Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information for

Supramolecular rulers enabling selective detection of short ssDNA *via* chiral self-assembly

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General. All reagents were obtained from commercial suppliers and used without further purification. ^{1}H NMR, were recorded on an ARX 300 MHz Bruker spectrometer in D_2O and MeOD with the use of the residual solvent peak as reference. Mass spectrometric studies were performed in the positive ion mode using a quadrupole mass spectrometer (Micromass,Platform 2+). Samples were dissolved in water and were continuously introduced into the mass spectrometer at a flow rate of 10 mL/min through a Waters 616HPLC pump. The temperature (60°C) and the extraction cone voltage (Vc=10-30V) were varied to avoid fragmentations in some cases. The Atomic Force Microscopy (AFM) images were obtained using an Ntegra Spectra instrument (NT-MDT, Russia) operated in tapping mode under ambient conditions. Silicon cantilever tips (NSG 10) with a resonance frequency of 140–390 kHz, a force constant of 5.5–22.5 mÀ1 and a tip curvature radius of 10 nm were used. Typically, to prepare AFM samples, 10 mL aliquots of sample solutions were deposited on freshly cleaved mica substrates and dried in air at room temperature prior to imaging. For agarose gel electrophoresis studies **1** was mixed with the corresponding amount of ssDNA and then incubated for 30 minutes at room temperature. The resulted solutions were loaded in 1% agarose gels, and electrophoresis experiments were carried out in TAE buffer solution (40 mM Tris-HCl, 1%, acetic acid, 1 mM EDTA, pH = 7.4) at 90 V for 120 minutes. The migration of ssDNA in free and complexed states was visualized under UV light, after gels staining with ethidium bromide. The CD spectra were recorded on a Bio-Logic MOS 450 CD spectrometer (France) using a 1.0 mm path length quartz cuvette. Each spectrum was recorded at wavelengths between 200 and 450 nm and a scan speed of 60 nm min−1. All observed CD spectra were baseline subtracted for deionized water.

Synthesis

((2E,2'E,2''E)-2,2',2''-((nitrilotris(benzene-4,1-diyl))tris(methanylylidene))tris(hydrazin-1-yl-2-ylidene))tris(aminomethaniminium) chloride (**1**) A mixture of Tris-(4-formylphenyl) amine (329 mg, 1 mmol) and aminoguanidinium hydrochloride (331.5 mg, 3 mmols) in $CHCl_3$:MeOH = 1:1 (20 ml) was heated under reflux for 48h. After cooling to r.t., the solvent was removed by *vacuo* to afford **1**: 600 mg (99%). Orange solid. ^{1}H-NMR (300 MHz, MeOD) 8.10 (s, 3H); 7.78 (d, J^3= 9 Hz, 6H); 7.16 (d, J^3= 9 Hz, 6H). ESI-MS: 498.2 (30, [M+H-3xHCl]).

2,2',2''-((2E,2'E,2''E)-2,2',2''-((nitrilotris(benzene-4,1-diyl))tris(methanylylidene))tris(hydrazin-1-yl-2-ylidene))tris(N,N,N-trimethyl-2-oxoethanaminium) chloride (**2**) A mixture of Tris-(4-formylphenyl) amine (329 mg, 1 mmol) and Girard T reagent (501 mg, 3 mmols) in dry $CHCl_3$:MeOH = 1:1 (20 ml) was heated under reflux for 48h. After cooling to r.t., the solvent was removed by *vacuo* to afford **2** as a mixture of *anti* and *syn* conformers : 775 mg (99%). Orange solid. ^{1}H-NMR (300 MHz, D_2O) 8.08-7.88 (s, *syn+anti*, 3H), 7.56 (d, J^3= 9 Hz, 6H), 7.03 (d, J^3= 9 Hz, 6H), 4.55-4.11 (s, *syn+anti*, 6H), 3.27-3.26 (s, *syn+anti*, 27H). ESI-MS: 223.9 (30, [M/3-3xCl]).

((2E,2'E,2''E)-2,2',2''-(benzene-1,3,5-triyltris(methanylylidene))tris(hydrazin-1-yl-2-ylidene)) tris(aminomethaniminium) chloride (**3**) A mixture of Benzene-1,3,5-tricarbaldehyde (162 mg, 1 mmol) and aminoguanidinium hydrochloride (331.5 mg, 3 mmols) in dry MeOH (20 ml) was heated under reflux for 24h. After cooling to r.t., the precipitate was filtered and washed with cold dry MeOH (3x10ml) followed by drying under *vacuo* to afford **3**: 316 mg (72%). Orange solid. ^{1}H-NMR (300 MHz, MeOD) 8.35 (s, 3H), 8.24 (s, 3H). ESI-MS: 331.1 (100, [M+H-3xHCl], 661.2 (60, [2M+H-6xHCl]).

