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Synthesis of homodimeric monomethine cyanine dyes as noncovalent nucleic acid labels and their absorption and fluorescence spectral characteristics

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Dedicated to Professor Dr. Karl-Heinz Drexhage on the occasion of his 65th birthday

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Abstract

Several novel homodimeric asymmetric monomethine cyanine dyes based on the thiazole orange (TO) chromophore were synthesised via an improved synthetic procedure. The two TO chromophores [1-(ω-bromoalkyl)-4-[(3-methyl-2-(3H)-benzothiazolilyden)methyl] quinolinium iodide], with different chain lengths of the methylene linker between the quinolinium ring and the quaternary ammonium nitrogen, were connected by bisquaternization with *N,N,N′,N′*-tetramethyl-1,3-propanediamine, *N,N,N′,N′*-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo- [2,2,2]octane and 1,4′-bipyridine. The homodimeric dyes have large molar absorptivity (ε 130 000–180 000 l mol^{-1} cm^{-1}) at 505–506 nm. In the presence of ds DNA, their fluorescence maxima were located at 530–534 nm and the fluorescence quantum yields were in the range 0.48–0.96. Fluorescence maxima between 560–650 nm and fluorescence quantum yields of 0.3–0.8 were observed in the presence of ss DNA.

Keywords: Monomethine cyanine dyes; Homodimers; Synthetic method; Absorption; Fluorescence; Noncovalent nucleic acid labels

1. Introduction

In recent years there has been extensive research on the syntheses and applications of polymethine dyes as noncovalent labels to nucleic acid detection [1–3]. Penta-, tri- and monomethine cyanine dyes have been synthesised and used as nucleic acid stains, the latter ones being the most numerous and extensively studied. Among cyanines, they are the best noncovalently binding nucleic acid labels with respect to their most important property, viz. high fluorescence signal. As part of our search of novel and improved methods for the preparation

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of asymmetric monomethine cyanine dyes, we have synthesised a series of noncovalent nucleic acid labels [4–7] and studied their spectral profiles [8,9]. Nonradioactive labelled DNA stains, stable under electrophoretic conditions, are found among homodimeric monomethine cyanines with four positive charges. These dyes, known as TOTO-1 [*N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden)methyl]quinolinium-1-yl]propyl}-1,3-propanediammonium tetraiodide] and YOYO-1 (*N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzoxazolilyden) methyl] quinolinium-1-yl] propyl}-1,3-propanediammonium tetraiodide], form highly fluorescent complexes with ds DNA [10]. Recently Staerk et al. [11] synthesised derivatives similar to TOTO-1, with extended methylene bridges between the quinolinium ring and the quaternary ammonium nitrogen. They observed that TOTO-1, and similar derivatives with extended linker, predominantly bisintercalate in the 5′-CTGAG-3′ binding site of oligonucleotides [11,12].

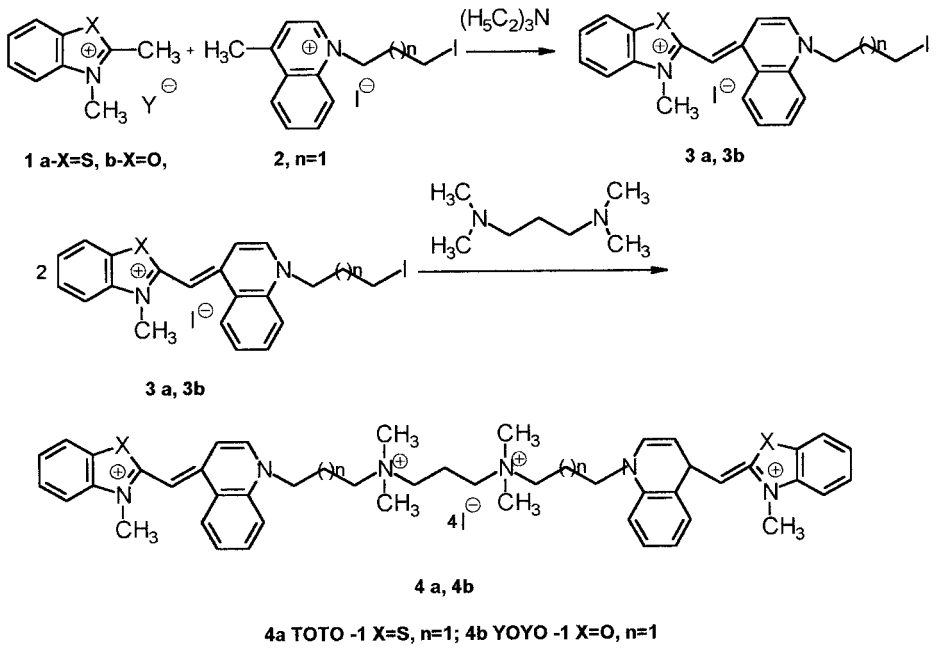
In this study we present an improved synthetic method of novel homodimeric asymmetric monomethine cyanines similar to TOTO-1 and their absorption and fluorescence spectral characteristics.

