SYNTHESIS OF UNSYMMETRIC CYANINE DYE VIA MEROCYANINE AND THEIR INTERACTION WITH DNA

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An effective method has been developed for synthesis of neutral merocyanine dye **4** from (4-nitrophenyl)malondialdehyde. Merocyanine **4** was used as a basic building block for preparation of unsymmetric cyanine dyes **9** and **10**, both derived from the same aromatic malondialdehyde. With the cyanine **10**, pH-dependent affinity to DNA has been studied. Unsymmetric cyanine dye **10** has been compared with symmetric dye **8**. The corresponding binding constants (*K*) have been calculated for various pH.

Keywords: Malondialdehyde; Merocyanine; Symmetric; Unsymmetric; Cyanine; DNA.

Cyanine dyes are very interesting and intensively studied family of chromophoric compounds[1,2]. They are widely used in industrial chemistry, in the chemistry of dyes, in medicine, and are used in specific branches of nonlinear optics[1,3] (NLO), optical recording[3], photodynamic therapy[1,3] (PDT), cancer treatment[1,3] and optical sensing[1,3] while new structures with interesting properties are still emerging every year.

Cyanine dyes from malondialdehydes are mentioned in literature only rarely. We have extended this area and prepared some symmetric cyanine dyes derived from 2-arylmalondialdehydes. We studied affinity and selectivity to anions and biologically important sulfated biopolymer heparin[4].

In our further research, we have focused on a study of affinity of the dyes to DNA as a most biologically important biopolymer with several binding sites for various guest molecules[5]. Cyanine dye–DNA interactions have been extensively studied and a variety of noncovalent binding modes have been

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identified[5,6]. It is well known that DNA can be used as a template for formation of supramolecular structures of cyanine dyes[7]; certain dyes can also influence physiological functions of DNA[5]. Guest molecules have a high potential as chemotherapeutic drugs which may suppress the gene replication or transcription in tumor cells. In addition, such a host–guest interaction may be used for detection of nucleic acids when the physical properties of the guest molecule change upon binding and may be easily monitored[8]. Especially useful along these lines is DNA staining, which is based on the color change of the dye upon binding to the macromolecule[7]. Despite of many different types of cyanine dyes which have been studied in interaction with DNA, cyanine dyes derived from aromatic malondialdehydes have been omitted. Moreover, from our previous research, dyes of this type in the presence of anionic biopolymer heparin exhibit significant spectral changes in the visible region[4]. Therefore, our goal was comparison of affinity of symmetric and unsymmetric cyanine dyes based on aromatic malondialdehydes to DNA and to try to evaluate the role of both-side heteroaromatic rings of the methinium chain.