2,2',2''-((2E,2'E,2''E)-2,2',2''-(benzene-1,3,5-triyltris(methanylylidene))tris(hydrazin-1-yl-2-ylidene))tris(N,N,N-trimethyl-2-oxoethanaminium) chloride (**4**) A mixture of Benzene-1,3,5-tricarbaldehyde (162 mg, 1 mmol) and Girard T reagent (501 mg, 3 mmols) in dry MeOH (20 ml) was heated under reflux for 24h. After cooling to r.t., the precipitate was filtered and washed with cold dry MeOH (3x10ml) followed by drying under *vacuo* to afford **3** as a mixture of *anti* and *syn* conformers: 550 mg (90%). Orange solid. ^{1}H-NMR (300 MHz, D_2O) 8.27-7.92 (s, *syn+anti*, 6H), 4.72-4.22 (s, *syn+anti,* 6H), 3.35-3.33 (s, *syn+anti*, 27H). ESI-MS: 167.9 (100, [M/3 - 3xCl]) 251.7 (51, [M/2 - 3Cl]).

Several methods have been employed to gather information regarding binding of **1**-**4** with ssDNA strands.

Interestingly, the ^{1}H-NMR spectra of **1** -4 are reminiscent with a slow exchange of the *syn* and *anti*-isomers of the amidic moiety in solution. ESI-MS analyses show that even at low voltage, that the compounds are strongly solvated by water molecules.

Circular dichroism

In order to monitor the interaction between the charged starshaped molecules and the ssDNA strand we followed the titration of a solution of DNA with a solution of the respective compounds in water, registering the signals they give through circular dichroism (CD). Interestingly from the compounds that had similar charged moieties only the ones that had the trisphenylamine(TPA) core induced a change in the signal (**1** and **2**). Stock solutions of ssDNA (0.2 mM), **1** and **2** (16.6 mM) were prepared in water. For an analysis, 10 µL of ssDNA solution was injected in 490 µL of water and the signal registered. For the titration 3 µL of solution of **1** or **2** were added to the previous solution at each step.

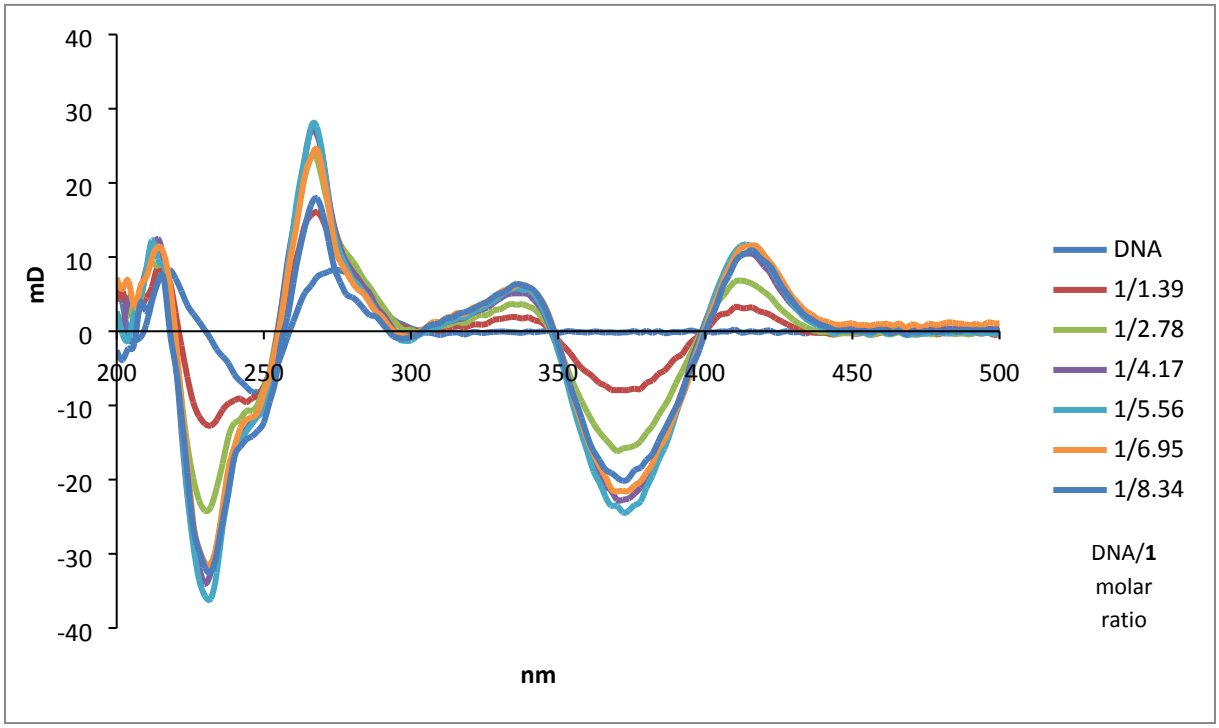

Figure S1. Circular dichroism experiment of 25b ssDNA titration with **1** up to charge equivalence point

