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Effect of N-substituent in 4-styrylpyridinium dyes on their binding to DNA

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Experimental Section

Materials

All solvents and other reagents are commercial products (Acros, Aldrich, Merck) and were used without further purification. Solvents were purified by standard procedures. Deionized water with resistivity ≥ 18 MΩ cm-1 was used for the preparation of buffer solutions and spectrometric measurements. BPE buffer (6.0 mM Na2HPO4, 2.0 mM NaH2PO4, pH=7.0) was used for photometric DNA titrations and for CD spectroscopic studies.

General Methods

Melting points are determined on a Melt-temp melting point electrothermal apparatus and are uncorrected.

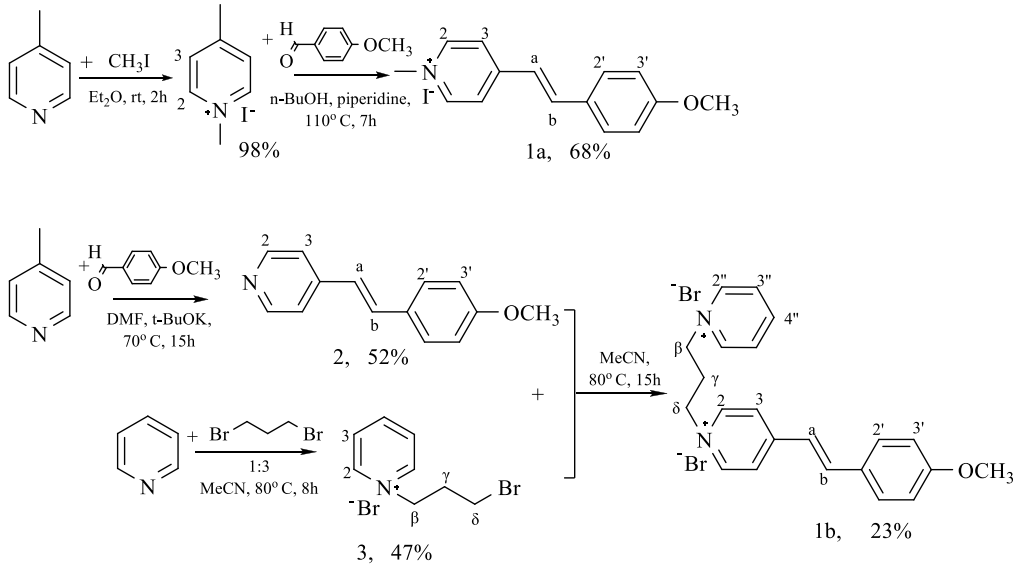
The reaction progress and purity of the final products were monitored by TLC on silica gel (DC-Alufolien Kieselgel 60 F254, Merck). Column chromatography was conducted on silica gel (Kieselgel 60, particle size 0.063-0.200 mm, Merck). Flash chromatography was performed using a Biotage IsoleraTM Prime system.

^{1}H and ^{13}C (APT method) NMR spectra were recorded on a Bruker AVANCE-400 spectrometer. The chemical shifts and spin-spin coupling constants were determined with accuracy of 0.01 ppm and 0.1 Hz, respectively.

Elemental analysis was performed at the A.N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences.

Electrospray ionization (ESI) mass spectra were detected in the mode of full mass scanning of positive ions on a tandem dynamic mass spectrometer equipped with a mass analyzer with an octapole ionic trap.

Synthesis of dyes 1a,b



1-Methyl-4-picolinium iodide

4-methylpyridine (1, 1 mL, 10.2 mmol) was added iodomethane (2.0 mL, 32 mmol) at room temperature with stirring in diethyl ether for 2h. The mixed liquids immediately became yellow and solidified. The obtained solid was washed with diethyl ether and dried on a filter to give the desired product 2 as lilac powder in 98% yield. M.p. 151-153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 2.59 (s, 3H, CH_3); 4.27 (s, 3H, CH_3-N^+); 7.96 (d, 2H, H-3, *J* 6.3 Hz); 8.82 (d, 2H, H-2, *J* 6.5 Hz). Anal. calcd for $C_7H_{10}IN$: C, 35.77%; H, 4.29%; N, 5.96%; found: C, 36.22%; H, 4.31%; N, 6.14%.

(E)-4-(4-Methoxystyryl)-1-methylpyridinium iodide (1a)

A mixture of 1-methyl-4-picolinium iodide (0.1g, 0.42 mmol), 4-methoxybenzaldehyde (0.086 g, 0.63 mmol) and piperidine (41 μL) in n-butanol (5 mL) was stirred at 110 °C for 4 h. The solvent was removed and the obtained residue was recrystallized from methanol and dried on a filter to give dark yellow solid in 68% yield.[S1] M.p. 218-219 °C ^{1}H NMR (400 MHz, DMSO-d_6): 3.83 (s, 3H, OCH_3); 4.23 (s, 3H, CH_3-N^+); 7.07 (d, 2H, H-3', *J* 8.9 Hz); 7.36 (d, 1H, H-b, *J* 16.3 Hz); 7.72 (d, 2H, H-2', *J* 8.6 Hz); 7.97 (d, 1H, H-a, *J* 16.4 Hz); 8.16 (d, 2H, H-3, *J* 6.9 Hz); 8.81 (d, 2H, H-2, *J* 6.6 Hz). Anal. calcd for $C_{15}H_{16}INO$: C, 51.01%; H, 4.57%; N, 3.97%; found: C, 51.1%; H, 4.64%; N, 3.85%.

(E)-4-(4-Methoxystyryl)pyridine (2)

A mixture of 4-picoline (0.214 ml, 2.2 mmol), potassium *tert* –butoxide (0.37 g, 3.3 mmol) and 4-methoxybenzaldehyde (0.3 g, 2.2 mmol) is introduced in 5 mL of anhydrous DMF and heated 6 h at 80°C under efficient magnetic stirring. After being cooled at room temperature, ice water was added and the white precipitate was poured into ice water, filtered out and isolated as a white solid in 52% yield.[S2] M.p. 124-126 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.85(s, 3H, OCH_3); 6.89 (d, 1H, b, *J* 16.2 Hz); 6.93 (d, 2H, H-3', *J* 8.5 Hz); 7.27 (d, 1H, a, *J* 16.2 Hz); 7.34 (d, 2H, H-3, *J* 5.7 Hz); 7.50 (d, 2H, H-2', *J* 8.6 Hz); 8.56 (d, 2H, H-2, *J* 5.4 Hz). Anal. calcd for $C_{14}H_{13}NO$: C, 79.59%; H, 6.2%; N, 6.63%; found: C, 76.18%; H, 6.27%; N, 6.2%.

