Available online at www.sciencedirect.com
SCIENCE DIRECT
Dyes and Pigments 66 (2005) 135–142
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Synthesis of novel monomeric and homodimeric cyanine dyes based on oxazolo[4,5-*b*]pyridinium and quinolinium end groups for nucleic acid detection

Aleksey Vasilev[a], Todor Deligeorgiev[a,*], Nikolai Gadjev[a], Karl-Heinz Drexhage[b]

[a]*Faculty of Chemistry, University of Sofia, 1164 Sofia, Bulgaria*
[b]*Department of Chemistry, University of Siegen, D-57068 Siegen, Germany*

Received 5 August 2004; received in revised form 10 September 2004; accepted 23 September 2004
Available online 24 November 2004

Abstract

Twelve novel di-, tri- and tetracationic monomeric and homodimeric monomethine cyanine dyes based on oxazolo [4,5-*b*]pyridinium and quinolinium end groups were synthesized by condensation of 4-methyl-2-methylmercapto-oxazolo [4,5-*b*]pyridinium methosulfate and 1-(ω-iodopropyl)-4-methylquinolinium or appropriate 1-(ω-bromoalkyl)-4-methylquinolinium compounds and subsequent quaternization with pyridine, 1-methyl-4-aza-1-azonia-bicyclo[2.2.2]octanium iodide, 4,4′-trimethylenebis(1-methylpiperidinium)methyl iodide, *N,N,N,N′,N′*-pentamethyl-1,3-propandiammonium iodide, or bisquaternization with *N,N,N′,N′*-tetramethyl-1,3-propanediamine, 4,4′-trimethylenebis(1-methylpiperidine). All dyes absorb at 521–522 nm in methanol and have a high molar absorptivity of about 106 000 (monomeric dyes) and 240 000 L mol^{-1} cm^{-1} (homodimeric dyes). The products were characterized by ^1H NMR spectra and elemental analysis. In the presence of nucleic acid a strong enhancement of the fluorescence was observed.

Keywords: Monomeric; Homodimeric cationic monomethine cyanine dye; Oxazolo[4,5-*b*]pyridinium; Nucleic acid; Fluorescence

1. Introduction

In recent years there has been a growing scientific and commercial interest in the synthesis and application of cyanine dyes that are suitable as non-covalently binding nucleic acids labels [1–7]. Our investigations in this area [8–11] have prompted us to search for new intermediates and dyes. Recently we synthesized [9] homodimeric dyes of the TOTO-1 family with extended methylene bridges between the two chromophores and showed [12] that such dyes have higher fluorescent quantum yield upon binding with nucleic acid than the commercially available dye TOTO-1. In another study [13] we described a synthetic pathway to novel homodimeric asymmetric monomethine cyanines related to YOYO-1. These dyes have extended methylene bridges between the two chromophores and show interesting absorption and fluorescence characteristics.

Several unsymmetrical monomeric and homodimeric cyanine dyes that incorporate an aza-benzazolium moiety (including the oxazolo[4,5-*b*]pyridine heterocycle) were described by Haugland and Yue [14]. These dyes are non-fluorescent in aqueous solution, but exhibit bright fluorescence when associated with DNA or RNA. These useful and interesting results provoked us to search for novel representatives of this dye class and to investigate their interaction with nucleic acids.

* Corresponding author. Tel.: +359 2 8161269; fax: +359 2 9625438.
E-mail address: toddel@chem.uni-sofia.bg (T. Deligeorgiev).

0143-7208/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2004.09.019

2. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Bruker 250 MHz instrument in DMSO-d_6. Absorption spectra were scanned on a Unicam 530 UV–VIS spectrophotometer (1×10^{-5} mol L^{-1} in MeOH) and the corrected fluorescent spectrum (excitation at 480 nm) on a Perkin Elmer MPF44 spectrofluorimeter. The fluorescence quantum yield was determined relative to that of Rhodamine 6G ($Q_f = 0.95$ in ethanol) [18]. Lepidine, pyridine, 1,3-diiodopropane, 1,3-, 1,4-, 1,5-dibromoalkanes, *N,N,N′,N′*-tetramethyl-1,3-propandiamine (TMPDA), 4,4′- trimethylenebis(1-methylpiperidine) (TMBMP) and 1,4-diazabicyclo[2.2.2]octane (DABCO) are commercial products.