RESULTS AND DISCUSSION

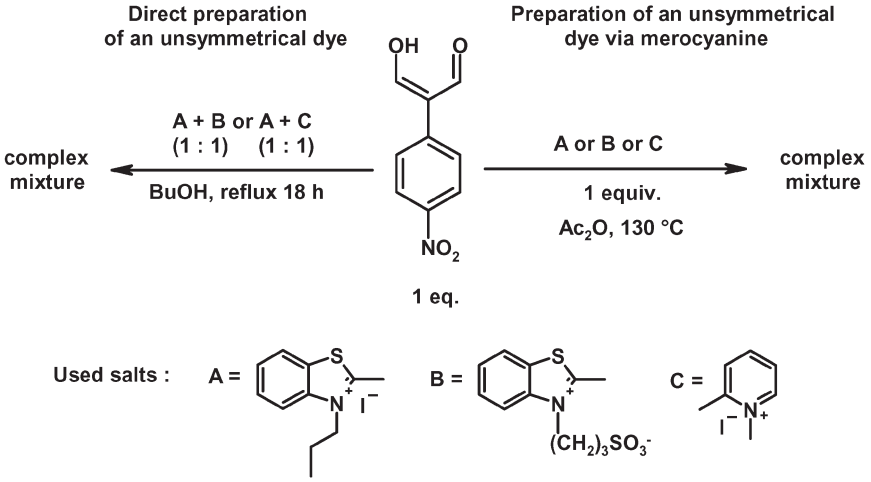
Preparation of Unsymmetric Cyanine Dyes

At the first stage, it was necessary to find a synthetic way for preparation of unsymmetric cyanine dyes with two different heteroaromatic rings on both sides of the chain. Preparation of unsymmetric dyes is well documented in literature[1,3]. On the other hand, the synthesis of unsymmetric cyanines from aromatic malondialdehydes has not been described.

Our first synthetic strategy for preparation of unsymmetric dyes was based on a previous method which was used for preparation of symmetric dyes[4]. We used two different salts of corresponding 2-methylbenzothiazoles in the 1:1 ratio and reacted them with one equivalent of selected malondialdehyde **1** (Scheme 1).

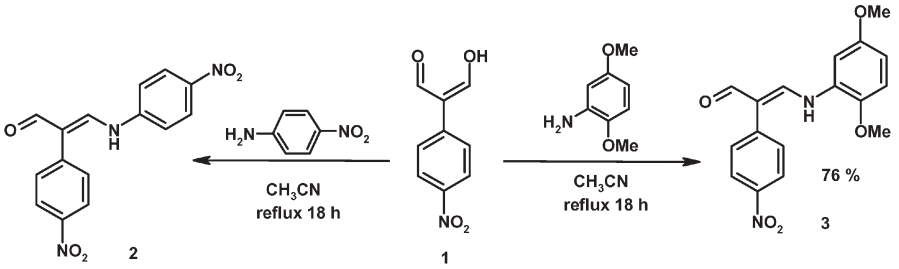
This method afforded complex mixtures containing probably traces of unsymmetric and symmetric dyes, which could not be separated and purified. Therefore we focused our effort on the second approach. It is described in the literature that unsymmetric cyanines can be prepared via neutral merocyanines. The method utilizes tetraalkoxy-2-alkylpropanes as basic building blocks[9,10] for condensation with various heteroaromatic salts. In the first step of the described method, a tetraalkoxypropane is reacted with one equivalent of heteroaromatic salt to afford corresponding neutral

merocyanine dye. The dye is then converted with a second, different heteroaromatic salt into final unsymmetric dye[10]. We have tried to apply this principle in the case of aromatic malondialdehyde (Scheme 1), but the method failed in synthesis of neutral merocyanine. We obtained only complex mixtures containing traces of the product. Moreover, the product was probably accompanied by a symmetric cyanine dye, which was formed by the reaction between nascent neutral merocyanine and starting heteroaromatic salt.



