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

Electronic Supplementary Information

"The interaction of a β-Fused isoindoline-porphyrin conjugate with nucleic acids"

Federica Mandoj,[a] Alessandro D'Urso,[b] Sara Nardis,[a] Donato Monti, [a] Manuela Stefanelli,[*a] Chiara M. A. Gangemi, [b] Rosalba Randazzo, [b] Frank R. Fronczek,[c] Kevin M. Smith,[c] and Roberto Paolesse[a]

*corresponding author: manuela.stefanelli@uniroma2.it

[a] *Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome, Italy*

[b] *Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Viale A. Doria 6, 95125 Catania Italy*

[c] *Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*

To be published in New Journal of Chemistry.

General

Reagents and solvents for syntheses (Aldrich, Merck or Fluka) were of the highest grade available and were used without further purification. Chromatographic purification was perfomed by column, using using silica gel 60 (70-230 mesh, Sigma Aldrich) as the stationary phase. ^{1}H NMR spectra were recorded on a Bruker AV300 spectrometer (300 MHz). FAB mass spectra were obtained on a VGQuattro spectrometer in the positive-ion mode using $CHCl_3$ as solvent and m-nitrobenzyl alcohol (Aldrich) as matrix. UV-vis spectra were measured on a Cary 50 spectrophotometer using chloroform as solvent.

Preparation of compound 2: Zinc dioxo porphyrin complex **1** (120 mg; 0,17 mmol) was dissolved in a solution of dichloromethane/ethanol/acetic acid (5:5:1) (44 mL) and an excess of DAMN (460 mg; 4,3 mmol) was added. The resulting solution was refluxed for 3 h, following the progress of the reaction by TLC analysis and UV-vis spectroscopy. After solvent evaporation, the crude product was purified on a short column of silica gel, eluting with dichloromethane. The first dichroic fraction was collected and crystallized from CH_2Cl_2/MeOH to afford the zinc porphyrin **2** as a green-violet powder (90 mg, 0.12 mmol; 68% yield).

Mp >300°C.

UV-vis ($CHCl_3$): λ_{max}, nm (log ε) nm 436 (5.06), 473 sh, 598 (4.61).

IR: ($CHCl_3$): ν_{max}/cm^{-1} 2235.

^{1}H NMR δ_H ($CDCl_3$, J [Hz]): 8.98 (q, 4H, β-pyrroles), 8.92 (s, 2H, β-pyrroles), 8.21 (d, 4H, J=7.6 Hz, phenyls), 8.02 (d, 4H, J=6.9 Hz, phenyls), 7.82 (m, 12H, phenyls).

MS (FAB) : m/z 780 (M^+). Anal. Calcd for $C_{48}H_{26}ZnN_8$: C, 73.90; H, 3.36; N, 14.36. Found C, 73.82; H, 3.29; N, 14.43 %.

Preparation of compound 3: compound **2** (80 mg; 0,10 mmol) was dissolved in 30 mL of formamide and an excess of sodium amide (50 wt. % suspension in toluene), washed with hexane, (120 mg; 3,08 mmol) was added. The mixture was heated at 70 °C for 7 h and then poured into water. The precipitate was filtered off, dissolved in chloroform and dried over anhydrous Na_2SO_4. After solvent evaporation, the product was directly crystallized from CH_2Cl_2/MeOH affording the desired product **(3)** as a green powder (49 mg, 0,061 mmol; 61% yield).

Mp >300°C. UV-vis ($CHCl_3$): λ_{max}, nm (log ε) nm 442 (4.97), 591 (4.15).

^{1}H NMR δ_H ($CDCl_3$, J [Hz]): 9.22 (br s, 1H, -NH), 9.01 (br s, 3H, β-pyrroles), 8.88 (br s, 3H, β-pyrroles), 8.18 (br m, 4H, phenyls), 7.56 (br m, 16H, phenyls), 6.65 (br s, 2H, -NH_2).