2.1. Preparation of quaternary ammonium salts *3e*, *3f* and *3g*

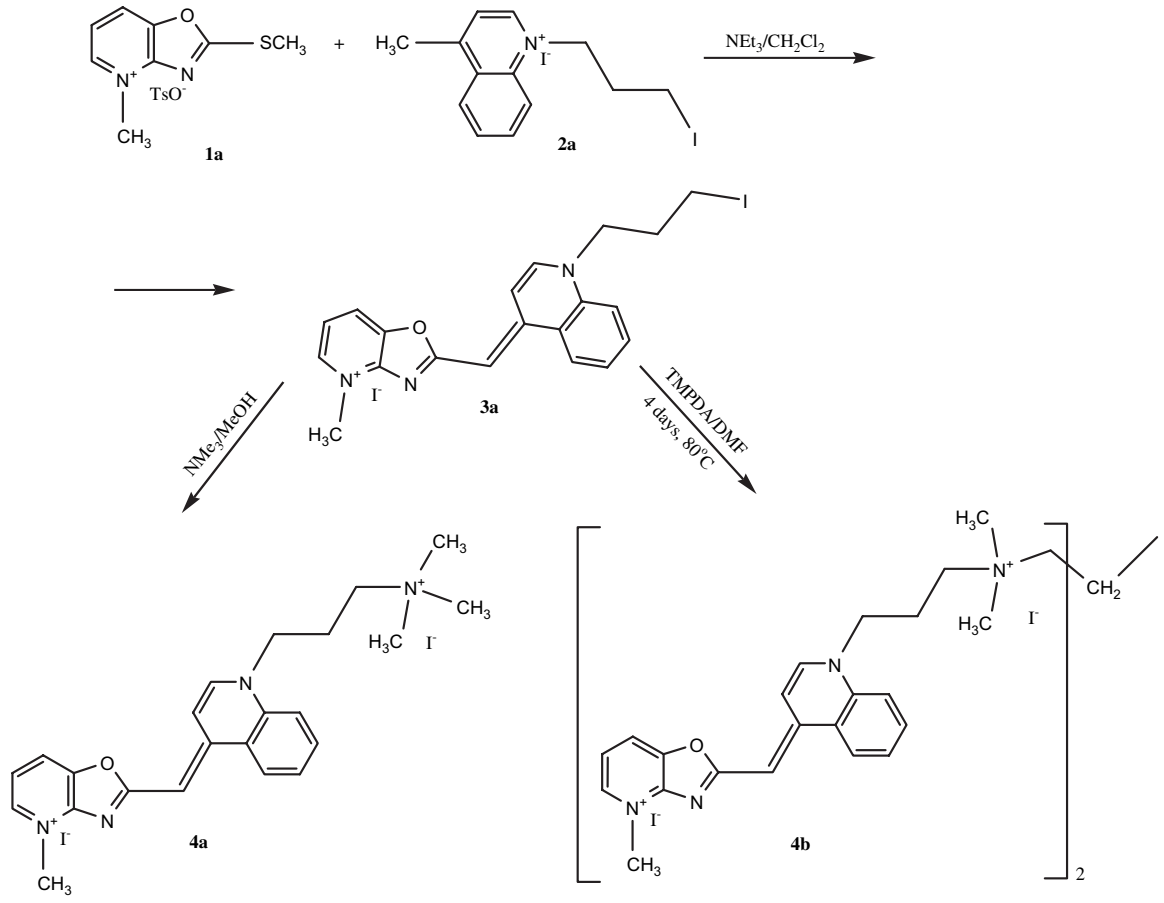
0.01 mol TMPDA, TMBMP or DABCO was added to 50 ml diethyl ether and the solution was cooled to 10 °C. 0.01 mol methyl iodide dissolved in 10 ml diethyl ether was added dropwise during 10–15 min. Thirty minutes after addition, the white precipitate was suction filtered, washed with ether and dried in a desiccator. The yields were quantitative.