1-(3-Bromopropyl)pyridinium bromide (3)

A solution of pyridine (0.066 ml, 0.82 mmol) in MeCN (4 mL) was very slowly added dropwise to a solution of dibromopropane (0.25 ml, 0.27 mmol) in MeCN (4 mL) under continuous stirring at 80 °C. After the addition was completed the obtained mixture was stirred at 80 °C during 5 h. Then the solvent was evaporated, the residue was purified by column chromatography (neutral Al_2O_3, gradient mixture as an eluent: dichloromethane–MeOH) to give

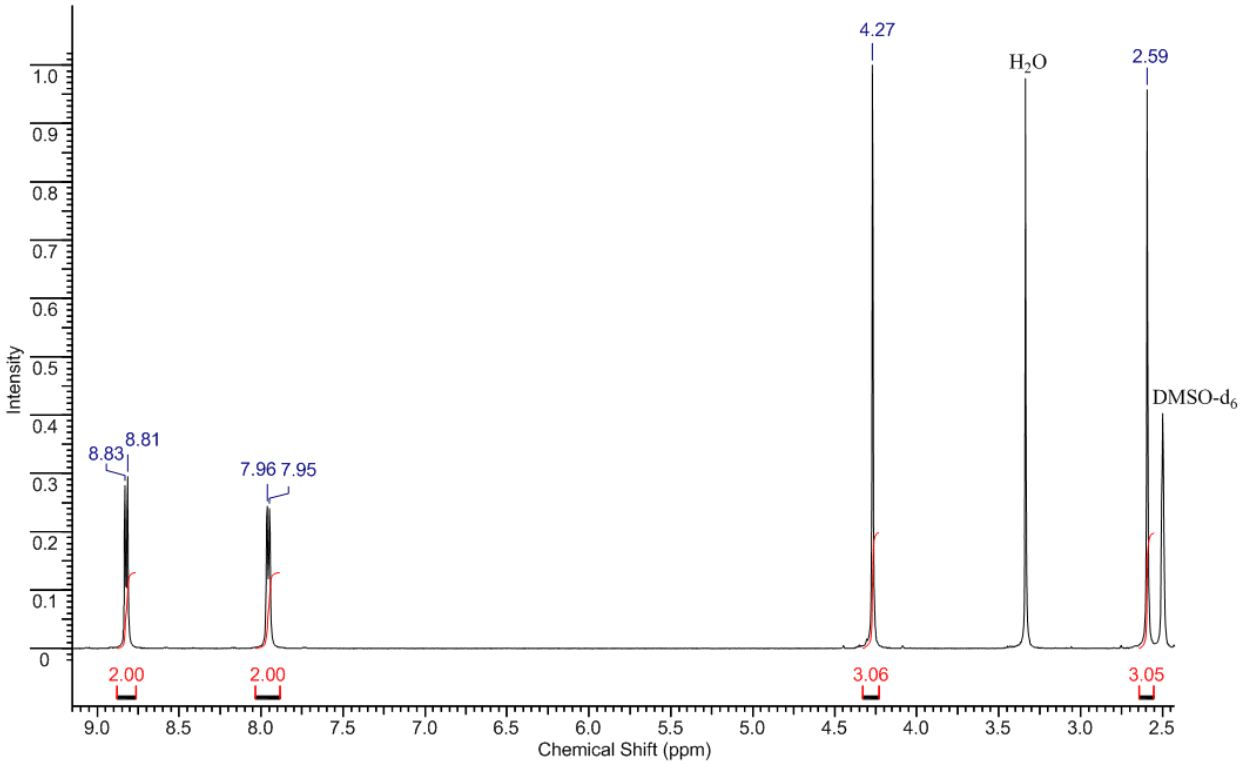
the desired product 6 as white solid in 47% yield. M.p. 128-130 °C ^{1}H NMR (400 MHz, CD_3CN): 2.55 (m, 2H, H-γ); 3.53 (t, 2H, H-δ, *J* 6.3 Hz); 4.78 (t, 2H, H-β, *J* 7.0 Hz); 8.06 (t, 2H, H-3, *J* 6.7 Hz); 8.53 (t, 1H, H-4, *J* 7.6 Hz); 8.94 (d, 2H, H-2, *J* 5.7 Hz). Anal. calcd for $C_8H_{11}Br_2N$: C, 34.20%; H, 3.95%; N, 4.98%; found: C, 32.98%; H, 4.08%; N, 5.2%.

(E)-4-(4-methoxystyryl)-1-(3-(pyridinio propyl)pyridinium bromide (1b)

