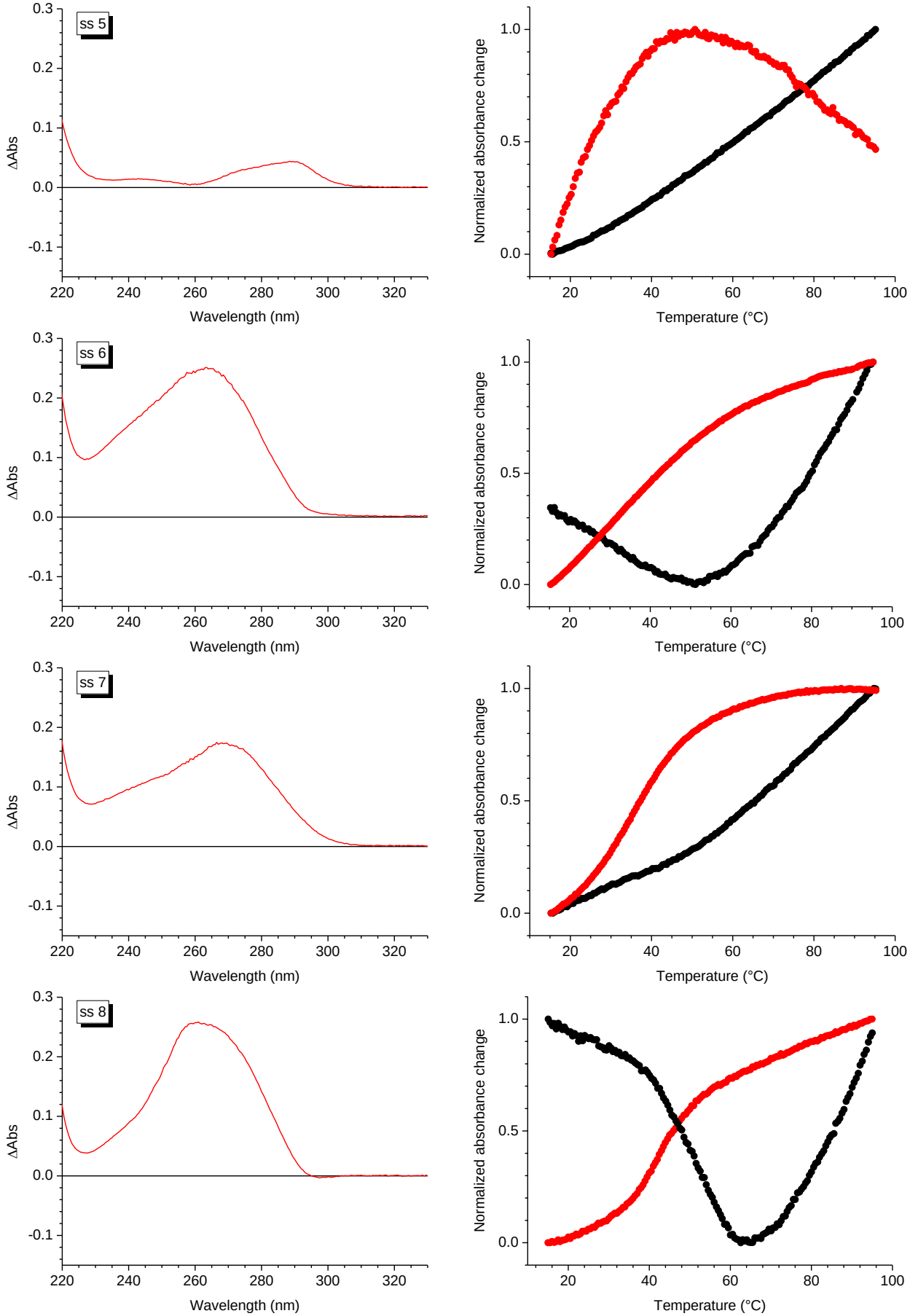


Fig. S8 TDS (left panels; ΔAbs = $A_{90\,°C} - A_{20\,°C}$) and UV melting profiles (right panels), monitored at 260 (red points) and 295 nm (black points), of selected nucleic acid samples (see legends). Experimental conditions: *c*(DNA) = 5 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2.