2.2. Preparation of dyes *5a–5l*

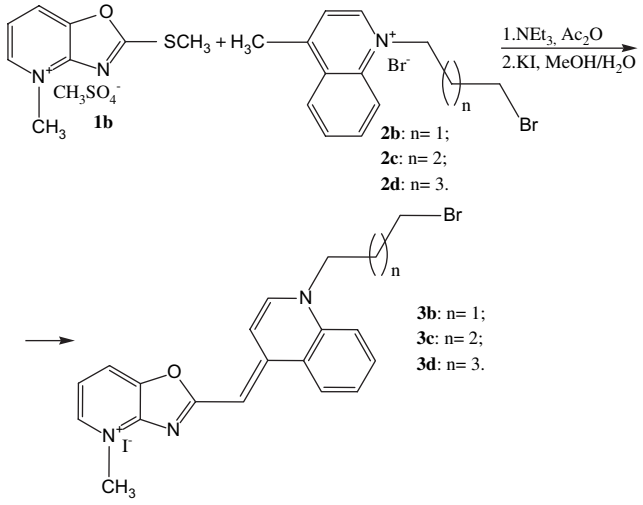
0.001 mol **3a**, **3b**, **3c** or **3d**, 10 ml methoxyethanol and 0.001 mol of the appropriate ammonium compound (or 0.0005 mol of TMPDA or TMBMP for the bisquaternization of compound **3c**) were vigorously stirred and refluxed for 6 h. The reaction mixture was cooled to room temperature and 30 ml diethyl ether was added. The precipitated dye was suction filtered, washed with ether and air dried. The formed bromide of the dye was dissolved in 15 ml methanol and 0.002 mol KI (or 0.004 mol KI for the bisquaternized dyes **5k** and **5l**) in 2 ml water was added. The mixture was kept at 5 °C in a refrigerator for several days. The resulting precipitate was suction filtered and air-dried. Dyes **5a–5l** were recrystallized from ethanol.

3. Results and discussion

Haugland et al. [14] have synthesized the now commercially available dyes JO-PRO-1 **4a** and JOJO-1



Scheme 1.



Scheme 2.

4b by first reacting 4-methyl-2-methylthiooxazolo[4,5-*b*]pyridinium tosylate **1a** with 1-(3-iodopropyl)-4-methylquinolinium iodide **2a** in methylene chloride in the presence of triethylamine (Scheme 1) to obtain dye **3a**. Then this compound was reacted in methanol solution with trimethylamine to yield **4a**. Likewise TMPDA was bisquaternized with **3a** in DMF to afford **4b**. In our synthesis we followed a similar route.

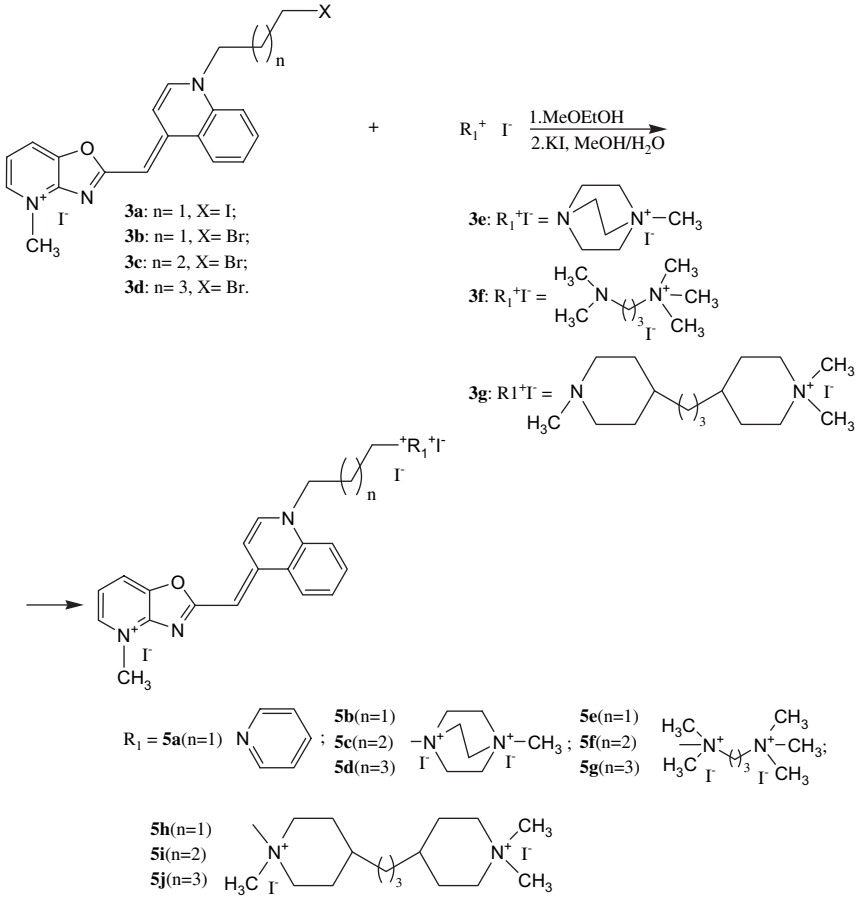
The intermediate 1-(3-iodopropyl)-4-methylquinolinium iodide **2a** was synthesized using a published procedure [16].

