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Synthesis of novel monomeric asymmetric tri- and tetracationic monomethine cyanine dyes as fluorescent non-covalent nucleic acid labels

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Abstract

Several novel tricationic and one tetracationic monomethine cyanine dyes belonging to the Thiazole Orange family have been prepared via an improved synthetic procedure. The dyes, useful for nucleic acid detection, bear trimethylamonium, triethylamonium, 1-methylpyrrolidinium, 4-methylmorpholinium, pyridinium, *N*,*N*-dimethylpyridinium and 3-(dimethylamonio)propan-1-ol substituents. All derivatives absorb in the region 501–514 nm and have a molar absorptivity of 62 000–93 000 l mol^{-1} cm^{-1}. The reaction products were characterized by ^{1}H NMR spectroscopy and elemental analysis.

Keywords: Monomeric; Cationic monomethine cyanine dyes; Nucleic acids

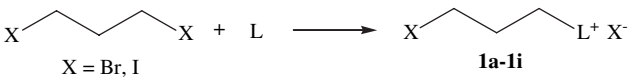
1. Introduction

Studies on binding of organic dyes with DNA are very important for designing of novel and more efficient drugs targeted to DNA [1,2], for exploring the biological function of nucleic acids and the interaction mechanism of some drugs. Therefore, in the recent decades the investigations of the reaction mechanism between small molecules and nucleic acids and the development of rapid and convenient assays for nucleic acids are an active area in bio-analytical chemical research [3,4]. The growing scientific and commercial interest in the field of synthesis and application of cyanine dyes as bio-probes [5–8] as well as our investigations in this area [9–14] has prompted us to search for new cyanine dyes as nucleic acid labels.

2. Result and discussion

Since the discovery of Lee and co-workers [15,16] that an old photographic dye (they called it Thiazole Orange – TO) is an excellent non-covalent nucleic acid probe, many new dyes of this class have been synthesized and investigated [17,18].

TO and related dyes can be synthesized according to the method of Brooker et al. [19,20] by the reaction of 2-methylthiobenzothiazolium salts with 1-alkyl-4-methylquinolinium salts. The disadvantages of this method are the evolution of toxic methylmercaptan and especially the possibility of interchange of the alkyl groups at the alkylthio group and nitrogen atoms in the quaternized 2-alkylthio starting materials, which leads to unexpected reaction products [20,21]. To avoid the aforementioned disadvantages we used an improved



Scheme 1.

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Table 1
Chemical structures of compounds **1a–1i**

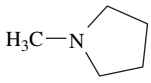
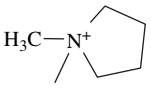
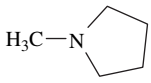
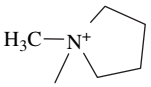
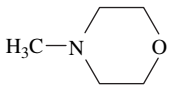
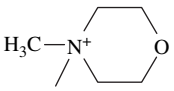
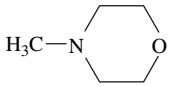
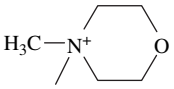
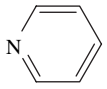
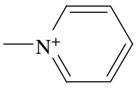
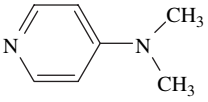
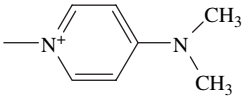
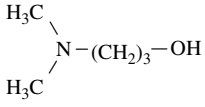
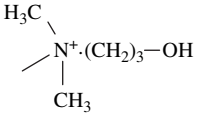
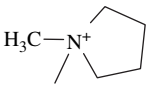
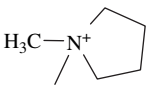
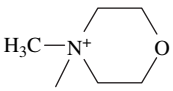
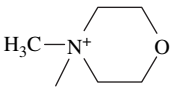
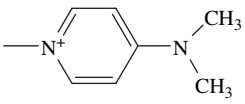
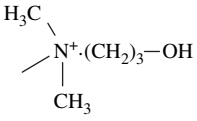
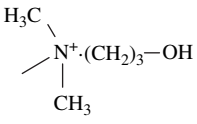
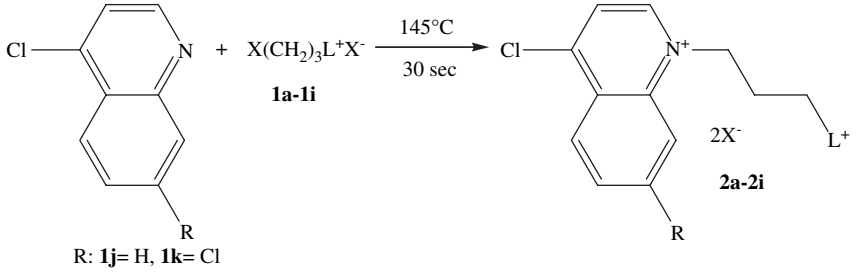
Compound no.	L	L^+	X
1a	Trimethylamine	$-N^+(CH_3)_3$	I
1b	Triethylamine	$-N^+(CH_2CH_3)_3$	Br
1c			Br
1d			I
1e			Br
1f			I
1g			I
1h			Br
1i			Br