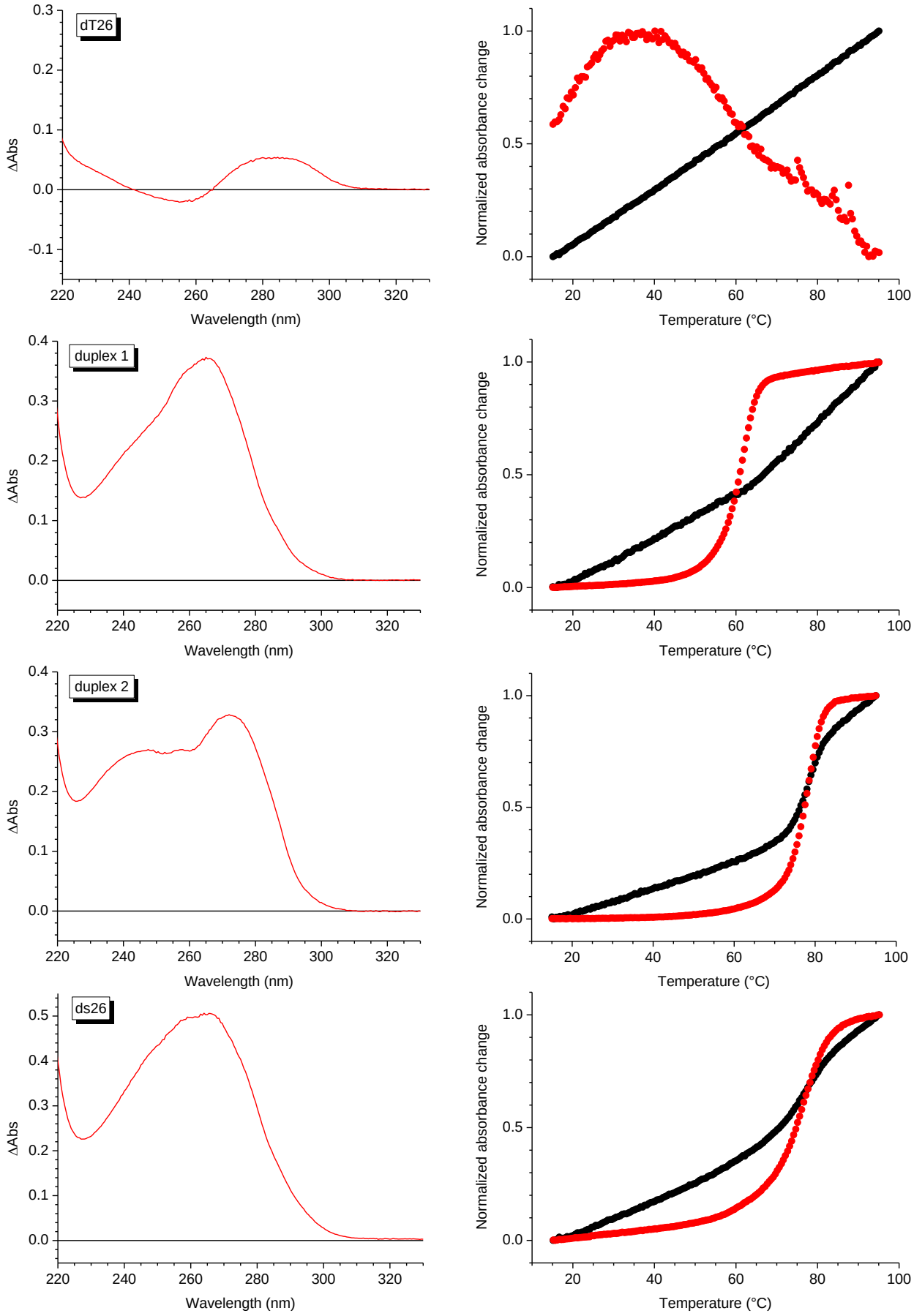
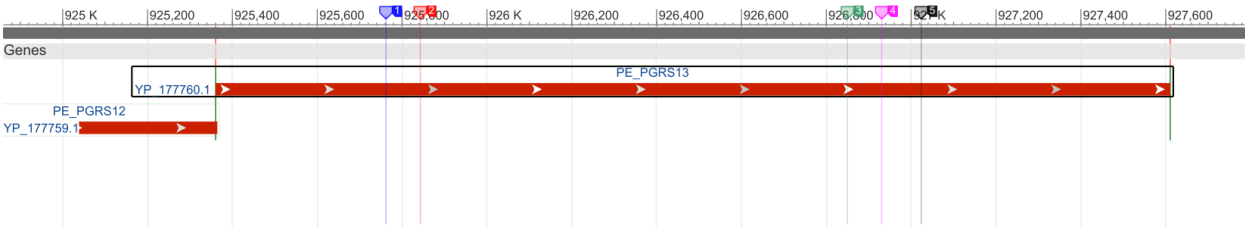