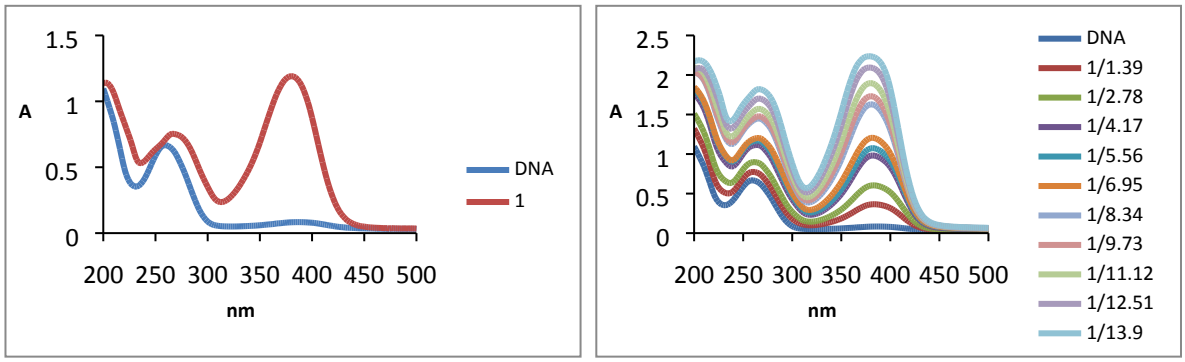

Figure S2. a) Absorbance spectrum of 25b ssDNA and **1** b) titration monitored by absorbance

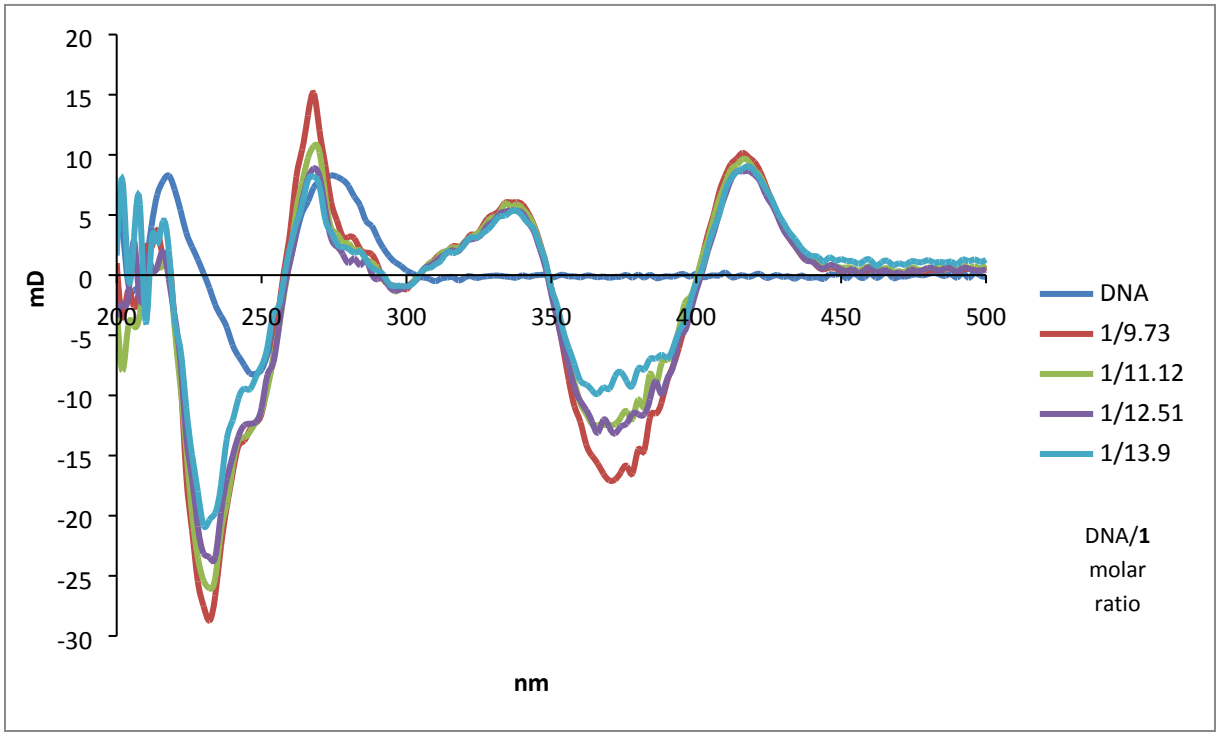

Figure S3. Circular dichroism experiment of 25b ssDNA titration with **1** after charge equivalence point