Table 2
Chemical structures of compounds **2a–2i**

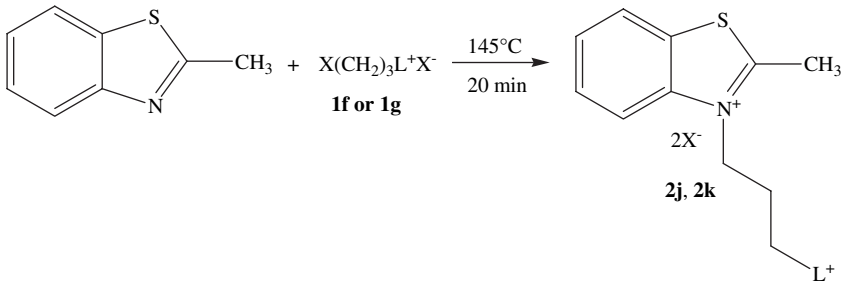
Compound no.	Starting compound no.	R	L^+	X
2a	**1a**, **1j**	H	$-N^+(CH_3)_3$	Br
2b	**1b**, **1j**	H	$-N^+(CH_2CH_3)_3$	Br
2c	**1c**, **1k**	Cl		Br
2d	**1d**, **1k**	Cl		I
2e	**1e**, **1j**	H		Br
2f	**1e**, **1k**	Cl		Br
2g	**1h**, **1j**	H		Br
2h	**1i**, **1j**	H		Br
2i	**1i**, **1k**	Cl		Br

procedure. The original method has been patented [22] for the synthesis of asymmetric and symmetric monomethine cyanine dyes. It involved condensation of quaternized 2-chloro-heterocycles with quaternized 2- or 4-methyl-heterocyclic compounds in the presence of a basic agent such as triethylamine. The method does not involve the evolution of any strong pollutant and offers more synthetic possibilities.

The dye synthesis requires some known and novel intermediates to be prepared. Thus 1,3-dibromopropane and 1,3-diiodopropane were quaternized with trimethylamine, triethylamine, 1-methylpyrrolidine, 4-methylmorpholine, pyridine, *N*,*N*-dimethylpyridine and 3-(dimethylamino)propan-1-ol to give quaternary salts **1a–i** (Scheme 1 and Table 1).



Scheme 2.



Scheme 3.

4-Chloroquinoline [23] and 4,7-dichloroquinoline were quaternized in solvent-free conditions for extremely short reaction time (30 s) with the quaternary salts **1a–i** to give intermediates **2a–i** (Scheme 2 and Table 2).

Intermediates **1i**, **2h** and **2i** are new and their structures were confirmed on the appropriate dyes.

The intermediates **2j** and **2k** were prepared according our earlier described procedure [14] by quaternization of 2-methylbenzothiazole with the quaternary salts **1f** or **1g** (Scheme 3 and Table 3). Compounds **2a–k** were synthesized according to the principles of green chemistry [24], under solvent-free conditions, with very high yields (90–98%), without undesired side reaction products and for extremely short reaction time.

The tricationic dyes **3a–h** (Scheme 4 and Table 4) were synthesized by the condensation of 2-methylbenzothiazolium salts **1f** or **1g** with 4-chloroquinolinium derivatives **2a–i** in the presence of the sterically hindered Hünig's base (*N*-ethyldiisopropylamine) in mild conditions (room temperature) and for relatively short reaction time (2 h), without evolution of any toxic pollutants.

