Homodimeric monomethine cyanine dyes SOSO-1 and TOTO-1-6C—synthesis and fluorescence properties in the presence of nucleic acids

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Abstract

Two new homodimeric asymmetric monomethine cyanine dyes SOSO-1 and TOTO-1- 6C bearing four and six positive charges respectively have been synthesized. The longest wavelength absorption maxima of both dyes lie between 495 and 520 nm; the molar absorptivities are between 110,000 and 150,000 $l\,mol^{-1}\,cm^{-1}$. SOSO-1 and TOTO-1-6C do not fluoresce in TE buffer and bidistilled water but become strongly fluorescent upon binding to dsDNA. The fluorescence maxima are at 540.4 and 534.4 nm and the fluorescence quantum yields are 0.25 and 0.35, respectively.
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1. Introduction

The application of new homodimeric asymmetric monomethine cyanine dyes and heterodimeric cyanine dyes as noncovalent fluorescent markers for biopolimers makes a substantial impact on nucleic acids research [1–10]. As a part of our investigations on novel and improved homodimers as fluorescent probes for nucleic acids detection [8] we have synthesized two new homodimeric asymmetric monomethine cyanine dyes *N,N,N′,N′* - tetramethyl - *N,N′* - bis - {[4[(3 - methyl - 2(*3H*)benzoselenazolylidene)methyl]quinolinium]-1,3-propanediyl}-1,3-propanediammonium tetraiodide **SOSO-1** and *N,N,N′,N′*-tetramethyl-*N,N′*-bis-{[4[(3-(1-pyridiniopropyl)-2(*3H*)benzothiazolylidene)methyl]quinolinium]-1,3-propanediyl}-1,3-propanediammonium hexaiodide **TOTO-1-6C**.

In this study, are presented the synthetic method for preparation of the dyes TOTO-1-6C and SOSO-1 as well as their absorption and fluorescence

spectral characteristics in the presence of nucleic acids. The influence of different factors (e.g. temperature, light, etc.) on the fluorescence properties and stability of the dye–DNA complexes was investigated.

2. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected.

Absorption spectra were scanned on a Specord M40 (Carl Zeiss, Jena) UV–VIS spectrophotometer and the corrected fluorescence spectra (excitation at 480 nm) on a Perkin-Elmer MPF44 spectrofluorimeter. The emission spectra were corrected using a standard Tungsten lamp, while the excitation spectra were corrected with Rhodamine B. The fluorescence quantum yield (Q_F) was determined relative to that of Rhodamine 6G [11].

Stock solutions were prepared by dissolving 1 mM of TOTO-1-6C and SOSO-1 in 1 ml DMSO and subsequent dilution with TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) to a final concentration of TOTO-1-6C 1.5×10^{-6} M and of SOSO-1 3×10^{-6} M. The fish sperm dsDNA was purchased from Sigma (USA).

2.1. Preparation of [1-(3-iodopropyl)-4-[(3-methyl-2(3H)benzoseleselenazolylidene)-methyl]quinolinium iodide (3a) and 1-(3-iodopropyl)-4-{[4-(3-(1-pyridiniopropyl)-2(3H)benzothiazolylidene)methyl]}quinolinium diiodide (3b)

0.0066 mol **1a** or **1b** and 3.03 g (0.0066 mol) 1-(3-iodopropyl)-4-chloroquinolinium iodide **2** [2] were dissolved in 160 ml methanol. 1.14 g (0.0136 mol) sodium bicarbonate, dissolved in 20 ml water, were added and stirred at room temperature for 30 min. The reaction mixture was then refluxed for 30 min. After cooling, the flask content was poured with stirring in a 500 ml aqueous solution of excess potassium iodide. The precipitate was filtered, washed first with acetone then with water and air-dried. The yields of **3a** and **3b** were 64 and 51%, respectively. After recrystallization from methanol **3a** had an m.p of 200–202 °C and **3b** had an m.p. of 250–252 °C.

Elemental analysis for **3a**: $C_{21}H_{20}I_2N_2Se$; (633.17)

	C%	H%	N%
Calculated:	39.84	3.18	4.42
Found:	40.00	3.58	4.56

^{1}H NMR (DMSO), δ (ppm): 8.82–7.22 (m, 10H, Ar); 7.12 (s, 1H, CH); 4.69 (t, 2H, NCH_2); 3.99 (s, 3H, N^+CH_3); 3.19–3.13 (m, 2H, CH_2I); 2.42–2.31 (m, 2H, CH_2).