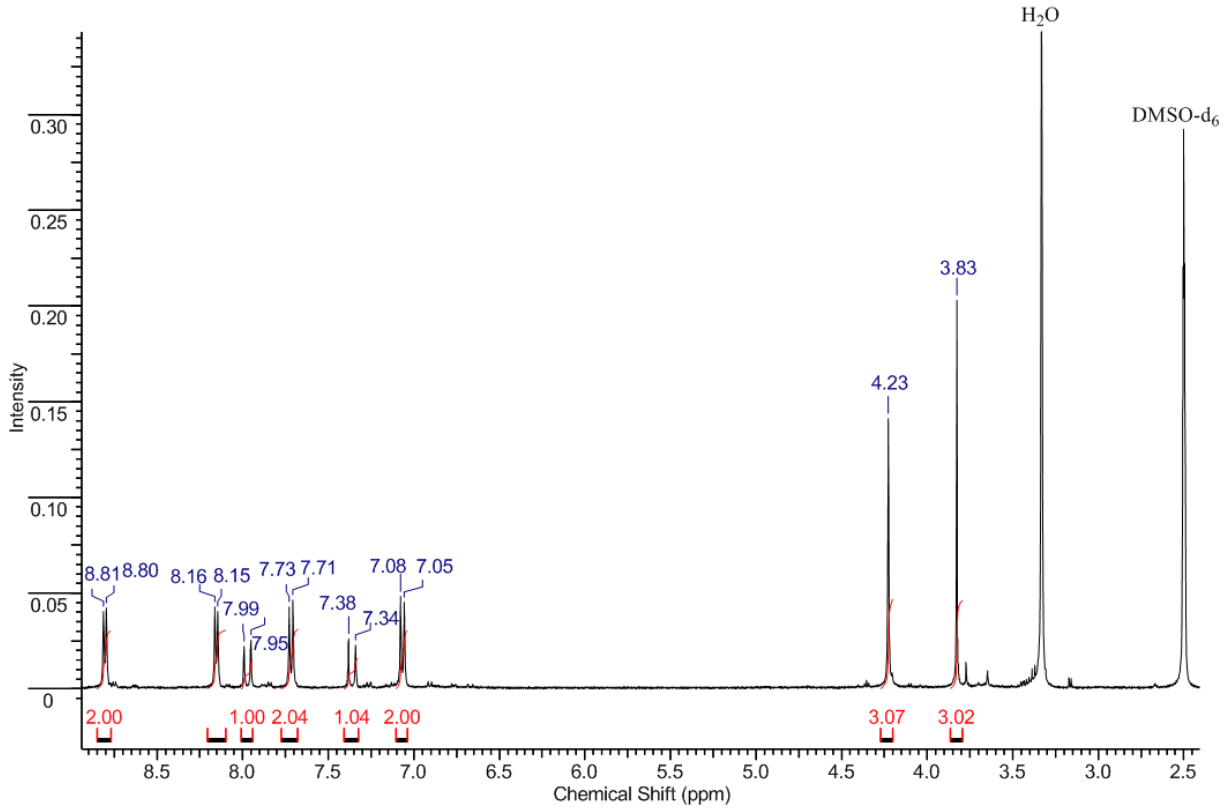
A mixture of 2 (0.08g, 0.4 mmol) and 3 (0.11 g, 0.4 mmol) in MeCN (4 mL) was stirred at 80 °C for 14 h. Then the solvent was evaporated, the residue was purified by column chromatography (neutral Al_2O_3, gradient mixture as an eluent: dichloromethane–MeOH) to give the desired product 1b as yellow oil in 23% yield. ^{1}H NMR (400 MHz, DMSO-d_6): 2.64 (m, 2H, H-γ); 3.83 (s, 3H, OCH_3), 4.63 (t, 2H, H-δ, *J* 7.0 Hz); 4.75 (t, 2H, H-β, *J* 7.0 Hz); 7.07(d, 2H, H-3', *J* 8.6 Hz); 7.41 (d, 1H, H-b, *J* 16.4 Hz); 7.73 (d, 2H, H-2', *J* 8.6 Hz); 8.04 (d, 1H, H-a, *J* 16.0 Hz); 8.21 (t, 2H, H-3", *J* 7.0 Hz); 8.22 (d, 2H, H-3, *J* 6.6 Hz); 8.65 (t, 1H, H-4", *J* 7.4 Hz); 8.96 (d, 2H, H-2, *J* 6.2 Hz); 9.17 (d, 2H, H-2", *J* 5.8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): 31.8, 55.5, 56.3, 57.6, 114.74, 120.83, 123.41, 128.2, 130.09, 141.20, 141.23, 144.06, 145.01, 145.8, 153.61, 161.36. Anal. calcd for $C_{22}H_{24}Br_2N_2O$: C, 53.68%; H, 4.91%; N, 5.69%; found: C, 54.02%; H, 4.52%; N, 5.53%. ESI-MS 7 in H_2O, m/z: calcd 166.09; found 166.1 $[7]^{2+}$

^{1}H and ^{13}C NMR spectra of compounds

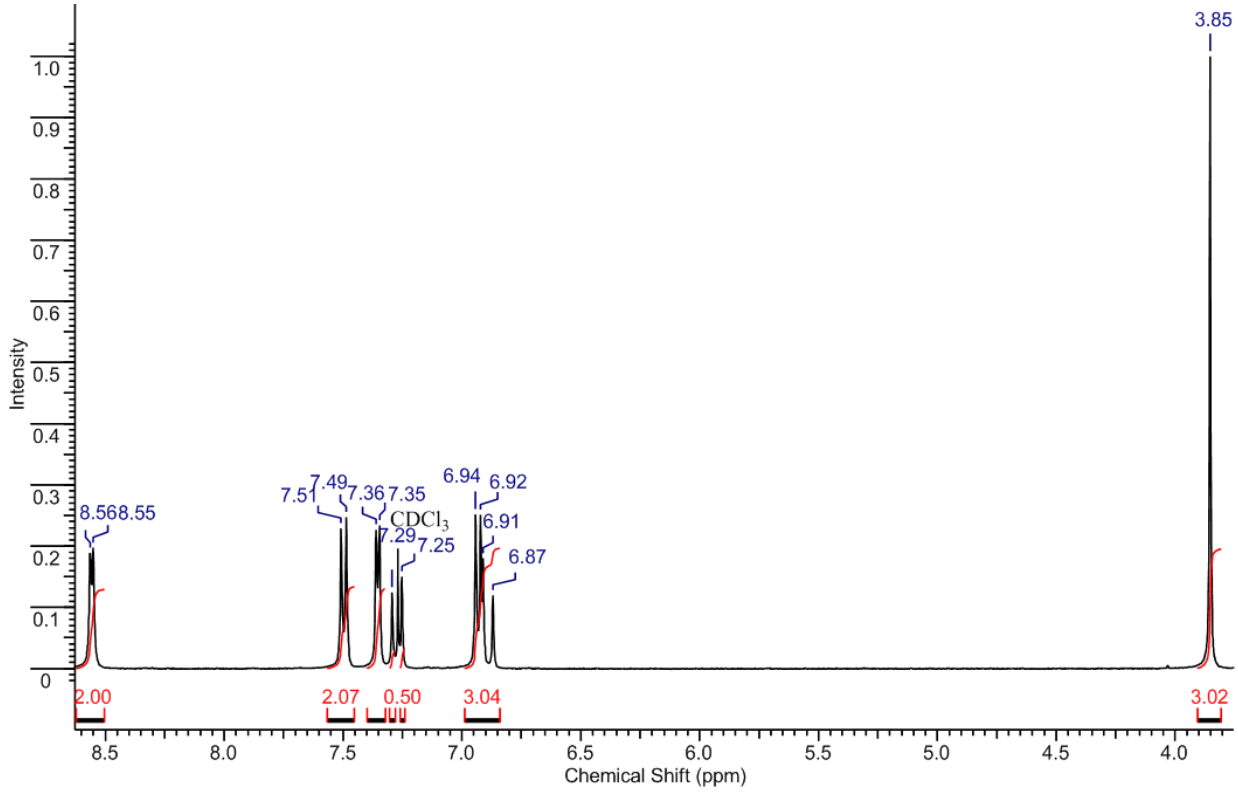
1-Methyl-4-picolinium iodide in DMSO-d_6