MS (FAB) : m/z 799 (M^+). Anal. Calcd for $C_{48}H_{29}ZnN_9$: C, 72.32; H, 3.67; N, 15.81. Found C, 72.51; H, 3.61; N, 15.63 %.

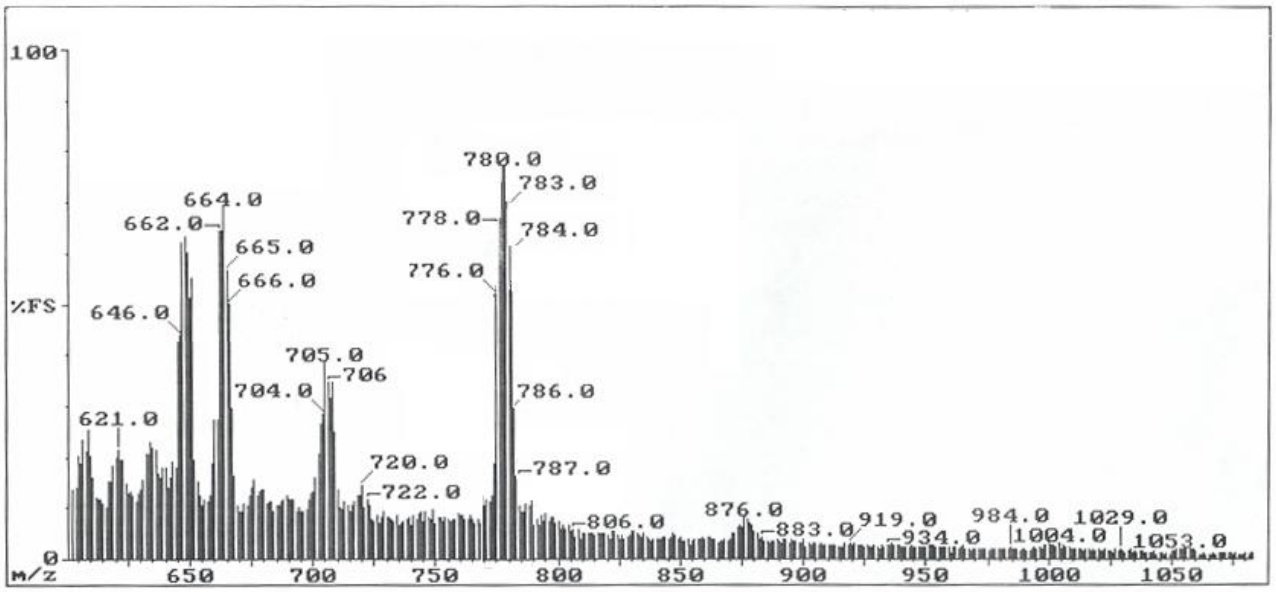

Figure S1: FAB mass spectrum of Zn-porphyrin **2**

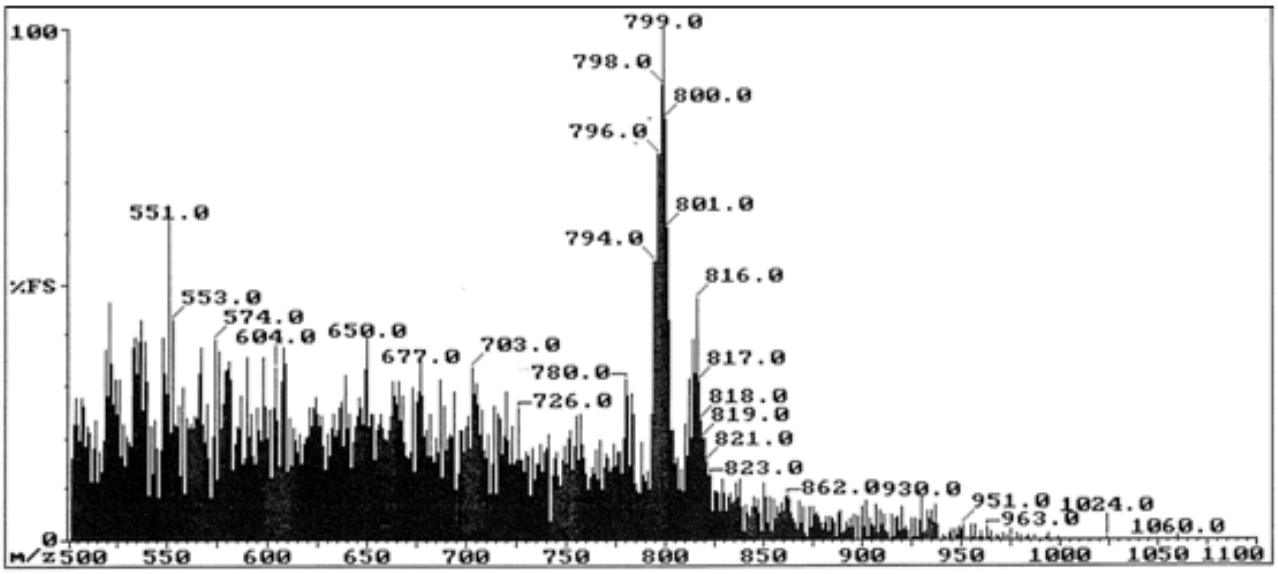

Figure S2: FAB mass spectrum of Zn-porphyrin **3**

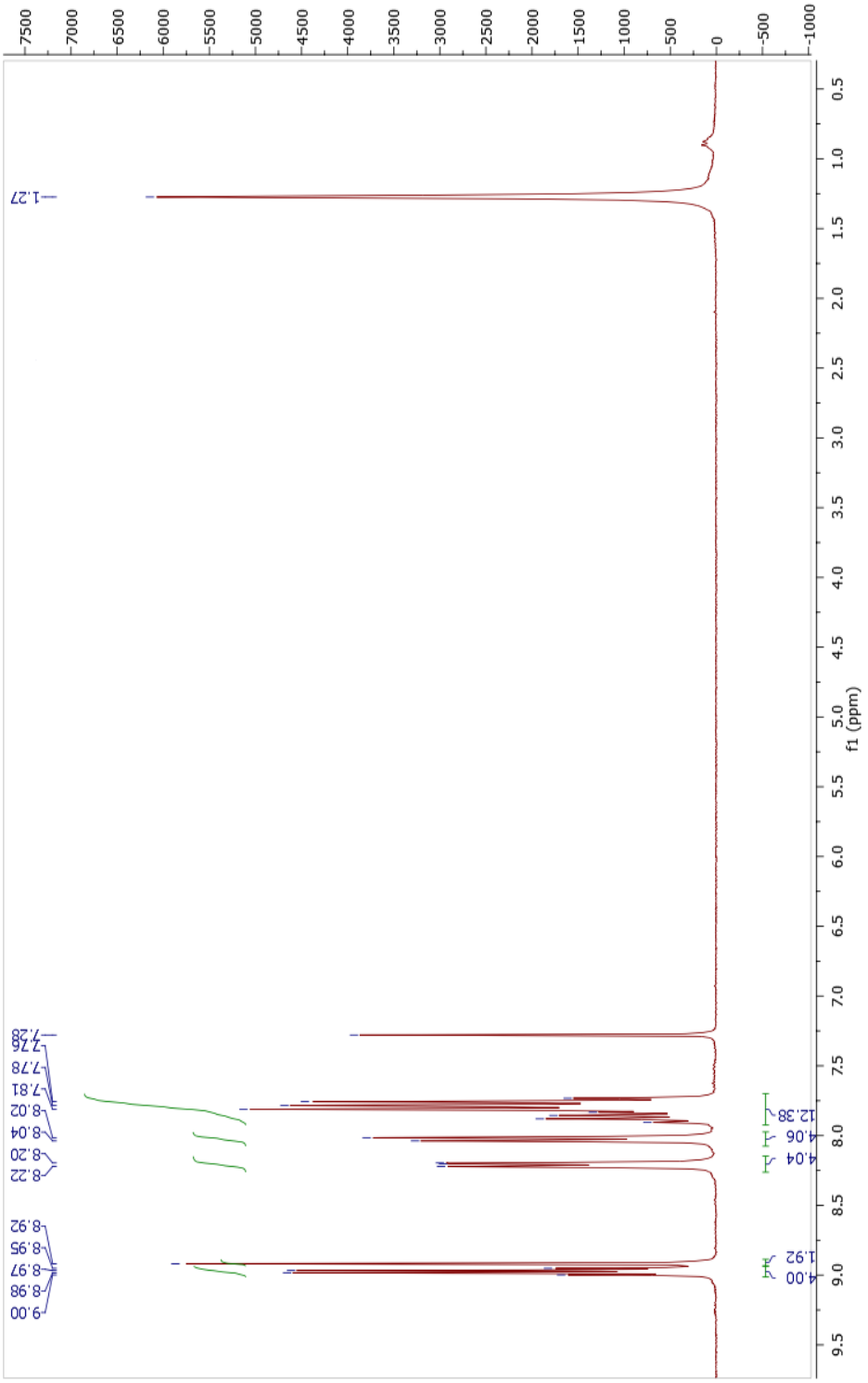

Figure S3: ^{1}H NMR spectrum in $CDCl_3$of Zn-porphyrin **2**

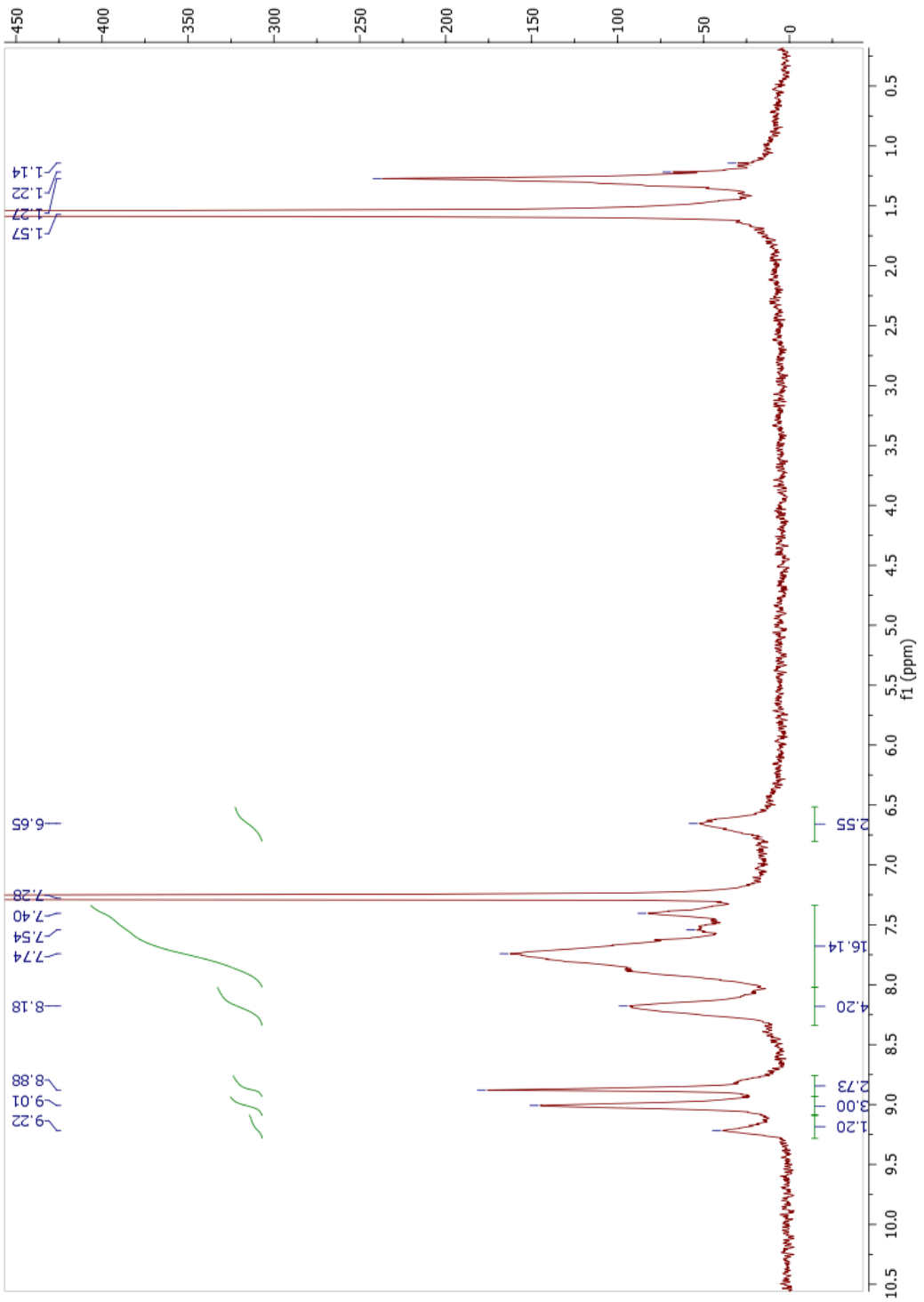

Figure S4: ^{1}H NMR spectrum in $CDCl_3$of Zn-porphyrin **3**

UV/Vis experiments: Porphyrin stock solutions were prepared dissolving solid compound in a mixture of DMSO:H_2O (4:1) to obtain [ZnTPPIsoind]= $3.03x10^{-4}$M. All