Elemental analysis for **3b**: $C_{28}H_{28}I_3N_3S$; (819.33)

	C%	H%	N%
Calculated:	41.04	3.44	5.13
Found:	41.33	3.93	5.40

^{1}H NMR (DMSO), δ (ppm): 9.13–7.35 (m, 15H, Ar); 6.94 (s, 1H, CH); 4.89 (t, 2H, NCH_2); 4.80 (t, 2H, NCH_2); 4.69–4.60 (m, 2H, CH_2); 4.03 (br s, 2H, NCH_2); 3.34 (m, $CH_2I + H_2O$); 2.39 (m, 2H, CH_2).

2.2. Preparation of N,N,N′,N′-tetramethyl-N,N′-bis-{[4[(3-methyl-2(3H)benzoselenazolylidene)methyl]-quinolinium]-1,3-propanediyl}-1,3-propanediammonium tetraiodide (SOSO-1) (5a) and N,N,N′,N′-tetramethyl-N,N′-bis-{3-[4[(3-(1-pyridiniopropyl)-2(3H)benzothiazolylidene)methyl]quinolinium]-1,3-propanediyl}-1,3-propanediammonium hexaiodide (TOTO-1-6C) (5b)

A 0.0018 mol of **3a** or **3b** and 0.12 g (0.0009 mol) N,N,N′,N′-teteramethyl-1,3-propanediamine **4** were mixed with 25 ml diethylene glycol monomethyl ether and heated with stirring on an oil bath at 140 °C for 4 h. Finally the reaction mixture was heated at 175 °C for 10 min and then rapidly cooled down to room temperature. The flask was refrigerated overnight and then filtered by suction, washed with diethyl ether and air-dried. The yield for **5a** was 46% and after recrystallization from methanol the pure product had an m.p. of 230–232 °C. The yield for **5b** was 42% and after recrystallization from water the pure product had an m.p of 237–238 °C.

Elemental analysis for **5a**: $C_{49}H_{58}I_4N_6Se_2$; (1396.62)

	C%	H%	N%
Calculated:	42.14	4.18	6.02
Found:	42.03	4.22	5.80

^{1}H NMR (DMSO), δ (ppm): 8.84–7.23 (m, 20H, Ar); 7.15 (s, 2H, 2×CH); 4.67 (br s, 4H, 2×NCH$_2$); 4.02 (br s, 6H, 2×N$^+$CH$_3$); 3.34 (br s, N$^+$(CH$_2$)$_2$+ H$_2$O); 3.12 (s, 12H, 2×N$^+$(CH$_3$)$_2$); 2.50 (m, DMSO+CH$_2$); 2.24 (br s, 4H, 2×CH$_2$).

Elemental analysis for **5b**: C$_{63}$H$_{74}$I$_6$N$_8$S$_2$; (1768.88)

	C%	H%	N%
Calculated:	42.78	4.22	6.33
Found:	42.65	4.21	6.53

^{1}H NMR (DMSO), δ (ppm): 8.83–7.32 (m, 30H, Ar); 6.92 (s, 2H, 2×CH); 4.69 (br s, 4H, 2×NCH$_2$); 4.02 (br s, 4H, 2×NCH$_2$); 3.69 (br s, 4H, 2×NCH$_2$); 3.35 (br s, 8H, N$^+$(CH$_2$)$_2$); 3.17 (s, 12H, 2×N$^+$(CH$_3$)$_2$); 2.54 (m, DMSO+CH$_2$); 2.36 (br s, 4H, 2×CH$_2$).
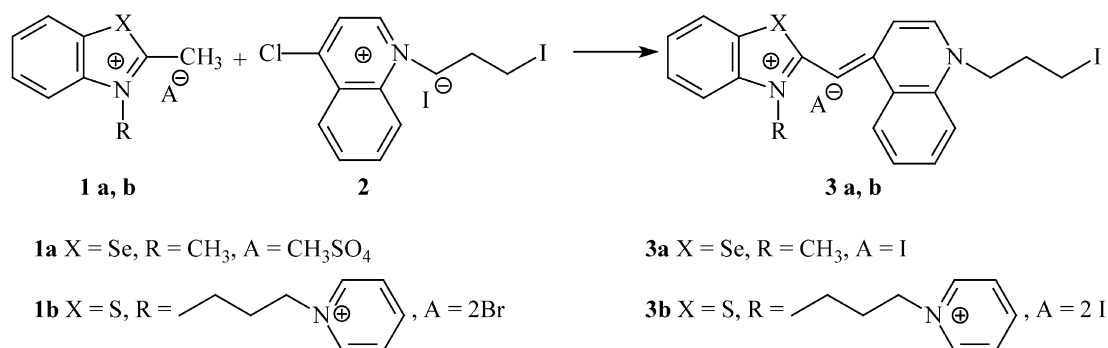
3. Results and discussion