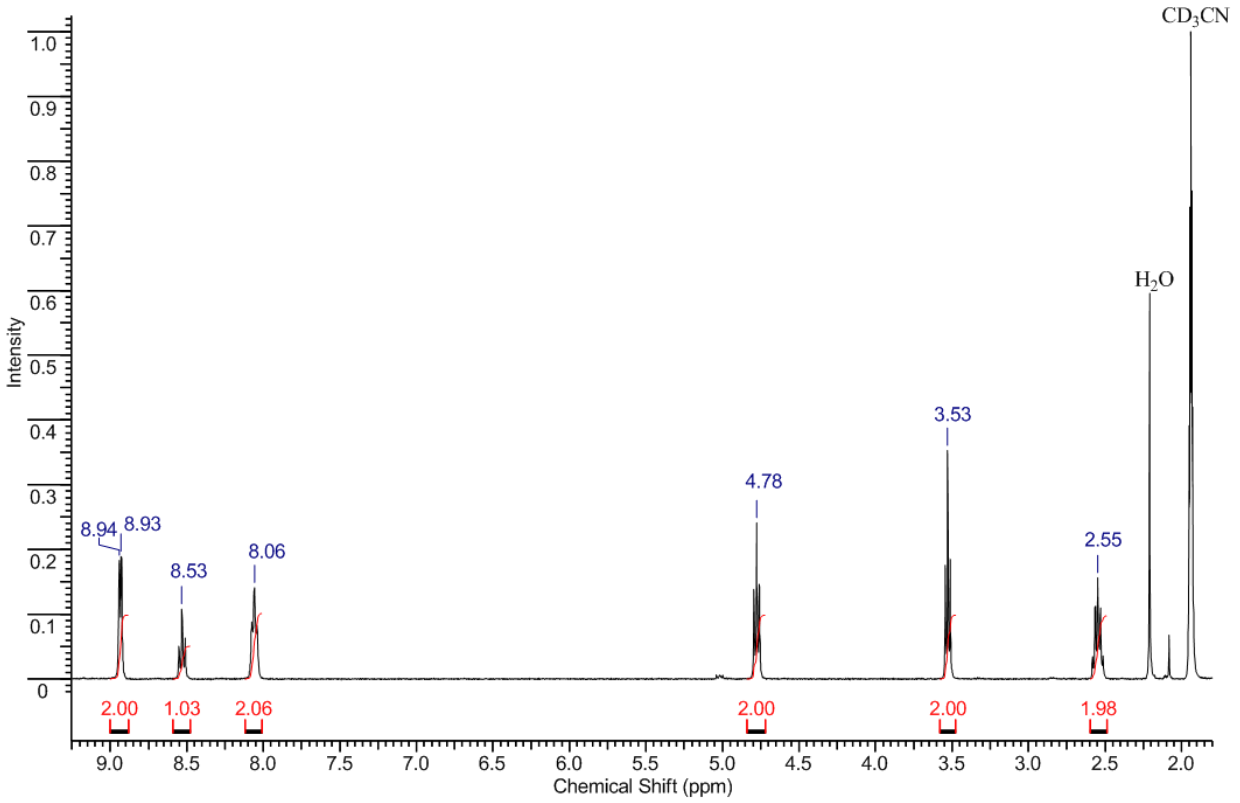
(E)-4-(4-Methoxystyryl)-1-methylpyridinium iodide (1a) in DMSO-d_6



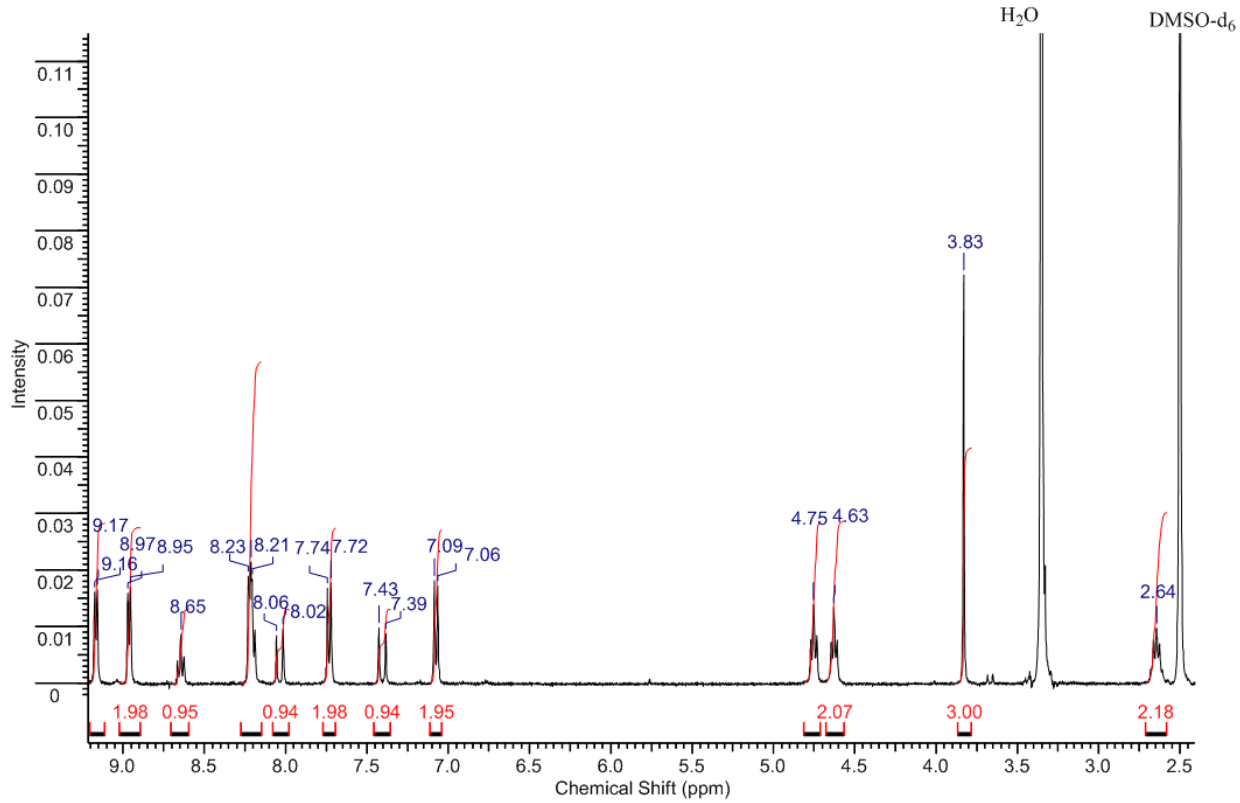
(E)-4-(4-Methoxystyryl)pyridine (2) in $CDCl_3$