2. Results and discussion

Rye et al. have synthesised TOTO-1 **4a** and YOYO-1 **4b** by reacting of 2-methylthio-3-methylbenzothiazolium **1a** or 2-methylthio-3-methylbenzoxazolium **1b** salts with 1-(3-iodopropyl)-4-methylquinolinium iodide **2** in the presence of a base. The obtained dyes **3a, 3b** were bisquaternized with *N,N,N′,N′*-tetramethyl-1,3-propanediamine (Scheme 1). Staerk et al. [11] have used the same preparation method to synthesise TOTO-1 derivatives by altering the length of the methylene linker between the quinolinium ring and the quaternary ammonium nitrogen (Scheme 1, X = S, *n* = 4,5).

The quaternized lepidines **7a, 7b** were prepared by the reaction of lepidine **5** with dibromoalkanes **6a, 6b**, in molar ratio 1:4, either neat or in a nonpolar solvent and at room temperature for 24–72 h [13] (Scheme 2).

We have recently proposed a new synthetic method for the preparation of monomethine cyanines [7] in which quaternary salts of heterocyclic 2- or 4-methyl compounds and *N*-heterocyclic 2- or 4-sulfobetainic compounds were reacted by melting together or by refluxing in different solvents in the



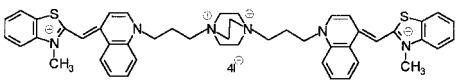
Scheme 1.

absence of a base. On this basis melting of anhydro-3-methyl-2-sulfobenzothiazolium hydroxide **8** with 1-(ω-bromoalkyl)-4-methylquinolinium bromides **7a, 7b** yielded the starting monocationic ω-bromoalkyl monomethine cyanines **9a, 9b** (Scheme 3).

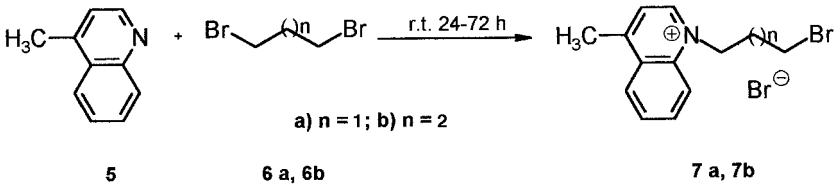
The dyes **9a, 9b** were bisquaternized with *N,N,N′, N′*-tetramethyl-1,3-propanediamine, *N,N,N′,N′*-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo[2,2,2] octane and 1,4′-bipyridine. The quaternization was carried out in 2-methoxyethanol for considerably shorter reaction time (15 min to 3 h) than previously described methods [10,11] (Scheme 4) giving the homodimeric dyes **10a–10f**.