nucleotides were purchased on Sigma Aldrich. The stock solutions were prepared dissolving the nucleotides in Na phosphate buffer (1mM, pH 7.0) in order to obtain a concentration ranging from 0,8 mM to 1 mM. The UV titration experiments were performed adding increasing amount of nucleotides (0,5 – 4,5 μM) to a 2 μM solution of porphyrin diluted in phosphate buffer (1mM, pH 7.0), using JASCO V-530 spectrophotometer. All UV titration experiments were conducted in 3 mL acrylic cuvettes, in which adsorption of porphyrin compounds on the cuvette walls was negligible.

Binding constant: The intrinsic binding constant K_b was determined by using the formula $[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b (\varepsilon_B - \varepsilon_F)$ where ε_A, ε_F, and ε_B correspond to A_{obsd}/[porphyrin], the extinction coefficient for the free porphyrin and the extinction coefficient for the porphyrin complex in fully bound form, respectively. A plot of $[DNA]/(\varepsilon_A - \varepsilon_F)$ vs [DNA] will have a slope of $1/(\varepsilon_B - \varepsilon_F)$ and a y-axis intercept equal to $1/K_b (\varepsilon_B - \varepsilon_F)$. The binding constant K_b was calculated from the ratio of the slope to the y-axis intercept.

UV and CD experiments with poly(dG-dC): polydG-dC is purchased on Sigma Aldrich. The stock solutions were prepared dissolving the sequence in Na cacodilate buffer (1mM, pH 7.0). The interaction between porphyrin and poly(dG-dC) was studied by spectropolarimeter Jasco J-710 equipped with a single-position Peltier temperature-control system and spectrophotometer JASCO V-630. A quartz cuvette with a 1 cm path length was used for these experiments. In order to force the interaction of **3** with poly(dG-dC) we induced the double helix formation in the presence of **3**. After performing UV (Figure S6) and CD (Figure 2) spectra of a solution containing poly(dG-dC) 10 μM and **3** 4 μM, we increased the temperature up to 90°C. After 15 minutes at 90° we decrease fast the temperature to 25° C and carried out the UV (Figure S6) and CD (Figure 2) spectra.