The dye SOSO-1 **5a** was synthesized by reacting 2,3-dimethylbenzselenazolium methosulfate **1a** with 1-(3-iodopropyl)-4-chloroquinolinium iodide **2** in mild alkaline media (Scheme 1) and the resulting monomethine cyanine dye **3a** was bis-quarternized with N,N,N′,N′-teteramethyl-1,3-propanediamine **4** (Scheme 2). The same reaction pathway was used for the preparation of the dye TOTO-1-6C **5b**. 2-Methyl-3-[(3-pyridinio)pro-

pyl]benzothiazolium dibromide **1b** [12] was reacted with 1-(3-iodopropyl)-4-chloroquinolinium iodide **2** giving the monomethine cyanine dye **3b** (Scheme 1).
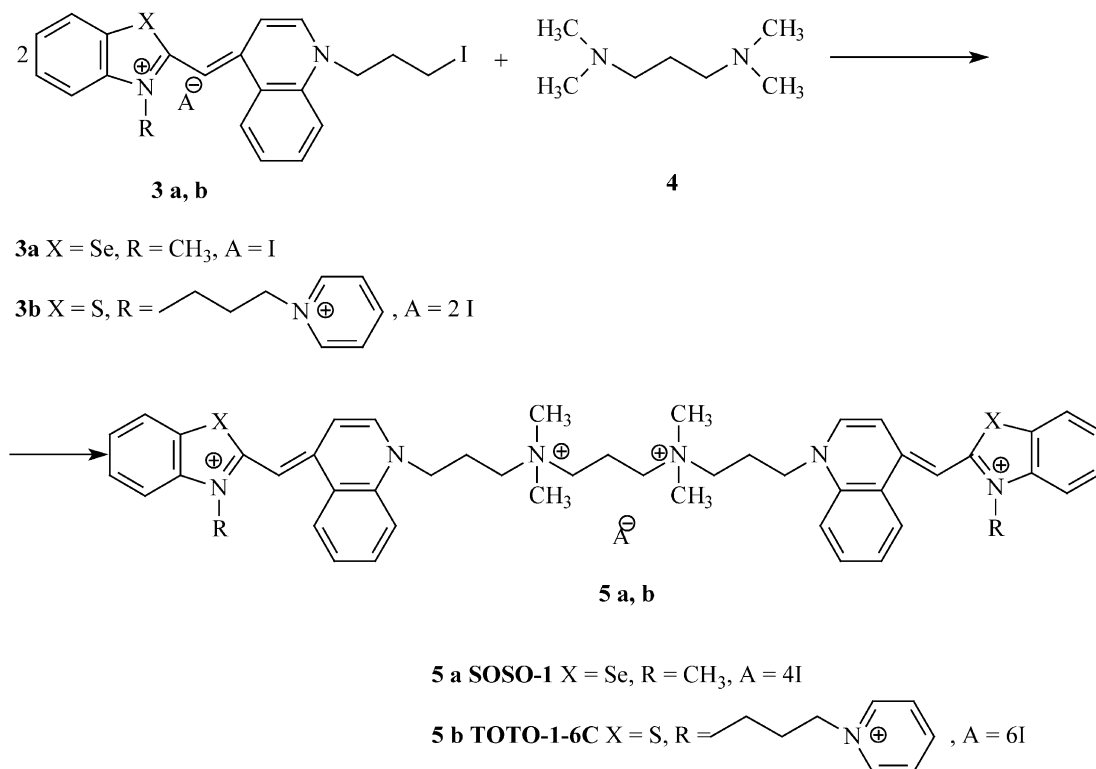
The preparation of TOTO-1-6C **5b** was carried out by bisquaternization of the monomeric monomethine cyanine dye **3b** with N,N,N′,N′-tetramethyl-1,3-propanediamine **4** (Scheme 2). The result was a homodimeric asymmetric monomethine cyanine dye bearing six positive charges.