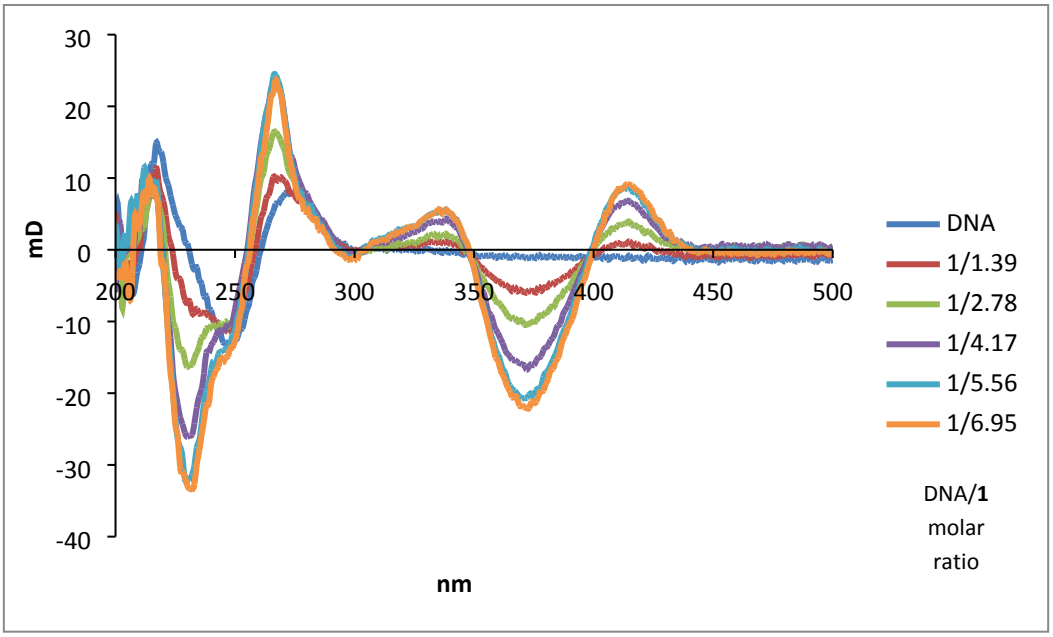

Figure S4. Titration of 16b ssDNA 5'-CAA-GCC-CTT-AAC-GAA-C-3' with **1**

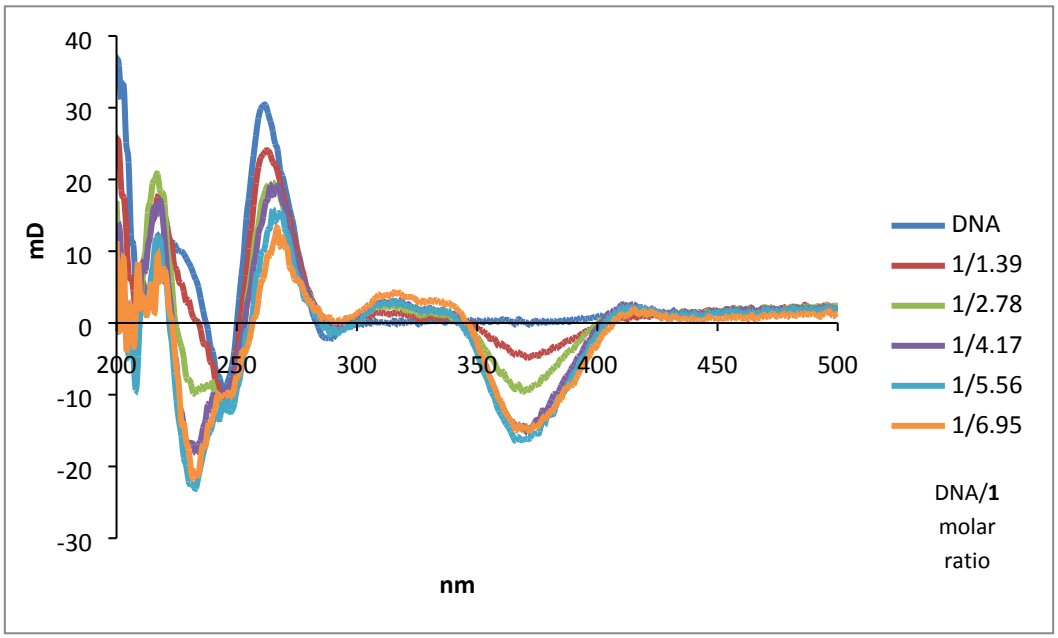

Figure S5. Titration of 16b ssDNA polyA with **1**