The following homodimeric monomethine cyanines were synthesised:

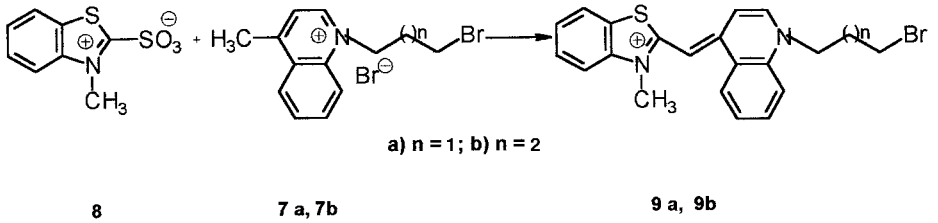
10a: *N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden) methyl]quinolinium-1-yl]propyl}-1,4- diazoniabicyclo[2,2,2]octane tetraiodide



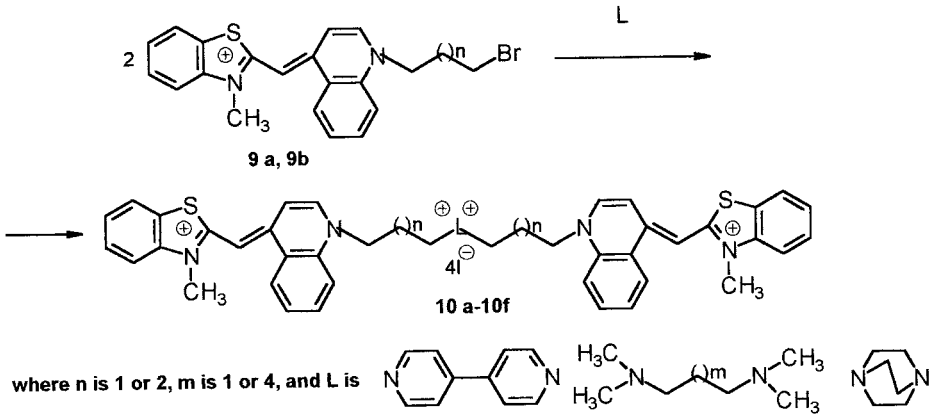
10b: *N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden) methyl]quinolinium-1-yl]butyl}-1,4- diazoniabicyclo[2,2,2]octane tetraiodide



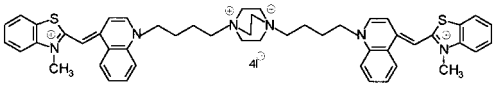
Scheme 2.



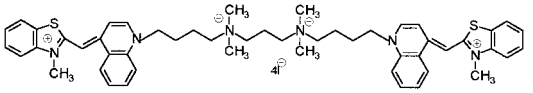
Scheme 3.



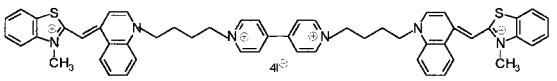
Scheme 4.



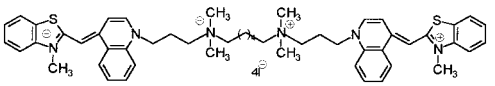
10c: *N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3H)benzothiazolilyden)methyl]quinolinium-1-yl]butyl}-1,3-propanediammonium tetraiodide



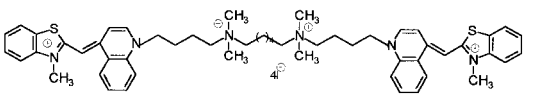
10d: *N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden) methyl]quinolinium-1-yl]butyl}-1,4-bipyridinium tetraiodide



10e: *N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden)methyl]quinolinium-1-yl]propyl}-1,6-hexanediammonium tetraiodide



10f. *N,N,N′N′*-tetramethyl-*N,N′*-bis-{3-[4-[(3-methyl-2(3*H*)benzothiazolilyden)methyl]quinolinium-1-yl]butyl}-1,6-hexanediammonium tetraiodide



Some characterisation data for the homodimeric dyes **10a–f** are given in Table 1. All dyes are new, except dye **10c** [11].

The synthesised dyes did not exhibit any fluorescence in solution at room temperature, with the exception of **10c** and **10f** whose Q_F value was less than 0.001. They all became strongly fluorescent upon binding to DNA. Our investigations showed that both the position and the intensity of the fluorescence maxima depend strongly on the dye structure and the type of DNA used (Table 2).