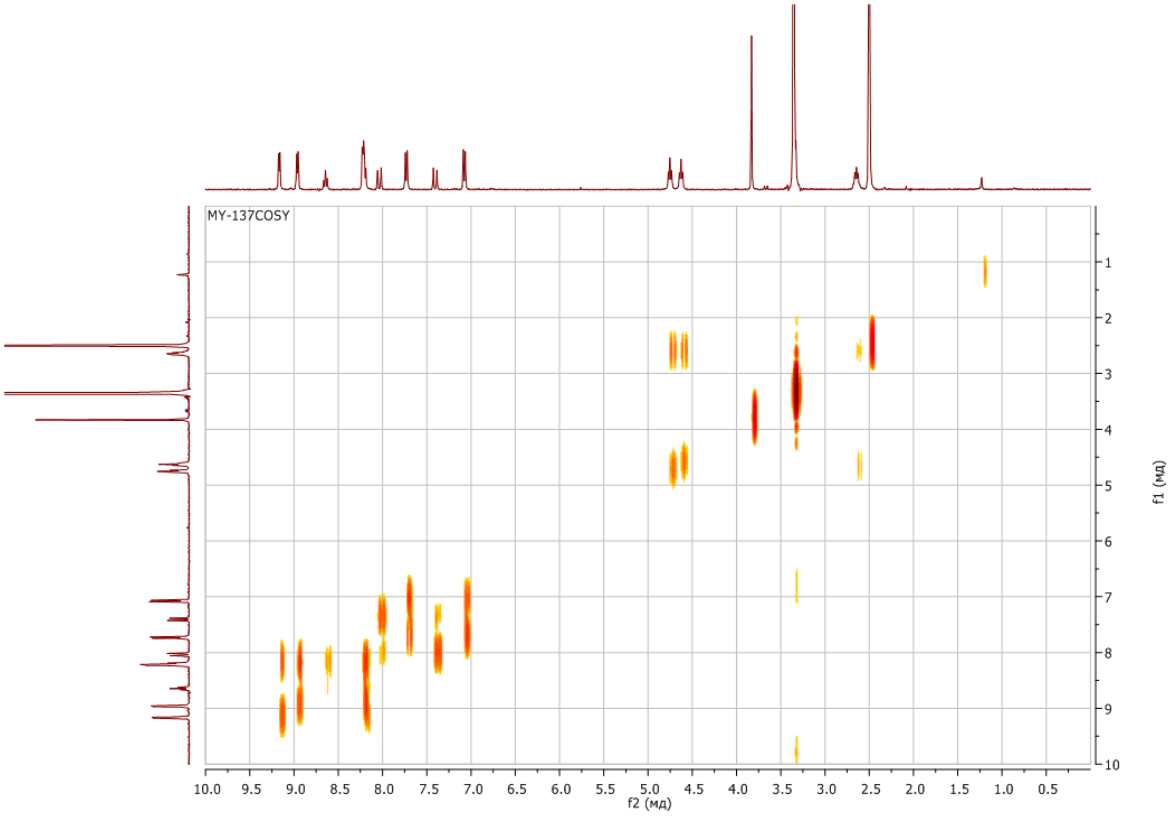
1-(3-Bromopropyl)pyridinium bromide (3) in CD_3CN