SCHEME 1

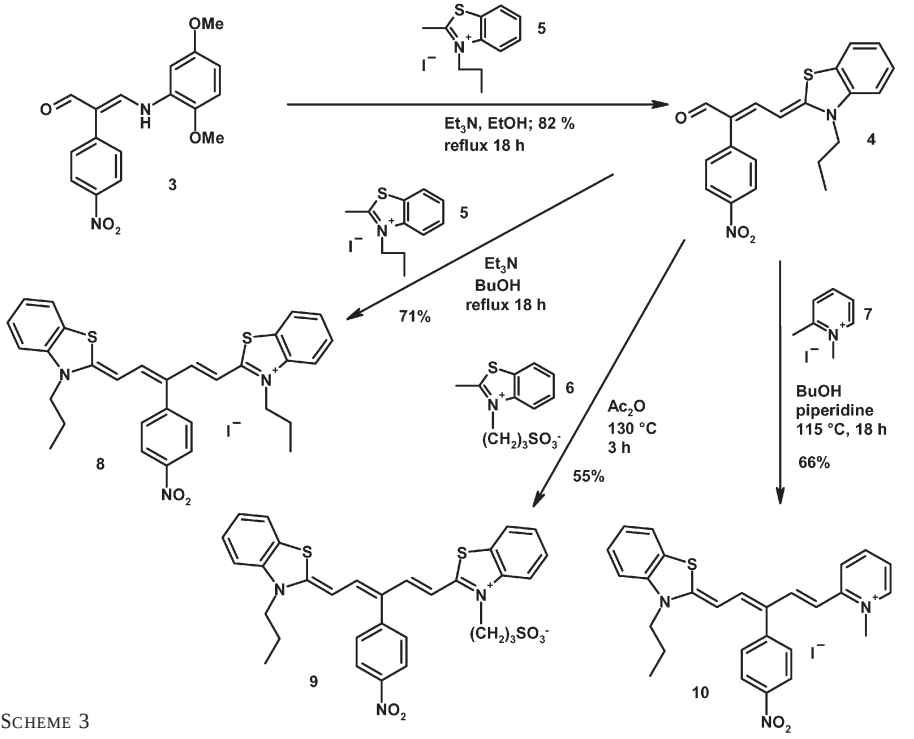
Our further idea how to reach neutral merocyanine from aromatic malondialdehyde, was based on the known[11,12] conversion of malondialdehyde with an aromatic amine into corresponding acrylaldehyde (Scheme 2). The subsequent reaction of the acrylaldehyde with benzothiazolium salt in alkaline medium should lead to the desired neutral merocyanine dye (Scheme 3).



SCHEME 2

In the first step, 2-(4-nitrophenyl)malondialdehyde (**1**) was reacted with several aromatic amines (Scheme 2). The condensation with aniline afforded the product accompanied by a complex mixture of other compounds which make separation and purification of acrylaldehyde difficult. In the case of 4-nitroaniline, as another amine which was used in condensation with malondialdehyde, the corresponding product **2**, 3-(4-nitroanilino)-2-(4-nitrophenyl)acrylaldehyde was clearly formed. Unfortunately, the solubility of the aldehyde was very limited under the conditions of the condensation with benzothiazolium salt and this fact complicated its utilization. The best results were obtained by condensation of malondialdehyde **1** with 2,5-dimethoxyaniline, which formed the corresponding acrylaldehyde **3** in the yield of 76%. Compound **3** was easily separated by mere filtration; it was sufficiently soluble for the next step.

The next step, condensation with 2-methyl-3-propylbenzothiazol-3-ium iodide (**5**), was accomplished under reflux in ethanol in the presence of triethylamine. The reaction smoothly afforded the desired merocyanine dye **4** in 82% yield (Scheme 3).



SCHEME 3

The final step was synthesis of unsymmetric charged cyanine dyes from neutral merocyanine **4**. First, we tried to convert the dye **4** into symmetric cyanine dye to justify the reactivity of **4** to benzothiazolium salt **5** (Scheme 3). Merocyanine **4** was reacted with the salt **5** and the corresponding symmetric cyanine dye **8** was clearly formed. Then we studied condensation of **4** with salt **6**, which should lead to unsymmetric cyanine dye **9**. In this case, under conditions successfully used for preparation of cyanine dye **8**, the salt **6** did not afford the product; starting compounds were found in the reaction mixture. Therefore we changed the reaction conditions. The temperature of the mixture was increased to 130 °C and acetic anhydride, which is frequently used in syntheses of cyanine dyes[10], was used as a solvent. Under these conditions, unsymmetric cyanine dye **9** with two different benzothiazolium units on both sides was formed in a good yield (Scheme 3). In the last step we decided to prepare an unsymmetric cyanine dye with benzothiazolium unit on one side and a unit with different heterocycle on the other. We chose 2-methylpyridinium iodide (**7**) as a salt for the condensation with merocyanine **4**. The reaction was accomplished in butan-1-ol with piperidine instead of triethylamine as a base. Compound **10** was formed in a good yield (Scheme 3).