Fig. S9 TDS (left panels; ΔAbs = $A_{90\,°C} - A_{20\,°C}$) and UV melting profiles (right panels), monitored at 260 (red points) and 295 nm (black points), of selected nucleic acid samples (see legends). Experimental conditions: *c*(DNA) = 5 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2.

a)



b)

Nr	Pos.	G-score	Sequence
1	925762	42	TGGGGTTGGTGGGACGGGTGGGGCCGGAGGGC
2	925843	42	TGGGGCTGGTGGGACGGGTGGGGCCGGCGGGC
3	926848	42	CGGGGCCGGTGGGCCCGGAGGGGCCGGTGGGC
4	926929	42	CGGGGCTGGCGGGGAGGGCGGGGCCGGCGGGG
5	927022	42	GGGGGCCGGCGGGGCCGGTGGGGTCGGTGGGC

Fig. S10 (a) Genomic mapping and (b) details of five repetitive 31-mer G-rich sequences (**1**–**5**) found in the PE_PGRS13 gene (CDS: YP 177760.1) of *Mycobacterium tuberculosis* H37Rv. The conserved guanines are shown in dark yellow; G-score is calculated using the default QGRS Mapper parameters.

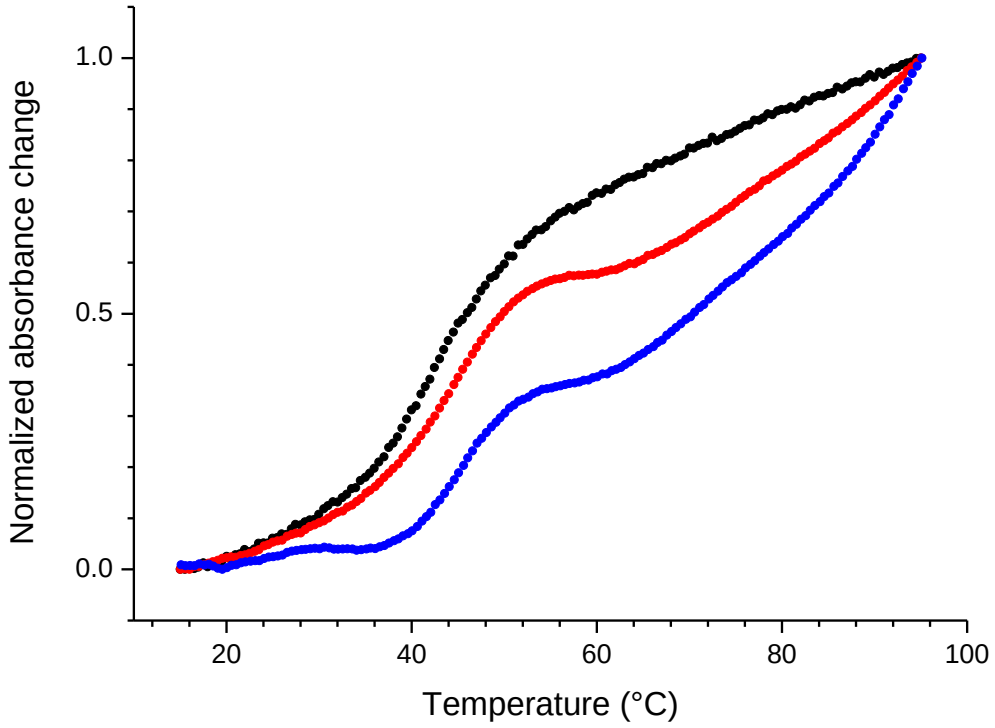


Fig. S11 UV melting profiles, monitored at 260 nm, of oligonucleotide ss 8 (c = 2 µM in 10 mM $LiAsMe_2O_2$, 100 mM KCl buffer, pH 7.2) in the absence (black) and in the presence of one (red) and two (blue) molar equivalents of **BCVP**.

References

1. *A Guide to Recording Fluorescence Quantum Yields*, HORIBA Jobin Yvon (Application note), URL: http://www.horiba.com/scientific/products/fluorescence-spectroscopy/application-notes/quantum-yields/.
2. G. He, X. Zhao, X. Zhang, H. Fan, S. Wu, H. Li, C. He and C. Duan, *New J. Chem.*, 2010, **34**, 1055.
3. K. W. Lim, P. Alberti, A. Guédin, L. Lacroix, J.-F. Riou, N. J. Royle, J.-L. Mergny and A. T. Phan, *Nucleic Acids Res.*, 2009, **37**, 6239–6248.