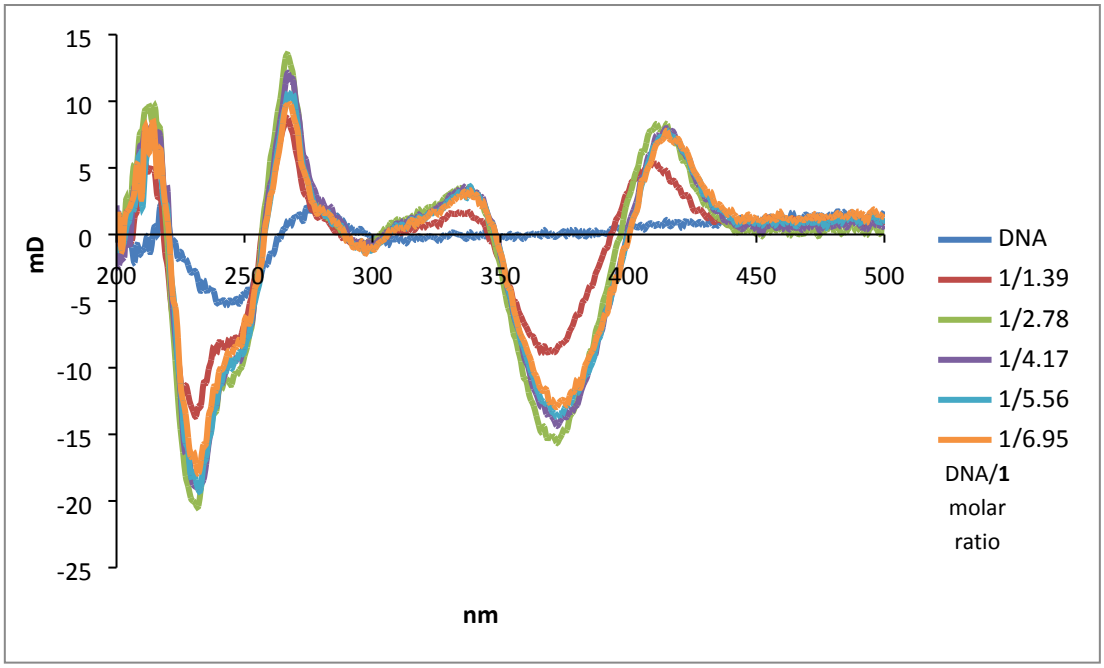

Figure S6. Titration of 10b ssDNA 5'-CAA-GCC-CTT-A-3' with **1**

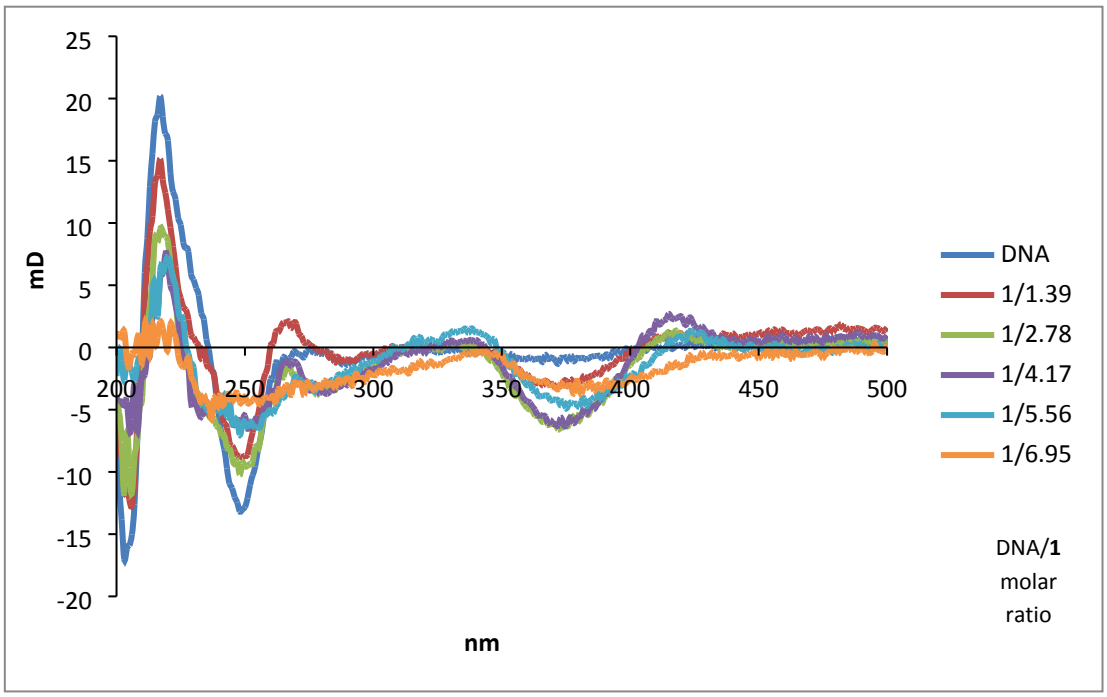

Figure S7. Titration of 10b ssDNApolyA with **1**