Study of the Affinity of Prepared Dyes to DNA

The prepared unsymmetric salt **10** has been studied and compared with symmetric salt **8**, which was already studied in detail as a optical senzor for sulfates[4]. In the case of unsymmetric dye **9**, strong aggregation was observed under conditions of the measurement and therefore the dye was excluded from further experiments. We have studied the influence of structure of cyanine dyes **8** and **10** on stability of their complex with DNA at various pH (Figs 1–4).

First of all, we compared dyes **8** and **10** in acidic medium, pH 5.50 (Figs 1 and 2). We have observed a stronger affinity of the symmetric dye **8** to DNA, whereas unsymmetric dye **10** interacted more weakly. In neutral medium, pH 7.00 (Figs 3 and 4), the situation was similar. Symmetric dye **8**, in comparison with dye **10**, showed a stronger affinity to DNA. Generally we have observed a stronger influence of pH on the value of K for symmetric cyanine dye **8** and small dependence for unsymmetric cyanine dye **10**. In the case of symmetric dye **8**, the 1:1 complex was observed in both types of the medium – at pH 5.50 and 7.00, respectively. Concerning the unsymmetric dye **10**, 1:1 and 2:1 complexes of DNA and the dye have been

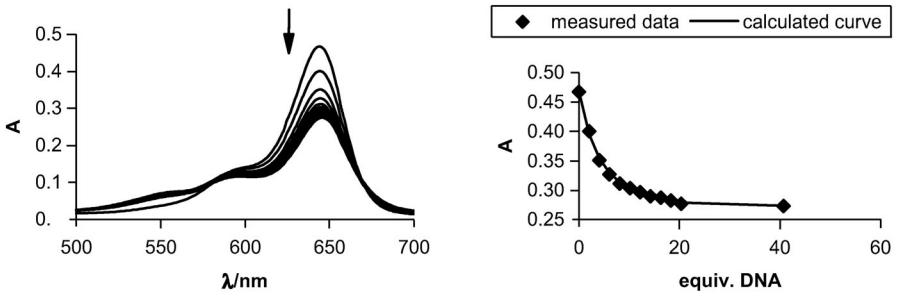


FIG. 1
Addition of DNA (0–40 equiv.) to the unsymmetric cyanine dye **8** in phosphate buffer, pH 5.50, wavelength 643 nm

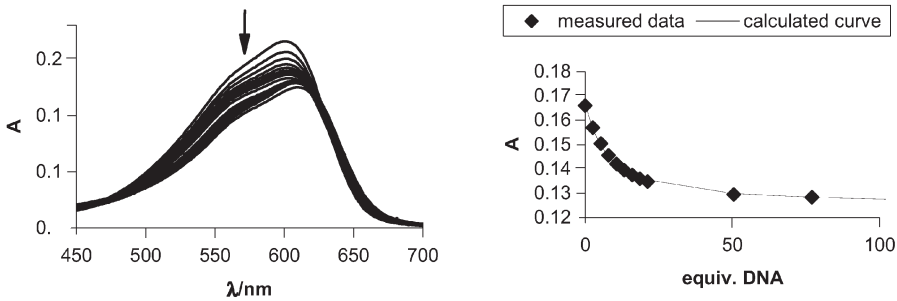


FIG. 2
Addition of DNA (0–80 equiv.) to the unsymmetric cyanine dye **10** in phosphate buffer, pH 5.50, wavelength 643 nm