The quaternized lepidines 1-(3-bromopropyl)-4-methylquinolinium bromide **2b**, 1-(4-bromobutyl)-4-methylquinolinium bromide **2c** and 1-(5-bromopentyl)-4-methylquinolinium bromide **2d** were prepared by room temperature reaction of lepidine with the appropriate dibromoalkanes in molar ratio 1:4 [17].

The monomethine cyanine dyes **3a**, **3b**, **3c** and **3d** were synthesized by the method of Brooker et al. [16] (Scheme 2).

The monomethylated ammonium compounds **3e**, **3f** and **3g** were prepared by the reaction of DABCO, TMPDA and TMBMP with methyl iodide in diethyl ether and were used without purification.

4-methyl-2-methylthiooxazolo[4,5-*b*]pyridinium methosulfate **1b** was prepared from 2-mercapto-oxazolo [4,5-*b*]pyridine [15] by heating with two equivalents of dimethylsulfate [14].



Scheme 3.

Table 1
Structures and starting compounds for dyes **5a–5l**

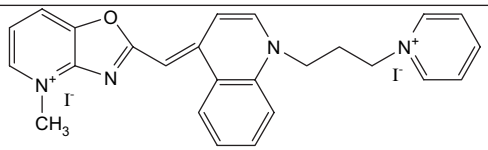
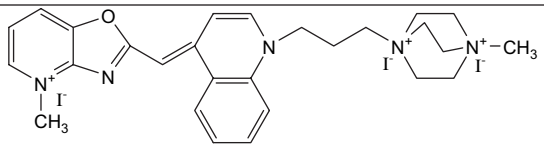
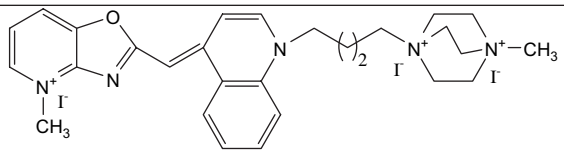
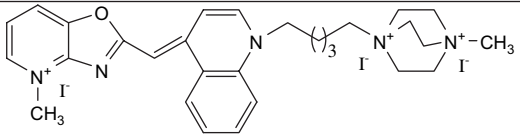
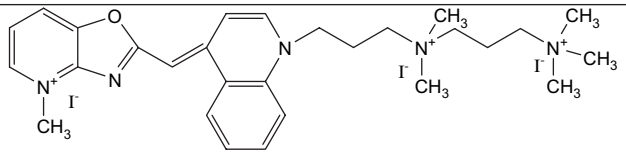
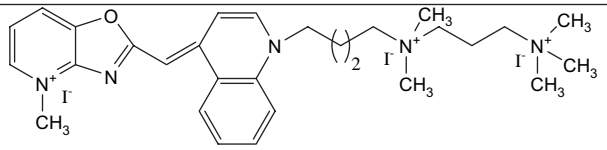
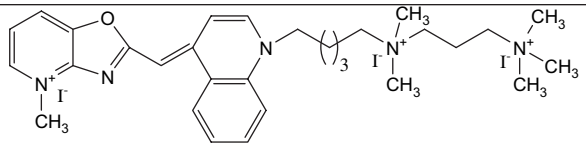
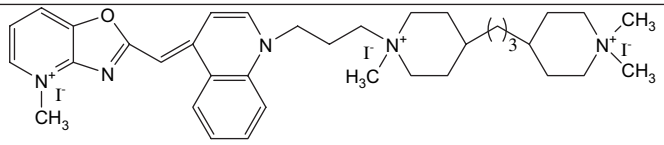
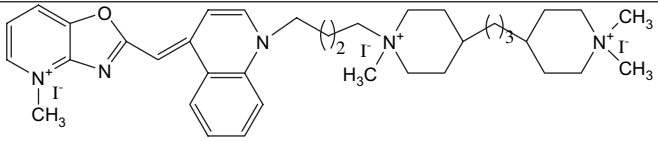
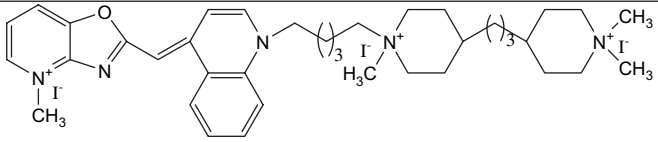
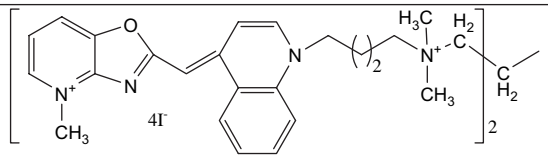
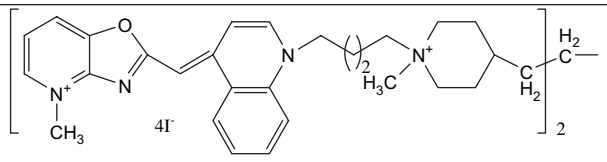
Dye no	Formula/Name	Starting compounds
5a	 1-[3-(*N*-pyridinio)propyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium diodide	**3a**, **pyridine**
5b	 1-[3-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))propyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3b, 3e**
5c	 1-[4-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))butyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3c, 3e**
5d	 1-[5-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))pentyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3d, 3e**
5e	 1-[3-(*N*-(1,3-pentamethylpropanediamonium))propyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3b, 3f**