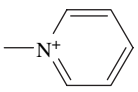
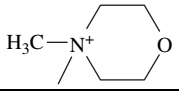
The dicationic dye **4b** [14] is the intermediate for the preparation of a four positive charged (tetracationic) dye **5a**. It was synthesized by the condensation of 2-methylbenzothiazolium and 4-chloroquinolinium quaternary salts **2j** and **4a** in the presence of *N*-ethyldiisopropylamine (Scheme 5).

In our previous work [14] we demonstrated that increasing of the positive charges in the dye molecule leads to increasing of the affinity of dyes to dsDNA and enhancing of the fluorescence of the complex dye–dsDNA. On the other hand, it is well known [25] that the successful preparation and purification of quaternary nitrogen-containing polycationic molecules is very difficult due to their instability because of undesired side reactions like Hofmann elimination for instance. Therefore we think that the successful preparation and purification of the monomeric tri- and tetracationic monomethine cyanine dyes **3a–h** and **5a** is a substantial advantage of this work. Dye **5a** was prepared by the quaternization of dye **4b** [14] and monoquaternized 1,4-diazabicyclo[2.2.2]octane (DABCO) in methoxyethanol as a solvent (Scheme 6).

All dyes **3a–h** and **5a** are new and their structures were proven by ^{1}H NMR spectroscopy (Table 5) and elemental analysis (Table 6).

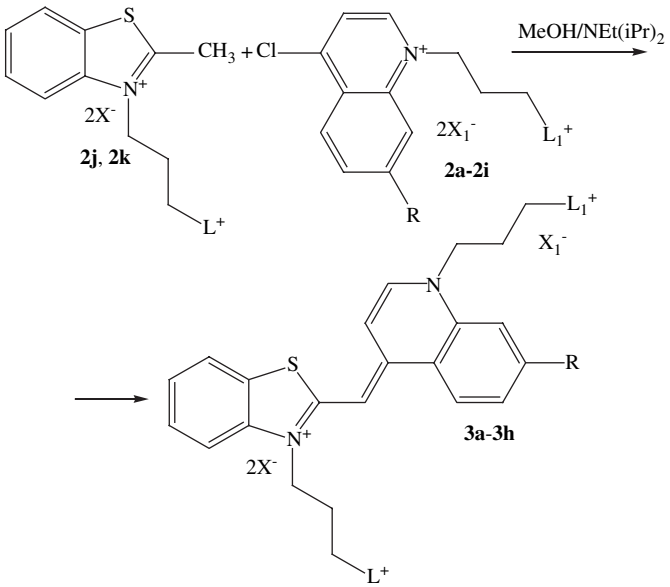
The longest wavelength absorption maxima of the studied dyes (Table 6) are in the region 501–514 nm. The corresponding molar absorptivities are high with values between 62 000 and 93 000 l mol^{-1} cm^{-1}. The dyes are practically non-fluorescent, but become strongly fluorescent after binding to dsDNA. More detailed studies on the photo-physical properties of dyes **3a–h** and **5a** in the presence of nucleic acids are in progress and will be published elsewhere.

Table 3
Chemical structures of compounds **2j**, **2k**

Compound no.	L^+	X
2j		I
2k		I

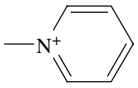
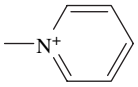
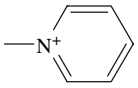
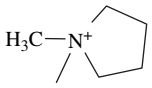
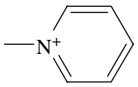
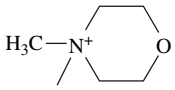
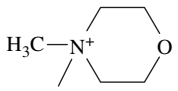
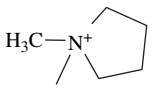
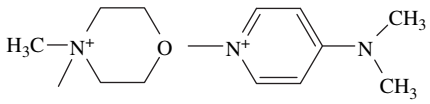
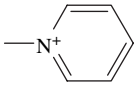
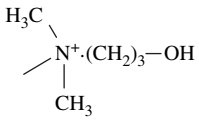
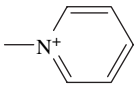
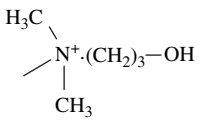
3. Experimental part

Melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Bruker



Scheme 4.