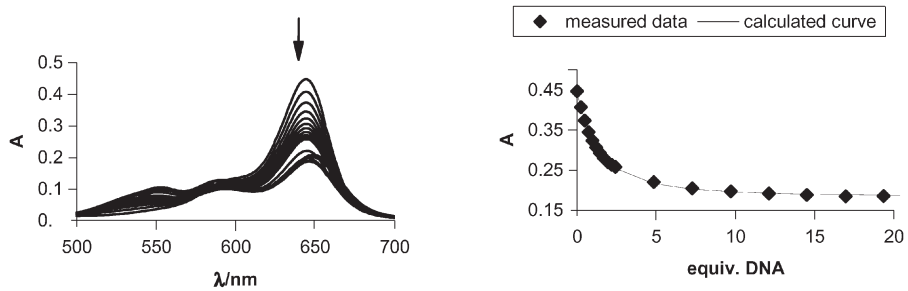


FIG. 3
Addition of DNA (0–20 equiv.) to the symmetric cyanine dye **8** in phosphate buffer, pH 7.00, wavelength 643 nm

observed. The calculated *K* and the observed complexes at various pH for dyes **8** and **10** are summarized in Table I.

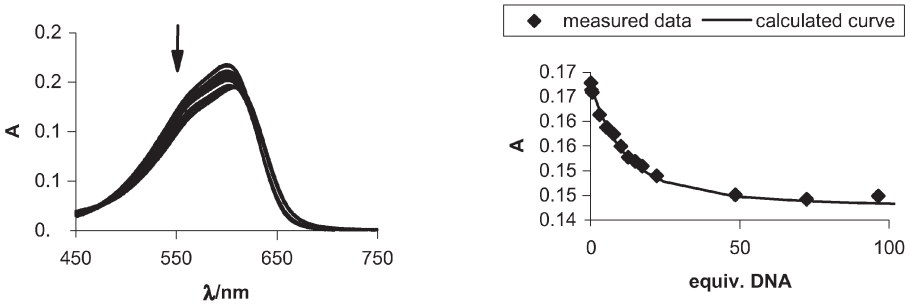


FIG. 4
Addition of DNA (0–100 equiv.) to the unsymmetric cyanine dye **10** in phosphate buffer, pH 7.00, wavelength 643 nm

TABLE I
Calculated values of *K* of symmetric and unsymmetric cyanine dyes **8** and **10** with DNA

K for complexes DNA–dye	pH 5.50		pH 7.00	
Stoichiometry of complex	1:1	2:1	1:1	2:1
K for symmetric cyanine dye **8**	5.0	–	–5.7	–
K for unsymmetric cyanine dye **10**	4.8	9.3	4.9	9.5

CONCLUSIONS

We have developed an efficient method for preparation of neutral merocyanine dye **4** from (4-nitrophenyl)malondialdehyde (**1**). Merocyanine **4** has been used as a key building block in the synthesis of unsymmetric cyanine dyes **9** and **10**, whereas dye **9** has been excluded from our study due to strong aggregation. We studied and compared interaction of symmetric dye **8** and unsymmetric dye **10** with DNA. Our pilot exploration showed difference between unsymmetric and symmetric dyes in affinity to DNA at various pH. Slightly higher affinity to DNA was evident in the case of symmetric dye **8** in comparison with unsymmetric dye **10**. Nevertheless, a precise evaluation of contributions of both heteroaromatic rings on the methinium chain to the affinity of the dyes to DNA is not possible at this stage of research. It is necessary to prepare larger series of unsymmetric and symmetric dyes and to study and compare their affinity to DNA.

EXPERIMENTAL

NMR spectra were obtained with a Varian 300 (300.077 MHz for ^{1}H and 75.460 MHz for ^{13}C) at 23 °C in DMSO-d_6. Chemical shifts (δ) are presented in ppm, coupling constants (*J*) in Hz. Mass spectra were obtained by electron impact (EI+) with a VG Analytical ZAB-EQ spectrometer.