Table 1 (*continued*)

Dye no	Formula/Name	Starting compounds
5f	 1-[4-(*N*-(1,3-pentamethylpropanediamonium))butyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3c, 3f**
5g	 1-[5-(*N*-(1,3-pentamethylpropanediamonium))pentyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3d, 3f**
5h	 1-[3-(*N*-(1-methyl-4-[3-((1,1-dimethylpiperidinium)-4-yl)propyl]piperidinium))propyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3b, 3g**
5i	 1-[4-(*N*-(1-methyl-4-[3-((1,1-dimethylpiperidinium)-4-yl)propyl]piperidinium))butyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3c, 3g**
5j	 1-[5-(*N*-(1-methyl-4-[3-((1,1-dimethylpiperidinium)-4-yl)propyl]piperidinium))pentyl]-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium triiodide	**3d, 3g**

(*continued on next page*)

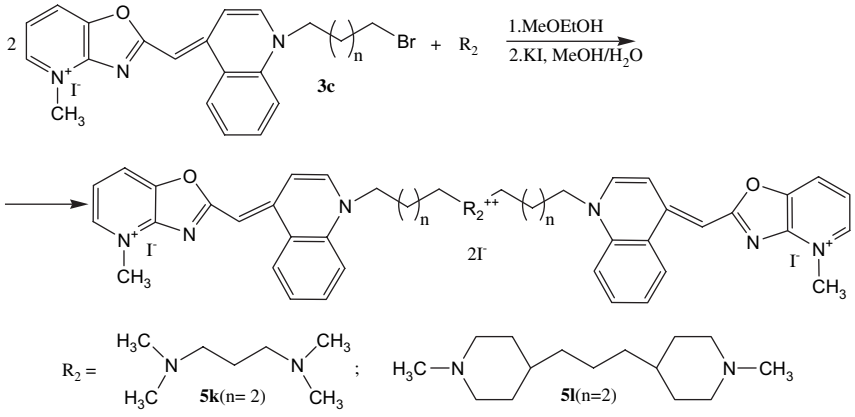
Table 1 (*continued*)

