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Synthesis and properties of YOYO-1-type homodimeric monomethine cyanine dyes as noncovalent nucleic acid labels

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In memory of Professor Masaru Matsuoka

Abstract

Several novel homodimeric asymmetric monomethine cyanine dyes based on the oxazole yellow (YO) chromophore were synthesized. The two YO chromophores [1-(ω-bromoalkyl)-4-[(3-methyl-2-(3H)-benzaxozolilydene)methyl]-quinolinium perchlorate with different chain lengths of the ω-bromoalkyl substituent were bisquaternized with N,N,N′,N′-tetramethyl-1,3-propanediamine, N,N,N′,N′-tetramethyl-1,6-hexanediamine, 1,4-diazabi-cyclo[2,2,2]octan and bipyridine.The homodimeric dyes have molar absorptivity 140,000–180,0000 l.mol^{-1}cm^{-1} at 479 nm upon binding to ds DNA. The fluorescence maxima of the complexes are around 505 nm and the fluorescence quantum yields (Q_F) are between 0.2 and 0.7

Keywords: Monomethine cyanines; Homodimers; Absorption spectra; Fluorescence; Noncovalent nucleic acid labels

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1. Introduction

The applications of polymethine dyes as noncovalent labels for nucleic acid detection and quantitation in science and clinical analysis have increased in recent years [1–3]. Among the homodimeric monomethine cyanines with four positive charges, highly sensitive DNA stains stable under electrophoretic conditions were found [4]. The commercially available [2] homodimers TOTO-1 N,N,N′,N′-tetramethyl-N,N′-bis{[4-[(3-methyl-2-(3*H*)-benzothiazolilydene)methyl]-quinolinium-1-yl]-1,3-propanediyl}-1,3-propandiammonium tetraiodide and YOYO-1 N,N, N′,N′-tetramethyl-N,N′-bis{3-[4-[(3-methyl-2-(3*H*)-benzoxazolilydene)-methyl]-quinolinium-1-yl]-1,3-propanediyl} - 1,3 - propanediammonium tetraiodide form highly fluorescent complexes with ds DNA. Recently we synthesized [5] homodimeric dyes of TOTO-1 family with extended methylene bridges between the quinolinium ring and the quaternary ammonium nitrogen and showed [6] that such dyes have higher fluorescent quantum yield upon

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binding with nucleic acid, than the commercially available TOTO-1 homodimeric dye.

In this study we describe a synthetic pathway to novel homodimeric asymmetric monomethine cyanines similar to YOYO-1 and report their absorption and report fluorescence spectral characteristics.

2. Results and discussion

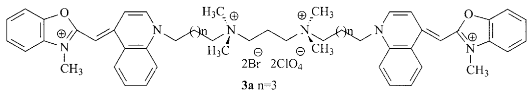
TOTO-1 and YOYO-1 have been synthesized by reacting of 1-(3-iodopropyl)-4-[(3-methyl-2-(3*H*)-benzothiazolilydene)methyl]quinolinium iodide or 1-(3-iodopropyl)-4-[(3-methyl-2-(3*H*)-benzoxazolilydene)methyl]quinolinium iodide with N,N,N′,N′-tetramethyl-1,3-propanediamine [4]. We have used the same pathway (Scheme 1). The starting benzoxazolic monomethine cyanine dyes **1a–d** were synthesized by the method of Brooker at al. [7] or by method used in our previous work [5].

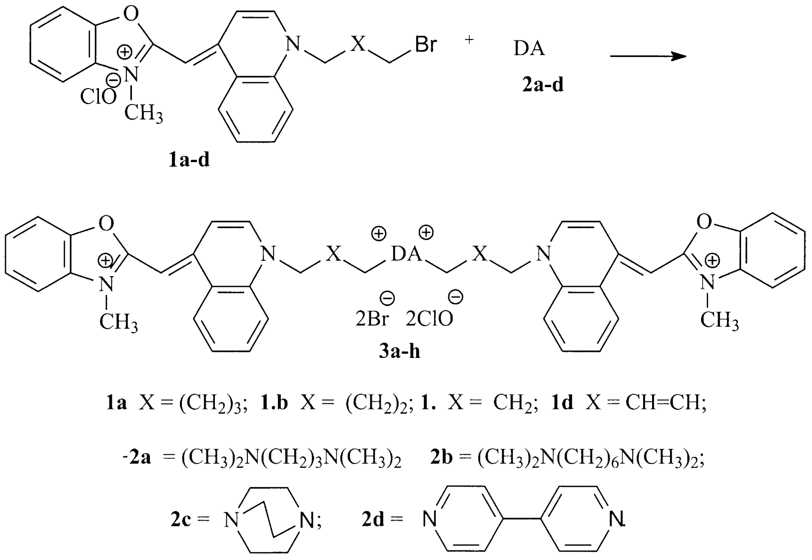
The benzoxazolic monomethine cyanines **1a–d** were bisquaternized with N,N,N′,N′-tetramethyl-1,3-propanediamine, N,N,N′,N′-tetramethyl-1, 6-hexandiamine, 1,4-diazabicyclo[2,2,2]octan and 1,4-bipyridine. Quaternization was carried out by refluxing the reaction components in methoxyethanol for 1–5 h giving homodimeric dyes **3a–h**. All dyes are new and some characterization data for the homodimeric dyes **3a–h** are given in Table 1.