3-(2,5-Dimethoxyanilino)-2-(4-nitrophenyl)acrylaldehyd (**3**)

The reaction flask was charged with 2-(4-nitrophenyl)malondialdehyde (200 mg, 1.04 mmol), 2,5-dimethoxyaniline (150 mg, 0.98 mmol) and dry acetonitrile (15 ml). The mixture was stirred at 75 °C for 18 h. Then the volume of the reaction mixture was reduced to a half and precipitated acrylaldehyde **3** was filtered off, washed with diethyl ether (3 ml) and dried in vacuo. The filtrate was allowed to crystallize and the product was separated as mentioned above. Total yield of compound **3** was 221 mg (76%). ^{1}H NMR (DMSO-d_6): 12.41 d, 1 H, *J* = 13.8; 9.78 d, 2 H, *J* = 3.8; 8.22 dt, 2 H, *J* = 8.8, *J* = 2.6; 7.74 dd, 1 H, *J* = 13.2, *J* = 3.8; 7.47 dt, 2 H, *J* = 8.8, *J* = 2.6; 6.88 d, 1 H, *J* = 8.8; 6.81 d, 1 H, *J* = 2.9; 6.64 dd, 1 H, *J* = 8.8, *J* = 2.6; 3.94 s, 3 H; 3.8 s, 3 H. ^{13}C NMR (DMSO-d_6): 189.2 (CH=O), 154.3 (C), 145.6 (C), 144.8 (C), 143.4 (C), 143.2 (CH), 129.3 (C), 125.5 (CH), 124.4 (CH), 112.3 (CH), 111.1 (C), 108.7 (CH), 101.7 (CH), 56.5 (CH_3), 55.9 (CH_3). HRMS (EI+): for $C_{17}H_{16}N_2O_5$ [MH] calculated 328.1046, found 328.1059.

2-(4-Nitrophenyl)-3-(3-propyl-2,3-dihydrobenzothiazol-2-ylidene)but-1-enal (**4**)

The reaction flask was charged with acrylaldehyde **3** (100 mg, 0.30 mmol), benzothiazolium salt **5** (97 mg, 0.30 mmol), dry triethylamine (0.5 ml), 1,2-dimethoxyethane (3 ml) and dry ethanol (7 ml). The stirred mixture was refluxed for 18 h. The mixture was reduced to half volume and placed in a fridge for 5 h. Precipitated merocyanine **4** was filtered off, washed with diethyl ether (1 ml) and dried in vacuo. The filtrate was allowed to crystallize and the solid was separated as mentioned above. Total yield of compound **4** was 91 mg (82%). ^{1}H NMR (DMSO-d_6): 9.39 s, 1 H; 8.25 d, 2 H, *J* = 8.8; 7.77 d, 1 H, *J* = 7.6; 7.63 d, 2 H, *J* = 8.9; 7.40 m, 3 H; 7.20 t, 1 H, *J* = 7.0; 5.94 d, 1 H, *J* = 13.0; 4.00 t, 2 H, *J* = 7.0; 1.64 sextet, 2 H, *J* = 7.3; 0.87 t, 3 H, *J* = 7.3. ^{13}C NMR (DMSO-d_6): 188.8 (CH=O), 160.1 (C), 145.3 (C), 142.4 (C), 141.8 (C), 130.5 (CH), 127.2 (CH), 125.4 (C), 123.6 (C), 123.2 (CH), 122.3 (CH), 111.6 (CH), 99.1 (CH), 89.0 (CH), 46.1 (CH_2), 19.9 (CH_2), 10.9 (CH_3). MS: for $C_{20}H_{18}N_2O_3S$ calculated 366, found (M + H) 367. For $C_{20}H_{18}N_2O_3S$ calculated: 65.56% C, 4.95% H; found: 65.72% C, 4.98% H.