Dye no	Formula/Name	Starting compounds
5k	 *N*,*N*,*N*',*N*'-tetramethyl-*N*,*N*'-bis-{[4-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium-1-yl]-1,4-butanediyl}-1,3-propanediamonium tetraiodide	**3c, TMPDA**
5l	 *N*,*N*'-dimethyl-*N*,*N*'-bis-{[4-[(4-methyl-4*H*-oxazolo[4,5-*b*]pyridine-2-ylidene)methyl]quinolinium-1-yl]-1,4-butanediyl}-4,4'-trimethylenebis(1-methylpiperidinium) tetraiodide	**3c, TMBMP**

Dicationic dye **5a** was synthesized from monomethine cyanine dye **3a** with pyridine in methoxyethanol. Tricationic dyes **5b**–**5j** were prepared in similar way from the appropriate dyes **3b**, **3c** and **3d** and compounds **3e**, **3f** and **3g** (Scheme 3 and Table 1).

The monomethine cyanine dye **3c** was reacted with TMPDA or TMBMP to give tetracationic homodimeric monomethine cyanine dyes **5k** and **5l**. Bisquaternization was carried out by refluxing the reaction mixture in methoxyethanol (Scheme 4 and Table 1). This resulted in a shorter reaction time (6 h) in comparison with the experiment reported earlier [14] (4 days). All dyes are new and their chemical structures were unequivocally proven by ^{1}H NMR spectra (Table 2) and elemental analysis (Table 3). Their UV–VIS spectral data are also given in Table 3.

The longest wavelength absorption maxima of the studied dyes are in the region 521–522 nm. The corresponding molar absorptivities are very high between 106 000 and 240 000 L mol^{-1} cm^{-1}. The dyes are



Scheme 4.