The ^{1}H NMR spectra are similar (see Section 2) to those reported earlier [8] for this type of homodimeric dyes. The dyes TOTO-1-6C and SOSO-1 do not show fluorescence at the concentrations used but become strongly fluorescent upon excitation at 480 nm after binding to dsDNA (Table 1, Fig. 1).

The longest wavelength absorption maximum of SOSO-1 is at 495 nm with a shoulder at 520 nm. The corresponding molar absorptivities are 110,000 and 74,000 respectively. For TOTO-1-6C this maximum is at 505.5 nm and the molar absorptivity is 146,700. At concentrations 1×10^{-6}M and lower, no evidence for aggregation of the dyes in the absorption spectra is found. The fluorescence maximum of the complex SOSO-1/dsDNA is at 540.4 nm, while this of TOTO-1-6C/dsDNA complex is at 534.4 nm. The fluorescence quantum yields are 0.25 and 0.35, respectively. A coincidence in position and intensity of the fluorescence maxima of the complexes with both dsDNA and it's denatured single stranded form is observed. The emission in the presence of oligo-nucleotide (20-mer) and bovine serum albumin

1a, b   **2**   **3 a, b**

1a X = Se, R = CH$_3$, A = CH$_3$SO$_4$ **3a** X = Se, R = CH$_3$, A = I

1b X = S, R = ⌇⌇⌇N$^+$⟨⟩, A = 2Br   **3b** X = S, R = ⌇⌇⌇N$^+$⟨⟩, A = 2 I

Scheme 1.

3 a, b

3a X = Se, R = CH$_3$, A = I

3b X = S, R = [structure], A = 2 I

5 a, b

5 a SOSO-1 X = Se, R = CH$_3$, A = 4I

5 b TOTO-1-6C X = S, R = [structure], A = 6I

Scheme 2.

Table 1
Absorption and fluorescence characteristics of the studied dyes: λ_{abs} (nm): absorption maximum; ϵ (l M^{-1} cm^{-1})—molar absorptivity; λ_F(nm)—fluorescence maximum; Q_F—fluorescence quantum yield

Dye	Without dsDNA		With dsDNA[a]		
	λ_{abs} (nm)	ϵ (l M^{-1} cm^{-1})	λ_{abs} (nm)	λ_F(nm)	Q_F
SOSO	495,520(sh)	110,000, (77,400)	496,522	540.4	0.25
TOTO	505.5	146,700	505.5	534.4	0.35

[a] The concentration of dsDNA is 3 μg/ml.

(BSA) is in the region 520–540 nm. The sensitivity of TOTO-1-6C to oligonucleotide is approximately 10-fold higher than that of SOSO-1.

The fluorescence measurements in bidistilled water at different salt concentrations (from 0.12 to 1 M NaCl) show a 30% quenching of emission in solution containing 1 M NaCl.

Investigations on the stability of the dye/dsDNA complexes have been done. As their formation is too fast the measurements are per-

formed immediately after mixing the dye and the dsDNA solution. The experimental results show that the complexes are stable at least for 48 h when kept at sunlight—no photobleaching was observed.

Melting curve analysis is performed by heating dsDNA in the presence of TOTO-1-6C or SOSO-1. The fluorescence intensity diminishes and the position of the fluorescence maximum shifts to the right with approximately 20 nm. After cooling for

1 min the fluorescence characteristics become identical with those of the starting solution.

The dye/dsDNA complexes are also stable when used for agarose gel electrophoresis. Clear gels (with no fluorescence background) are obtained; the color of the bright bands is green. The brightness of the band obtained with TOTO-1-6C is much higher than that with SOSO-1.