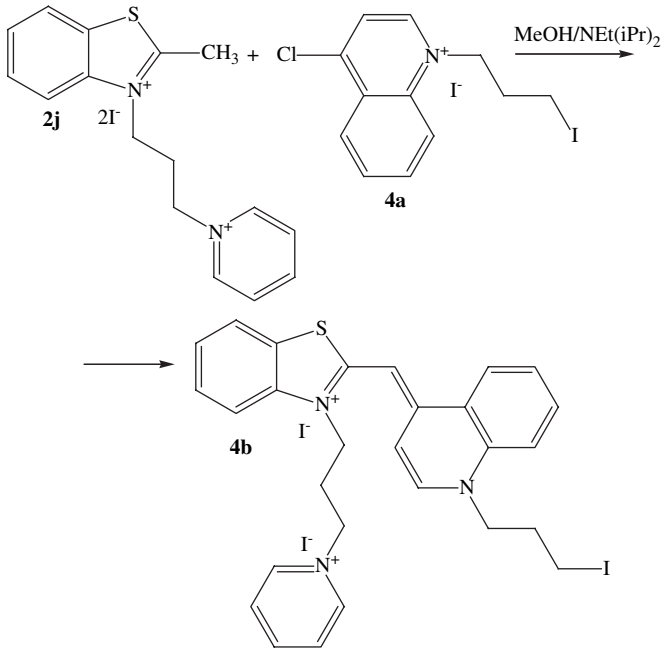
Table 4
Chemical structures of dyes **3a–3h**

Dye no.	Starting compound no.	L^+	L_1^+	R	X/X_1
3a	**2a**, **2j**		$-N^+(CH_3)_3$	H	I/I
3b	**2b**, **2j**		$-N^+(CH_2CH_3)_3$	H	I/Br
3c	**2c**, **2j**			Cl	I/Br
3d	**2e**, **2j**			H	I/Br
3e	**2d**, **2k**			Cl	I/I
3f	**2g**, **2k**			H	I/Br
3g	**2h**, **2j**			H	I/Br
3h	**2i**, **2j**			Cl	I/Br

250 MHz instrument in DMSO-d_6. Absorption spectra were scanned on a Unicam 530 UV–vis spectrophotometer (1×10^{-5} mol/l in MeOH). 1,3-Diiodopropane, 1,3-dibromopropane, 4-methylmorpholine, 1-methylpyrrolidine, *N*-ethyldiizopropylamine, 4,7-dichloroquinoline, *N*,*N*-dimethylpyridine, pyridine, 2-methylbenzothiazole and 1,4-diazabicyclo[2.2.2] octane (DABCO) are commercial products.

3.1. Synthesis of intermediates *1a–i*

The appropriate nitrogen-containing base (trimethylamine, triethylamine, 1-methylpyrrolidine, 4-methylmorpholine, pyridine, *N*,*N*-dimethylpyridine and 3-(dimethylamino)propan-1-ol)

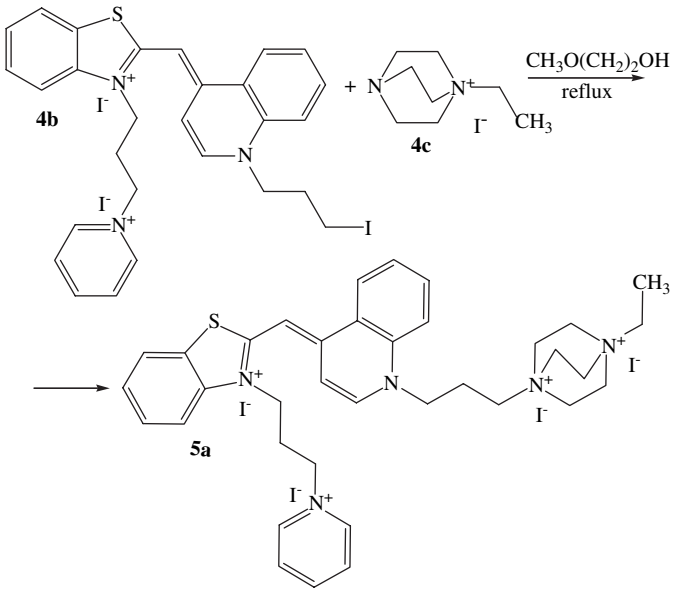


Scheme 5.