Table 2
^{1}H NMR data of dyes **5a–5l**

Dye no.	^{1}H NMR (δ, ppm, DMSO-d_6)
5a	2.49–2.52 m (2H, CH_2); 4.22 s (3H, N^+CH_3); 4.62 t (2H, N^+CH_2); 4.80 t (2H, N^+CH_2); 6.54 s (1H, CH); 7.35–9.12 m (14H, Ar)
5b	2.06–2.1 m (2H, CH_2); 3.28 s (3H, N^+CH_3); 3.71 t (2H, N^+CH_2); 3.85 br s (12H, N^+CH_2); 4.23 s (3H, N^+CH_3); 4.55 t (2H, N^+CH_2); 6.57 s (1H, CH); 7.37–8.06 m (9H, Ar)
5c	1.85 brs (4H, 2× CH_2); 3.29 s (3H, N^+CH_3); 3.58 brs (2H, N^+CH_2); 3.88 brs (12H, N^+CH_2); 4.22 s (3H, N^+CH_3); 4.57 brs (2H, N^+CH_2); 6.54 s (1H, CH); 7.34–8.59 m (9H, Ar)
5d	1.33–1.37 m (2H, CH_2); 1.77–1.88 m (4H, 2× CH_2); 3.28 s (3H, N^+CH_3); 3.50 t (2H, N^+CH_2); 3.87 br s (12H, N^+CH_2); 4.22 s (3H, N^+CH_3); 4.54 t (2H, N^+CH_2); 6.53 s (1H, CH); 7.33–8.58 m (9H, Ar)
5e	2.24–2.26 m (4H, CH_2); 3.10 s (6H, 2× N^+CH_3); 3.13 s (9H, 3× N^+CH_3); 3.28 t (2H, CH_2); 3.44 br s (4H, 2× N^+CH_2); 3.55 t (2H, N^+CH_2); 4.23 s (3H, N^+CH_3); 4.56 t (2H, N^+CH_2); 6.57 s (1H, CH); 7.37–8.60 m (9H, Ar)
5f	1.87 brs (4H, 2× CH_2); 2.21–2.23 m (2H, CH_2); 3.09 (6H, 2× N^+CH_3); 3.13 s (9H, 3× N^+CH_3); 3.28–3.41 m (6H, 3× N^+CH_2); 4.22 s (3H, N^+CH_3); 4.57 brs (2H, N^+CH_2); 6.54 s (1H, CH); 7.34–8.58 m (9H, Ar)
5g	1.32–1.38 m (2H, CH_2); 1.75–1.90 m (4H, CH_2); 2.14–2.17 m (2H, CH_2); 3.05 s (6H, 3× N^+CH_3); 3.11 s (9H, 2× N^+CH_3); 3.23–3.37 m (6H, 2× N^+CH_2); 4.22 s (3H, N^+CH_3); 4.54 t (2H, N^+CH_2); 6.53 s (1H, CH); 7.33–8.58 m (9H, Ar)
5h	1.23 brs (6H, CH_2); 1.52–1.63 m (10H, CH_2 + CH); 2.22–2.25 m (2H, CH_2); 1.79 m (2H, CH_2); 3.00 s (6H, N^+CH_3); 3.11 s (3H, N^+CH_3); 3.33–3.49 m (10H, N^+CH_2); 4.23 s (3H, N^+CH_3); 4.56 t (2H, N^+CH_2); 6.36 s (1H, CH); 7.36–8.60 m (9H, Ar)
5i	1.28 brs (6H, CH_2); 1.51–1.86 m (14H, CH_2 + CH); 3.03 s (6H, N^+CH_3); 3.10 s (3H, N^+CH_3); 3.39 m (10H, N^+CH_2); 4.22 s (3H, N^+CH_3); 4.55 t (2H, N^+CH_2); 6.54 s (1H, CH); 7.33–8.58 m (9H, Ar)
5j	1.26 brs (6H, CH_2); 1.36–1.89 m (16H, CH_2 + CH); 2.99 s (6H, N^+CH_3); 3.09 s (3H, N^+CH_3); 3.26–3.43 m (10H, N^+CH_2); 4.22 s (3H, N^+CH_3); 4.53 t (2H, N^+CH_2); 6.54 s (1H, CH); 7.33–8.56 m (9H, Ar)
5k	1.89 brs (8H, CH_2); 2.23 m (2H, CH_2); 3.12 s (12H, N^+CH_3); 3.37 t (4H, N^+CH_2); 3.48 t (4H, N^+CH_2); 4.19 s (6H, N^+CH_3); 4.56 brs (4H, N^+CH_2); 6.54 s (2H, 2× CH); 7.30–8.52 m (18H, Ar)
5l	1.29 brs (6H, CH_2); 1.55–2.49 m (18H, CH_2); 3.02 brs (6H, 2× N^+CH_3); 3.36–3.43 m (12H, N^+CH_2); 4.21 s (6H, N^+CH_3); 4.54 t (4H, N^+CH_2); 6.53 s (2H, CH); 7.33–8.58 m (18H, Ar)

Table 3
Characterization data for dyes **5a–5k**

Dye	m.p. (°C)	Yield (%)	λ_{max} nm (ε L mol^{-1} cm^{-1})	Molecular formulae (Mm)	Analysis	Found	
					Calc.		
					C	H	N
5a	206–208	60	522 (148 000)	$C_{25}H_{24}I_2N_4O$ (650.30)	46.21	3.69	–
					46.17	3.72	–
5b	239–241	70	521 (125 500)	$C_{27}H_{34}I_3N_5O{\cdot}H_2O$ (843.33)	38.27	4.55	8.80
					38.45	4.30	8.30
5c	223–225	79	522 (130 000)	$C_{28}H_{36}I_3N_5O{\cdot}EtOH{\cdot}1.5H_2O$ (1824.86)	39.28	5.03	8.07
					39.49	4.97	7.68
5d	234–236	75	522 (170 000)	$C_{29}H_{38}I_3N_5O$ (853.37)	40.72	4.89	8.60
					40.82	4.49	8.21
5e	170–172	50	522 (113 000)	$C_{28}H_{40}I_3N_5O$ (843.37)	39.60	4.61	8.11
					39.88	4.78	8.30
5f	169–171	50	522 (113 000)	$C_{29}H_{42}I_3N_5O{\cdot}2H_2O$ (893.43)	39.24	5.51	7.50
					38.99	5.19	7.84
5g	164–166	52	521 (106 000)	$C_{30}H_{44}I_3N_5O{\cdot}2H_2O$ (907.46)	39.90	5.49	7.44
					39.71	5.33	7.72
5h	180–182	54	521 (126 000)	$C_{36}H_{52}I_3N_5O$ (951.56)	45.38	5.46	7.26
					45.44	5.51	7.36
5i	185–187	60	522 (149 000)	$C_{37}H_{54}I_3N_5O{\cdot}3H_2O$ (1019.63)	43.72	6.20	6.95
					43.59	5.93	6.87
5j	190–192	65	522 (139 000)	$C_{38}H_{56}I_3N_5O$ (979.61)	–	–	7.53
					–	–	7.15
5k	215–218	77	522 (240 000)	$C_{49}H_{60}I_4N_8O_2{\cdot}6H_2O$ (1408.78)	40.84	5.39	7.96
					41.78	5.25	7.95
5l	220–222	75	522 (237 000)	$C_{57}H_{72}I_4N_8O_2{\cdot}6H_2O$ (1516.26)	45.38	5.94	7.67
					45.13	5.58	7.39