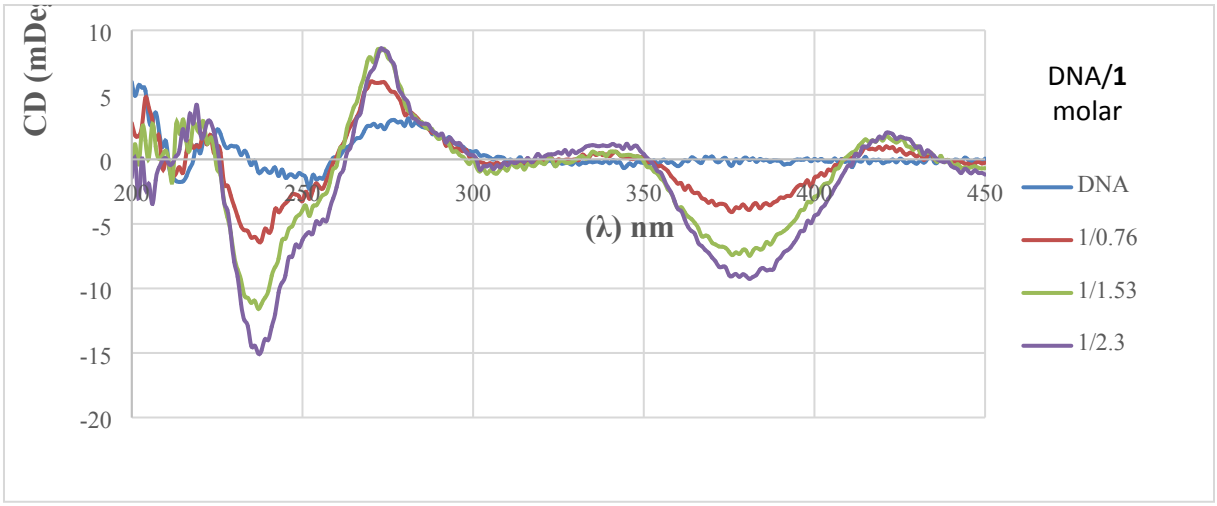

Figure S8. Titration of 7b ssDNApolyA with **1**

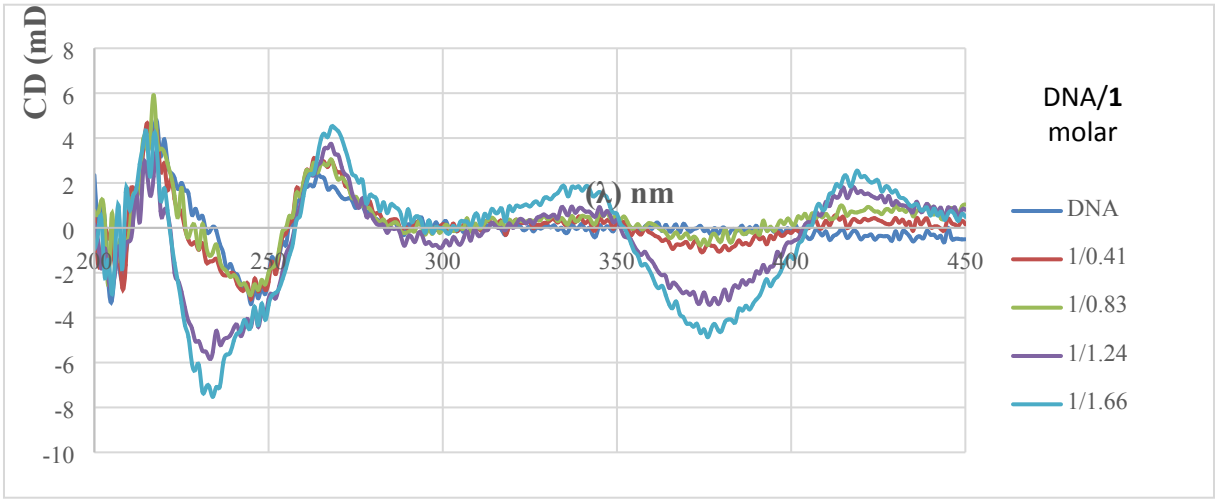

Figure S9. Titration of 5b ssDNApolyA with **1**