The longest wavelength absorption maxima of the studied benzoxazolic dyes are in the region 478–485 nm. The corresponding molar absorptivities are between 140,000 and 180,000 l mol^{-1} cm^{-1}. The dyes have no fluorescence on their own but become strongly fluorescent after binding to ds DNA. The fluorescence maxima of the complexes are around 505 nm and the fluorescence quantum yields (Q_F) are between 0.3 and 0.7 except for **3f** (Table 2). More detailed studies on the photophysical properties of the novel homodimeric dyes **3a–h** in the presence of nucleic acids are in progress.

The following homodimeric monomethine cyanines were synthesized:

3a: N, N,N′, N′-tetramethyl-N, N′-bis-{[4-[(3-methyl-2(3*H*)-benzoxazolilydene)methyl]quinolinium-1-yl]-1,5-pentanediyl}-1,3-propanediammonium dibromide diperchlorat.



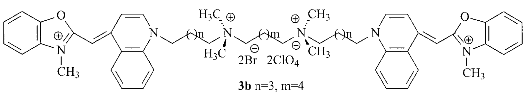


1a X = (CH$_2$)$_3$; **1.b** X = (CH$_2$)$_2$; **1.** X = CH$_2$; **1d** X = CH=CH;

-2a = (CH$_3$)$_2$N(CH$_2$)$_3$N(CH$_3$)$_2$ **2b** = (CH$_3$)$_2$N(CH$_2$)$_6$N(CH$_3$)$_2$;

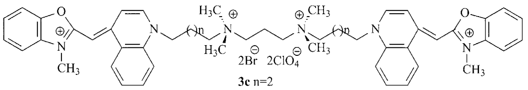
Scheme 1.

3b: N, N,N′, N′-tetramethyl-N, N′-bis-{[4-[(3-methyl-2(3*H*)-benzoxazolilydene)-methyl]quinolinium-1-yl]-1,5-pentanediyl}-1,6-hexanediammonium dibromide diperchlorat.



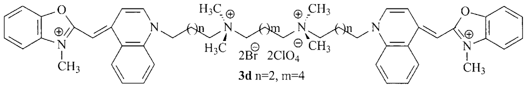
3b n=3, m=4

3c: N, N,N′, N′-tetramethyl-N, N′-bis-{[4-[(3-methyl-2(3*H*)-benzoxazolilydene)-methyl]quinolinium-1-yl]-1,4-butanediyl}-1,3-propanediammonium dibromide diperchlorat.



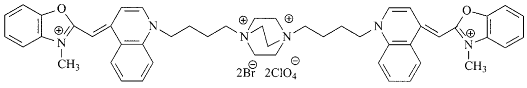
3c n=2

3d: N, N,N′, N′-tetramethyl-N, N′-bis-{[4-[(3-methyl-2(3*H*)-benzoxazolilydene)-methyl]quinolinium-1-yl]-1,4-butanediyl}-1,6-hexanediammonium dibromide diperchlorat.



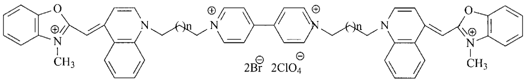
3d n=2, m=4

3e: N, N′-bis-{4[4-[(3-methyl-2(3*H*)-benzoxazolilydene)methyl]quinolinium-1-yl]-1,4-butanediyl}-1,4-diazoniabicyclo[2,2,2]octane dibromide diperchlorat.



3e

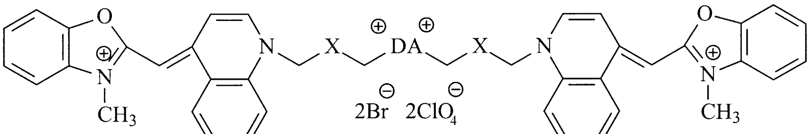
3f: N, N′-bis-{[4-[(3-methyl-2(3*H*)-benzoxazolilydene)methyl]quinolinium-1-yl]-1,4-butanediyl}-1,4′-bipyridinium dibromide diperchlorat.