All dyes showed similar fluorescence characteristics in TE buffer (pH 7.0) in the presence of ds DNA (5 μg/ml). Their fluorescence maxima were located at 530–534 nm and their quantum yields were in the range of 0.48–0.96. In the presence of ss DNA (5 μg/ml) the fluorescence maxima were between 560 and 650 nm and the fluorescence quantum yields were 0.3–0.8. The large difference (more than 40 nm) between the fluorescence maxima of compounds **10c** and **10f** in the presence of double- and single-stranded DNA (see Table 2) suggests the possible use of the two dyes for distinguishing between ds DNA and ss DNA in solution. The detection limit of ds DNA with compound **10f** is less than 20 ng. Investigations on the fluorescence properties of these dyes, in the presence of different types of DNA and RNA, as well as studies on the mechanism of their interaction with nucleic acids, are in progress.

Table 1
Characterisation data for dyes **10a–f**

Dye	Yield % [reaction time (h)]	m.p. (°C)	λ_{max} (nm) ε (l mol^{-1} cm^{-1})	Molecular formula	Analysis (%) found/calc. C	H	N
10a	53 (3)	278–280	506 (129 600)	$C_{48}H_{52}I_4N_6S_2$	–	–	6.8
							6.5
10b	93 (2)	220–222	506 (162 300)	$C_{50}H_{56}I_4N_6S_2$	5.9	94.4	97.0
					45.7	4.3	96.4
10c	54 (1.5)	204–206	505 (151 100)	$C_{51}H_{62}I_4N_6S_2$	45.8	4.7	6.5
					46.0	4.7	6.3
10d	54 (2)	215–217	506 (179 400)	$C_{54}H_{52}I_4N_6S_2.3\ CH_3OH$	46.7	4.3	5.7
					47.1	4.4	5.8
10e	59 (1)	266–268	505 (170 100)	$C_{52}H_{64}I_4N_6S_2.CH_3OH$	46.5	5.1	6.0
					96.2	4.9	6.1
10f	60 (0.25)	222–223	505 (169 000)	$C_{54}H_{68}I_4N_6S_2.5\ CH_3OH$	46.0	5.6	5.8
					46.2	5.7	5.5

Table 2
Fluorescence characteristics of the dyes in the presence of nucleic acids[a]

Dye	ds DNA		ss DNA	
	λ_F (nm)	Q_F	λ_F (nm)	Q_F
10b	529.2	0.69	575.6	0.42
10c	530.4	0.94	568.0	0.80
10d	531.2	–	582.0	–
10e	532.4	0.48	644.4	0.31
10f	529.0	0.94	567.0	0.88

[a] λ_F, position of the fluorescence maximum in nanometers; Q_F, fluorescence quantum yield; (–), no measurement; the numbers of the dyes correspond to those given in the text; the fluorescence characteristics of dye **10a** were not measured.

3. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. The ^{1}H NMR spectra of the dyes were obtained on a Bruker MSL 300 MHz in DMSO-d_6. The absorption spectra were recorded on a Perkin–Elmer Lambda 17 UV/VIS spectrophotometer (1×10^{-5} l mol^{-1} cm^{-1} in methanol). Fluorescence spectra (excitation at 480 nm) were scanned on a Perkin–Elmer MPF44 spectrofluorimeter. The fluorescence quantum yield (Q_F) was determined relative to that of dye TO ($Q_F = 0.2$) [2]. Stock solutions were prepared by dissolving 1 mg of the dye in 1 ml DMSO and subsequent dilution with TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 7.0) to a final concentration of $0.5.10^{-5}$ mol/l. Salmon sperm DNA (native — d_s, and denaturated — ss) was purchased from Sigma (USA).

3.1. Preparation of 1-(ω-bromopropyl)-4-methylquinolinim bromide **7a** *and 1-(ω-bromobutyl)-4-methylquinolinim bromide* **7b** *[13]*

Lepidine and 1,3-dibromopropane or 1,4-dibromobutane in molar ratio 1:4 were left for 24–72 h in the dark at room temperature. The resultant precipitate was filtered, washed with ether and air dried. **7a**: 68%, yield, m.p. 136–140°C, literature m.p. 139–141 [13]; **7b**: 50%, yield, m.p. 148–152°C, literature m.p. 150–152 [13].