The results from the study of the relationship between the fluorescence intensity (I_F) and the amount of DNA at constant dye concentration (5×10^{-6} M) show that it is linear in the range 0.3–5 μg/ml for SOSO-1 and 0.075-10 μg/ml for TOTO-1-6C (Fig. 2a and b).
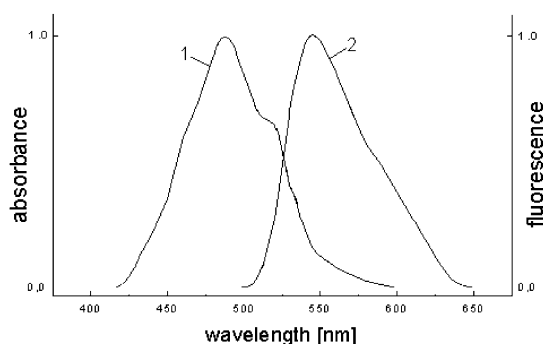

Fig. 1. Absorption and fluorescence spectra of SOSO-1 in TE buffer (concentration 5×10^{-6}M) in the presence of 5 μg/ml dsDNA.

In our previous investigation [13] it was shown that for monomethine cyanine dyes the increasing of the number of positive charges (1, 2 and 3) leads to a rise in the sensitivity as well as in the range of the linear relationship between the fluorescence intensity and the concentration of dsDNA. The same effect is observed also with the newly synthesized homodimers. The detection limit of dsDNA in solution with dye concentration 5×10^{-7} M is 100 ng/ml with SOSO-1 and 20 ng/ml with TOTO-1-6C.

4. Conclusions

Two new homodimeric monomethine cyanine dyes SOSO-1 and TOTO-1-6C bearing four and six positive charges respectively were synthesized using an improved synthetic procedure.

SOSO-1 and TOTO-1 6C do not fluoresce in solution but make strongly fluorescent complexes with nucleic acids. The fluorescence quantum yield is higher when TOTO-1-6C bearing six positive charges is bound to dsDNA. Therefore TOTO-1-6C provides higher sensitivity for DNA detection compared to SOSO-1. The detection limit of dsDNA in solution with dye concentration 5×10^{-7} M is 100 ng/ml for SOSO-1 and 20 ng/ml for TOTO-1-6C. The fluorescence intensity shows
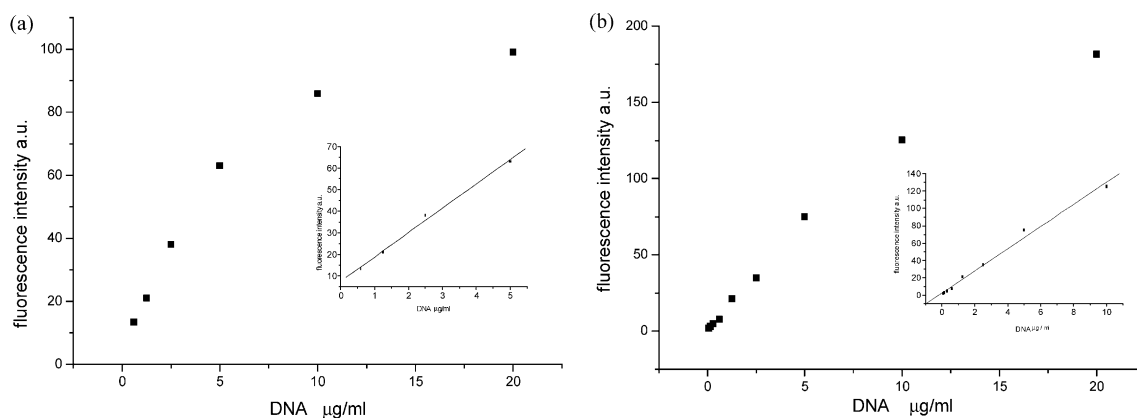

Fig 2. (a) Fluorescence intensity in arbitrary units of the complex SOSO-1 (5×10^{-6}M)/dsDNA (0.6–20 μg/ml) as a function of the dsDNA concentration; in the insert is given the linear dependence in the range 0.6–5 μg/ml dsDNA at the same dye concentration. (b) Fluorescence intensity in arbitrary units of the complex TOTO-1-6C (5×10^{-6}M)/dsDNA (0.075–20 μg/ml) as a function of the dsDNA concentration; in the insert is given the linear dependence in the range 0.075–10μg/ml dsDNA at the same dye concentration.

a linear dependence over a broad range of DNA concentrations, allowing quantitative determination of dsDNA. This fact together with the stability of the formed complexes makes the investigated dyes appropriate for application in gel electrophoresis as well as in developing new methods for quantitation and visualization of nucleic acids.

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