(0.01 mol), 0.04 mol 1,3-dibromopropane or 1,3-diiodopropane and 30 ml acetone were mixed in reaction vessel and vigorously stirred for 20 min. The reaction mixture was left for 7 days at room temperature in the dark. The formed precipitate was suction filtered and air dried. The yields were quantitative.

3.2. Synthesis of intermediates *2a–i* [14]

Intermediates **1a–i** (0.01 mol) and 0.015 mol 4-chloroquinoline or 4,7-dichloroquinoline were mixed and heated together in a reaction vessel to 140–145 °C for 30 s. After



Scheme 6.

Table 5
^{1}H NMR spectra and yields (%) of dyes **3a–h** and **5a**

Dye no.	Yield (%)	^{1}H NMR (DMSO-d_6, δ (ppm))
3a	56	2.49–2.55 m (4H, CH_2); 3.40 br s (9H, N^+CH_3); 4.96–5.07 m (8H, N^+CH_2); 6.90 s (1H, CH); 7.51–9.22 m (15H, Ar)
3b	72	1.22 t (9H, CH_3); 2.24 br s (2H, CH_2); 2.54 br s (2H, CH_2); 3.24–3.33 q (6H, N^+CH_2); 3.43 t (2H, N^+CH_2); 4.73 t (2H, N^+CH_2); 4.79 t (2H, N^+CH_2); 4.96 t (2H, N^+CH_2); 6.93 s (1H, CH); 7.42–9.18 m (15H, Ar)
3c	67	2.12 br s (4H, $2 \times CH_2$); 2.34 br s (2H, CH_2); 2.50–2.52 m (DMSO + CH_2); 3.05 s (3H, N^+CH_3); 3.55 br s (6H, $3 \times N^+CH_2$); 4.85 t (2H, N^+CH_2); 4.96 br s (2H, N^+CH_2); 5.02 t (2H, N^+CH_2); 6.90 s (1H, CH); 7.37–9.22 m (14H, Ar)
3d	74	2.36 br s (2H, CH_2); 2.54 br s (2H, CH_2); 3.18 s (3H, N^+CH_3); 3.48 t (4H, CH_2O); 3.77 t (2H, N^+CH_2); 3.91–4.04 m (4H, N^+CH_2); 4.70 t (2H, N^+CH_2); 4.83 t (2H, N^+CH_2); 4.95 t (2H, N^+CH_2); 6.94 s (1H, CH); 7.46–9.20 m (15H, Ar)
3e	58	2.12 br s (4H, CH_2); 2.32 br s (4H, CH_2); 3.04 s (3H, N^+CH_3); 3.19 s (4H, OCH_2); 3.53 br s (6H, N^+CH_2); 3.96–4.86 m (5H, $N^+CH_3 + N^+CH_2$); 4.62 br s (2H, N^+CH_2); 4.76 br s (2H, N^+CH_2); 6.89 s (1H, CH); 7.27–8.92 m (9H, Ar)
3f	45	2.27 br s (2H, CH_2); 2.43 m (2H, CH_2); 3.15 br s (4H, OCH_2); 3.17 s (6H, NCH_3); 3.35–3.48 m (4H, N^+CH_2); 3.98 t (2H, N^+CH_2); 4.41 t (4H, N^+CH_2); 4.72 t (4H, N^+CH_2); 6.95–8.90 m (15H, CH + Ar)
3g	43	2.56–2.58 m (DMSO + CH_2); 4.21 s (6H, $N^+(CH_3)_2$); 4.75–4.95 m (10H, N^+CH_2); 6.89 s (1H, CH); 7.42–9.15 m (15H, Ar)
3h	59	2.49–2.55 m (DMSO + CH_2); 3.07 s (1H, OH); 4.15 s (6H, N^+CH_3); 4.78–4.83 m (4H, N^+CH_2); 4.90–5.01 m (6H, $N^+(CH_3)_2$); 6.86 s (1H, CH); 7.35–9.16 m (14H, Ar)
5a	51	2.10 t (3H, CH_3); 2.39 br s (2H, CH_2); 2.57 br s (2H, CH_2); 3.58–3.62 q (2H, N^+CH_2); 3.89–3.92 m (12H, N^+CH_2); 4.71–4.95 m (8H, N^+CH_2); 6.94 s (1H, CH); 7.45–9.18 m (15H, Ar)

cooling to room temperature was added 30 ml acetone. The formed precipitate was suction filtered and dried in a desiccator.