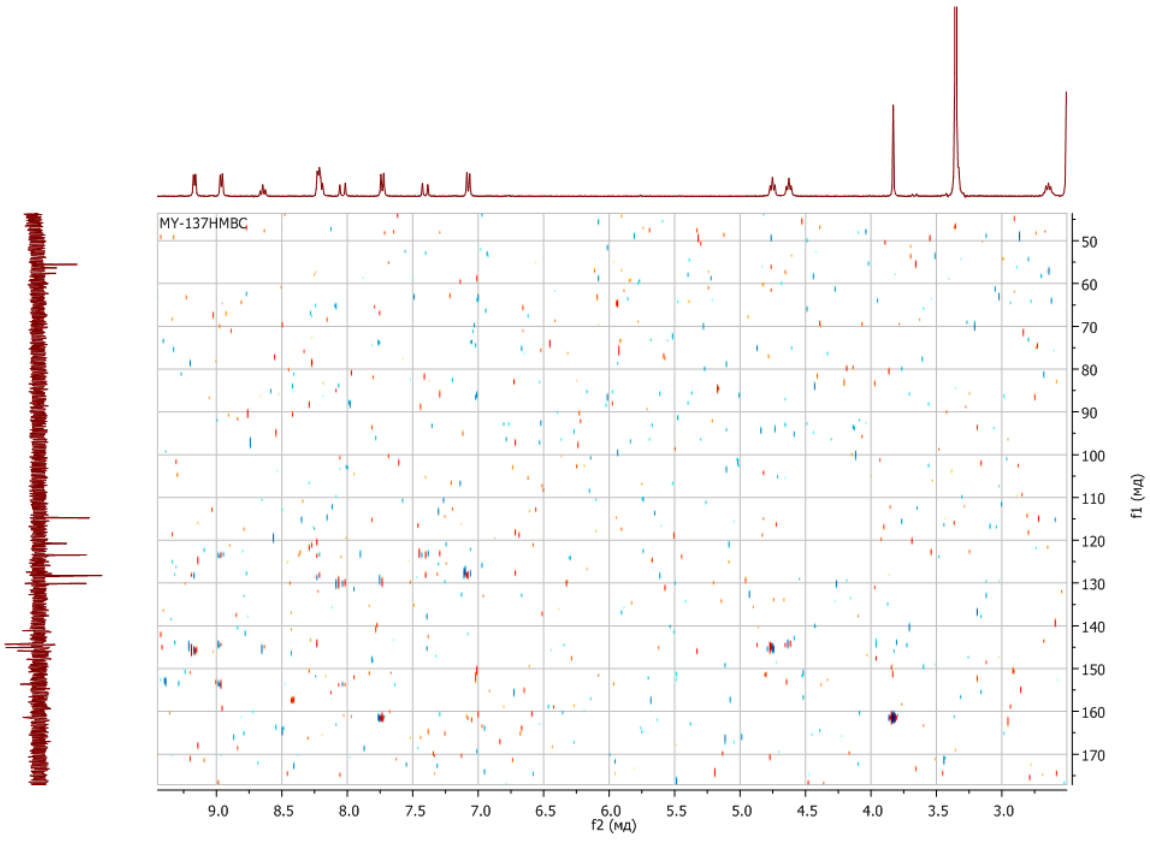
(E)-4-(4-methoxystyryl)-1-(3-(pyridinio propyl)pyridinium bromide (1b) in DMSO-d_6



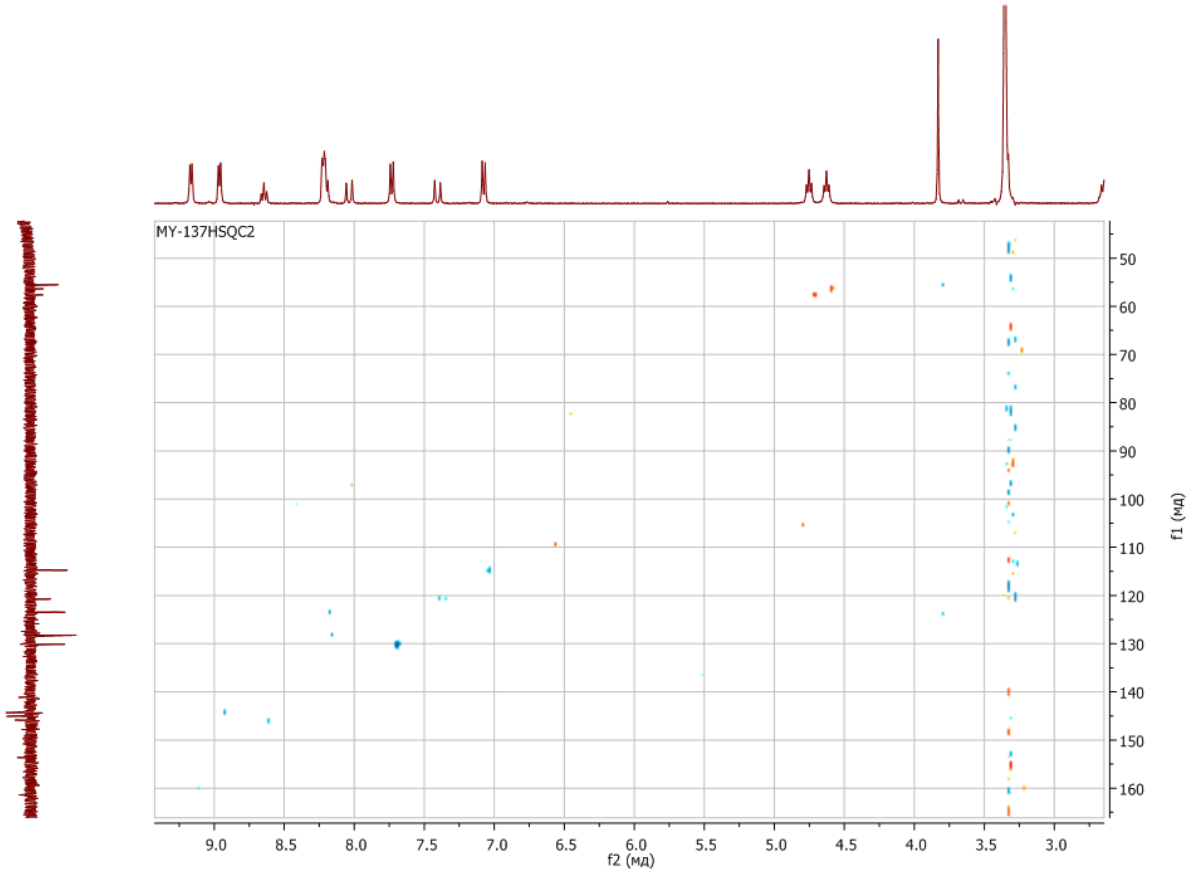
(E)-4-(4-methoxystyryl)-1-(3-(pyridinio propyl)pyridinium bromide (1b) in DMSO-d_6 COSY



(E)-4-(4-methoxystyryl)-1-(3-(pyridinio propyl)pyridinium bromide (1b) in DMSO-d_6 HMBC



(E)-4-(4-methoxystyryl)-1-(3-(pyridinio propyl)pyridinium bromide (1b) in DMSO-d_6 HSQC