practically non-fluorescent (very low fluorescence quantum yield), but become strongly fluorescent after binding to dsDNA. For example, the fluorescence quantum yield of the complex **5g**–dsDNA is 0.45 (fluorescence maximum at 549 nm), compared to 0.01 for the dye itself. More detailed studies on the photo-physical properties of the described novel dyes **5a–5l** in the presence of nucleic acids are in progress.

4. Conclusions

Twelve novel di-, tri- and tetracationic monomeric and homodimeric monomethine cyanine dyes based on oxazolo[4,5-*b*]pyridinium and quinolinium end groups were synthesized using an improved synthetic procedure.

The reaction time was shorter (6 h) in comparison with the experiment reported earlier [14] (4 days).

All dyes have extremely high molar absorptivity of about 120 000 (monomeric dyes) and 240 000 L mol^{-1} cm^{-1} (homodimeric dyes).

The fluorescence quantum yield of the complex **5g**–dsDNA is 0.45 (fluorescence maximum at 549 nm), compared to 0.01 for the dye itself. This fact together with the stability of the formed complexes makes the investigated dyes appropriate for application in nucleic acids analysis.

References

[1] Haugland RP. Handbook of fluorescent probes and research chemicals. 9th ed. Eugene, OR: Molecular Probes; 2002.
[2] Yue S, Haugland RP. US patent 5 410 030; 1995.
[3] Yue S, Johnson I, Haugland RP. US patent 5 582 977; 1996.
[4] Rye HS, Yue S, Wemmer DE, Quesada MA, Haugland RP, Mathiesa RA, et al. Nucleic Acids Res 1992;20:2803–12.
[5] Benson SC, Singh P, Glazer AN. Nucleic Acids Res 1993;21:5727–35.
[6] Benson SC, Mathies RA, Glazer AN. Nucleic Acids Res 1993;21:5720–6.
[7] Benson SC, Zheng Z, Glazer AN. Anal Biochem 1995;231(1): 247–55.
[8] Deligeorgiev TG, Timcheva I, Maximova V, Gadjev N, Drexhage KH. J Fluoresc 2002;12(2):225–9.
[9] Deligeorgiev TG, Gadjev N, Timcheva I, Maximova V, Katerinopoulos HE, Foukaraki E. Dyes Pigments 2000;44:131–6.
[10] Gadjev N, Deligeorgiev TG, Kim SH. Dyes Pigments 1999;40:181–6.
[11] Deligeorgiev TG, Gadjev N. Dyes Pigments 1995;29(4): 315–22.
[12] Timcheva I, Maximova V, Deligeorgiev T, Gadjev N, Drexhage KH, Petkova I. J Photochem Photobiol B Biol 2000;58(2–3):130–5.
[13] Gadjev N, Deligeorgiev T, Timcheva I, Maximova V. Dyes Pigments 2003;57:161–4.
[14] Haugland RP, Yue S. WO 00/66664; 2000.
[15] Davidkov K, Simov D. Khim Geterotsikl Soedin 1981;50:608–10.
[16] Brooker LGS, Keyes GH, Williams WW. J Am Chem Soc 1942;64:199.
[17] Varbanova S. Nauch Trud Vissh Veterinarnomed Inst 1973;23:211 [in Bulgarian].
[18] Zander C, Drexhage KH. Adv Photochem 1995;20:59–78.