3.3. Synthesis of intermediates *2j* and *2k* [14]

2-Methylbenzothiazol (0.015 mol) and 0.01 mol **1f** or **1g** were mixed and heated at 140–145 °C for 20 min. After cooling to room temperature, 30 ml acetone was added. The formed precipitate was suction filtered and air dried. Yields: **2j** (89%), **2k** (74%).

3.4. Synthesis of tricationic dyes *3a–h*

Intermediates **2j** or **2k** (0.002 mol) and 0.0022 mol **2a–i** were grounded together and suspended in 15 ml methanol. *N*-Ethyldiisopropylamine (0.004 mol) was added dropwise for 1–2 min and the reaction mixture was vigorously stirred at room temperature for 2 h. The formed red precipitate was suction filtered and air dried. The analytical samples were obtained after two recrystallizations in ethanol.

3.5. Synthesis of tetracationic dye *5a*

Dye **4b** (0.82 g, 0.001 mol) [14], 0.29 g (0.0011 mol) **4c** and 10 ml methoxyethanol were mixed in 50 ml round bottom flask, equipped with mechanical stirrer and reflux condenser. The reaction mixture was vigorously stirred and refluxed for 5 h. After cooling to room temperature the precipitated dye was suction filtered, washed with ethanol and air dried. The analytical sample was prepared after three recrystallizations from ethanol.

4. Conclusions

Nine novel tri- and tetracationic monomeric monomethine cyanine dyes, based on the Thiazole Orange chromophore were successively synthesized in good to excellent yields using an improved synthetic procedure and purified by fractional recrystallization.

The corresponding molar absorptivities at 501–514 nm are high with values between 62 000 and 93 000 l mol^{-1} cm^{-1}. The dyes are practically non-fluorescent, but become strongly fluorescent after binding to dsDNA. The investigations of the

Table 6
Spectral characteristics, melting points and elemental analysis of dyes **3a–h** and **5a**

Dye no.	λ_{max} (nm) (ε l mol^{-1} cm^{-1})	Mp (°C)	Molecular formulae (M_w)	Analysis (%) calcd./found	
				C	H
3a	502 (55 600)	>260 dec	$C_{31}H_{37}I_3N_4S$ (878.43)	42.39/42.70	4.25/4.46
3b	507 (74 900)	220–221	$C_{34}H_{43}BrI_2N_4S$ (873.51)	46.75/46.97	4.96/4.85
3c	501 (73 600)	256–258	$C_{33}H_{38}BrClI_2N_4S$ (891.91)	44.44/44.61	4.29/4.37
3d	507 (75 300)	229–230	$C_{33}H_{39}BrI_2N_4OS \cdot 4H_2O$ (877.65)	41.92/42.40	5.01/4.84
3e	514 (87 300)	244–246	$C_{33}H_{44}ClI_3N_4OS$ (960.96)	41.25/41.17	4.62/4.82
3f	508 (76 000)	190–191	$C_{35}H_{44}BrI_2N_5OS$ (916.53)	45.87/45.78	4.84/4.78
3g	501 (76 700)	196–198	$C_{33}H_{41}BrI_2N_4OS$ (875.48)	45.27/45.38	4.27/4.47
3h	508 (93 200)	276–278	$C_{33}H_{40}BrClI_2N_4OS$ (909.93)	43.56/43.67	4.43/4.05
5a	506 (62 600)	244–246	$C_{36}H_{45}I_4N_5S$ (1087.46)	39.80/40.48	4.17/4.67

dec, decomposition.

dyes as nucleic acid probes are in progress and the results will be reported elsewhere.

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