3-(4-Nitrophenyl)-1-(3-propyl-2,3-dihydrobenzothiazol-2-ylidene)-
5-[3-(3-sulfonatopropyl)benzothiazol-2-yl]pentamethinium (**9**)

The reaction flask was charged with merocyanine **4** (50 mg, 0.14 mmol), benzothiazolium salt **6** (37 mg, 0.14 mmol) and acetic anhydride (7 ml). The reaction mixture was heated to 130 °C for 3 h. The mixture was then cooled to laboratory temperature and the precipitated cyanine dye **9** was filtered off, washed with cold ethanol (1 ml) and dried in vacuo. Compound **9** was obtained as a green crystaline powder (47 mg, 55%). ^{1}H NMR (DMSO-d_6): 8.40 d, 2 H, *J* = 8.8; 7.39–8.08 m, 12 H; 6.17 d, 1 H, *J* = 14.0; 5.96 d, 1 H, *J* = 13.5; 4.41 t, 2 H, *J* = 7.0; 4.16 t, 2 H, *J* = 7.0; 2.46 t, 2 H, *J* = 6.7; 1.96 quintet, 2 H, *J* = 5.9; 1.64 sextet,

2 H, J = 7.0; 0.82 t, 3 H, J = 7.3. ^{13}C NMR has not been obtained due to very limited solubility of dye **9**. HRMS (EI+): for $C_{31}H_{29}N_3O_2S_2$ [M – SO_3] calculated 539.1698, found 539.1701.

1-(1-Methylpyridin-1-ium-2-yl)-3-(4-nitrophenyl)-5-(3-propyl-2,3-dihydrobenzothiazol-2-ylidene)-3-(4-nitrophenyl)pentamethinium Iodide (**10**)

The reaction flask was charged with merocyanine **4** (54 mg, 0.15 mmol), pyridinium salt **7** (35 mg, 0.15 mmol), piperidine (0.3 ml) and dry butan-1-ol (7 ml). The reaction mixture was heated to 115 °C for 18 h. The mixture was then cooled to laboratory temperature and the precipitated compound **10** was filtered off, washed with diethyl ether (2 × 20 ml) and dried in vacuo. The product **10** was obtained as a dark green crystalline powder (57 mg, 66%). ^{1}H NMR (DMSO-d_6): 8.59–8.10 m, 6 H; 7.75 d, 1 H, J = 7.7; 7.64 d, 2 H, J = 8.3; 7.48 bs, 1 H; 7.38 m, 3 H; 7.17 t, 1 H, J = 7.7; 5.94 m, 1 H; 5.49 m, 1 H; 3.90 s, 5 H; 1.57 bs, 2 H; 0.81 bs, 3 H. ^{13}C NMR (DMSO-d_6): 156.8 (C), 153.0 (C), 146.7 (CH), 146.3 (C), 144.6 (CH), 144.2 (C), 142.8 (CH), 141.9 (C), 141.7 (CH), 131.3 (CH), 127.2 (CH), 126.8 (C), 124.1 (2 × CH), 123.6 (C), 122.9 (CH), 122.3 (CH), 120.8 (CH), 111.3 (CH), 106.8 (CH), 91.2 (CH), 46.0 (CH_2), 44.7 (CH_3), 19.9 (CH_2), 11.0 (CH_3). HRMS (EI+): for $C_{27}H_{25}N_3O_2S$ [M – H] calculated 455.1668, found 455.1667. UV-Vis (30% MeOH): λ_{max} = 600 nm, ε_{max} = 6.4 × 10^4 mol^{-1} l cm^{-1}.

Study of Binding Cyanine Dyes **8** and **10** to DNA

The association of the cyanine dyes **8** and **10** with DNA was studied by UV-Vis spectrometry according to the method reported previously[4]. Binding constants (K) were calculated from changes in absorbance of cyanine dyes (ΔA) by nonlinear regression using the program Letagroup spefo 2005. As polymer DNA chains have various lengths, K for dyes **8** and **10** were calculated using concentrations of each repeating base pairs. Dyes **8** and **10** were used as 2.7 μM solutions in 1 mM phosphate buffer in H_2O–MeOH–DMSO (68:30:2, v/v/v). DNA was used as a 1 mM solution in 1 mM phosphate buffer in H_2O–MeOH–DMSO (68:30:2, v/v/v), and the solution was added to a solution of the cyanine dye.

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