4. J. Dai, D. Chen, R. a Jones, L. H. Hurley and D. Yang, *Nucleic Acids Res.*, 2006, **34**, 5133–5144.
5. A. T. Phan, V. Kuryavyi, J. Ma, A. Faure, M.-L. Andréola and D. J. Patel, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 634–639.
6. S. Amrane, M. Adrian, B. Heddi, A. Serero, A. Nicolas, J. Mergny and A. T. Phan, *J. Am. Chem. Soc.*, 2012, **134**, 5807–5816.
7. V. Kuryavyi, A. T. Phan and D. J. Patel, *Nucleic Acids Res.*, 2010, **38**, 6757–6773.
8. P. Schultze, R. F. Macaya and J. Feigon, *J. Mol. Biol.*, 1994, **235**, 1532–1547.
9. A. T. Phan, V. Kuryavyi, S. Burge, S. Neidle and D. J. Patel, *J. Am. Chem. Soc.*, 2007, **129**, 4386–4392.
10. A. Ambrus, D. Chen, J. Dai, R. A. Jones and D. Yang, *Biochemistry*, 2005, **44**, 2048–2058.
11. A. Ambrus, D. Chen, J. Dai, T. Bialis, R. a Jones and D. Yang, *Nucleic Acids Res.*, 2006, **34**, 2723–2735.
12. M. Adrian, D. J. Ang, C. J. Lech, B. Heddi, A. Nicolas and A. T. Phan, *J. Am. Chem. Soc.*, 2014, **136**, 6297–6305.
13. S. Amrane, R. W. L. Ang, Z. M. Tan, C. Li, J. K. C. Lim, J. M. W. Lim, K. W. Lim and A. T. Phan, *Nucleic Acids Res.*, 2009, **37**, 931–938.
14. Y. Wang and D. J. Patel, *Structure*, 1994, **2**, 1141–1156.
15. S. Amrane, A. Kerkour, A. Bedrat, B. Vialet, M.-L. Andreola and J.-L. Mergny, *J. Am. Chem. Soc.*, 2014, **136**, 5249–5252.
16. A. Membrino, S. Cogoi, E. B. Pedersen and L. E. Xodo, *PLoS One*, 2011, **6**, e24421.
17. M. Trajkovski, M. W. da Silva and J. Plavec, *J. Am. Chem. Soc.*, 2012, **134**, 4132–4141.
18. A. T. Phan, V. Kuryavyi, H. Y. Gaw and D. J. Patel, *Nat. Chem. Biol.*, 2005, **1**, 167–173.