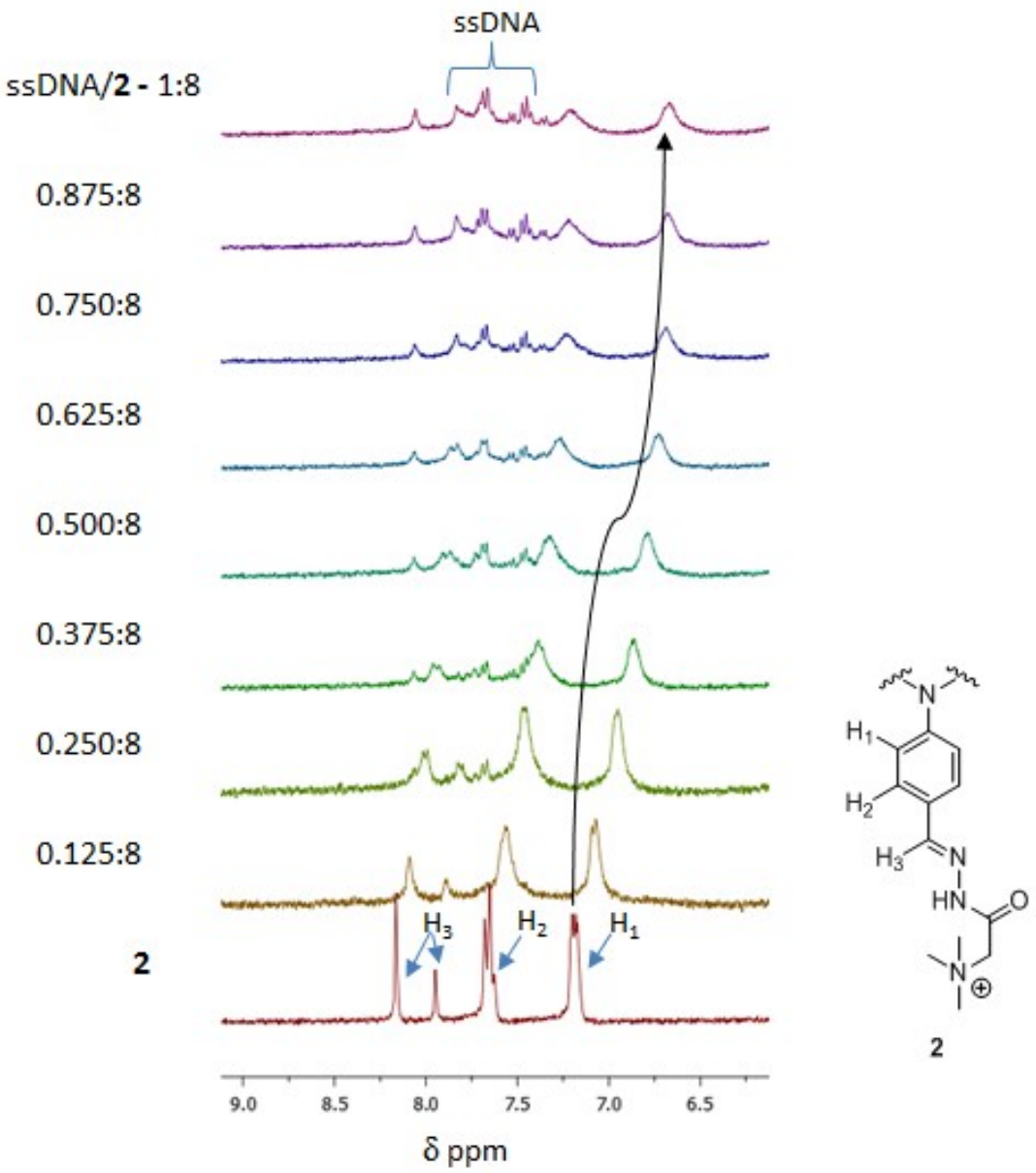

Figure **S10**. Fragment of NMR spectra in D_2O showing the shift of the aromatic signals given by compound **2** when undergoing titration with 25 base ssDNA solution.