Steady-state optical measurements

UV-vis spectra were measured using a two channel spectrophotometer Varian-Cary 300. Fluorescence spectra were measured at 20±1 °C with a Cary Eclipsespectrofluorometer (Agilent). Coumarin 6 in ethanol (φ^{fl} = 78.0%)[S3] and quinine in 1.0N sulfuric acid (φ^{fl} = 54.6%)[S4] were used as a reference for the fluorescence quantum yield measurements. The fluorescence quantum yields[S5] were calculated using equation:

$$\varphi_i = \varphi_0 \frac{\left(1 - 10^{-A_0}\right) * S_i * n_i^2}{\left(1 - 10^{-A_i}\right) * S_0 * n_0^2},$$

where φ_i and φ_0 are the fluorescence quantum yields of the studied solution and the standard compound, respectively; A_i and A_0 are the absorptions of the studied solution and the standard, respectively; S_i and S_0 are the areas underneath the curves of the fluorescence spectra of the studied solution and the standard, respectively; and n_i and n_0 are the refractive indices of the solvents for the substance under study and the standard compound (n_i = 1.33, water; n_0 = 1.361, ethanol; n_0 = 1.397, sulfuric acid).

CD spectra were obtained using a J-500C spectropolarimeter (Japan), when the studied solutions were placed in a 1-cm rectangular cuvette in the cuvette compartment of the device, recording was performed at a speed of 50 nm/min and with standard sensitivity in different wavelength ranges.

McGhee and von Hippel method.

SpecFit32 program was used to estimate stability constant from spectrophotometric titration data. Approximate stability constant and corresponding concentrations of reagents were used to calculate the complex concentration at the last titration point:

$$L + DNA \Leftrightarrow \{DNA - L\}$$

$$K = \frac{[Complex]}{[DNA] \times [L]} \quad (1),$$

where: $[Complex] \equiv x \equiv C_{bind\ DNA}$, $C_{free\ DNA} \equiv [DNA]=(C_{DNA}-x)$, $C_{free\ Lig} \equiv [L]=(C_L-x)$,

C_{DNA} and C_L is total DNA and ligand concentrations respectively (free+binded).

After removing brackets and some transformation of equation (1) we have got square-type equation:

$$x^2 - x \times \left(C_{DNA} + C_L + 1/K\right) + C_{DNA} \times C_L = 0 \quad (2)$$

Standard solving, count coefficients, calculate the discriminate and roots:

$$a \times x^2 + b \times x + c = 0$$

$$a = 1; \quad b = -\left(C_{DNA} + C_L + 1/K\right); \qquad c = C_{DNA} \times C_L; \qquad D = b^2 - 4 \times a \times c$$

$$x = \frac{-b \pm \sqrt{D}}{2 \times a} \quad (3)$$

To get the correct concentration of the complex the root based on $-\sqrt{D}$ was used. Other root, based on $+\sqrt{D}$ is always yields negative [DNA] and [L] concentrations so it was rejected).

Complex percentage was calculated from concentration of the complex, with ligand concentration use in denominator (in the case of the ligand is titrated by DNA):

$$\text{complex}\% = \frac{C_{bind\ DNA_i}}{C_L} \times 100\% \quad (4)$$

More accurate absorption of the complex was calculated from last titration point data by this formula (the wavelength with maximum optical changes was selected for the absorbance):

$$A_{\text{compl}} = \frac{A_{\text{max DNA conc}} - A_{lig} \times (100\% - \text{complex}\%)}{\text{complex}\%} \quad (5),$$

where $A_{\text{max DNA conc}}$ - is the absorption of the solution with maximum DNA concentration. Complex concentrations in all points of spectrophotometric titration were calculated by this equation:

$$C_{K_i} = C_{lig.} \cdot \frac{A_{lig.} - A_i}{A_{lig.} - A_{compl.}} \quad (6)$$

Scatched plot[S6,S7] (with x = r_i and y = $r_i / C_{free.lig._i}$ where $r_i = \frac{C_{K_i}}{C_{DNA_i}}$) was used with fit function to calculate stability constant *K* and binding site size *n*:

$$\frac{r_i}{C_{free.lig._i}} = K \cdot (1 - n \cdot r) \cdot \left(\frac{1 - n \cdot r}{1 - (n-1) \cdot r} \right)^{n-1} \quad (7)$$

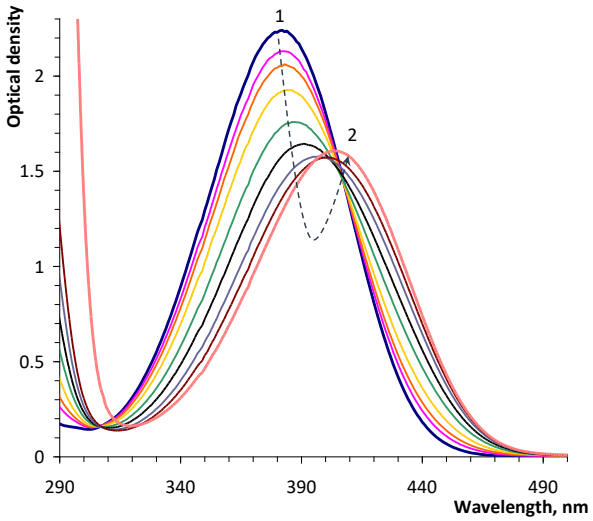


Figure S1a Change of absorption spectra upon addition of DNA C_{DNA} = 0 – (1), $3.2 \cdot 10^{-3}$ mol·L^{-1}– (2), C_{1b} = $7.6 \cdot 10^{-5}$ mol·L^{-1} in BPE water buffer (pH=7).