3f n=2

3g: N, N,N′, N′-tetramethyl-N, N′-bis-{3-[4-[(3-methyl-2(3*H*)-benzoxazolilydene)-methyl]quinoli-

Table 1
Characterization data for homodimeric dyes **3a–h**



3a-h

Dye	X	DA	m.p. (°C)	Yield (%)	λ_{max} nm[a] (ε 1 mol^{-1}cm^{-1})	Molecular formulae	Analysis $\frac{\text{Found}}{\text{Calc.}}$ C	H	N
3a	$(CH_2)_3$	$(CH_3)_2N(CH_2)_3N(CH_3)_2$	182–184	76	481(462sh) (138,900)	$C_{53}H_{66}Br_2Cl_2N_6O_{10}\cdot 2H_2O$	52.0 52.4	5.7 5.8	7.05 6.9
3b	$(CH_2)_3$	$(CH_3)_2N(CH_2)_6N(CH_3)_2$	256–258	69	480 (164,300)	$C_{56}H_{72}Br_2Cl_2N_6O_{10}\cdot 2H_2O$	53.55 53.55	5.8 6.05	6.6 6.7
3c	$(CH_2)_2$	$(CH_3)_2N(CH_2)_3N(CH_3)_2$	180–182	53	478(438sh) (133,000)	$C_{51}H_{62}Br_2Cl_2N_6O_{10}\cdot H_2O$	52.0 52.4	5.65 5.5	6.8 7.2
3d	$(CH_2)_2$	$(CH_3)_2N(CH_2)_6N(CH_3)_2$	228–231	64	482(458sh) (166,700)	$C_{54}H_{56}Br_2Cl_2N_6O_{10}$	54.2 54.4	5.7 5.7	6.9 7.05
3e	$(CH_2)_2$	$N(CH_2CH_2)_3N$	236–238	49	480 (186,300)	$C_{50}H_{66}Br_2Cl_2N_6O_{10}$	52.8 53.05	5.6 4.95	7.5 7.4
3f	$(CH_2)_2$	$NC_5H_4—C_5H_4N$	268–270	42	481 (162,900)	$C_{54}H_{52}Br_2Cl_2N_6O_{10}\cdot H_2O$	54.3 54.3	4.6 4.5	7.2 7.0
3g	CH_2	$(CH_3)_2N(CH_2)_6N(CH_3)_2$	128–131	70	480 (148,100)	$C_{52}H_{64}Br_2Cl_2N_6O_{10}\cdot H_2O$	52.7 52.8	5.9 5.6	6.8 7.1
3h	CH=CH	$(CH_3)_2N(CH_2)_3N(CH_3)_2$	214–217	82	485 (140,200)	$C_{51}H_{58}Br_2Cl_2N_6O_{10}\cdot 4H_2O$	50.2 50.3	4.9 5.4	6.7 6.9

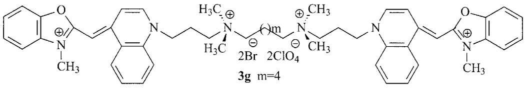
[a] Solvent CH_3CN, except in the case of **3e** and **3h**, which were measured in MeOH.

Table 2
Experimental spectral characteristics of the investigated dyes in presence of ds DNA

Dye	With ds DNA[a]		
	λ_{abs} (nm)	λ_{fl} (nm)	Q_F
3a	497.2	508.6	0.43
3b	497.0	504.8	0.65
3c	496.1	509.0	0.32
3d	498.2	506.0	0.52
3e	440.5, 480 sh	507.4	0.17
3f	499.2	504.0	0.005
3g	495.1	506.2	0.54
3h	463.8, 490 sh	512.2	0.30

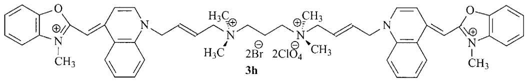
[a] The concentration of ds DNA is 10 μg/ml.

nium-1-yl]-1,3-propanediyl}-1,6-hexanediammonium dibromide diperchlorat.



3g m=4

3h: N, N,N′, N′-tetramethyl-N, N′-bis-{4-[4-[(3-methyl-2(3*H*)-benzoxazolilydene)-methyl]quinolinium-1-yl]-1,4-but-2-enediyl}-1,3-propanediammonium dibromide diperchlorat.



3h

3. Experimental

3.1. General

Melting points were determined on a Koffler apparatus and are uncorrected. Absorption spectra were scanned on a Specord M40 (Carl Zeiss, Jena) UV–Vis spectrophotometer (1×10^{-5} M/l in CH_3CN or C_2H_5OH for **3e**,**h**) 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 Rhodamin B. The fluorescence quantum yield (Q_f) was determined relative to that of Rhodamin 6G ($Q_f = 0.95$ in Ethanol) [8].

Stock solutions were prepared by dissolving 1mM of each dye in 1 ml DMSO and subsequent dilution with TE buffer (10 mM Tris-HCl, pH 7.0, 1mM EDTA) to a final concentration of 1×10^{-6} M. The fish sperm dsDNA was purchased from Sigma (USA).

3.2. General procedure for the preparation of dimers

The monomethine cyanine dyes **1a–d** 2 mM were dissolved in 10–20 ml methoxyethanol with stirring and heating. The diamine 1 mM was then added and the reaction mixture was refluxed for 1–5 h (TLC control). The flask contents were then cooled down to room temperature or in some cases left overnight in refrigerator.

The resultant precipitate was filtered off by suction, washed with ethanol/ether 1:1 and air-dried. Characterization data for dyes **3a**–**h** are shown in Table 1.

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