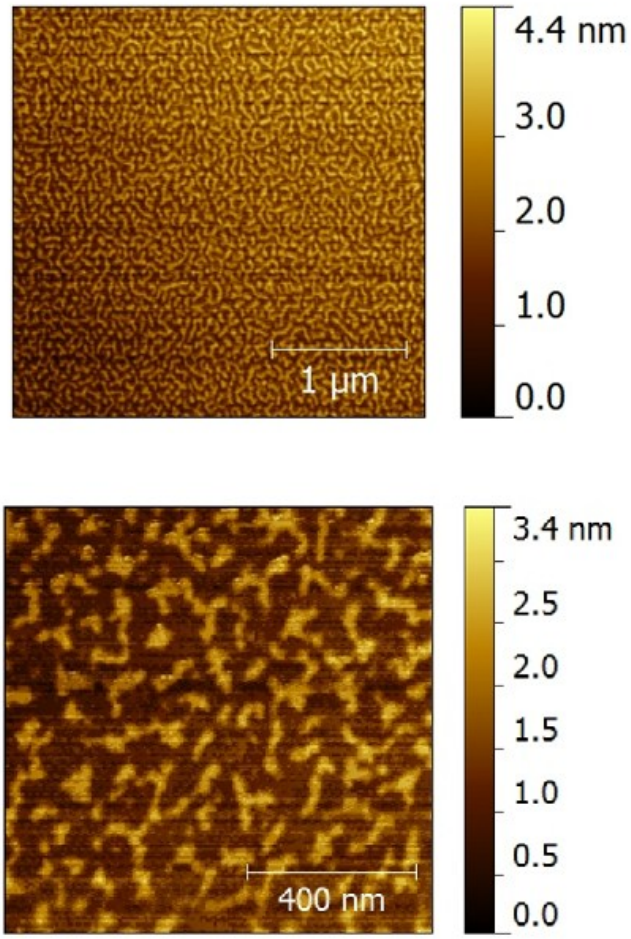

Figure **S11**. AFM images of 25 bases ssDNA/**2** at 1/8.34 molar ratio obtained by deposition on mica surface, dried and imaged in air

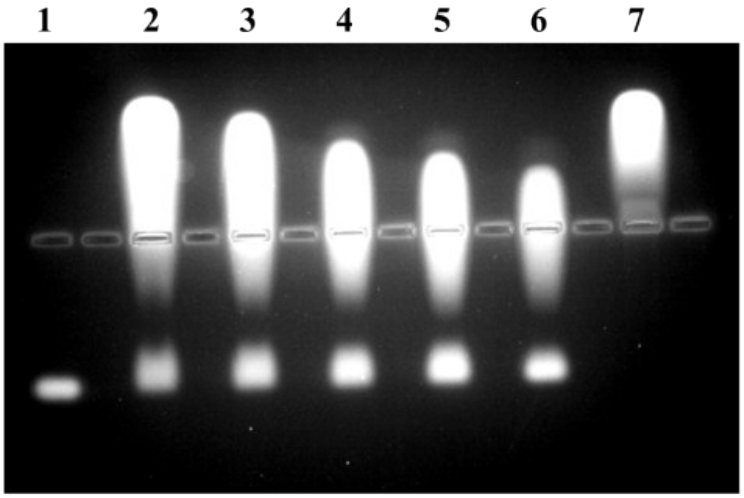

Figure **S12**. Gel electrophoresis of 25 base ssDNA/**2** in different molar ratios: (1)-DNA/**2** 1:0 (2)-DNA/**2** 1:25 (3)-DNA/**2** 2:25 (4)-DNA/**2** 3:25; (5)-DNA/**2** 4:25 (6)-DNA/**2** 5:25 (7)-DNA/**2** 0:1

(1) C. Allain,F. Schmidt, R. Lartia, G. Bordeau, C. F.-Debuisschert, F. Charra, P. Tauc, and M.-P. T.-Fichou,*ChemBioChem*, **2007**, *8*, 424.
(2) J. Kypr, I. Kejnovska , D. Renciuk, and M. Vorlıckova, *Nucleic Acids Research*, **2009**, *37*, 1713.
(3) J. Sponer,P. Jurecka,I. Marchan,F.J. Luque, M. Orozco,and P. Hobza,*Chem. Eur. J.***2006**,*12*, 2854.
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