*3.2. Preparation of 1-(3-bromopropyl)-4-[(3-methyl-2(3*H*)benzothiazolilyden)methyl] quino-linium iodide* **9a** *and 1-(3-bromobutyl)-4-[(3-methyl-2 (3*H*)benzothiazolilyden) methyl] qunolinium iodide* **9b**

0.01 mol **7a or 7b** and 0.01 mol anhydro 3-methyl-2-sulfobenzothiazolium hydroxide **8** were mixed and finely ground in a mortar. The reaction mixture was transferred into a flask and melted. The reaction mixture was then stirred for several minutes until evolution of sulphur dioxide ceased. The solidified melt was cooled to 70–80°C and sufficient methanol was added to dissolve the dye. The hot solution was filtered and the dye was precipitated by the addition of potassium iodide and cooling. The dyes were recrystallized from methanol. **9a**: 56%, yield, m.p. 191–201°C, literature m.p. 204–206 [6]; **9b**: 56%, yield, m.p. 230–235°C, literature m.p. 235–237 [6].

3.3. Preparation of homodimeric monomethine cyanine dyes **10a–f**

0.002 mol **9a or 9b** and 0.001 mol of *N,N,N′,N′*-tetramethyl-1,3-propanediamine, *N,N,N′,N′*-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo[2,2,2] octane or 1,4′-bipyridine were refluxed in 5–9 ml 2-methoxyethanol for 15 min to 3 h. After cooling the precipitated dye was filtered and dried. The dye was dissolved in hot methanol and an excess of aqueous potassium iodide solution was added to the hot solution. The solution was cooled and the precipitated dye was filtered and dried. The dyes were recrystallized from methanol. Some data for dyes **10a–f** are given in Table 1.

3.3.1. NMR spectra of dyes **10b–f**

3.3.1.1. Dye **10b**. 7.39–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2×CH); 4.65 (br s, 4H, 2×N–CH_2); 4.04 (s, 6H, 2×N^+–CH_3); 3.04–3.43 (m, 16H, CH_2N ($CH_2CH_2)_3$ N^+CH_2); 1.86 (br s, 8H, 2×CH_2–CH_2).

3.3.1.2. Dye **10c**. 7.28–8.78 (m, 20H, Ar); 6.88 (s, 2H, 2×CH); 4.66 (br s, 4H, 2×N–CH_2); 4.00 (s, 6H, 2×N^+–CH_3); 3.18–3.48 (m, 8H, $N^+(CH_2)_2$); 3.13 (s, 12H, 2×$N^+(CH_3)_2$); 2.32 (br s, 2H, CH_2); 1.88 (br s, 8H, 2×CH_2–CH_2).

3.3.1.3. *Dye* **10d**. 7.36–9.40 (m, 28H, Ar); 6.97 (s, 2H, 2×CH); 4.76 (m, 4H, 2×bipyrN–CH_2); 4.68 (m, 4H 2×N–CH_2); 4.04 (s, 6H, 2×N^+–CH_3); 2.14 (br s, 4H, 2×CH_2); 1.96 (br s, 4H, 2×CH_2).

3.3.1.4. *Dye* **10e**. 7.37–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2×CH); 4.65 (br s, 4H, 2×N–CH_2); 4.05 (s, 6H, 2×N^+–CH_3); 3.34–3.57 (m, 8H, $N^+(CH_2)_2$); 3.12 (s, 12H, 2×$N^+CH_3)_2$); 2.32 (br s, 4H, 2×CH_2); 1.75 (br s, 4H, 2×CH_2); 1.33 (br s, 4H, 2×CH_2).

3.3.1.5. *Dye* **10f**. 7.26–8.77 (m, 20H, Ar); 6.86 (s, 2H, 2×CH); 4.65 (br s, 4H, 2×N–CH_2); 3.99 (s, 6H, 2×N^+–CH_3); 3.29–3.40 (m, 8H, $N^+(CH_2)_2$); 3.07 (s, 12H, 2×$N^+(CH_3)_2$); 1.84 (br s, 8H, 2×CH_2–CH_2); 1.77 (br s, 4H, 2×CH_2); 1.39 (br s, 4H, 2×CH_2).

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