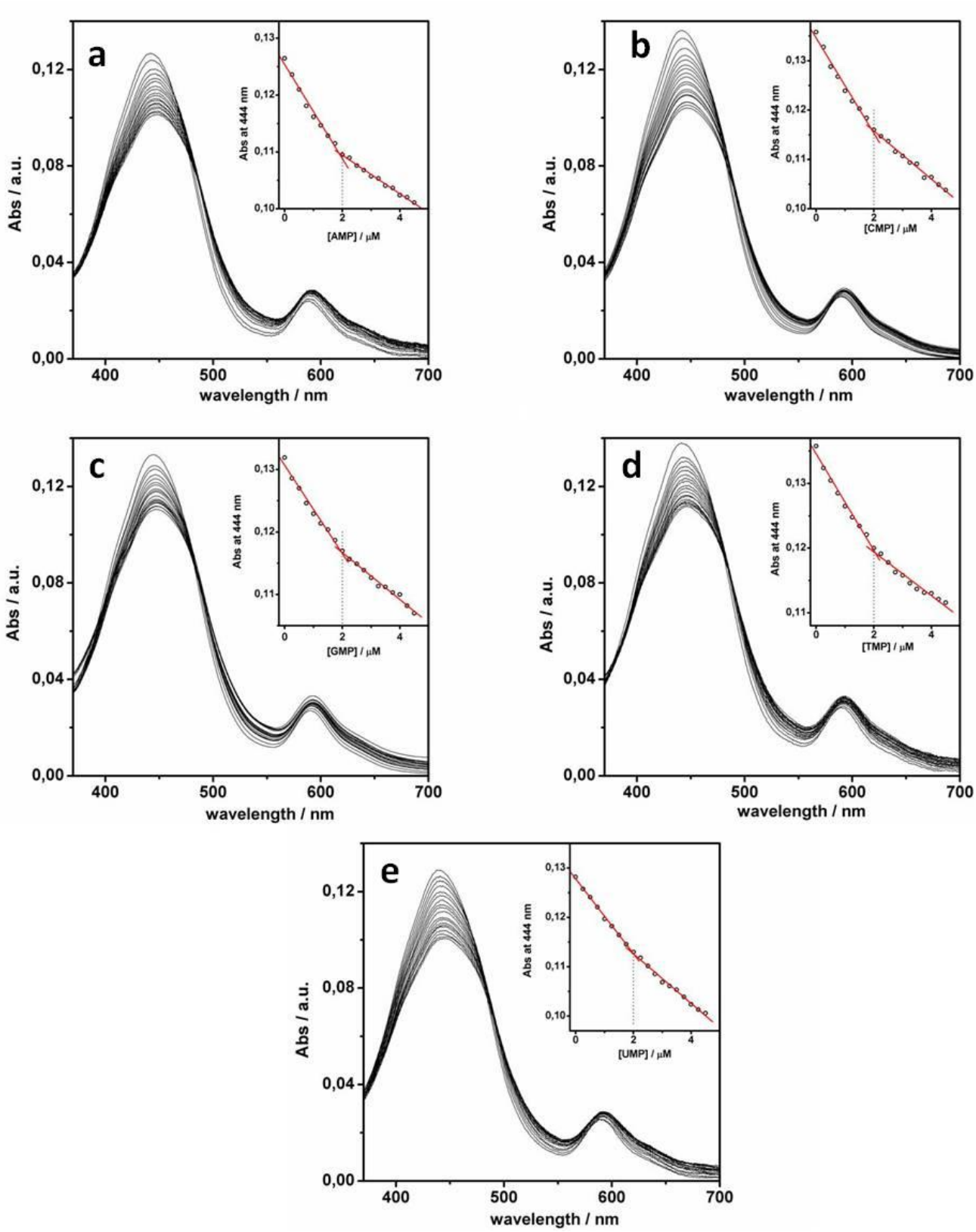

Figure S5: UV-vis titration of 3 2µM solution with increasing concentration of five nucleotides (from 0,25 µM to 4,5 µM): a) AMP, b) CMP, c) GMP, d)TMP, e) UMP.