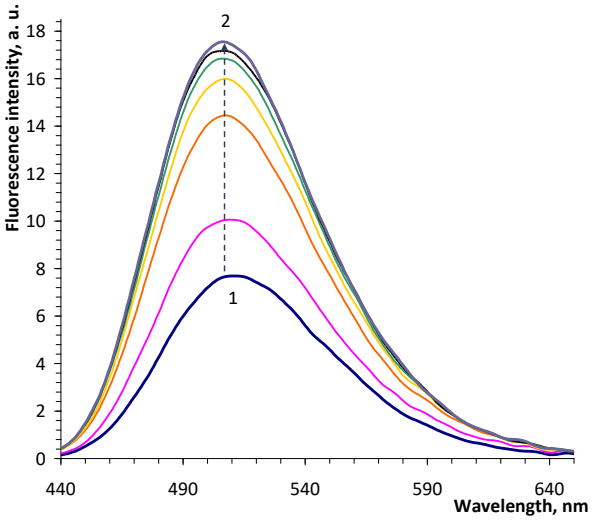


Figure S1b Change fluorescence spectra (λ_{ex} = 408 nm) of **1** upon addition of DNA CDNA = 0 – (1), $5.9 \cdot 10^{-4}$ mol·L^{-1}– (2), C_{1b}= $2.9 \cdot 10^{-6}$ mol·L^{-1}, in BPE water buffer (pH=7).

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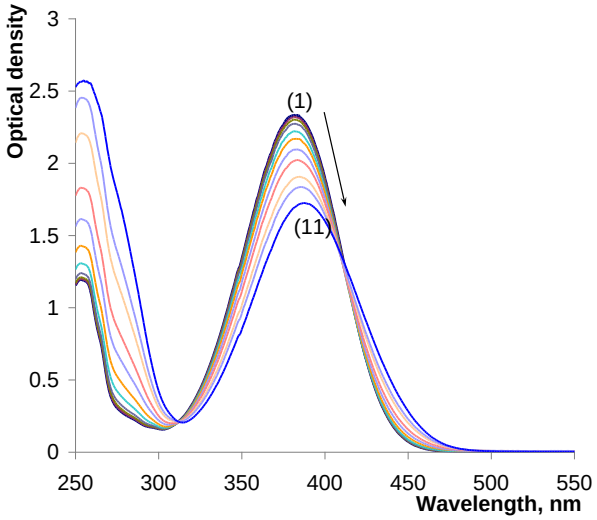

Figure S2a Change of absorption spectra upon addition of poly(dG-dC)$_2$, C $_{dG\text{-}dC}$ = 0 (1), $2.8\cdot10^{-4}$ mol·L^{-1} (11), C$_{\mathbf{1b}}$ = $7\cdot10^{-5}$ – $4.8\cdot10^{-5}$mol·L^{-1} in BPE water buffer (pH=7).

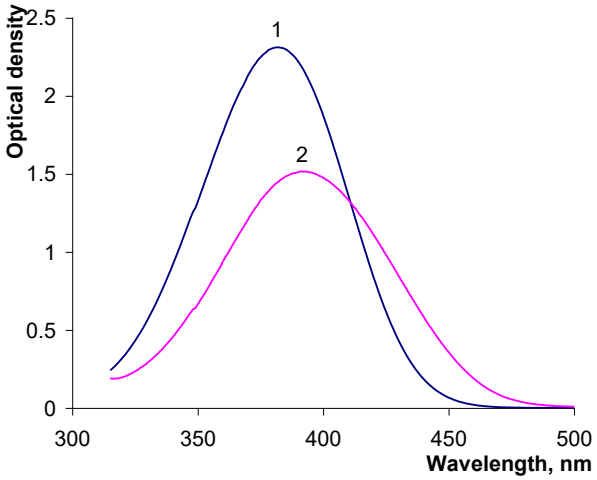

Figure S2b Calculated absorption spectra of **1b** (1) and its complex with poly(dG-dC)$_2$ (2), C$_{\mathbf{1b}}$ = $7\cdot10^{-5}$ mol·L^{-1} in BPE water buffer (pH=7).

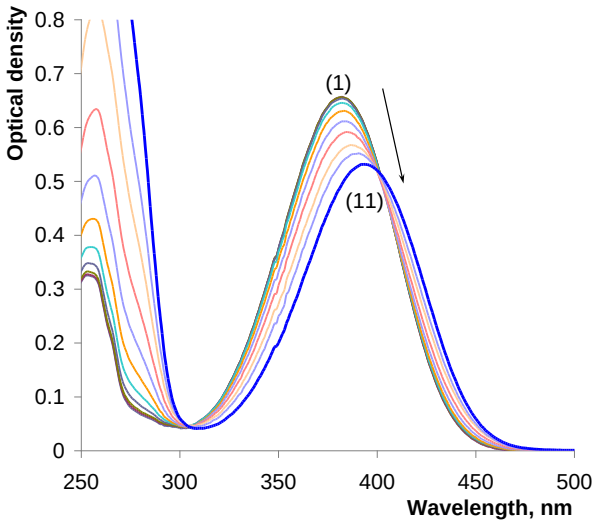

Figure S3a Change of absorption spectra upon addition of poly(dA-dT)$_2$, $C_{dA\text{-}dT}$ = 0 (1), $1.9 \cdot 10^{-4}$ mol·L^{-1}– (11), $C_{\mathbf{1b}} = 2 \cdot 10^{-5} – 1.3 \cdot 10^{-5}$mol·L^{-1} in BPE water buffer (pH=7).

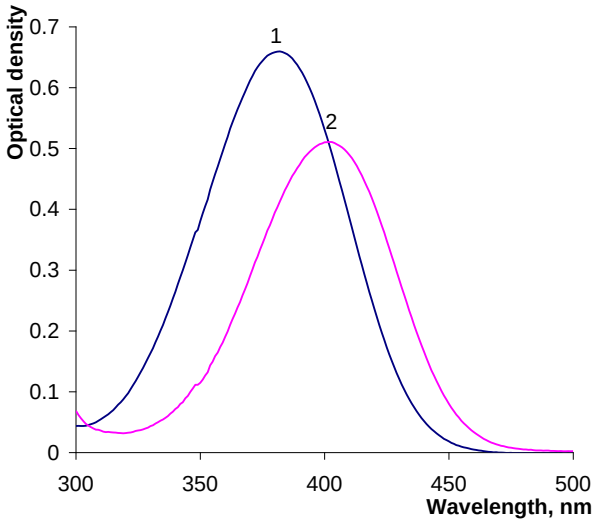

Figure S2b Calculated absorption spectra of **1b** (1) and its complex with poly(dA-dT)$_2$ (2), $C_{\mathbf{1b}} = 2 \cdot 10^{-5}$ mol·L^{-1} in BPE water buffer (pH=7).

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