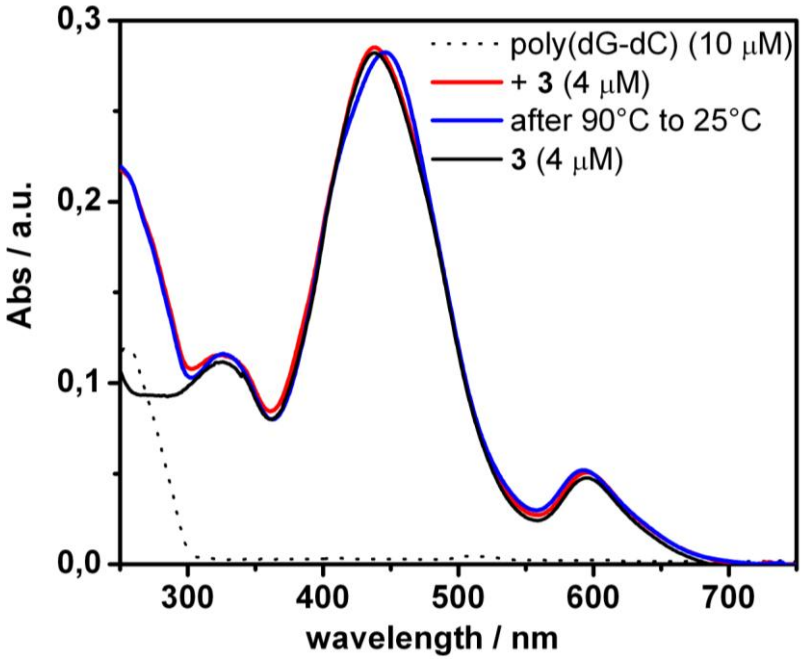

Figure S6: UV-vis titration of poly(dG-dC) 10 μM in in Na cacodilate buffer (1mM, pH 7.0) dotted curve, in the presence of **3** 4μM, red curve, and after melting/annealing experiment (blue curve). The black solid curve is UV spectrum of **3** 4μM in in Na cacodilate buffer (1mM, pH 7.0).

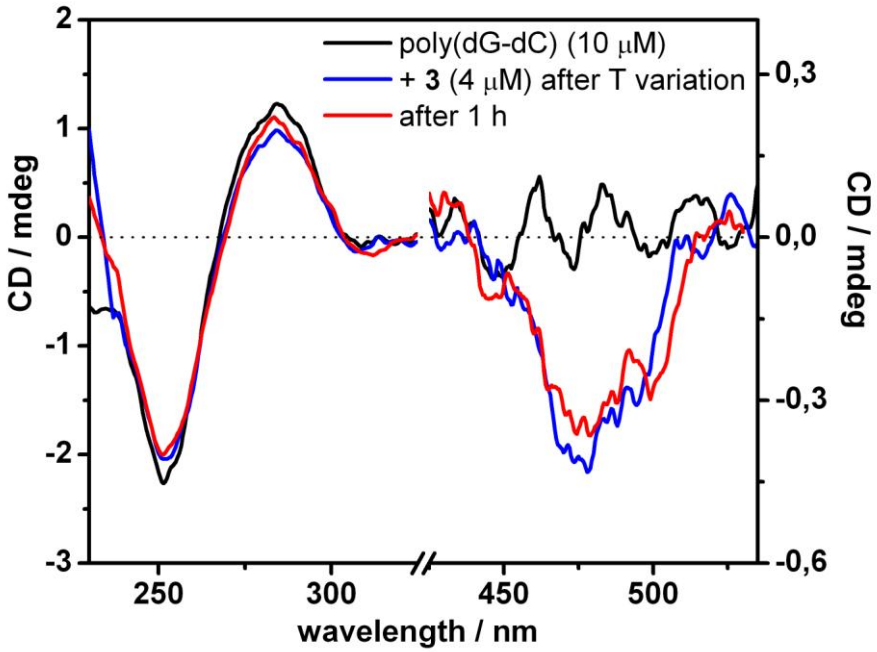

Figure S7: CD spectra of poly(dG-dC) 10 μM solution in cacodilate buffer 1mM (black curve) in the presence of **3** 4μM after increasing the temperature to 90°C and cooling down fast to 25°C (blue curve), and after 1 h (red curve).